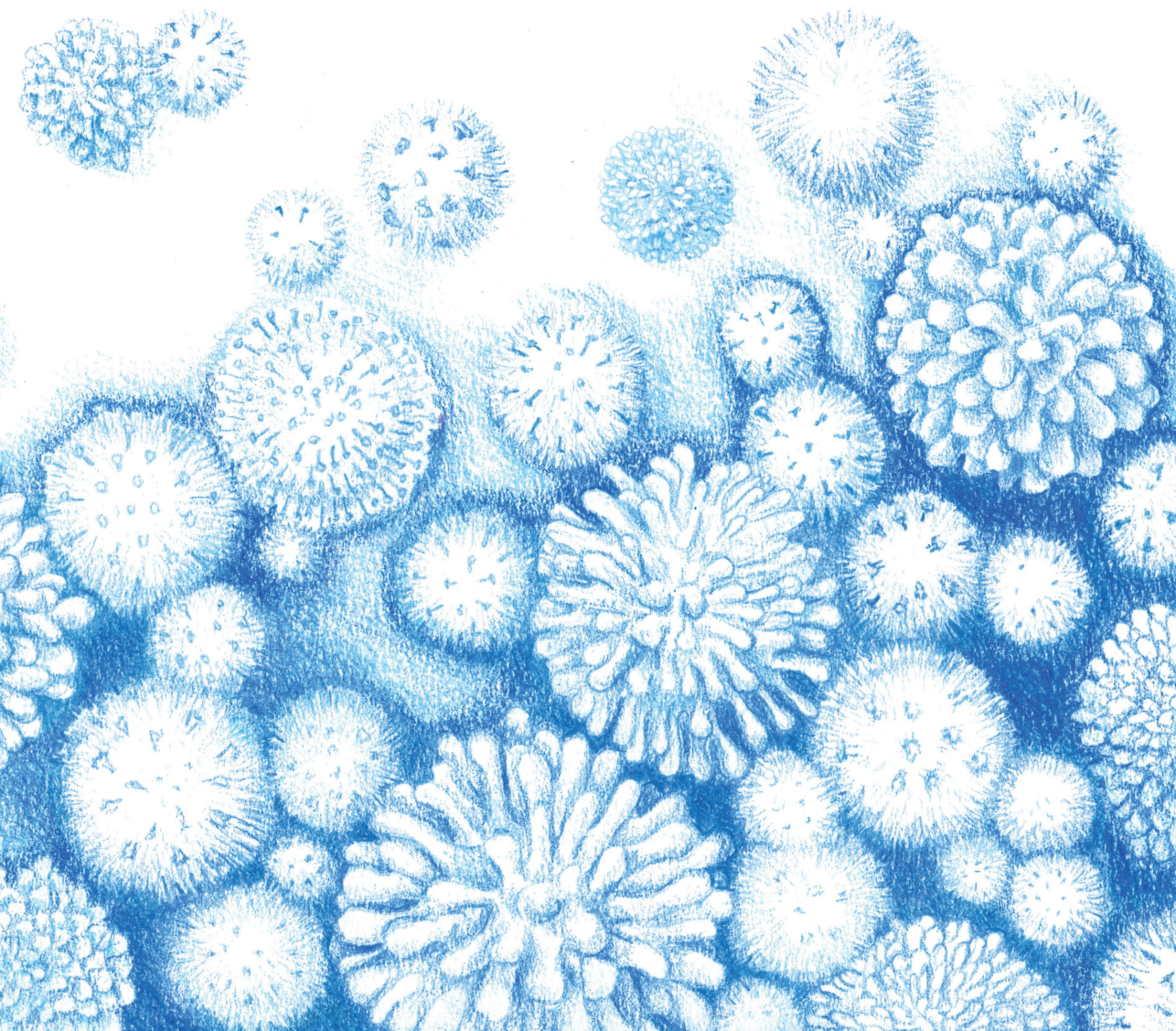
A PHARMACIST'S CONTRIBUTION TO ERADICATE HEPATITIS C

Elise J. Smolders



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Promotoren: Prof. dr. D.M. Burger
Prof. dr. J.P.H. Drenth
Copromotor: Dr. C.T.M.M. de Kanter (Prinses Máxima Centrum voor kinderoncologie)

Manuscriptcommissie:
Prof. dr. R. van Crevel
Prof. dr. R.A. de Man (Erasmus Medisch Centrum)
Dr. M. Van Luin (Rijnstate Ziekenhuis)

Paranimfen:
Drs. G.E. Benoist
Drs. P.D.J. Bollen
Drs. K. van Steeg

Voor oma

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General introduction

INTRODUCTION TO HEPATITIS C VIRUS INFECTIONS

Hepatitis C virus (HCV) infections have a major impact on global healthcare, since it is estimated that 130-150 million people are infected with HCV worldwide. The major long-term complication caused by the virus is liver cirrhosis, which can eventually lead to hepatocellular carcinoma (HCC), liver transplantation, or death[1].

HCV is a positive, single stranded RNA virus, which replicates in the hepatocyte. Around 10^{10} to 10^{12} HCV particles can be generated per day and these particles remain available in the body for 2-3 hours. There is a high occurrence of resistant-associated substitutions (RAS), which appear naturally or are caused by drug selective pressure. These RAS are caused by the high replication rate and the low fidelity of the HCV polymerase (prone to error)[2].

HCV is transmitted through blood-blood contact and only transferred among humans. The main routes for transmission are intravenous drug use or high risk sexual activity[3]. Other routes are mother-to-child transmission, medical procedures (e.g. dialysis), and transfusion of unscreened blood and blood products[1]. After infection, there is an acute phase in which approximately 20% of infected patients clear the virus. The other 80% of patients becomes chronically infected[3].

HCV causes permanent damage to the liver, which can eventually lead to cirrhosis. Development of cirrhosis is a slow process. Around 15-30% of patients develops cirrhosis within 20 years of infection[1]. Compensated cirrhosis is an asymptomatic phase in which there is already function loss of the liver (Child-Pugh [CP] class A). This can transfer to a state of decompensated cirrhosis (CP-B/C or Model for End-Stage Liver Disease [MELD] score >10) which includes complications such as ascites, hepatic encephalopathy, and portal hypertension[4, 5] (Figure 1). Decompensated patients with a MELD score >15 have an indication for liver transplantation in the Netherlands[6]. Lastly, cirrhosis caused by a viral infection is a major risk factor for development of HCC[5].

Aside from hepatic disease, HCV patients can suffer from several extra hepatic complications, such as insulin resistance (Type-2 diabetes mellitus [T2DM]), renal diseases (mixed cryoglobulinemia), depression, and/or cardiovascular events[7, 8] (Figure 1).

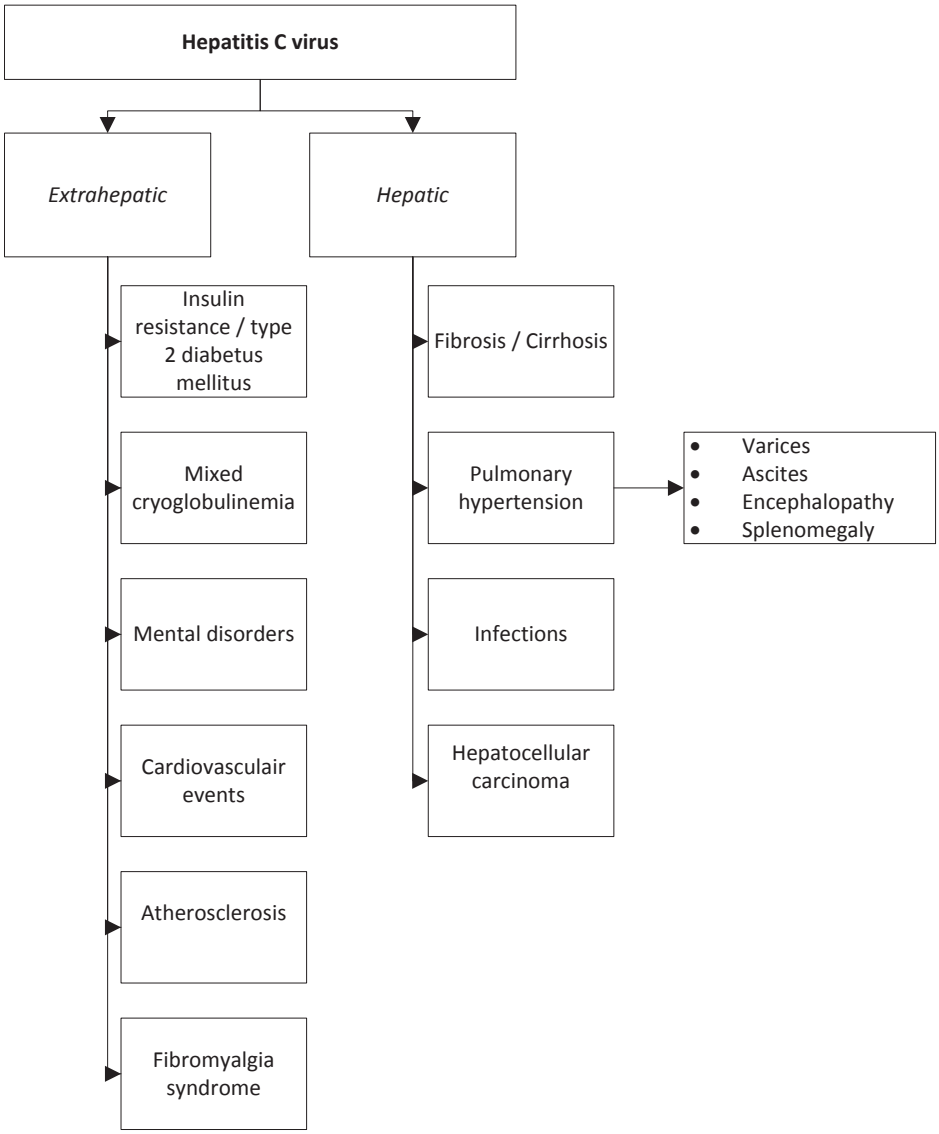

Figure 1: Overview of hepatic and extra hepatic complications of HCV infection. Data adapted from Negro[7] and Moller et al[4].

TREATMENT OF THE HEPATITIS C VIRUS

The goal of HCV treatment is to eradicate the virus and therefore preventing progression of liver disease and its complications. If the patient already has cirrhosis the progression to a worse state of disease is attempted to be prevented. Clearance of the virus 12 weeks after completion of treatment is called sustained virological response (SVR12) and is the primary outcome measure in HCV treatment.

Treatment options and efficacy of HCV treatment has rapidly changed over the last years. As shown in Figure 2, a major increase in patients reaching SVR12 was accomplished with the introduction of telaprevir and boceprevir[9]. Telaprevir and boceprevir were used in combination with peg-interferon alfa 2a/2b (subcutaneous injection) and ribavirin.

Telaprevir and boceprevir are oral drugs that directly target HCV and are therefore called direct-acting antivirals (DAAs). From 2014 onwards, more oral DAAs have become available with even higher response rates, shorter treatment duration (8-12 weeks instead of 24-48 weeks), and better safety profiles[10]. To date, the drugs listed in Table 1 are licensed in Europe[11-15] and the United States[16, 17].

Table 1: Overview of the available DAAs in 2017.

Class	Drug name
Protease inhibitors	Telaprevir[a], boceprevir, simeprevir, grazoprevir, paritaprevir/ritonavir
Nonstructural protein 5A (NS5A) inhibitors	Daclatasvir, ledipasvir, elbasvir, velpatasvir, ombitasvir
Nonstructural protein 5B (NS5B) inhibitors	Sofosbuvir, dasabuvir

[a]Telaprevir was withdrawn from the market by the manufacturer in 2015.

In general, patients are treated with at least two DAAs. The options are 1) an NS5B polymerase inhibitor in combination with an NS5A inhibitor and/or a protease inhibitor or 2) a protease inhibitor with an NS5A inhibitor (Figure 2, x-axis)[18].

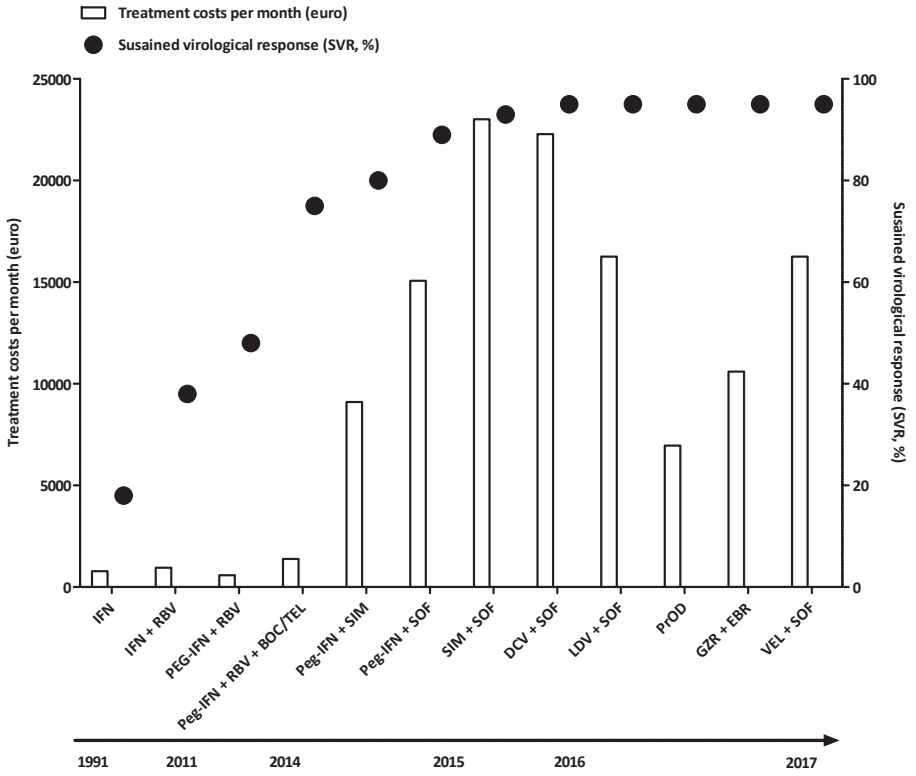

Figure 2: Overview of the various treatment regimens for chronic HCV genotype 1 infection, SVR-rates, and costs per month (based on genotype 1).

Data adapted from EASL guidelines 2016[18] and http://www.medicijnkosten.nl (April 2017).

IFN: Interferon; Peg-IFN: Peg-interferon alfa 2a or 2b; RBV: Ribavirin; BOC/TEL: Boceprevir or telaprevir; SOF: Sofosbuvir; SIM: Simeprevir; DCV: Daclatasvir; LDV: Ledipasvir; PrOD: Paritaprevir/ritonavir, ombitasvir, and dasabuvir; GZR: Grazoprevir; EBR: Elbasvir; VEL: Velpatasvir.

SELECTING THE RIGHT COMBINATION OF DIRECT-ACTING ANTIVIRALS

We could state that all combinations of DAAs are equally effective and in real-life SVR-rates >90% can be reached[19-21]. However, for selection of the right DAA regimen, the physician must deal with several aspects of DAA treatment. First, it is necessary to determine the genotype of the virus. There are 6 genotypes and various numbers of subtypes (a, b, c, etc.)[2] and only a few approved drugs are effective against all genotypes (pan-genotypic). For example, dasabuvir can only be used in genotype 1 patients and velpatasvir is considered pan-genotypic[13, 17].

Secondly, the physician must be informed whether the patient is treatment-experienced and/or has cirrhosis. Both these characteristics influence the chance of reaching SVR. When treating these patients, it is recommended to prolong treatment from 12 to 24 weeks or to add ribavirin (12 weeks of treatment). Of course, other patient characteristics such as renal function and co-morbidities (hemophilia) must also be taken into account when selecting a regimen[18].

Another aspect that complicates treatment are (baseline) RAS which could influence treatment-outcome[22]. RAS testing could help us with selecting the right regimen, deciding on treatment duration, and adding ribavirin yes or no. However, the exact role in daily practice is still under debate as international guidelines give conflicting recommendations[18, 23].

The last aspect that I would like to discuss is the use of co-medication and the risk of drug-drug interactions (DDIs). These DDIs must be recognized and managed before initiation of DAA therapy, since drug-interactions could cause unnecessary toxicity or loss of efficacy[24, 25].

A final note is that novel DAA therapy is extremely expensive and that we are obliged to society to treat these patients effectively, both in terms of costs and treatment success (Figure 2).

OUTLINE OF THIS THESIS

As discussed, we have highly effective drugs, and if we use these drugs wisely we can probably eradicate HCV if the medical community is willing and able to detect all the infected individuals[26, 27]. However, there are still gaps in our knowledge when it comes to the optimal use of DAAs. Examples of knowledge gaps are: the use of DAAs in special patient populations, DAAs in combination with co-medication, and the use of ribavirin. As a pharmacist investigating the pharmacology of DAAs and ribavirin, I aimed to contribute to the optimization of HCV therapy and therefore eradication of the virus. The aim of this thesis was to *answer pharmacological issues concerning current HCV therapy including novel DAAs and ribavirin, which can be used for the optimization of HCV treatment*.

As a result, this thesis focuses on four major topics:

1) DDIs involving the DAAs. DAAs can both be victims and perpetrators of drug-interactions and these interactions are relatively new in the field of hepatology. In

addition, despite studies described in the drug label, there is still information missing about drug-interactions and DAAs.

2) Ribavirin pharmacokinetics and therapeutic drug monitoring (TDM). Ribavirin is still part of HCV therapy and the pharmacokinetics of ribavirin have not yet been studied with the novel DAAs.

3) Treatment of HCV with DAAs in special patient populations. With introduction of the novel DAAs, little information about special patients was available and therefore studied in this thesis.

4) A pharmacist's contribution to eradicate HCV is the last part of this thesis and describes five special patients where we illustrate that knowledge of clinical pharmacology is helpful to select the right treatment for individual patients.

Part 1: Drug-drug interactions involving direct-acting antivirals

DAAs are extensively metabolized by liver enzymes which makes them potential victims of drug-interactions. This means that the plasma concentration of the DAA can be affected by another drug (perpetrator, the cause of the drug-interaction). Altered drug levels could cause toxicity in case of increased drug levels, or loss of efficacy in case of decreased drug levels[24]. With respect to DAAs, lower exposure could mean that the HCV virus becomes resistant to the drug, probably leading to treatment failure and therefore harder to re-treat the infection in the future[22]. On the other hand, DAAs can also be perpetrators of DDIs themselves: they influence several drug-transporters and metabolizing enzymes altering plasma concentrations of co-medication[25].

Psychoactive drugs undergo extensive hepatic metabolism and are widely used in HCV-infected patients. Use of psychoactive drugs in this population is high, as there is a high prevalence for mental disorders[28-30]. This makes it reasonable that both psychoactive drugs and DAAs are combined in HCV patients with a risk for DDIs as a consequence. For the various DAAs little information about DDIs with psychoactive drugs is available and therefore, we aimed to give an overview of the available information about DDIs between psychoactive drugs and DAAs in **Chapter 1**.

When drugs are licensed by the regulatory agencies, drug labels become available. These labels contain information about pre-marketing studies concerning DDIs. This DDI information is derived from both *in vitro* and *in vivo* DDI studies. After release, the label is often the only source of DDI information for physicians and pharmacists. From a clinical, academic point of view, information in the drug label concerning DDIs is

discussed using two recently approved agents, i.e. grazoprevir plus elbasvir[16] and velpatasvir plus sofosbuvir[17] **(Chapter 2)**.

HCV and insulin resistance/type 2 diabetes mellitus are associated, so drugs for both diseases are commonly co-administered[31-34]. Daclatasvir is a DAA used for HCV treatment and metformin is the first drug of choice for T2DM. Metformin is not metabolized but a substrate of various drug-transporters, namely plasma membrane monoamine transporter (PMAT), organic cation transporter (OCT) 1, 2 and 3 and multidrug and toxin extrusion protein (MATE) 1 and 2K[35]. When co-administered with daclatasvir, there might be a DDI as daclatasvir inhibits OCT1/2, at least *in vitro*[14]. This interaction had not been studied *in vitro* nor *in vivo*. In **Chapter 3** we performed a randomized, cross-over study in healthy volunteers to determine the effect of daclatasvir on metformin pharmacokinetics. Our hypothesis was that daclatasvir would increase metformin exposure.

An HIV/HCV co-infection is commonly seen[36]. HIV is treated with antiretroviral therapy, which includes different classes of drugs such as integrase inhibitors, protease inhibitors, and (non)-nucleoside reverse transcriptase inhibitors. Atazanavir is a protease inhibitor that is combined with the CYP3A4 boosters ritonavir or cobicistat[37, 38]. Daclatasvir is substrate of CYP3A4 and in combination with atazanavir/ritonavir[39, 37] the dose of daclatasvir must be reduced with 50% due to CYP3A4 inhibition of atazanavir/ritonavir[40]. However, we do not know what the impact of atazanavir/cobicistat is on daclatasvir[41]. Therefore, we conducted a randomized, cross-over study in healthy volunteers to study whether atazanavir/ritonavir and atazanavir/cobicistat have comparable influence on the pharmacokinetics of daclatasvir **(Chapter 4)**.

As previously described, there is a genuine risk on DDIs when HCV patients, both mono- and co-infected, are treated with DAAs[42-44]. We studied these DDIs extensively and, on a daily basis, we help physicians managing these DDIs. However, we do not know what the actual risk is on DDIs in Dutch patients. We tried to answer this question retrospectively analyzing data from a nationwide, real-life mono-infected cohort **(Chapter 5)** and an HIV/HCV co-infected cohort **(Chapter 6)**. The question that follows, when our patients are at risk, what is the clinical relevance of these DDIs and are they handled accurately? This question is answered in **Chapter 7** using an HIV/HCV co-infected cohort.

Part 2: Ribavirin pharmacokinetics and therapeutic drug monitoring

The goal of TDM is to optimize a patient's clinical outcome by managing the drug dose with the assistance of measured plasma drug concentrations[45]. These concentrations are interpreted by pharmacists and dose advises are given.

To decide whether TDM is useful for a particular drug, the drug must meet certain criteria. For example, there must be an appropriate drug assay available, adequate pharmacokinetic data must be available, the drug has to have a narrow therapeutic range, and the drug should have a well-described concentration-effect relationship[46, 47]. Ribavirin meets these criteria and is therefore a possible candidate for TDM.

As said, an appropriate drug assay must be available. A number of analytical assays for ribavirin has been developed worldwide. To assure the quality of these assays, a quality control program was developed to evaluate the accuracy of these assays. In **Chapter 8** we present the first results of this worldwide quality control program involving ribavirin assays.

Another requirement for TDM is the need for a therapeutic range. The therapeutic range for ribavirin combined with peg-interferon and boceprevir/telaprevir was 2.2-3.6 mg/L[48]. With the introduction of the modern DAAs, the need arose to redefine a therapeutic range. It is unclear if we should use the range of 2.2-3.6 mg/L for ribavirin plasma concentrations when combined with, for instance, sofosbuvir plus simeprevir/daclatasvir/ledipasvir. Can we maybe strive to lower plasma concentrations as the new DAAs are highly active? This hypothesis is discussed in **Chapter 9** where we present the results of a Dutch cohort in which ribavirin concentrations are studied in combination with modern DAA therapy.

HIV/HCV co-infected patients usually have lower ribavirin plasma concentrations than mono-infected patients[49]. This was previously described in a Western cohort; however, this was never studied in Thai patients. Therefore, in collaboration with the HIV-NAT (HIV Netherlands, Australia, Thailand) research group a prospective study was conducted evaluating the current Thai treatment regimen of peg-interferon alfa plus ribavirin in HIV/HCV co-infected Thai patients. The results are presented in **Chapter 10**.

Part 3: Treatment of hepatitis C virus in special patient populations

Special patient populations are in this thesis defined as patients with a specific co-morbidity which could alter the pharmacokinetics of an agent. For these patients in depth knowledge about the clinical pharmacology of drugs is necessary. For example, hepatic or renal impairment could influence the clearance of drugs which are eliminated by the liver or kidney, respectively[50, 51]. Both conditions can be related to HCV infection[50, 52] and potentially influence pharmacokinetics of various DAAs. In **Chapter 11** we give an overview of data available in literature about the efficacy, safety, and pharmacokinetics of DAAs in context of these two special patient populations.

Physiology-based pharmacokinetic modeling (PBPK) is a technique where physicochemical properties and *in vitro* data of drugs are used to simulate absorption, distribution, metabolism, and excretion (ADME)[53, 54]. In other words, *in vitro* to *in vivo* extrapolation (IVIVE) is used to predict the pharmacokinetics of a drug in a defined population, for instance healthy volunteers or cirrhotic patients. An advantage of these computer models is that there is less need for full pharmacokinetic studies in human subjects.

It is known that cirrhotic patients have altered liver clearance, possibly affecting the pharmacokinetics of daclatasvir which is primarily hepatically metabolized[55]. A clinical study in cirrhotic subjects showed that patients receiving a single dose of daclatasvir had a decreased maximum plasma concentration compared with non-cirrhotic subjects, but based on the unaffected unbound daclatasvir concentration the authors concluded that no dose adjustment was necessary[56]. In **Chapter 12** we describe a PBPK model of daclatasvir in cirrhotic patients in which we aimed to simulate and confirm these *in vivo* findings. We used Simcyp®, a PBPK tool, that contains a model of physiological changes in cirrhotic patients[57, 55].

Part 4: A pharmacist's contribution to eradicate hepatitis C

The last part of the thesis consists of four case reports **(Chapter 13)**. These were all special patients in which we used our pharmacological knowledge to optimize treatment.

Finally, I finish with a general discussion on the various topics discussed in this thesis.

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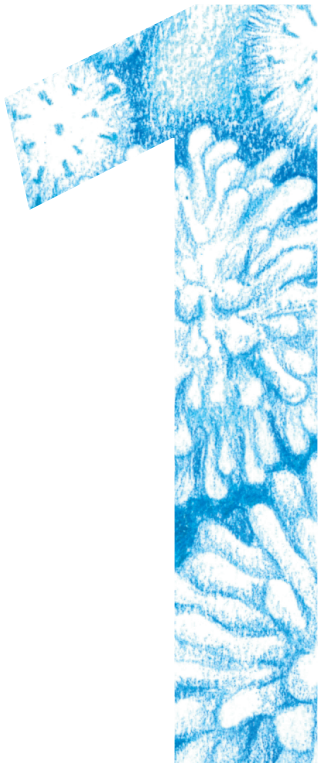
Part 1

Drug-drug interactions involving direct-acting antivirals

Drug-drug interactions between direct-acting antivirals and psychoactive medications

EJ Smolders
CTMM de Kanter
RJ de Knegt
M van der Valk
JPH Drenth
DM Burger



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ABSTRACT

Treatment options for chronic hepatitis C virus (HCV) infection have drastically changed since the development and licensing of new potent direct-acting antivirals (DAAs). The majority of DAAs are extensively metabolized by liver enzymes and have the ability to influence cytochrome P450 (CYP) enzymes. Additionally, these same DAAs are both substrates and inhibitors of drug-transporters, which makes the DAAs both possible victims or perpetrators of drug-drug interactions (DDIs). There is a high prevalence of mental illnesses such as depression or psychosis, in HCV-infected patients; therefore, psychoactive medications are frequently co-administered with DAAs. The majority of these psychoactive medications are also metabolized by CYP enzymes but remarkably little information is available on DDIs between psychoactive medications and DAAs. Hence, the aim of this review is to provide an overview of the interaction mechanisms between DAAs and psychoactive agents. In addition, we describe, evidenced-based interactions between DAAs and psychoactive drugs and identify safe options for the simultaneous treatment of mental illnesses and chronic HCV infection.

KEY POINTS

- Escitalopram and citalopram have been studied in combination with most direct-acting antivirals and either of these drugs can be safely combined with hepatitis C virus (HCV) treatment.
- No formal interaction studies between psychoactive agents and sofosbuvir or ledipasvir have been performed in humans. However, these DAAs are generally neither victims nor perpetrators of drug-interactions and can therefore be safely used in combination with psychoactive drugs.
- Boceprevir, simeprevir, and the combination paritaprevir/ritonavir plus ombitasvir with dasabuvir are most likely to cause drug-interactions via the inhibition of cytochrome P450 (CYP) 3A4. Therefore, caution must be exercised when CYP3A4 substrates such as midazolam and/or quetiapine are co-administered with these DAAs.

INTRODUCTION

One of the components previously used in the treatment regimen for hepatitis C virus (HCV) is peg-interferon; however, it has major adverse effects on mental health and depression was a commonly seen adverse event[1]. Since the development of novel direct-acting antivirals (DAAs), peg-interferon is no longer used in the treatment of HCV infections in resource-rich settings. However, the prevalence of mental disorders remains high among untreated HCV-infected patients[2]. For example, a retrospective study reported that 86% of HCV-infected patients had at least one psychiatric, drug-, or alcohol use-related disorder recorded in their patient charts. The most common conditions were depressive disorders (50%), psychosis (50%), anxiety disorders (41%), post-traumatic stress disorders (34%), and bipolar disorders (16%)[3]. Another study reported a prevalence of 41% for anxiety and 27% for depression in HCV-infected individuals (n = 395)[4]. One explanation for this high prevalence was that patients with mental disorders are more likely to have a drug addiction, because intravenous drug use is a major route of HCV transmission[5].

The results from a cross-sectional study were in agreement with the high prevalence of mental disorders. In that study, 16% of the HCV-infected patients were on antidepressants and 10% were on antipsychotics (n = 3,716)[6]. This corresponds with data from a Dutch nationwide survey in which benzodiazepines, drugs used for treating opioid dependence, and selective serotonin reuptake inhibitors (SSRIs) were among the drugs most frequently used by chronic HCV-infected patients[7].

The use of antipsychotics and antidepressants during DAA therapy increases the risk of drug-drug interactions (DDIs). Both DAAs and psychoactive agents are extensively metabolized in the liver and have the ability to affect the activities of various enzymes (e.g., cytochrome P450 [CYP]) and drug-transporters (e.g., P-glycoprotein [P-gp]). This makes DAAs as well as psychoactive agents possible victims (objects of DDIs) and perpetrators (causes of DDIs) of drug-interactions, which could negatively affect treatment outcomes as the result of adverse effects (increased plasma concentrations) or treatment failure (decreased plasma concentrations)[8, 9]. In order to interpret the DDIs between DAAs and psychoactive agents, it is important to have sufficient knowledge of their therapeutic ranges. Benzodiazepines, tricyclic antidepressants (TCAs), and antipsychotics have a narrow therapeutic range while SSRIs have a broad therapeutic range. Generally, drugs with narrow therapeutic ranges are more likely to have clinically relevant DDIs than drugs with large therapeutic ranges[10]. DAAs have a large therapeutic range, which makes them less susceptible to the effects of an increase or decrease

in their plasma concentrations caused by, for example, CYP inhibition or induction. However, extremely low plasma concentrations could lead to virologic failure.

Little information is available on interactions between DAAs and psychoactive agents. Therefore, the aim of this review is to provide an overview of the interaction mechanisms of DAAs and psychoactive agents. In addition, we describe evidenced-based interactions between DAAs and psychoactive drugs and identify safe options for treatment of the simultaneous treatment of mental illnesses and HCV infection.

METHODS

We searched PubMed (1946-January 2016) and EMBASE (1947-January 2016) to identify peer-reviewed studies. The search covered all DAAs recommended in European and US guidelines[11, 12] and licensed by the European Medicines Agency (EMA) and US Food and Drug Administration (FDA). The DAAs included protease inhibitors (PIs) (boceprevir, simeprevir, paritaprevir, and grazoprevir), NS5A inhibitors (daclatasvir, ledipasvir, ombitasvir, and elbasvir), and NS5B polymerase inhibitors (sofosbuvir and dasabuvir). Telaprevir was excluded from the review because it has limited use in current therapy. We also referred to the work published by Kiser and colleagues[13] for more information about telaprevir and DDIs between DAAs and psychoactive drugs.

The psychoactive agents included were SSRIs, TCAs, typical and atypical antipsychotics, benzodiazepines, monoamine oxidase inhibitors, lithium, and St John's wort.

The Google and Google Scholar search engines, and ClinicalTrials.gov (http://www.clinicaltrials.gov) website and the Liverpool drug-interaction database (http://www.hep-druginteractions.org) were used to identify conference papers and abstracts. All searches were performed in English. The search items contained generic and/or brand names of the drugs and included terms such as antidepressant, antipsychotic, sedative, and tranquilizer.

Information about the pharmacokinetics and metabolism of the DAAs and psychoactive agents were obtained from the Summary of Product Characteristics (SmPC) and FDA Prescribing Information for each drug as well as from the Lexicomp database (available via http://www.uptodate.com). Enzyme inhibitors and inducers were defined as being strong, moderate, or weak if they changed the area under the concentration-time curve (AUC) of a substrate by 5-fold, >2 to <5-fold, and <2-fold, respectively. Substrates were also grouped as being minor and major substrates of enzymes. These groupings were

based on the clinical relevance of the potential interaction described by Lexicomp (http://www.uptodate.com)[14, 15].

DRUG-DRUG INTERACTION MECHANISMS: DIRECT-ACTING ANTIVIRALS

In this section, we elaborate on the mechanisms by which DAAs can be perpetrators and/or victims of DDIs. We focused on interactions through hepatic drug-metabolism and drug-transporters because this are the most important pathways underlying interactions between DAAs and psychoactive agents. These mechanisms are illustrated using examples of drug-interactions between DAAs and psychoactive agents or non-psychoactive drugs, which were studied in healthy volunteers.

Tables 1 and 2 give an overview of the involvement of enzymes and drug-transporters in the metabolism of DAAs and psychoactive agents respectively. Table 3 shows the results of drug-interaction studies performed between DAAs and psychoactive drugs.

Phase I and II reactions: direct-acting antivirals as perpetrators

Drugs that influence drug-metabolizing enzymes (perpetrators) such as CYP and uridine 5'-diphospho-glucuronosyltransferase (UGT) have the ability to affect the plasma concentration of substrates of the enzymes (victims). Inhibitors of CYP and UGT generally cause an increased plasma concentration of the victim, while inducers usually lower the plasma concentration of the victim.

Ritonavir is included in the fixed-dose combination with paritaprevir, ombitasvir, and dasabuvir to 'boost' the pharmacokinetic characteristics of paritaprevir through the inhibition of CYP3A4; this opens the door for DDIs to occur. For example, the AUC and maximum plasma concentration (C_{max}) of orally administered midazolam, a CYP3A4 substrate, increased ~26-fold and ~4-fold, respectively, when midazolam was co-administered with ritonavir (note: the interaction between ritonavir and midazolam is studied in the absence of paritaprevir, ombitasvir, or dasabuvir)[16]. Increases in the plasma concentration of midazolam have also been observed when the drug was administered with boceprevir, simeprevir, and grazoprevir, which are also CYP3A4 inhibitors. Boceprevir is a strong inhibitor of CYP3A4, while simeprevir and grazoprevir are mild CYP3A4 inhibitors (Table 1)[17-19].

Table 1: Overview of the route of metabolism, effects on enzymes, and transporters of direct -acting antivirals.

Drug	Enzyme		Transporter		Comments	Reference
	Substrate	Inhibitor	Substrate	Inhibitor		
Protease inhibitors						
Boceprevir	AKR-mediated pathway CYP3A4/5	CYP3A4/5		P-gp (mild)		[17]
Simeprevir	CYP3A4	Intestinal CYP3A4 CYP1A2 (weak)		OATP1B1/3 P-gp		[18]
Paritaprevir	CYP3A4/5	UGT1A1	P-gp OATP1B1 BCRP	OATP1B1/3 OATP2B1 BRCP		[20]
Ritonavir	CYP3A4 CYP2D6 (lesser extend)	CYP3A4 CYP2D6 (?) Inducer CYP2C19 Inducer CYP1A		OATP2B1 OCT1 (?) BRCP		[20]
Grazoprevir	CYP3A4	CYP3A4 (mild)	OATP1B1 OATP1B3	BCRP		[19]
NS5A inhibitors						
Daclatasvir	CYP3A4		P-gp	P-gp OATP1B1 OCT1 BCRP		[22]
Ledipasvir	Metabolism unknown, unchanged ledipasvir is the major compound in feces		P-gp BCRP	P-gp BCRP		[23]
Elbasvir	CYP3A4			BCRP		[19]
Ombitasvir	Hydrolysis followed by oxidative metabolism	UGT1A1				[20]

Table 1: Overview of the route of metabolism, effects on enzymes, and transporters of direct -acting antivirals. *(continued)*

Drug	Enzyme		Transporter		Comments	Reference
	Substrate	Inhibitor	Substrate	Inhibitor		
NS5B polymerase inhibitors						
Sofosbuvir	Hepatic non-enzymatic metabolism Elimination renally		Sofosbuvir: P-gp Sofosbuvir: BCRP		Sofosbuvir is a nucleoside analog triphosphate	[24]
GS-331007			GS-331007 is not substrate of P-gp or BCRP		Sofosbuvir (prodrug) is metabolized in GS-461203 and GS-331007	
Dasabuvir	CYP2C8 CYP3A4	UGT1A1		BCRP	Main active metabolite is dasabuvir M1 which is formed by CYP2C8	[25]

Only drug-metabolizing enzymes and drug-transporters that are thought to be involved in clinically relevant drug-drug interactions, according to the Summary of Product Characteristics or Prescribing Label are given.

AKR: Aldoketoreductase; BCRP: Breast cancer resistance protein; CYP: Cytochrome P450; DAA: Direct-acting antiviral; OATP; Organic anion-transporting polypeptide; OCT: Organic cation transporter; P-gp: P-glycoprotein; UGT: Uridine 5'-diphospho-glucuronosyltransferase.

UGTs are inhibited by DAAs such as paritaprevir, ombitasvir, and dasabuvir. Lorazepam (benzodiazepine) is a UGT substrate; however, the interaction between lorazepam and DAAs has not has not been studied. Interaction studies between furosemide (UGT1A1 substrate) and combination regimen paritaprevir/ritonavir, ombitasvir, and dasabuvir have indicated the importance of UGT inhibition. Results from these studies showed that the AUC and C_{max} of furosemide were increased by 8 and 42%, respectively. As a result of this, a reduction in the dose of furosemide of up to 50% might be required if the drugs have to be co-administered[20].

Phase I and II reactions: direct-acting antivirals as victims

DAAs, e.g., daclatasvir, simeprevir, grazoprevir, and elbasvir are predominantly metabolized by CYP3A4/5 in the liver and gastrointestinal tract. Thus, caution is needed when DAAs are administered with strong inducers or inhibitors of CYP3A4. A reduced plasma concentration of DAAs creates a potential risk of resistance of the virus to the administered drug and/or virologic failure, while elevated drug concentrations increase the risk of adverse events. Most psychoactive agents do not strongly inhibit or induce CYP3A4 and thus, we do not expect DAAs to be victims of psychoactive agents. St John's wort, which is a psychoactive agent, is the exception; it is a strong CYP3A4 inducer. However, co-administration of boceprevir and St John's wort showed only a slight decrease in the plasma concentration of boceprevir (9%)[21].

DAAs are not metabolized by UGT or other phase-II enzymes; therefore, phase-II mediated DDIs with DAAs as victims are not usually expected.

Drug-transporters: direct-acting antivirals as perpetrators

DAAs inhibit various drug-transporters such as efflux transporters P-gp and breast cancer resistance protein (BCRP) (Table 1), which are, among other, located at the blood-brain barrier (BBB). Little information is available on DDIs between psychoactive agents and drug-transporters. However, it is known that amitriptyline and risperidone are P-gp substrates (Table 2). Accordingly, inhibition of these transporters increases substrate concentrations in the cerebrospinal fluid[26]. Since the pharmacological action of psychoactive drugs takes place in the brain, inhibition of P-gp can result in an increased pharmacological effect.

No formal interaction studies have been conducted between the P-gp substrates and DAAs. The effect of P-gp inhibition by DAAs has been studied using digoxin, which is a known P-gp substrate. Boceprevir had a minor influence on plasma digoxin concentrations (an increase in AUC and C_{max} by 19 and 18%, respectively)[17]. Daclatasvir and simeprevir also affected digoxin plasma concentrations; the AUC of digoxin was

increased by 27 and 39%, and C_{max} was increased by 65 and 31%, respectively[18, 22]. It should be noted that these interactions between the P-gp inhibitors and digoxin were driven by the concentration of digoxin in the intestinal lumen, which is high after oral intake. For psychoactive drugs, interactions with P-gp inhibitors take place at the BBB. This is affected by the systemic concentration of the P-gp substrate instead of the concentration in the lumen. Therefore, it is difficult to predict possible interactions between DAAs and psychoactive drugs from these results. Additionally, the clinical relevance of P-gp inhibition by DAAs depends on the inhibitory potential of the perpetrator and the therapeutic range of the victim.

Many DAAs are inhibitors of organic anion-transporting polypeptide (OATP) 1B1 and OATP1B3, which are uptake transporters. None of the psychoactive drugs is a substrate of OATPs; hence, these transporters are not discussed in this review.

Drug-transporters: direct-acting antivirals as victims

Most DAAs are substrates of P-gp and OATPs; therefore, DAAs are possible victims when psychoactive agents inhibit or induce these transporters. However, there is limited data available on psychoactive agents and transporters. An example demonstrating the importance of a transporter-mediated interaction, is the contra-indication of grazoprevir with OATP1B1/3 inhibitors.

DRUG-DRUG INTERACTION MECHANISMS: PSYCHOACTIVE AGENTS

Table 2 provides an overview of the enzymes and transporters involved in the metabolism of psychoactive agents. In this section, we describe the mechanisms by which psychoactive agents can be victims and perpetrators of DDIs.

Psychoactive agents as perpetrators

In general, psychoactive agents are more often victims of DDIs than perpetrators. For example, benzodiazepines have limited influence on drug-metabolizing enzymes and transporters (Table 2).

Various antipsychotics, SSRIs, and TCAs have the potential to inhibit CYP2D6, which makes these drugs perpetrators of drug-interactions. However, the currently available DAAs are not metabolized by CYP2D6 and therefore DDIs via this pathway are not expected (Tables 2, 3).

Table 2 : Overview of the route of metabolism, effects on enzymes, and transporters of psychoactive agents.

Drug	Enzyme		Transporter		References
	Substrate	Inhibitor	Substrate	Inhibitor	
Benzodiazepines					
Alprazolam	CYP3A4 (major)	CYP3A4 (weak)			[28]
Bromazepam	CYP1A2 (major) CYP3A4 (minor)	CYP2E1 (weak)			
Brotizolam	CYP3A4				
Chlordiazepoxide	CYP3A4 (major)				[29]
Clobazam	CYP2C19 (major) CYP2B6 (minor) CYP3A4 (minor)	CYP2D6 (moderate) CYP2C19 (weak) Inducer: CYP3A4 (weak)	P-gp		[30]
Clorazepate	CYP3A4 (major)				[31]
Diazepam	CYP3A4 (major) CYP2C19 (major) CYP1A2 (minor) CYP2B6 (minor) CYP2C9 (minor)	CYP2C19 (weak) CYP3A4			[32]
Flurazepam	CYP3A4 (major)	CYP2E1 (weak)			[33]
Lorazepam	Conjugation (UGT)				
Lormetazepam	Conjugation (UGT)				
Midazolam	CYP3A4 (major) CYP2B6 (minor)	CYP2C8 (weak) CYP2C9 (weak) CYP3A4 (weak)			[34]
Oxazepam	UGT				

Table 2 : Overview of the route of metabolism, effects on enzymes, and transporters of psychoactive agents. (*continued*)

Drug	Enzyme		Transporter		References
	Substrate	Inhibitor	Substrate	Inhibitor	
Temazepam	UGT (major) CYP2B6 (minor) CYP2C19 (minor) CYP3A4 (minor) CYP2C9 (minor)				[35]
Zolpidem	CYP3A4 (major) CYP1A2 (minor) CYP2C19 (minor) CYP2D6 (minor)				[36]
Zopiclone	CYP3A4 (major) CYP2C8 (minor)				
Selective Serotonin Reuptake Inhibitors (SSRIs)					
Citalopram	CYP3A4 (major) CYP2C19 (major) CYP2D6 (minor)	CYP2D6 (weak) CYP2C19 (weak) CYP1A2 (weak) CYP2B6 (weak)			[37]
Duloxetine	CYP1A2 (major) CYP2D6 (major)	CYP2D6 (moderate)			[38]
Escitalopram	CYP3A4 (major) CYP2C19 (major)	CYP2D6 (weak)			[39]
Fluoxetine	CYP2C9 (major) CYP2D6 (major) CYP1A2 (minor) CYP2B6 (minor) CYP2C19 (minor) CYP2E1 (minor) CYP3A4 (minor)	CYP2D6 (strong) CYP2C19 (moderate) CYP1A2 (weak) CYP2B6 (weak) CYP2C9 (weak)			[40]

Table 2 : Overview of the route of metabolism, effects on enzymes, and transporters of psychoactive agents. (*continued*)

Drug	Enzyme		Transporter		References
	Substrate	Inhibitor	Substrate	Inhibitor	
Fluvoxamine	CYP2D6 (major) CYP1A2 (major)	CYP2C19 (strong) CYP1A2 (strong) CYP2B6 (weak) CYP2C9 (weak) CYP2D6 (weak) CYP3A4 (weak)			[41]
Paroxetine	CYP2D6 (major)	CYP2D6 (strong) CYP2B6 (moderate) CYP2C19 (weak) CYP2C9 (weak) CYP1A2 (weak)			[42]
Sertraline	CYP2C19 (minor) CYP3A4 (minor) CYP2B6 (minor) CYP2D6 (minor) CYP2C9 (minor)	CYP2B6 (moderate) CYP2C19 (moderate) CYP2D6 (moderate) CYP1A2 (weak) CYP2C8 (weak) CYP2C9 (weak)			[43]
Trazodone	CYP3A4 (major) CPY2D6 (minor)			Inducer: P-gp	
Venlafaxine	CYP2D6 (major) CYP3A4 (major) CYP2C9 (minor) CYP2C19 (minor)	CYP2B6 (weak) CYP2D6 (weak) CYP3A4 (weak)			[44]
Vortioxetine	CYP2D6 (major) CYP3A4/5 (major) CYP2C9 (minor) CYP2C19 (minor) CYP2C8 (minor) CYP2B6 (minor) CYP2A6 (minor)		P-gp (minor)	P-gp (weak)	[45]

Table 2 : Overview of the route of metabolism, effects on enzymes, and transporters of psychoactive agents. (*continued*)

Drug	Enzyme		Transporter		References
	Substrate	Inhibitor	Substrate	Inhibitor	
Tricyclic antidepressants (TCAs)					
Amitriptyline	CYP2D6 (major) CYP1A2 (minor) CYP2B6 (minor) CYP2C19 (minor) CYP2C9 (minor) CYP3A4 (minor)	CYP1A2 (weak) CYP2C19 (weak) CYP2C9 (weak) CYP2D6 (weak) CYP2E1 (weak)	P-gp		
Clomipramine	CYP1A2 (major) CYP2C19 (major) CYP2D6 (major) CYP3A4 (minor)	CYP2D6 (moderate)			[46]
Dosulepin	COMT				
Doxepin	CYP2D6 (major) CYP1A2 (minor) CYP2C19 (minor) CYP3A4 (minor)				
Imipramine	CYP2C19 (major) CYP2D6 (major) CYP1A2 (minor) CYP2B6 (minor) CYP3A4 (minor)	CYP2D6 (moderate) CYP1A2 (weak) CYP2C19 (weak) CYP2E1 (weak)			
Maprotiline	CYP2D6 (major)				
Nortriptyline	CYP2D6 (major) CYP1A2 (minor) CYP2C19 (minor) CYP3A4 (minor)	CYP2D6 (weak) CYP2E1 (weak)	P-gp		

Table 2 : Overview of the route of metabolism, effects on enzymes, and transporters of psychoactive agents. (*continued*)

Drug	Enzyme		Transporter		References
	Substrate	Inhibitor	Substrate	Inhibitor	
Other antidepressants					
Agomelatine	CYP1A2 CYP2C9 CYP2C19				
Bupropion	CYP2B6 (major) CYP1A2 (minor) CYP2A6 (minor) CYP2C9 (minor) CYP2D6 (minor) CYP2E1 (minor) CYP3A4 (minor)	CYP2D6 (strong)		OCT2	
Mianserin	CYP2D6				
Mirtazapine	CYP1A2 (major) CYP2D6 (major) CYP3A4 (major) CYP2C9 (minor)	CYP1A2 (weak)			
Moclobemide	CYP2C19 (major) CYP2D6 (minor)	CYP2C19 (moderate) CYP1A2 (weak) CYP2D6 (weak) MAO			
St. Johns wort		Inducer: CYP3A4 and CYP1A2 (possible various CYP enzymes)			

Table 2 : Overview of the route of metabolism, effects on enzymes, and transporters of psychoactive agents. (*continued*)

Drug	Enzyme		Transporter		References
	Substrate	Inhibitor	Substrate	Inhibitor	
Antipsychotics					
Aripiprazole	CYP2D6 (major) CYP3A4 (major)				[47]
Bromperidol	CYP3A4 CYP2D6				
Clozapine	CYP1A2 (major) CYP2A6 (minor) CYP2C19 (minor) CYP2C9 (minor) CYP2D6 (minor) CYP3A4 (minor)	CYP2D6 (moderate) CYP1A2 (weak) CYP2C19 (weak) CYP2C9 (weak) CYP2E1 (weak) CYP3A4 (weak)			
Flupentixol	CYP2D6				
Fluphenazine	CYP2D6 (major) CYP1A2	CYP1A2 (weak) CYP2C9 (weak) CYP2D6 (weak) CYP2E1 (weak)			
Haloperidol	CYP2D6 (major) CYP3A4 (major) CYP1A2 (minor)	CYP2D6 (moderate)			
Lurasidone	CYP3A4 (major)	CYP3A4 (weak)	P-gp		[48]
Olanzapine	CYP1A2 (major) CYP2D6 (minor) UGT	CYP1A2 (weak) CYP2C19 (weak) CYP2C9 (weak) CYP2D6 (weak) CYP3A4 (weak)			[49]

Table 2 : Overview of the route of metabolism, effects on enzymes, and transporters of psychoactive agents. (*continued*)

Drug	Enzyme		Transporter		References
	Substrate	Inhibitor	Substrate	Inhibitor	
Paliperidone			P-gp (weak)		[50]
Perphenazine	CYP2D6 (major) CYP1A2 (minor) CYP2C19 (minor) CYP2C9 (minor) CYP3A4 (minor)	CYP1A2 (weak) CYP2D6 (weak)			
Pimozide	CYP3A4 (major) CYP1A2 (major) CYP2D6 (major)	CYP2C19 (weak) CYP2D6 (weak) CYP2E1 (weak)			
Quetiapine	CYP3A4 (major) CYP2D6 (minor)				
Risperidone	CYP2D6 (major) CYP3A4 (minor)	CYP2D6 (weak)	P-gp		
Sertindole	CYP2D6 CYP3A4				
Zuclopenthixol	CYP2D6 (major) CYP3A4 (minor)				

Note that most of these drugs have older registration files, and therefore, possible involvement of transporters and drug-metabolizing enzymes may not be studied in sufficient detail per the current standards.

Information in this table was compiled from the following sources: Europeans Medicines Association Summary of Product Characteristics, US Food and Drug Administration Prescribing Information, and data from Lexicomp, available through http://www.uptodate.com (October 2015).

Note: Assignment of major/minor substrate status based on their clinically relevant drug-interaction potential (http://www.uptodate.com).

No information about hepatic metabolism and/or drug-transporters were available for: Flunitrazepam, loprazolam, nitrazepam, prazepam, lithium salts, chlorprothixene, fluspirilene, penfluridol, pericyazine, pipamperone, sulpiride, and tiapride.

COMT: Catechol-O-methyl transferase; CYP: Cytochrome P450; MAO: Monoamine oxidase; P-gp: P-glycoprotein; UGT: Uridine 5'-diphospho-glucuronosyltransferase.

Psychoactive agents as victims

Most benzodiazepines are substrates of various CYP enzymes, such as CYP3A4, CYP2B6, CYP2C19, and CYP1A2; therefore, benzodiazepines are potential victims of DDIs. Benzodiazepines have a narrow therapeutic range and a strong concentration-effect relationship[27]; thus, increased plasma concentrations are likely to cause increased toxicity.

Midazolam is a model substrate of CYP3A4; therefore, interactions between midazolam and DAAs have been extensively studied. For example, oral co-administration of midazolam and boceprevir resulted in an increase in the midazolam AUC of by 430% and an increase in its C_{max} of 177%. As a result, co-administration of midazolam and boceprevir is contra-indicated[17]. Similarly, an increase in the plasma concentration of midazolam is expected when it is administered with paritaprevir/ritonavir, ombitasvir, and dasabuvir; therefore, this co-administration is also contra-indicated[20]. Interactions between midazolam and simeprevir or grazoprevir have both been studied and increased midazolam AUC and C_{max} values were observed; thus, caution is needed with co-administration[18, 19]. On the other hand, daclatasvir has been shown to have little effect on midazolam exposure[22].

SSRIs are hepatically metabolized by various CYP enzymes (e.g., CYP3A4, CYP2D6, CYP2C9, and CYP2C19), particularly CYP3A4. Theoretically, this puts patients at risk when they are also taking DAAs. However, SSRIs have a broad therapeutic range; therefore, increased plasma concentrations of SSRIs are not likely to result in significant toxicities[10]. For instance, the co-administration of escitalopram (CYP3A4 substrate) and DAAs such as boceprevir, simeprevir, or the combination of paritaprevir/ritonavir, ombitasvir, and dasabuvir did not result in a clinically relevant increase in the escitalopram plasma concentration[17, 18, 20].

Antipsychotics are metabolized in the liver by a variety of CYP enzymes, as given in Table 2. CYP3A4 and CYP2D6 are involved in this metabolism; however, they can be inhibited by DAAs. Most antipsychotics have a narrow therapeutic range. Therefore, DDIs involving antipsychotics can result in clinically relevant outcomes, especially with strong CYP3A4 inhibitors such as boceprevir and ritonavir. However, no interaction studies have been conducted so far.

Table 3: Overview of studied interactions between direct-acting antivirals and psychoactive agents.

	DAA	Drug	DAA AUC	DAA C_{max}	DAA C_{min}	Drug AUC	Drug C_{max}	Drug C_{min}	Recommendation	Ref
Protease inhibitor	Boceprevir	Escitalopram (10 mg)	0.91 (0.81-1.02)	1.02 (0.96-1.08)	-	0.79 (0.72-0.87)	0.81 (0.76-1.87)	-	No dose adjustment DAA/drug	[17, 51]
		Midazolam po (4 mg)	-	-	-	430%↑	177%↑	-	Contra-indicated	[17, 52]
		St. Johns wort (600 mg)	0.91 (0.87-0.96)	0.94 (0.81-1.07)	1.00 (0.79-1.26)	1.23 (1.10-1.38)	1.32 (1.16-4.52)	1.37 (1.19-1.58)	No dose adjustment DAA/drug	[21]
	Simeprevir	Escitalopram (10 mg)	0.75 (0.68-0.83)	0.80 (0.71-0.89)	0.68 (0.59-0.79)	1.00 (0.97-1.03)	1.03 (0.99-1.07)	1.00 (0.95-1.05)	No dose adjustment DAA/drug	[18, 53]
		Midazolam po (0.075 mg/kg)	-	-	-	1.45 (1.35-1.57)	1.31 (1.19-1.45)	-	Caution when co-administered	[18]
		Midazolam IV (0.025 mg/kg)	-	-	-	1.10 (0.95-1.26)	0.78 (0.52-1.17)	-	No dose adjustment DAA/drug	[18]
	Grazoprevir[a]	Midazolam (2 mg/mL)	-	-	-	1.34 (1.29-1.39)	1.15 (1.01-1.31)	-	-	[19]
NS5A inhibitor	Daclatasvir	Escitalopram (10 mg)	1.12 (1.01-1.26)	1.14 (0.98-1.32)	1.23 (1.09-1.38)	1.05 (1.02-1.08)	1.00 (0.92-1.08)	1.10 (1.04-1.16)	No dose adjustment DAA/drug	[22]
		Midazolam (5 mg)	-	-	-	0.87 (0.83-0.92)	0.95 (0.88-1.04)	-	No dose adjustment DAA/drug	[22]
	Ledipasvir	No DDIs studied								
NS5B polymerase inhibitor	Sofosbuvir	No DDIs studied								

Table 3: Overview of studied interactions between direct-acting antivirals and psychoactive agents. (*continued*)

	DAA	Drug	DAA AUC	DAA C_{max}	DAA C_{min}	Drug AUC	Drug C_{max}	Drug C_{min}	Recommendation	Ref
Fixed-dose combinations	PTV/ritonavir, OBV, and DSV	Escitalopram (10 mg)	PTV: 0.98 (0.85-1.14) OBV: 1.02 (1.00-1.05) DSV: 1.01 (0.93-1.10)	PTV: 1.12 (0.88-1.43) OBV: 1.09 (1.01-1.18) DSV: 1.10 (0.95-1.27)	PTV: 0.71 (0.56-0.89) OBV: 0.97 (0.92-1.02) DSV: 0.89 (0.79-1.00)	0.87 (0.80-0.95)	1.00 (0.96-1.05)	-	No dose adjustment DAA/drug	[20, 54]
		Duloxetine (60 mg)	PTV: 0.83 (0.62-1.10) OBV: 1.00 (0.95-1.06) DSV: 0.92 (0.81-1.04)	PTV: 0.79 (0.53-1.16) OBV: 0.98 (0.88-1.08) DSV: 0.94 (0.81-1.09)	PTV: 0.77 (0.65-0.91) OBV: 1.01 (0.96-1.06) DSV: 0.88 (0.76-1.01)	0.75 (0.67-0.83)	0.79 (0.67-0.94)	-	No dose adjustment DAA/drug	[20, 54]
		Zolpidem (5 mg)	PTV: 0.68 (0.55-0.85) OBV: 1.03 (1.00-1.07) DSV: 0.95 (0.84-1.08)	PTV: 0.63 (0.46-0.86) OBV: 1.07 (1.00-1.15) DSV: 0.93 (0.84-1.03)	PTV: 1.23 (1.10-1.38) OBV: 1.04 (1.00-1.08) DSV: 0.92 (0.83-1.01)	0.95 (0.74-1.23)	0.94 (0.76-1.16)	-	No dose adjustment DAA/drug	[20, 54]
		Alprazolam (0.5 mg)	PTV: 0.96 (0.73-1.27) OBV: 1.00 (0.96-1.04) DSV: 0.98 (0.87-1.11)	PTV: 0.91 (0.64-1.31) OBV: 0.98 (0.93-1.04) DSV: 0.93 (0.83-1.04)	PTV: 1.12 (1.02-1.23) OBV: 0.98 (0.93-1.04) DSV: 1.00 (0.87-1.15)	1.34 (1.15-1.55)	1.09 (1.03-1.15)	-	No dose adjustment DAA/drug	[20, 54]
		Diazepam (2 mg)	PTV: 0.91 (0.78-1.07) OBV: 0.98 (0.93-1.03) DSV: 1.01 (0.94-1.06)	PTV: 0.95 (0.77-1.15) OBV: 1.00 (0.93-1.08) DSV: 1.05 (0.98-1.13)	PTV: 0.92 (0.82-1.03) OBV: 0.93 (0.68-0.98) DSV: 1.05 (0.98-1.12)	0.78 (0.73-0.82)	1.18 (1.07-1.30)	-	No dose adjustment DAA/drug	[55]
	PTV/ritonavir and OBV	Duloxetine (60 mg)	PTV: 0.96 (0.70-1.32)	PTV: 1.07 (0.63-1.81)	PTV: 0.93 (0.76-1.14)	-	-	-	-	[20]

When possible geometric mean ratios (with 90% confidence intervals in parentheses) are presented; otherwise, percentages are presented.

[a]The interactions with midazolam are studied without elbasvir. No interaction studies with grazoprevir/elbasvir were performed.

AUC: Area under the concentration-time curve; C_{max}: Maximum plasma concentration; C_{min}: Minimum plasma concentration; DAA: Direct-acting antiviral; DDI: Drug-drug interaction; PTV: Paritaprevir; OBV: Ombitasvir; DSV: Dasabuvir; IV: Intravenous; po: Per os; Ref: Reference.

CLINICAL GUIDANCE

In this section, we provide guidance for clinical decision making regarding the use of a combined treatment of DAAs and psychoactive drugs. Most potential drug-interactions have not been subjected to rigid pharmacokinetic testing in humans, and recommendations are often based on theoretical interpretations of the pharmacokinetic characteristics of drugs.

We believe that a relevant interaction only occurs when a drug (victim) is metabolized to a 'major' or 'moderate' extent. Major or moderate substrate status is based on the potential clinically relevant drug-interaction as described by Lexicomp[15]. A major status indicates that the regimen should be modified, whereas a moderate status implies that the therapy should be monitored. Consequently, a drug should have strong (>5-fold increase in substrate AUC) or moderate (2-to 5-fold increase in substrate AUC) influence on an enzyme/transporter (perpetrator) in order to cause an interaction (Tables 4, 5, 6, 7, 8, 9, 10).

Protease inhibitors

Boceprevir

Table 4 shows benzodiazepines, antidepressants, and antipsychotics that are safe to combine with boceprevir. Of the currently available DAAs, boceprevir is one of the most potent CYP3A4 inhibitors. Therefore, we do not recommend combining boceprevir and drugs primarily metabolized by CYP3A4, especially if they have a narrow therapeutic range (a contra-indication).

Co-administration of midazolam and boceprevir (both oral and parenteral) is contra-indicated since the midazolam AUC and C_{max} are both significantly increased[17]. This exceptional increase was not observed with other DAAs, which emphasizes the strong inhibitory potential of boceprevir on CYP3A4.

On the other hand, no dose adjustment is required when escitalopram is administered with boceprevir. This is unexpected as escitalopram is a CYP3A4 substrate. It is possible that there is involvement of other unknown enzymes or transporters; hence, the underlying mechanism cannot be explained[17].

Boceprevir is also a P-gp inhibitor. Theoretically, this inhibition could have an impact on P-gp substrates; however, it seems to have minimal clinical relevance due to the mild inhibition of P-gp by boceprevir[17, 26]. Boceprevir may not be a victim of any DDIs with

benzodiazepines, SSRIs, TCAs, or antipsychotics, as studies with midazolam and escitalopram have shown[17]. Additionally, interaction studies between St John's wort and boceprevir showed no alterations in the plasma concentration of boceprevir; hence, this combination is safe to use[17, 21].

Finally, physicians should take care when prescribing boceprevir in combination with drugs that might prolong the QT interval and are metabolized by CYP3A4[17]. For instance, SSRIs and TCAs may influence the QT interval and serious pharmacodynamic interactions may occur when they are administered with boceprevir (Table 4).

Simeprevir

Table 5 shows psychoactive medications that can be safely combined with simeprevir. Simeprevir inhibits intestinal CYP3A4 and therefore only interactions with orally administered medications are relevant. Thus, intravenous midazolam can be used safely with simeprevir but oral midazolam should be used with caution, as the AUC and C_{max} of midazolam are increased by 45 and 31%, respectively, when the two are co-administered[18].

Studies have been conducted of escitalopram, and it can be safely used in combination with simeprevir[18].

Simeprevir inhibits P-gp and OATP1B1. Simeprevir has a higher impact than boceprevir on the transport activity of P-gp as indicated before. Therefore, inhibition of P-gp by simeprevir may lead to small increase in concentrations of P-gp substrates (e.g., risperidone and nortriptyline) in the brain. However, the clinical relevance seems limited[18].

Simeprevir is a possible victim of DDIs as it is primarily metabolized by CYP3A4[18]. St John's wort may therefore cause a decrease in the plasma concentration of simeprevir as it is a CYP3A4 inducer. Nevertheless, this change might not be clinically relevant since simeprevir exhibits high inter- individual variability in its plasma concentrations[56].

Table 4: Psychoactive agents that are safe, have a potential interaction, or are contra-indicated with boceprevir.

Class of psychoactive agents	Safe options	Contra-indicated	Potential interaction	Unknown
Benzodiazepine	Bromazepam Clobazam Flunitrazepam Lorazepam Lormetazepam Nitrazepam Oxazepam Prazepam Temazepam	Midazolam (oral and intravenous)	Alprazolam Brotizolam Chlordiazepoxide Clorazepate Diazepam Flurazepam Zolpidem Zopiclone	Loprazolam
Antidepressants	**SSRIs** Citalopram[a] Duloxetine Escitalopram[a] Fluoxetine[b] Fluvoxamine Paroxetine[b] Sertraline[b] **TCAs** Amitriptyline[b] Clomipramine[c] Dosulepin Doxepin[b] Imipramine[c] Maprotiline Nortriptyline[c] **Others** Agomelatine Bupropion Lithium salts[c] Mianserin Moclobemide Mirtazapine[c] St. Johns wort	N/A	**SSRIs** Trazodone Venlafaxine Vortioxetine **TCAs** N/A **Others** N/A	N/A
Antipsychotics	Clozapine[c] Flupentixol Fluphenazine Fluspirilene Olanzapine[c] Paliperidone[c] Perphenazine Sulpride[a] Tiapride Zuclopenthixol	Pimozide[a] Quetiapine	Aripiprazole[c] Bromperidol Haloperidol[a] Lurasidone Risperidone[c] Sertindole[c]	Chlorprothixene Penfluridol Pericyazine Pipamperone

[a]Known risk for prolongation of the QT interval (http://www.crediblemeds.org)

[b]Conditional risk for prolongation of the QT interval (http://www.crediblemeds.org)

[c]Possible risk for prolongation of the QT interval (http://www.crediblemeds.org)

N/A: Not applicable; SSRI: Selective serotonin reuptake inhibitors; TCA: Tricyclic antidepressants.

Table 5: Psychoactive agents that are safe, have a potential interaction, or are contra-indicated with simeprevir.

Class of psychoactive agents	Safe options	Contra-indicated	Potential interaction	Unknown
Benzodiazepine	Bromazepam Flunitrazepam Lorazepam Lormetazepam Midazolam iv Nitrazepam Oxazepam Prazepam Temazepam	N/A	Alprazolam Brotizolam Chlordiazepoxide Clobazam Clorazepate acid Diazepam Flurazepam Midazolam po Zolpidem Zopiclone	Loprazolam
Antidepressants	**SSRIs** Citalopram Duloxetine Escitalopram Fluoxetine Fluvoxamine Paroxetine Sertraline **TCAs** Clomipramine Dosulepin Doxepin Imipramine Maprotiline **Others** Agomelatine Bupropion Lithium salts Mianserin Moclobemide	N/A	**SSRIs** Venlafaxine Trazodone Vortioxetine **TCAs** Amitriptyline Nortriptyline **Others** Mirtazapine St. Johns wort	N/A
Antipsychotics	Clozapine Flupentixol Fluspirilene Fluphenazine Olanzapine Perphenazine Sulpride Tiapride Zuclopenthixol	N/A	Aripiprazole Bromperidol Haloperidol Lurasidone Paliperidone Pimozide Quetiapine Risperidone Sertindole	Chlorprothixene Penfluridol Pericyazine Pipamperone

IV: Intravenous; N/A: Not applicable; po: Per os; SSRI: Selective serotonin reuptake inhibitors; TCA; Tricyclic antidepressants.

NS5A inhibitor

Daclatasvir

Daclatasvir has a negligible influence on the activities of CYP3A4 and other CYP enzymes and no dose adjustments were required when it was studied with midazolam[22]. Based on this information, it is expected that most benzodiazepines, antidepressants, and antipsychotics can be used safely in combination with daclatasvir, as given in Table 6. Daclatasvir is metabolized by CYP3A4 and thus inducers and inhibitors of CYP3A4 have the ability to affect the plasma concentrations of daclatasvir[22]. Most psychoactive drugs do not influence CYP3A4; with the exception of St John's wort; therefore, co-administration of St John's wort and daclatasvir is contra-indicated[22].

NS5B polymerase inhibitor

Sofosbuvir

Sofosbuvir is an NS5B inhibitor and not a perpetrator of DDIs as it has no influence on CYP enzymes or drug-transporters; therefore, it has no impact on the plasma concentrations of psychoactive drugs[24]. However, an unexpected interaction has occurred involving sofosbuvir and the antiarrhythmic agent amiodarone[57, 58]. This indicates that not every DDI can be predicted based on the activities of CYP, UGT, or drug-transporters. The mechanism and the specific role of sofosbuvir in the interaction was uncertain because other DAAs (daclatasvir, simeprevir, and ledipasvir) were simultaneously administered and could have been involved in causing the interaction[57, 58]. Additionally, it could also be a pharmacodynamic interaction.

Sofosbuvir is metabolized in the liver and intestine; the drug is not a victim of enzymatic DDIs because it is not metabolized by, for example, CYPs or UGTs[24]. Sofosbuvir is a substrate for P-gp and BCRP; hence, interactions may occur with inducers and inhibitors of P-gp. P-gp inducers, e.g., St John's wort, could potentially decrease plasma concentrations of sofosbuvir and result in a decrease in the pharmacological effects of sofosbuvir. Therefore, co-administration of the two drugs is contra-indicated. Trazodone is also a possible P-gp inducer and may affect the plasma concentration of sofosbuvir (Table 7). Inhibition of P-gp could increase the plasma concentration of sofosbuvir; however, the interactions studies have not been performed in humans[24]. Lastly, the main (inactive) metabolite of sofosbuvir (GS-331007) is not a P-gp substrate[24].

Table 6: Psychoactive agents that are safe, have a potential interaction, or are contra-indicated with daclatasvir.

Class of psychoactive agents	Safe options		Contra-indicated	Potential interaction		Unknown
Benzodiazepine	Alprazolam	Lorazepam	N/A	Clobazam		Loprazolam
	Bromazepam	Lormetazepam				
	Brotizolam	Midazolam				
	Chlordiazepoxide	Oxazepam				
	Clorazepate	Nitrazepam				
	Diazepam	Prazepam				
	Flunitrazepam	Temazepam				
	Flurazepam	Zolpidem				
		Zopiclone				
Antidepressants	**SSRIs**	**TCAs**	St. Johns wort	**SSRIs**	**TCAs**	N/A
	Citalopram	Clomipramine		N/A	Amitriptyline	
	Duloxetine	Dosulepin			Nortriptyline	
	Escitalopram	Doxepin				
	Fluoxetine	Imipramine			**Others** N/A	
	Fluvoxamine	Maprotiline				
	Paroxetine					
	Sertraline	**Others**				
	Trazodone	Agomelatine				
	Venlafaxine	Bupropion				
	Vortioxetine	Lithium salts				
		Mianserin				
		Mirtazapine				
		Moclobemide				
Antipsychotics	Aripiprazole	Perphenazine	N/A	Lurasidone		Chlorprothixene
	Bromperidol	Pimozide		Paliperidone		Penfluridol
	Clozapine	Quetiapine		Risperidone		Pericyazine
	Flupentixol	Sertindole				Pipamperone
	Fluphenazine	Sulpride				
	Fluspirilene	Tiapride				
	Haloperidol	Zuclopenthixol				
	Olanzapine					

N/A: Not applicable; SSRI: Selective serotonin reuptake inhibitors; TCA: Tricyclic antidepressants.

Table 7: Psychoactive agents that are safe, have a potential interaction, or are contra-indicated with sofosbuvir.

Class of psychoactive agents	Safe options		Contra-indicated	Potential interaction		Unknown
Benzodiazepine	Alprazolam	Loprazolam	N/A	N/A		Loprazolam
	Bromazepam	Lorazepam				Nitrazepam
	Brotizolam	Lormetazepam				
	Chlordiazepoxide	Midazolam				
	Clobazam	Oxazepam				
	Clorazepate	Prazepam				
	Diazepam	Temazepam				
	Flunitrazepam	Zolpidem				
	Flurazepam	Zopiclone				
Antidepressants	**SSRIs**	**TCAs**	St. Johns wort	**SSRIs**	**TCAs**	N/A
	Citalopram	Amitriptyline		Trazodone	N/A	
	Duloxetine	Clomipramine				
	Escitalopram	Dosulepin			**Others**	
	Fluoxetine	Doxepin			N/A	
	Fluvoxamine	Imipramine				
	Paroxetine	Maprotiline				
	Sertraline	Nortriptyline				
	Venlafaxine					
	Vortioxetine	**Others**				
		Agomelatine				
		Bupropion				
		Lithium salts				
		Mianserin				
		Mirtazapine				
		Moclobemide				
Antipsychotics	Aripiprazole	Perphenazine	N/A	Lurasidone		Chlorprothixene
	Bromperidol	Pimozide				Penfluridol
	Clozapine	Quetiapine				Pericyazine
	Flupentixol	Risperidone				Pipamperone
	Fluphenazine	Sertindole				
	Fluspirilene	Sulpride				
	Haloperidol	Tiapride				
	Olanzapine	Zuclopenthixol				
	Paliperidone					

N/A: Not applicable; SSRI: Selective serotonin reuptake inhibitors; TCA: Tricyclic antidepressants.

Table 8: Psychoactive agents that are safe, have a potential interaction, or are contra-indicated with ledipasvir.

Class of psychoactive agents	Safe options		Contra-indicated	Potential interaction		Unknown
Benzodiazepine	Alprazolam	Lormetazepam	N/A	Clobazam		Loprazolam
	Bromazepam	Midazolam				
	Brotizolam	Prazepam				
	Chlordiazepoxide	Nitrazepam				
	Clorazepate	Oxazepam				
	Diazepam	Temazepam				
	Flunitrazepam	Zolpidem				
	Flurazepam	Zopiclone				
	Lorazepam					
Antidepressants	**SSRIs**	**TCAs**	St. Johns wort	**SSRIs**	**TCAs**	
	Citalopram	Clomipramine		N/A	Amitriptyline	
	Duloxetine	Dosulepin			Nortriptyline	
	Escitalopram	Doxepin				
	Fluoxetine	Imipramine			**Others**	
	Fluvoxamine	Maprotiline			N/A	
	Paroxetine	**Others**				
	Sertraline	Agomelatine				
	Trazodone	Bupropion				
	Venlafaxine	Lithium salts				
	Vortioxetine	Mianserin				
		Mirtazapine				
		Moclobemide				
Antipsychotics	Aripiprazole	Perphenazine	N/A	Lurasidone		Chlorprothixene
	Bromperidol	Pimozide		Paliperidone		Penfluridol
	Clozapine	Quetiapine		Risperidone		Pericyazine
	Flupentixol	Sertindole				Pipamperone
	Fluphenazine	Sulpride				
	Fluspirilene	Tiapride				
	Haloperidol	Zuclopenthixol				
	Olanzapine					

N/A: Not applicable; SSRI: Selective serotonin reuptake inhibitors; TCA: Tricyclic antidepressants.

Fixed-Dose combinations

Ledipasvir and sofosbuvir

Ledipasvir inhibits P-gp and BCRP and may cause interactions with P-gp and BCRP substrates (e.g., risperidone, and nortriptyline)[23]. P-gp inhibition at the BBB could potentially increase the exposure of these P-gp substrates in the brain. No interaction studies have been performed between ledipasvir and psychoactive agents. Table 8 shows the psychoactive agents that can be safely used with or potentially interact with ledipasvir.

The metabolism of ledipasvir is unknown but it is mainly excreted unchanged through bile. Thus, ledipasvir is not expected to be a victim of DDIs[23].

Paritaprevir/ritonavir, ombitasvir, and dasabuvir

Ritonavir is a strong CYP3A4 inhibitor but it also influences other CYP enzymes and drug-transporters. Consequently, caution is needed combining drugs metabolized by CYP3A4 with this fixed-dose regimen (paritaprevir/ritonavir, ombitasvir, and dasabuvir). Psychoactive agents such as duloxetine, escitalopram, zolpidem, alprazolam, and diazepam can be safely administered with this regimen as previous studies have not given any clinically relevant interactions (Table 9)[20, 59, 54, 55].

The plasma concentrations of duloxetine did not alter when it was co-administered with the combination regimen[20]. Duloxetine is a substrate for CYP2D6 and CYP1A2, and ritonavir inhibits CYP2D6 and induces CYP1A2. As no effect was observed when combined with paritaprevir/ritonavir, ombitasvir and dasabuvir, it was suggested that the inhibition of CYP2D6 and induction of CYP1A2 occurred to similar extents. We recommend that this combination regimen is used with care in patients receiving medications that are metabolized by CYP2D6 and/or CYP1A2, especially as a previous interaction study on the co-administration of olanzapine (CYP1A2 and CYP2D6 substrate) and ritonavir resulted in decreased olanzapine concentrations[60].

It is important to note that CYP2D6 inhibition by ritonavir is dose dependent[61]. Low-dose ritonavir (100 mg twice daily) had only a mild effect on CYP2D6, as shown with the CYP2D6 substrate desipramine (26% increase AUC) but the therapeutic dose of ritonavir (600 mg twice daily) had a stronger effect (desipramine AUC increase of 145%) [61-63]. However, this fixed-dose HCV regimen contains only 100 mg of ritonavir. Therefore, we expect DDIs only with co-administered drugs that are primarily metabolized by CYP2D6 and that have a narrow therapeutic range. Such drugs are not contra-indicated with this combination regimen; however, drug plasma concentrations and adverse events should be monitored after co-administration[61].

Ritonavir also inhibits P-gp; therefore, ritonavir may modify plasma concentrations of P-gp substrates such as risperidone and amitriptyline.

Paritaprevir, ombitasvir, and dasabuvir are inhibitors of UGT and thus benzodiazepines (e.g., lorazepam, lormetazepam, and oxazepam) conjugated by UGT could be victims of interactions (Table 9)[20].

Grazoprevir and elbasvir

Table 10 shows safe options for psychoactive drugs that can be administered with grazoprevir and elbasvir. Grazoprevir is a mild CYP3A inhibitor as it was observed to increase the plasma concentration of midazolam by only 30%[19]. Therefore, CYP3A4 substrates are not contra-indicated with grazoprevir. However, we recommend that prescribers be aware of possible interactions with drugs that are primarily metabolized by CYP3A4 and have a narrow therapeutic range. There are no reported studies on drug-interactions between the grazoprevir/elbasvir combination and psychoactive agents[19]. Grazoprevir and elbasvir are mainly metabolized by CYP3A4; thus, they should not be administered with St John's wort and other CYP3A4 inducers or inhibitors[19] (Table 10).

Table 9: Psychoactive agents that are safe, have a potential interaction, or are contra-indicated with paritaprevir, ritonavir, ombitasvir plus dasabuvir.

Class of psychoactive agents	Safe options	Contra-indicated	Potential interaction	Unknown
Benzodiazepine	Alprazolam Bromazepam Clobazam Diazepam Flunitrazepam Prazepam Nitrazepam Zolpidem	Midazolam	Brotizolam Clorazepate Chlordiazepoxide Flurazepam Lorazepam Lormetazepam Oxazepam Temazepam Zopiclone	Loprazolam
Antidepressants	**SSRIs** Citalopram Escitalopram Duloxetine[a] **TCAs** Dosulepin **Others** Lithium salts Moclobemide	St. Johns wort	**SSRIs** Fluoxetine[a] Fluvoxamine[a] Paroxetine[a] Sertraline Trazodone Venlafaxine[a] Vortioxetine[a] **TCAs** Amitriptyline[a] Clomipramine[a] Doxepin[a] Imipramine[a] Nortriptyline[a] Maprotiline[a] **Others** Agomelatine Bupropion Mirtazapine Mianserin[a]	N/A
Antipsychotics	Fluspirilene Sulpride Tiapride	Pimozide Quetiapine	Aripiprazole[a] Bromperidol[a] Clozapine Flupentixol[a] Fluphenazine[a] Haloperidol[a] Lurasidone Olanzapine Paliperidone Perphenazine[a] Risperidone[a] Sertindole[a] Zuclopenthixol	Chlorprothixene Penfluridol Pericyazine Pipamperone

[a]Substrates of CYP2D6.

N/A: Not applicable; SSRI: Selective serotonin reuptake inhibitors; TCA: Tricyclic antidepressants.

Table 10: Psychoactive agents that are safe, have a potential interaction, or are contra-indicated with grazoprevir plus elbasvir.

Class of physchoactive agents	Safe options		Contra-indicated	Potential interaction		Unknown
Benzodiazepine	Alprazolam Brotizolam Chlordiazepoxide Bromazepam Clobazam Flunitrazepam Lorazepam Lormetazepam Clorazepate Flurazepam	Nitrazepam Oxazepam Temazepam Prazepam Zolpidem Zopiclone	N/A	Diazepam Midazolam		Loprazolam
Antidepressants	**SSRIs** Citalopram Duloxetine Escitalopram Fluoxetine Fluvoxamine Paroxetine Sertraline Trazodone Venlafaxine Vortioxetine	**TCAs** Amitriptyline Clomipramine Dosulepin Doxepin Imipramine Maprotiline Nortriptyline **Others** Agomelatine Bupropion Lithium salts Mianserin Moclobemide Mirtazapine	St. Johns wort	**SSRIs** N/A	**TCAs** N/A **Others** N/A	N/A
Antipsychotics	Aripiprazole Bromperidol Clozapine Flupentixol Fluphenazine Fluspirilene Haloperidol Olanzapine Paliperidone	Perphenazine Pimozide Quetiapine Risperidone Sertindole Sulpride Tiapride Zuclopenthixol	N/A	Lurasidone		Chlorprothixene Penfluridol Pericyazine Pipamperone

N/A: Not applicable; SSRI: Selective serotonin reuptake inhibitors; TCA: Tricyclic antidepressants.

CONCLUSION

In this review, we have shown that there is a paucity of experimental data on drug-interactions between psychoactive agents and DAAs. Many mechanisms are involved in the metabolism and transport of both classes of drugs, making it difficult to predict which drugs can be safely co-administered to patients.

In our opinion, safe options for concomitant administration should be combinations that have actually been studied in humans, or combinations that are not based on theoretical pharmacokinetic interactions. In addition, all medications used at the start of and during HCV treatment should be inventoried, so that possible DDIs can be evaluated before clinically relevant effects arise. Physicians should also be aware of possible interactions and their consequences. These may include adverse effects caused by increased plasma drug concentrations or reduced efficacy due to decreases in drug exposure. These are of great importance as such issues may also affect adherence to both DAAs and psychoactive agents. Our final recommendation is that physicians contact pharmacists or clinical pharmacologists for support in managing these interactions.

This review provides an overview of the mechanisms of interactions between DAAs and psychoactive agents. Based on interaction studies, we give recommendations for the co-administration of DAAs and psychoactive agents. The administration of various combinations of drugs results in different potential interactions. It is therefore necessary that theoretical predictions of DDIs be backed with actual drug-interaction studies, in order to obtain more conclusive and useful data for clinical applications.

CONFLICTS OF INTERESTS

EJS and CTMMK declare that they have no conflicts of interest that are directly relevant to the content of this review. RJK received sponsorship/research grants from BMS, and Janssen; is a consultant for AbbVie, BMS, Gilead, Roche, and Janssen; and has delivered lectures for AbbVie, Janssen, Gilead, and Roche. JPHD is on the advisory boards for AbbVie, BMS, Gilead, Janssen, and Merck and received sponsorship/research grants from AbbVie and Janssen. DMB is on the advisory boards for AbbVie, BMS, Gilead, Janssen, and Merck and received sponsorship/research grants from BMS, Janssen, Merck, and ViiV.

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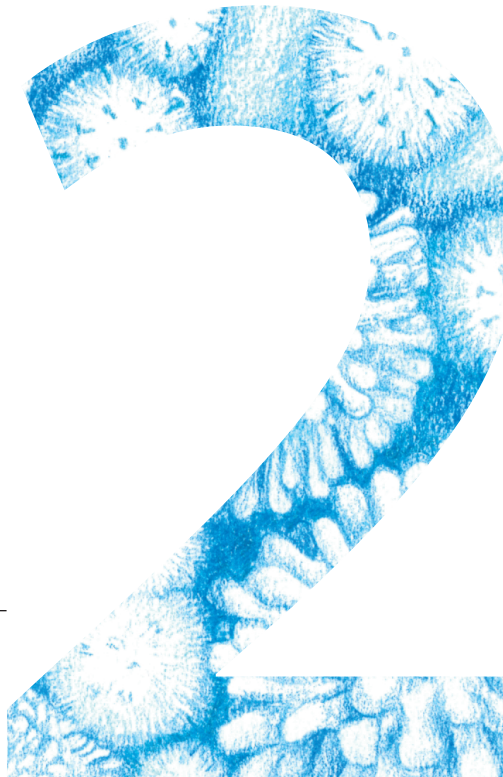
A call for a consortium for optimal management of drug-drug interactions in patient care

DM Burger
EJ Smolders
J Schapiro
JPH Drenth
DJ Back



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INTRODUCTION

During clinical development of medicines, manufacturers are obliged to assess the risk of drug-drug interactions (DDIs) with their new drug. There is no doubt that product labels of drugs that are nowadays introduced to the market contain much more information on DDIs than in the past. Indeed, the drug label is often the first source for DDIs available to physicians and pharmacists. But how informative are the data presented in the drug labels?

THE IMPORTANCE OF DRUG-DRUG INTERACTIONS

There is increased awareness of the importance of DDIs as they may be associated with clinical toxicity or treatment failure. This is fueled by a better understanding of mechanisms of DDIs, particularly when drug-transporters are involved. Also, the recognition that increased medication use in our ageing patient population leads to poly-pharmacy which is associated with an elevated risk of DDIs. Regulatory authorities such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have issued guidelines to support manufacturers in evaluating DDIs of not yet licensed drugs[1, 2]. A snapshot of that information will be channeled to the description of a drug's DDI potential in the product label.

Here we briefly describe the process of pre-licensure DDI evaluation. Second, we address four issues relating to DDI information in the product label from an academic/clinical point of view. These issues have been discussed by other stakeholders[3]. We illustrate this by commenting on two recently FDA and EMA-approved drug labels of direct acting antivirals (DAAs) for the treatment of chronic hepatitis C virus (HCV) infection, i.e. grazoprevir/elbasvir (Zepatier®) and velpatasvir/sofosbuvir (Epclusa®). These examples were chosen as they (1) reflect the current state of DDI reporting; (2) belong to a group of drugs with a high risk of DDIs[4]; and (3) are used in the treatment of chronic HCV patients who are known to use multiple concomitant medications[5].

ASSESSING A DRUG'S INTERACTION POTENTIAL DURING DRUG DEVELOPMENT

Generally speaking, the process of assessing a drug's potential to be a victim or a perpetrator of a (clinically significant) DDI during drug development involves three phases. DDIs can be assessed based on the plausible DDI mechanisms (via *in vitro* assessment or other knowledge) and also based on likely co-medications, or a combination of both a mechanistic and clinical relevance consideration. These three phases are not neces-

sarily conducted sequentially, but for the purpose of this commentary we describe them separately.

First, *in vitro* studies are conducted to determine a drug's substrate affinity and inhibitory/inducer capacity guided by a predefined list of preferred agents in these test systems[1, 2, 6]. A summary of the findings of the tests for sofosbuvir, velpatasvir, grazoprevir and elbasvir is listed in Table 1.

Table 1: Substrate affinity and inhibitory/inducer capacity of the DAAs discussed in this paper.

	Victim (= substrate of)		Perpetrator			
	Metabolism (enzyme)	Transporter	Metabolism (enzyme)		Transporter	
DAA			Inhibitor	Inducer	Inhibitor	Inducer
Sofosbuvir		P-gp BCRP				
Velpatasvir	CYP3A CYP2B6 CYP2C8	P-gp BCRP OATP1B1/3			P-gp BCRP OATP1B1/3	
Grazoprevir	CYP3A	OATP1B1/3	CYP3A		BCRP	
Elbasvir	CYP3A				BCRP	

DAA: Direct acting antiviral; P-gp: P-glycoprotein; BCRP: Breast cancer resistance protein; OATP: Organic anion transporter polypeptide; CYP: Cytochrome P450.

A second stage consists of clinical DDI studies with co-medications that have the potential to interact with the drug, for which both (probe) substrates and established perpetrators are used. For example, with grazoprevir, elbasvir, and velpatasvir, which were found *in vitro* to be cytochrome P450 (CYP3A) substrates, *in vivo* DDI studies with the established CYP3A inhibitor ketoconazole have been conducted[7, 8]. Additionally, both grazoprevir and velpatasvir have been studied with the CYP3A inducer rifampin and grazoprevir/elbasvir was studied with the CYP3A substrate midazolam, as grazoprevir is a weak CYP3A inhibitor[7, 8].

A third phase of DDI evaluations can be defined as a set of studies with frequently used co-medications in the target patient population for which the drug is being developed. Taking the DAAs again as examples, studies were planned with multiple antiretroviral agents and immunosuppressive agents, with the potential to examine both perpetrators and victims of a DDI.

Combining data from *in vitro* and clinical studies as part of physiologically-based pharmacokinetic (PBPK) modeling has also found its place into product labels, thereby extending our knowledge on DDIs without actually having to do all the clinical studies.

After completing these three pre-licensing phases, several pages of the product label are now dedicated to DDI information. The presentation of DDI data in product labels can be a challenge. One way to do this is by using Forest plots in which geometric mean ratios for area under the concentration-time curve (AUC) and maximum plasma concentration (C_{max}) are summarized from all DDI studies conducted.

One could also define a fourth, post-marketing, phase where yet undiscovered DDIs may be detected through clinical vigilance such as individual case reports and data mining of spontaneous reporting databases. In addition, controlled pharmacoepidemiologic studies are performed to examine the health effects of DDIs. These and other studies could also be requested or required by the regulatory agencies. This may lead to revisions of the product label, as was recently the case with amiodarone and sofosbuvir-based DAAs[9].

HOW INFORMATIVE IS THE DRUG-DRUG INTERACTION INFORMATION IN THE PRODUCT LABEL?

The purpose of a drug label is to assist a prescriber in the use of a specific medicine. However, a seemingly 'grey area' is how physicians should use (new) medications in patients on concomitant medications that are not mentioned in the product label. We recognize that it is unrealistic to expect product labels to have information on all therapeutic agents. The (online) available product label is a relatively static document that, despite regular updates, can be soon be outdated once it is published, as new data become available in the public domain. In our electronic age, we need to do better. Many healthcare providers will expect that the product label contains the relevant warnings for co-medications, and therefore assume that drugs not specifically mentioned in the label can be safely combined with the new drug. But to what degree is that true?

We now focus on four examples where we think that the drug label could be more informative:

1. In case a new drug is a CYP3A substrate, with which CYP3A inducers is an interaction mentioned in the label?
2. In case of interaction with CYP3A inducers, when will this lead to a contra-indication or labeled as 'not recommended'; in this the same or not?
3. In case a new drug is a (moderate) CYP3A inhibitor, which CYP3A substrates with a narrow therapeutic range are listed in the drug label?
4. New drugs are usually tested with relevant co-medication in the target patient population, but how is this set of 'relevant' medication selected?

To address the first issue, we have noticed that there is a clear warning in the product labels of both grazoprevir/elbasvir and velpatasvir/sofosbuvir that (strong) CYP3A inducers are contra-indicated with both DAA combinations. Guidance is given by the FDA providing a list of strong and moderate enzyme inducers[6]. However, comparison of the product labels with this list reveals important discrepancies (Table 2)[6]. Although both product labels state that the list of not recommended co-medications is 'not all inclusive' and the FDA warns that their examples of CYP3A inducers is not 'an exhaustive list' one wonders how many agents are missed? We acknowledge that there is no accepted list of CYP3A inducers, so this is an important knowledge gap. A well-known website (http://www.medicine.iupui.edu/clinpharm/ddis/) includes in addition to the drugs mentioned in Table 2 the following agents as CYP3A inducers: glucocorticoids, nevirapine, pioglitazone, and troglitazone. It must be noted that we could not find information how these agents have been selected and whether any external review process has been included.

Table 2: CYP3A inducers mentioned in product labels of grazoprevir/elbasvir and velpatasvir/sofosbuvir[7, 8] and in information provided by FDA[6].

Inducer	Grazoprevir/ elbasvir FDA label	Grazoprevir/ elbasvir EMA label	Velpatasvir/ sofosbuvir FDA label	Velpatasvir/ sofosbuvir EMA label	FDA table[b]
Phenytoin	X[a]	X	X	X	X
Carbamazepine	X	X	X	X	X
Rifampin	X	X	X	X	X
St John's wort	X	X	X	X	X
Efavirenz	X	X	X	X	X
Nafcillin	X				X
Bosentan	X	X			X
Etravirine	X	X			X
Modafinil	X	X		X	X
Phenobarbital			X	X	
Oxcarbazepine			X	X	
Rifabutine			X	X	
Rifapentin			X	X	

EMA guideline only reports rifampin as an inducer.
[a]Mentioned in label/table.
[b]Restricted to moderate and strong CYP3A inducers.

A second issue relates to what we think the goal of presenting DDI data in a product label is to assist clinicians in accurately weighing the risk/benefit ratio in view of the combination of drugs that a particular patient will be using. However, it is challenging for physicians to define such an individualized risk/benefit ratio. This is especially true

for combinations of drugs where information is often lacking or conflicting. In addition, the use of pharmacokinetic parameters and other mechanistic information in the label might be difficult to evaluate without some understanding of the concentration-effect relationship of a specific drug (pharmacokinetic-pharmacodynamic [PK-PD]). Therefore, terms such as 'contra-indicated', 'avoid use', or 'use with caution' are used in the product labels; however, these are suboptimal surrogates for a quantifiable risk/benefit ratio. Although clearly this guidance is far better than none at all – one needs to keep in mind its limitations when determining criteria and exact terms used. For example, rifampin is 'contra-indicated' with grazoprevir/elbasvir in both the EMA and FDA labels and velpatasvir/sofosbuvir is contra-indicated with rifampin in the EMA label but it is 'not recommended' in the FDA label. This is remarkable because trough levels of velpatasvir and grazoprevir are reduced by rifampin to a similar degree (i.e., 82 and 90%, respectively). Based on these pharmacokinetic results we would interpret the two terms as identical or should we see them as two different recommendations? Is there a suggestion that in some specific patients on rifampin velpatasvir/sofosbuvir might be used?

A third example relates to a drug being a CYP3A inhibitor and the selection of CYP3A substrates to be named in the product label. Here we only use the data from the grazoprevir/elbasvir label as velpatasvir/sofosbuvir does not possess CYP3A inhibitory capacity. Grazoprevir is a weak CYP3A inhibitor and premarketing *in vivo* studies demonstrated a 34% increase in midazolam AUC and a 43% increase in tacrolimus AUC[7]. How does this translate into warnings in the product label for other CYP3A substrates? At the time the grazoprevir/elbasvir file was reviewed, the FDA had specified a list of CYP3A substrates with narrow therapeutic ranges which are defined as 'those drugs where a small increase in exposure caused by CYP3A inhibition may lead to serious safety concerns'[6]. This list included the following marketed agents: alfentanil, ciclosporin, dihydroergotamin, ergotamin, fentanyl, pimozide, quinidine, sirolimus, and tacrolimus. We find it surprising that, with the exception of ciclosporin and tacrolimus, none of these CYP3A substrates with a narrow therapeutic range are included in the grazoprevir/elbasvir FDA and EMA labels[7].

More recently, the FDA website was updated and now a table is included with sensitive CYP450 substrates being drugs that demonstrate an increase in AUC of ≥5-fold with strong index inhibitors of a given metabolic pathway in clinical DDI studies. As grazoprevir/elbasvir is not a strong index inhibitor of CYP3A, this table does not apply.

A fourth and final comment relates to the selection of 'frequently used co-medications in the target patient population'. Antiretrovirals, immunosuppressive agents, statins, methadone, oral contraceptives, and acid-reducing agents are all part of common test

panels. But does this reflect the most widely used co-medication in the HCV patient population? To address this question, we used data from a paper by Lauffenberger et al who reviewed the most utilized medications in US patients with HCV from a large commercial insurance database[10]. It is remarkable that in the top-20 only omeprazole/pantoprazole is mentioned in the grazoprevir/elbasvir and velpatasvir/sofosbuvir FDA and EMA labels (Table 3).

Table 3: References in grazoprevir/elbasvir and velpatasvir/sofosbuvir product labels to most utilized medications in US patients with HCV based on a large commercial insurance database[10] .

Medication	Grazoprevir/elbasvir label	Velpatasvir/sofosbuvir label
Acetaminophen + combinations		
Zolpidem		
Levothyroxine		
Alprazolam		
Lisinopril		
Oxycodone		
Furosemide		
Amlodipine		
(Es)Omeprazole, Pantoprazole	F, E	F, E
Metformin		
Escitalopram		
Spironolactone		
Hydrochlorothiazide		
Bupropion		
Tramadol		
Metoprolol		
Lorazepam		
Azithromycin		
Atenolol		
Sertraline		

F: FDA label; E: EMA label

HOW TO MANAGE DRUG-DRUG INTERACTIONS WHEN PRODUCT LABELS DO NOT INTEND TO BE COMPLETE

DDI data are traditionally included in electronic prescribing systems for physicians and computerized drug-interaction surveillance systems for pharmacists. One could expect that these systems are up-to-date, which means that as soon as a new drug reaches the market contra-indicated drugs and warnings are added to these systems to guarantee

patient safety. This will be primarily based on the information provided by the drug label, as this is the only official communication on DDIs at such time point.

Various online DDI sources are available, such as Micromedex, First databank, in addition to the drug labels. The Working Group on Pharmacotherapy and Drug Information from the Royal Dutch Association for the Advancement of Pharmacy, take a step further by making predictions of DDIs based on similarities in pharmacokinetic profiles to other drugs not mentioned in the drug label[11]. In addition, the Interaction Checkers developed by the University of Liverpool (http://www.hiv-druginteractions.org & http://www.hep-druginteractions.org) have evolved to be an indispensable source for management of DDIs with antiretroviral agents and DAAs, and are now also included in international treatment guidelines. Over the years, >800 individual co-medications have been carefully evaluated and added to the websites. But one could also question why such external resources are necessary to fill a knowledge gap. Are all the evaluations of DDIs in such web resources being done as rigorously as the FDA and EMA do when reviewing a new drug application?

FINAL CONCLUSIONS AND RECOMMENDATIONS

We wish to stress that this commentary was not written to criticize either specific pharmaceutical companies who write drug labels or the regulatory authorities who approve these labels, but rather to start a discussion on how we can better inform healthcare providers about DDIs. We believe that more complete information on DDIs is important for safer patient management, and accept this is not only the responsibility of manufacturers and regulators. We suggest an initiative that includes all relevant stakeholders and addresses a systematic evaluation of DDI data, defines research gaps, and writes consensus documents. When will the Consortium for Optimal Management of drug-drug Interactions in patient Care (COMIC) see daylight?

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Metformin and daclatasvir: absence of a pharmacokinetic-pharmacodynamic drug-interaction in healthy volunteers

EJ Smolders
EPH Colbers
CTMM de Kanter
K Velthoven-Graafland
LT Wolberink
N van Ewijk-Beneken Kolmer
JPH Drenth
RE Aarnoutse
CJ Tack
DM Burger

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ABSTRACT

Aim

The aim of this study was to evaluate the effect of the proposed organic cation transporter (OCT) inhibitor daclatasvir on the pharmacokinetics and pharmacodynamics of the OCT substrate metformin.

Methods

This was an open-label, two-period, randomized, cross-over trial in 20 healthy subjects. Treatment A consisted of metformin and treatment B consisted of metformin + daclatasvir. Pharmacokinetic curves were recorded at steady-state. Geometric mean ratios (GMRs) with 90% confidence intervals (CIs) were calculated for metformin area under the concentration-time curve from 0 to 12 hours (AUC_{0-12}), maximum plasma concentration (C_{max}), and final plasma concentration (C_{last}). An oral glucose tolerance test was performed, measuring insulin, glucose and lactate levels.

Results

The GMRs (90% CI) of metformin AUC_{0-12}, C_{max}, and C_{last} (B versus A) were 109% (102-116%), 108% (101-116%), and 112% (103-122%). The geometric mean AUC_{0-2} for insulin, glucose, and lactate during treatments A and B were 84 and 90 h·mE/L, 13.6 and 13.4 h·mmol/L, and 3.4 and 3.5 h·mmol/L, respectively.

Conclusions

Bioequivalence analysis showed that daclatasvir does not influence the pharmacokinetics of metformin in healthy subjects. Pharmacodynamic parameters were also comparable between treatments.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is associated with insulin resistance, which might cause type 2 diabetes mellitus (T2DM)[1]. It is estimated that 150-170 million people are infected with HCV worldwide and 422 million people were living with T2DM in 2014[2, 3]. In addition, both conditions have a high impact on international healthcare because of the high morbidity and mortality rates of T2DM and HCV[4, 5].

The association between HCV and insulin resistance/T2DM has been studied extensively[6, 7]. Compared with controls, there is an increased prevalence of diabetes mellitus (DM) in HCV patients[8]. T2DM is two- to threefold more prevalent in HCV-infected patients compared with hepatitis-B-infected individuals[1]. Insulin resistance itself causes liver disease[1], and in combination with HCV, patients have an increased risk of developing cirrhosis and hepatocellular carcinoma[8, 9]. Furthermore, insulin resistance in HCV patients is correlated with reduced efficacy of HCV treatment, and viral clearance is associated with improved insulin sensitivity[1, 10]. In the literature, there is consensus about the relationship between HCV and insulin resistance/T2DM; however, the mechanisms behind this relationship are still under debate[7].

Metformin is a biguanide used for the treatment of T2DM, as it has the ability to lower the blood glucose concentrations in these patients. In Western counties, metformin is the first choice in treatment of T2DM. It is not metabolized but it is a substrate of several membrane transporters – namely, plasma membrane monoamine transporter (PMAT), organic cation transporter (OCT) 1, 2 and 3, and multidrug and toxin extrusion protein (MATE) 1 and 2K. The oral absorption and hepatic uptake of metformin is mediated by PMAT, OCT1, and OCT3. However, the involvement of the OCTs in intestinal absorption remains controversial[11, 12]. Metformin is excreted renally through glomerular filtration (protein binding is negligible) and active tubular secretion[11]. Tubular secretion is facilitated by uptake into the tubular cells via OCT2 and excretion into the urine via MATE1 and MATE2K[13]. Tubular reabsorption might be facilitated by OCT1 and PMAT[11, 14, 15]. As the renal clearance of metformin is higher than creatinine clearance, it is deduced that tubular secretion plays an important role in its excretion[16].

Drug-interactions influencing metformin pharmacokinetics (PK) are a result of inhibition or induction of the previously mentioned drug-transporters. The human immunodeficiency virus (HIV) integrase inhibitor dolutegravir increases the metformin exposure by 79%, probably via inhibition of OCT2[17]. Rifampicin, an OCT inducer, causes increased renal clearance and tubular secretion of metformin[18].

Similarly, a potential interaction may exist between the nonstructural protein 5A (NS5A) inhibitor daclatasvir and metformin. Daclatasvir is used for the treatment of HCV infection in combination with sofosbuvir and is licensed for the treatment of genotype 1, 3, and 4[19]. It is metabolized by cytochrome P450 (CYP) 3A4 and is a substrate of P-glycoprotein (P-gp). It does not influence drug-metabolizing enzymes but, at least *in vitro*, it seems to inhibit the activity of several drug-transporters, such as P-gp, OCT1, OCT2, organic anion transporting polypeptide 1B1, and breast cancer resistance protein[19]. However, the clinical relevance of OCT1 and OCT2 inhibition was unknown at the time of the present study.

Our hypothesis is that daclatasvir could decrease metformin tubular excretion, through inhibition of OCT2, and therefore causes increased plasma concentrations and increased glucose-lowering activity. Inhibition of OCT1 in the liver could also lead to increased plasma concentrations of metformin. The proposed *in vivo* PK interaction and the net pharmacodynamic (PD) effect are unknown, and therefore we conducted a PK-PD study to evaluate the potential drug-drug interaction between daclatasvir and metformin.

METHODS

Study design

This was an open-label, two-period, randomized, cross-over trial in healthy subjects. Subjects were randomized in treatment sequences AB and BA. Treatment A (reference) consisted of 500 mg metformin twice daily (BID) on day 1 and 2 (Metformin HCL Actavis 500 mg, Aurobindo Pharma - Milpharm Ltd, Middlesex, United Kingdom). The dose was increased to 1,000 mg BID on days 3-8. This gradual dose step-up was chosen to limit adverse events (AEs), as subjects used metformin without food for 8 days.

Treatment B (test) consisted of 500 mg metformin BID (day 1-2) and 1,000 mg metformin BID (days 3-8). From day 1 to day 8, 60 mg daclatasvir once daily was added (Daklinza®, Bristol-Myers Squibb, Uxbridge, United Kingdom[20]). Between treatments there was a washout period of 13 days.

To study metformin and daclatasvir exposure, at day 8 of treatment (steady-state), blood samples for a PK curve were obtained up to 12 hours and 24 hours after intake of metformin and daclatasvir, respectively. Secondly, to study metformin excretion, 12-hour urine was collected for the determination of metformin renal clearance.

The PD of metformin was studied using an oral glucose tolerance test (OGTT), which was also performed at day 8 of treatment. During this 2-hour test, venous blood was withdrawn to determine the plasma concentrations of glucose, lactate, and insulin.

Study participants

Healthy males and females were included. Subjects eligible for inclusion were 18-55 years of age and had a body mass index (BMI) of 18-36 kg/m^2. Subjects had to be in good age-appropriate health condition (physical examination, electrocardiography, biochemical, hematologic, and urinalysis testing). No concomitant medication was allowed, except for acetaminophen <2,000 mg/day. Main exclusion criteria were a positive HIV, hepatitis B, or HCV test, pregnancy, and estimated glomerular filtration rate (eGFR) <60 mL/min.

Dosing and adherence

During study visits at days 1, 2, 3, 5, and 8, medication was administered at 08:00 hour, supervised by the study personnel. In between study visits, subjects took the medication at home, and adherence was assessed as follows: (1) tablets were counted by the trial nurses; (2) Medication Event Monitoring System (MEMS) caps (Aardex Ltd, Zug, Switzerland) were used to monitor the opening of the metformin-containing bottles; and (3) subjects were instructed to record the time of medication intake (and any AE) in a diary.

PK sampling and oral glucose tolerance test

The study was conducted at the Clinical Research Centre Nijmegen in the Radboud university medical center, Nijmegen, the Netherlands.

At steady-state (day 8), blood samples were withdrawn to measure the plasma concentrations of metformin (A and B) and daclatasvir (B). Drugs were taken concomitantly after an overnight fast, and blood was withdrawn in ethylene diamine tetra-acid tubes at $t = 0$ (pre-dose), 0.5, 1, 1.5, 1.9, 2.5, 3, 4, 6, 8, 10, and 12 hours after metformin intake. During treatment B (daclatasvir), an additional sample was collected at 24 hours. Blood samples were stored in a refrigerator until centrifuged (5 minutes at 1,900 g). Plasma was transferred into polypropylene tubes and stored at -40°C until bioanalysis. To study metformin excretion at steady-state and to assess the renal clearance of metformin, urine was collected for 12 hours at intervals of 4 hours. Prior to the start of collection, morning urine was voided before the administration of metformin. Participants were asked to drink 200 mL water every 4 hours. Volume and pH of urine were noted, and it was stored at -40°C until further bioanalysis.

For the OGTT, the participants were instructed to avoid strenuous exercise and to follow a carbohydrate-controlled diet (at least 200-250 gram carbohydrates per day) for 3 days prior to day 8. The OGTT was performed after an overnight fast for at least 14 hours. At 10:00 hour, the subjects drank 75 g glucose in 200 mL water. Following the glucose intake, venous blood was withdrawn at $t = 0$ (pre-dose), 30, 60, 90, and 120 minutes to determine the plasma concentrations of glucose, lactate, and insulin.

Data were collected using Castor EDC (Castor Electronic Data Capture, Ciwit BV, Amsterdam, the Netherlands).

Bioanalytical methods

Metformin and daclatasvir were analyzed in the laboratory of the Department of Pharmacy of the Radboud university medical center, Nijmegen, the Netherlands. Metformin in the plasma and urine were determined using two different validated ultra-performance liquid chromatography (UPLC) assays with ultraviolet (UV) detection (236 nm).

Metformin was extracted from 200 µL plasma using 80 µL 4M sodium hydroxide and 3 mL 1-butanol/ (n-) hexane (50:50, *v/v*). This solution was vortexed for 1 minute at 1,600 rpm and centrifuged for 5 minutes at 1,900 *g*. The aqueous phase was frozen for 1 minute at -40°C before the organic phase was poured into a vial. Metformin was then back-extracted from the organic phase by adding 200 µL 0.1% phosphoric acid. This solution was vortexed for 1 minute at 1,600 rpm and centrifuged for 5 minutes at 1,900 *g*.

Metformin was extracted from 20 µL urine following the same procedures after adding 200 µL blank plasma. After back-extraction, 100 µL of the water phase was diluted with 900 µL water before injection.

Chromatography was performed using an Acquity UPLC HSS T3 analytical column (1.8 µm, 2.1 × 100 mm; Waters, Milford, MA, USA) with a mobile phase of 0.02 M phosphate buffer, pH 3.23. The flow rate was set on 0.6 mL/min. After every injection, the column was rinsed with a combination of eluent and acetonitrile (50:50, *v/v*) before equilibrating back to the initial eluent.

Accuracy across five metformin quality-control samples measured in three runs (n = 15) over 2 days ranged from 101 to 103% in plasma and 98 to 101% in urine. Interday precision ranged from 0.0 to 2.4% in plasma and 0.0 to 3.9% in urine (n = 15). Intraday precision ranged from 1.2 to 5.8% in plasma and 2.3 to 8.9% in urine (n = 5). For met-

formin in plasma, the calibration range was 0.01-5.00 mg/L and for urine the range was 2.0-2,100 mg/L.

Daclatasvir was measured using a validated UPLC method with UV detection (314 nm). Daclatasvir was extracted from 100 µL plasma using 200 µL acetonitrile/methanol (50:50, *v/v*) with 0.1% formic acid. This solution was vortexed for 5 minutes at 2,500 rpm and centrifuged for 5 minutes at 1,910 *g*. The supernatant (170 µL) was poured into a vial and centrifuged for 5 minutes at 1,910 *g*; 10 µL was then injected onto an Acquity UPLC BEH C18 analytical column (1.7 µm, 2.1 × 50 mm; Waters, Milford, MA, USA). The flow rate was set to 0.550 mL/min, and daclatasvir was eluted by using a gradient 0.05 M phosphate buffer and 0.05 M phosphate buffer/acetonitrile 30/70 *v/v*.

Accuracy across five daclatasvir quality-control samples, measured in three runs over 2 days, ranged from 98 to 107%. Interday precision ranged from 0.0 to 1.3% and intraday precision ranged from 1.3 to 6.0%. The calibration range of the method was 0.03-10 mg/L.

Insulin samples were collected in lithium-heparinized tubes and determined at the clinical chemistry laboratory of Radboud university medical center, Nijmegen, the Netherlands (random access analyzer, Roche E170 modular immunoassay, Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Glucose and lactate (blood gas tube Pro-Vent 4646E, lithium-heparin coating, Smiths Medical, MN, USA) were determined directly after sampling, using a glucose enzymatic-amperometric method (Biosen C-line GP, EKF-diagnostic GmbH, Barleben, Germany).

Pharmacokinetic analysis

A non-compartmental approach was used (WinNonlin/Phoenix version 6.3, Pharsight Corporation, St. Louis, MO, USA) to assess the area under the concentration-time curve from 0 to 12 hours ($AUC_{0\text{-}12}$) and 12-hour plasma concentration (C_{12}) for metformin and from 0 to 24 hours ($AUC_{0\text{-}24}$) and 24-hour plasma concentration (C_{24}) for daclatasvir. In addition, maximum plasma concentration (C_{max}), time to reach C_{max}, and apparent elimination half-life of metformin and daclatasvir were determined. Metformin renal clearance was calculated by dividing the total amount metformin excreted (0-12 hours) by the $AUC_{0\text{-}12}$. The secretion of metformin was calculated by subtracting the metformin clearance from the creatinine clearance, which was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula:

$$eGFR\ (mL/min/1.73\ m^2) = 141 \times \text{minimum}\ (Scr/\kappa)^{\alpha} \times \text{maximum}\ (Scr/\kappa, 1)\text{-}1.209 \times 0.993^{Age} \times 1.018\ [\text{if female}] \times 1.159\ [\text{if black}])$$

With Scr as standardized serum creatinine in mg/dL, α as -0.329 and -0.411 for females and males, κ as 0.7 and 0.9 for females and males, respectively, and age in years.

In addition, the geometric mean ratios (GMRs) with 95% confidence intervals (CIs) (treatment B versus A) for metformin secretion and eGFR were calculated.

Pharmacodynamic analysis

The plasma concentrations of glucose, lactate, and insulin were used to calculate the area under the concentration-time curve from 0 to 2 hours (AUC_{0-2}) for which WinNonlin/Phoenix was used. The insulin and glucose concentrations were also used to calculate the homeostatic model assessment insulin resistance (HOMA-IR) score, which is used to quantify insulin resistance (HOMA-IR = [glucose × insulin]/22.5).

Statistical analysis

The AUC_{0-12} values of metformin for treatments A and B were compared using the bioequivalence approach, which is recommended by the European Medicines Agency (EMA) to evaluate PK drug-interactions[21]. GMRs with 90% CIs of AUC_{0-12}, C_{max}, and C_{12} were calculated for metformin, comparing treatment B and treatment A. We used a linear mixed-effect model with fixed parameters to calculate the GMR with 90% CI. Fixed parameters were treatment, period, sequence, and subjects within sequence according to EMA guidelines[21].

For bioequivalence between treatments A and B, the AUC_{0-12} GMR with 90% CI should fall within the range of 80-125%.

Based on a previously observed inter-subject coefficient of variation (CV%) of 22% for metformin AUC_{0-12}[22], we expected the intra-subject CV% to be lower: 15%. For the sample-size calculation, we used a power calculation in SAS® 9.2 (SAS Institute Inc. 2011. Cary, NC, USA) using a paired t-test for lognormal distribution for showing equivalence). For 80% power to prove bioequivalence, a sample size of 17 subjects should be included in the study. To account for possible dropouts, 20 subjects were to be included.

Metformin renal clearance was log-transformed and compared between treatments using a paired t-test.

Glucose, lactate, and insulin AUC_{0-2} values were log-transformed and compared between treatments using a paired t-test. All statistical analyses were performed in IBM

SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

Safety and tolerability

During all study visits, AEs and laboratory safety (biochemistry and hematology) were monitored by the study nurses and physicians. AEs were graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events ('DAIDS AE Grading Table'); version 1.0, December 2004, clarification August 2009[23]).

Ethics

The trial was approved by the Investigational Review Board of Radboud university medical center, Nijmegen, the Netherlands. The trial was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki and registered at ClinicalTrials.gov (NCT02565862). All participants signed informed consent forms before screening evaluations.

RESULTS

Baseline characteristics

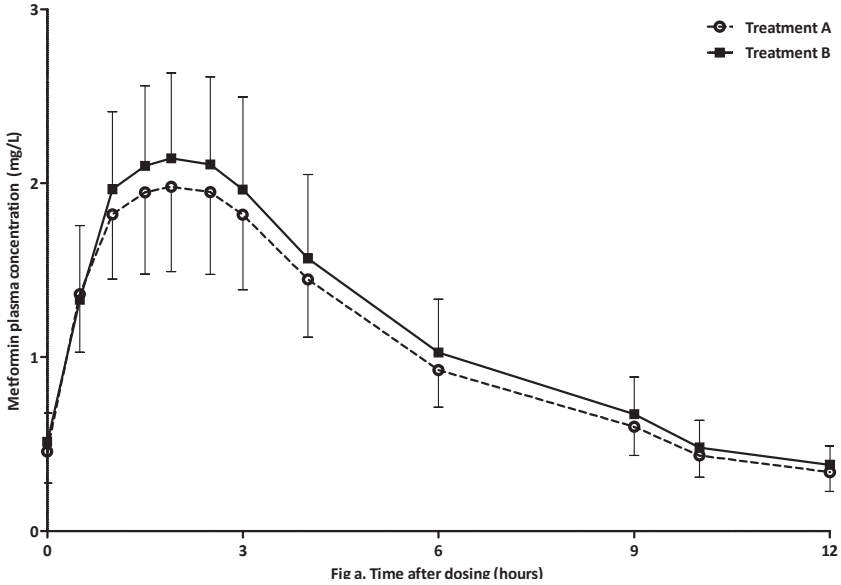
Twenty subjects (nine male) were enrolled, and all subjects completed the study. All subjects were white; their median (range) age was 47.5 (20-55) years and the median (range) BMI was 26.6 (22.9-36.0) kg/m^2. The subjects were in normal health, based on medical history, physical examination, vital signs, and biochemical and hematology data.

In general, adherence to the study medication was good, as proven by pill count, monitoring of the MEMS caps and the registration in the diary. Two subjects took a double dose of daclatasvir, and three subjects forgot one or two tablets of 500 mg metformin. These deviations did not lead to exclusion of any of the study participants.

Pharmacokinetics of metformin and daclatasvir

Steady-state geometric mean (GM) concentration-time curves of metformin are shown in Figure 1a and the PK parameters are given in Table 1.

One subject vomited during treatment A; therefore, the results of 19 subjects are presented. The GMR with 90% CI of the metformin $AUC_{0\text{-}12}$, C_{max}, and C_{12} of metformin with and without daclatasvir (B versus A) were 109% (102-116%), 108% (101-116%), and 112% (103-122%), respectively. As the CIs of all parameters fell within the range of 80-125%, absence of an interaction was confirmed.



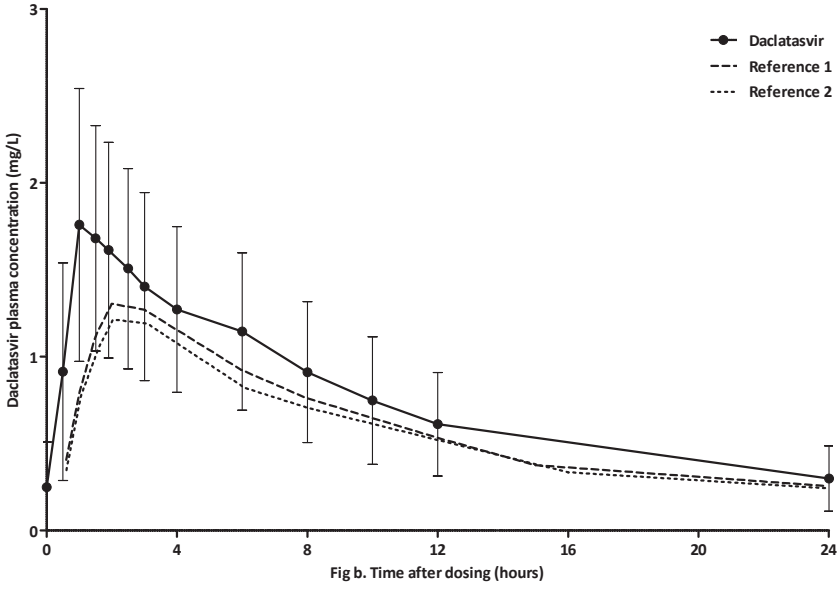


Figure 1: Pharmacokinetic curves of metformin for both treatments (A). Pharmacokinetic curve of daclatasvir (and reference) (B).

Data shown are geometric means with geometric coefficient of variation. Reference curves for daclatasvir were adapted from Gandhi et al[24].

A: Data of 19 subjects were used.

B: Data of 20 subjects were used.

Table 1: Steady-state pharmacokinetic parameters of metformin (*n* = 19) and daclatasvir (*n* = 20).

Metformin	Treatment A (n = 19[a])	Treatment B (n = 20)	GMR % (90% CI)
AUC_{0-12} (h·mg/L)	12.41 (22)	13.54 (25)	109 (102-116)
C_{max} (mg/L)	2.06 (23)	2.23 (23)	108 (101-116)
C_{12} (mg/L)	0.34 (32)	0.38 (29)	112 (103-122)
T_{max} (h)[b]	1.9 (1-2.5)	1.9 (1-3.0)	-
$T_{1/2}$ (h)[c]	4.77 (19)	4.86 (22)	-
Daclatasvir	**Treatment B**	**Reference[d]**	
AUC_{0-24} (h·mg/L)	18.38 (44)	12.7 (41); 13.8 (26)	
C_{max} (mg/L)	1.85 (40)	1.34 (38); 1.41 (28)	
C_{24} (mg/L)	0.30 (63)	0.225 (54); 0.225 (36)	
T_{max} (h)[a]	1 (1-2.5)	2.0 (1.0; 6.0)	
$T_{1/2}$ (h)[b]	11.23 (23)	-	

Geometric means are presented with geometric coefficient of variation. Also, the geometric mean ratios of treatment B (with daclatasvir) versus treatment A (without daclatasvir) are presented.

[a]For treatment A, 19 subjects are used for the pharmacokinetic analysis as 1 subject vomited during treatment.

[b]Values presented are medians (range).

[c]The apparent $T_{1/2}$ is calculated.

[d]The reference values from Gandhi et al are presented[24].

AUC_{0-12}: Area under the concentration-time curve from 0 to 12 hours; AUC_{0-24}: Area under the concentration-time curve from 0 to 24 hours; C_{12}: 12-hour plasma concentration; C_{24}: 24-hour plasma concentration; 90% CI: 90% confidence interval; C_{max}: Maximum plasma concentration; GMR: Geometric mean ratio; $T_{1/2}$: Elimination half-life; T_{max}: Time to reach C_{max}.

Urine was collected to estimate renal metformin clearance. Treatment B included 19 subjects because urine was not correctly stored for one subject. The GM (range) renal clearance of metformin for treatment A was 351 (148-646) mL/min and for treatment B was 333 (166-537) mL/min (p = 0.504). The GM (range) for metformin secretion during treatments A and B was 275 (25-538) mL/min and 269 (91-445) mL/min (p = 0.3822), respectively. The GMR (95% CI) for metformin secretion (B versus A) was 98% (70-137%) and 98% (95-101%), respectively, for eGFR (Figure 2).

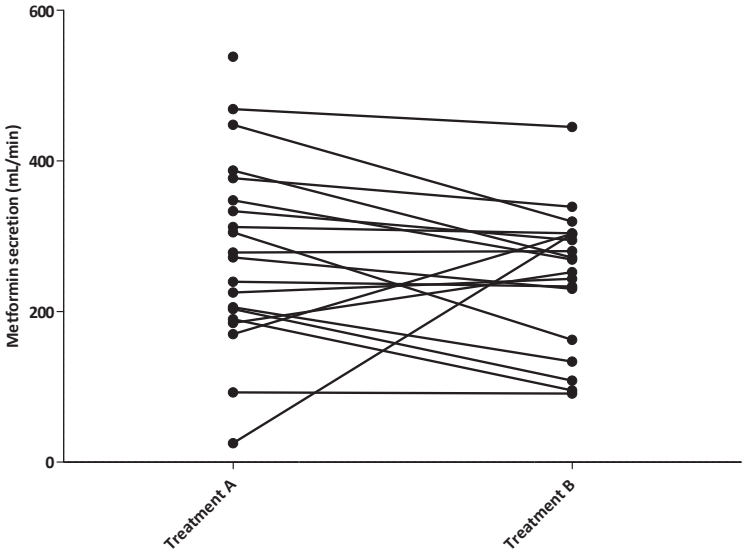


Figure 2: The 12-hour metformin secretion during treatments A and B shown per patient.
The urine of one subject was discarded during the trial; therefore, the metformin secretion of 19 subjects is shown.

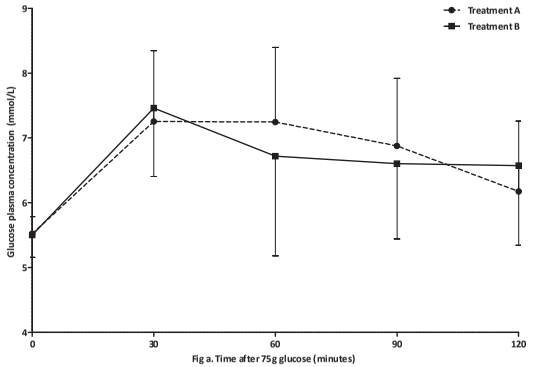
The GM concentration-time curve of daclatasvir and PK parameters are shown in Figure 1b and Table 1, respectively, combined with references values[24]. The GMs with geometric coefficient of variation (GCV%) $AUC_{0\text{-}24}$, C_{max}, and C_{24} values of daclatasvir were 18.38 (44) h·mg/L, 1.85 (40) mg/L, and 0.30 (63) mg/L.

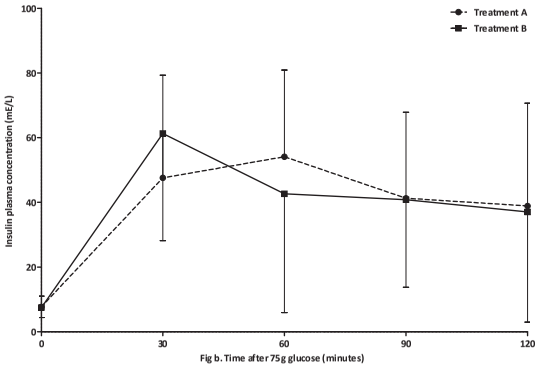
Pharmacodynamics of metformin

The OGTT was used to study PD endpoints (insulin, lactate, glucose). Treatment A contained data from 19 subjects as one subject was not able to tolerate the glucose drink during treatment A. The subject vomited and was excluded from the analysis.

The GM (GCV%) for $AUC_{0\text{-}2}$ for insulin for treatments A and B were 86 (49) and 87 (54) h·mE/L, respectively ($p = 0.430$). The glucose and lactate GM (GCV%) $AUC_{0\text{-}2}$ values during treatments A and B were 13.7 (10) and 13.4 (14) h·mmol/L and 3.4 (15) and 3.4 (18) h·mmol/L, respectively ($p = 0.919$; $p = 0.779$, respectively) (Figure 3). In Figure 4, we show the $AUC_{0\text{-}2}$ ratios (treatment B/treatment A) per subject for glucose, insulin, and lactate.

The HOMA-IR score was calculated for the individual subjects (treatments A and B), showing the variation of insulin resistance in the study population. The HOMA-IR varied from 0.73 to 4.81 during treatment A and 0.94 to 4.19 during treatment B.





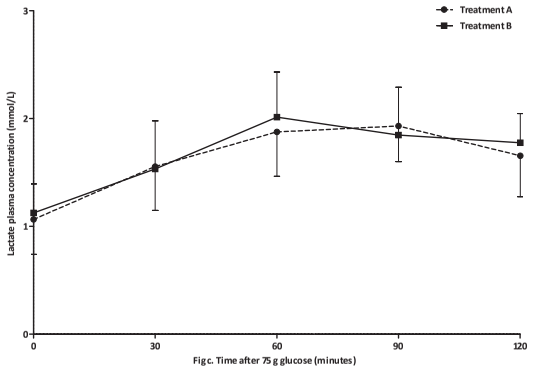


Figure 3: Results of the pharmacodynamic analysis. Glucose (A), insulin (B), and lactate (C) area under the concentration-time curves from 0 to 2 hours are shown.

Presented values are geometric means with geometric coefficients of variation (n = 19).

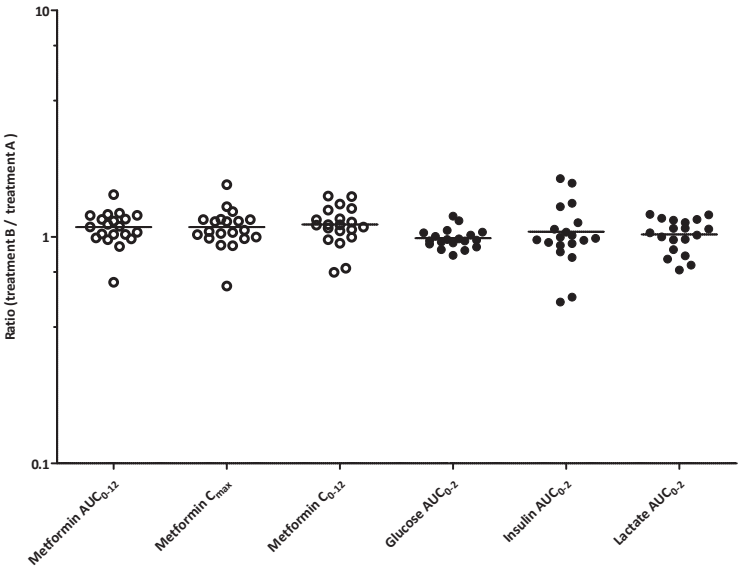


Figure 4: Ratios for treatment B/treatment A shown per subject for metformin area under the concentration-time curves from 0 to 12 hours, metformin maximum plasma concentration, metformin 12-hour plasma concentration, all open circles. In addition, the closed circles show the ratio for treatment B/treatment A per subject for the glucose area under the concentration-time curves from 0 to 2 hours (AUC_{0-2}), insulin AUC_{0-2}, and lactate AUC_{0-2}.

One subject did not tolerate the oral glucose tolerance test during treatment A; therefore, the ratio of 19 subjects is shown for glucose, insulin and lactate.

AUC_{0-12}: Area under the concentration-time curve from 0 to 12 hours; C_{max}: Maximum plasma concentration; C_{12}: 12-hour plasma concentration; AUC_{0-2}: Area under the concentration-time curve from 0 to 2 hours.

Safety and tolerability

A total of 129 AEs were reported during the trial, varying from three to 11 AEs per subject. No serious adverse events (SAEs) were reported. Only one grade 3 AE was reported: elevated amylase. The majority of the AEs were 'probably' related to the use of study medication (55%) and were reported during the combined treatment of metformin and daclatasvir (59%). Six AEs (four subjects) were reported directly after the intake of the study medication.

Most commonly reported AEs were diarrhea (n = 26), stomach ache/stomach cramps (n = 15), nausea (n = 11), headache (n = 10), and fatigue (n = 9). The gastrointestinal AEs are most likely caused by metformin. Subjects recovered from all AEs after the end-of-treatment. AEs reported (≥5%) per treatment are given in Table 2.

Table 2: Adverse events (AEs) reported during the trial, per treatment. Only AEs that were reported ≥5.0 % per treatment are given.

	Treatment A (Total number AEs: 53)			Treatment B (Total number AEs: 76)		
	Subjects, n	AEs, n	AEs, %	Subjects, n	AEs, n	AEs, %
Diarrhea	10	11	21	12	15	20
Fatigue	5	5	9	4	4	5
Stomach ache/cramps	5	5	9	6	10	13
Nausea	4	4	8	5	7	9
Sore throat	3	3	6	-	-	-
Vomiting	2	3	6	2	2	3
Common cold	3	3	6	-	-	-
Headache	2	2	4	7	8	11

Treatment A: 1,000 mg metformin twice daily.
Treatment B: 1,000 mg metformin twice daily and 60 mg daclatasvir once daily.
Toxicity grades were judged by the trial physician and graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events ('DAIDS AE Grading Table'); version 1.0, December 2004, clarification August 2009[23].
AEs: Adverse events.

DISCUSSION

We studied the potential interaction between the NS5A inhibitor daclatasvir and the biguanide metformin in healthy volunteers. We hypothesized that the exposure to metformin could possibly be increased due to OCT1 and/or OCT2 inhibition by daclatasvir, with altered glucose plasma concentrations as a result.

The results of the PK analysis did not support this hypothesis: no interaction was observed when metformin was administered with daclatasvir. In addition, there was no difference in metformin renal secretion between treatments. Therefore, we concluded that daclatasvir does not affect systemic exposure to metformin. Similarly, the PD analysis showed no difference between treatments, so we concluded that there was no PD interaction between daclatasvir and metformin.

The apparent absence of an effect of daclatasvir on metformin PK is confirmed by *in vitro* studies that were carried out later. Comparing the maximum therapeutic concentration of daclatasvir (C_{max}) of 1.85 mg/L found in the present study with the reported half-maximum inhibitory concentration (IC_{50}) showed that the *in vivo* unbound C_{max} was indeed lower than the *in vitro* data, as daclatasvir is highly bound to plasma proteins (99%). The IC_{50} of daclatasvir for OCT2 was 7.3 μM[25] and for OCT1 it was 1.4 μM[26], representing plasma concentrations of ~5.4 mg/L and ~1.0 mg/L, respectively, of unbound daclatasvir. We should note that, at the site of action (intestine, hepatocyte),

the daclatasvir concentration might be different to the C_{max} used, as the latter is the plasma concentration after systemic absorption. Daclatasvir concentrations could be higher in the intestine and portal vein, possibly inhibiting OCTs. This could be an explanation for the statistically significantly increased metformin plasma concentration when combined with daclatasvir (GMR and CI >100%). We argue that this increase is not clinically relevant for patients with normal metformin clearance but it might be clinically relevant in special populations with reduced metformin clearance, such as patients with renal impairment.

In daily practice, metformin is administered with food. In the present trial, we deviated from this recommendation, as subjects had to fast overnight for the execution of the OGTT. The systemic exposure of metformin is decreased in a fed state (C_{max}: 40%; AUC: 25%)[27]. In our study, C_{max} and AUC were elevated when compared with a previous study in healthy volunteers where metformin was taken with food: C_{max} 1.32 mg/L and $AUC_{0\text{-}24}$ 20.5 h·mg/L[28]. The high number of metformin-related AEs could be explained by these increased metformin exposures. Intake of metformin without food might cause additional AEs[27].

We used an OGTT to study the PD effect of metformin on glucose regulation with and without daclatasvir. The OGTT was conducted because the PK drug-interaction was only clinically relevant when also the glucose regulation (PD) was be altered. In addition, we did not want to exclude the possibility that there was a PD effect without a PK effect. In the present study, we showed that both PK and PD were related, as neither the systemic metformin concentrations nor OGTT results were affected by daclatasvir. The relation between the OGTT and metformin PD was shown previously in healthy volunteers, whereas the blood glucose levels were not altered[18, 29, 30].

Daclatasvir PK was studied only in treatment group B, in the presence of metformin; therefore, the PK of daclatasvir was compared with literature (Table 1). Daclatasvir was not studied separately, as metformin was thought not to influence any drug-metabolizing enzymes or transporters, and therefore we did not expect that metformin would influence daclatasvir PK[31]. Daclatasvir exposure was increased compared with reference values, as shown in Figure 1b[24]. In our study, subjects took daclatasvir while fasted, whereas daclatasvir was taken with food in the reference study. This might be an explanation for the elevated daclatasvir plasma concentrations because food decreases daclatasvir AUC by 23% and C_{max} by 28%[20]. However, daclatasvir plasma concentrations were somewhat higher than we would expect based on the food effect alone. Daclatasvir PK is increased solely by CYP3A4 and/or P-gp inhibitors, and metformin is neither of these. It could be that metformin induces other unidentified drug-transporters

or drug-metabolizing enzymes that contribute to the metabolism or distribution of daclatasvir[32]. Another explanation could be that the fasted healthy volunteers in our study had better absorption of daclatasvir, possibly caused by a more acidic gastric pH, increasing the solubility of daclatasvir.

No unexpected AEs or SAEs were reported in the present study. The study medication was well tolerated overall; however, almost all subjects reported diarrhea and/or stomach ache/cramps, which were related to the use of metformin. One subject did not tolerate the glucose solution, but, overall, the OGTT was well tolerated by the fasted participants. However, we should point out that the number of AEs was 76 with combined treatment of daclatasvir and metformin, versus 53 when metformin was given alone. This might have been caused by the relatively high daclatasvir plasma concentrations combined with the small increase of metformin plasma concentrations. Therefore, our recommendation is that daclatasvir and metformin can be combined, although we recommend that physicians monitor for (altered) AEs during treatment.

The limitations of our study were that daclatasvir PK was not studied separately and that we included healthy, white subjects who might not completely reflect the HCV/T2DM patient population that will use these drugs. Therefore, we included subjects with a wide range of age, BMI, and insulin resistance (HOMA-IR).

We did not determine OCT genotypes because all the PK curves for the subjects were in the same concentration range; we observed a low inter-subject variability for metformin; and the sample size was limited.

In conclusion, the establishment of bioequivalence in the present study showed that daclatasvir did not influence the PK of metformin in healthy subjects. PD parameters were also comparable between treatments. An increased number of AEs was reported when daclatasvir was combined with metformin; however, no unexpected AEs were reported. We recommend monitoring for altered AEs during treatment when daclatasvir and metformin are combined in HCV-infected patients with T2DM.

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CONFLICTS OF INTEREST

EJS, AC, CTMMK, KVG, LTW, NEBK, JPHD, REA and CJT declare that they have no conflicts of interest that are directly relevant to the content of this manuscript. DMB is a member of advisory boards of AbbVie, Bristol-Myers Squibb, Gilead, Janssen, Merck and ViiV Healthcare. He received sponsorship and research grants from Bristol-Myers Squibb, Janssen, Merck, and ViiV Healthcare. However, this did not influence the content of this manuscript.

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Daclatasvir 30 mg/day is the correct dose for patients taking atazanavir/cobicistat

EJ Smolders
EPH Colbers
CTMM de Kanter
K Velthoven-Graafland
JPH Drenth
DM Burger

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ABSTRACT

Background

Atazanavir is boosted with cytochrome P450 (CYP) 3A4 inhibitor ritonavir. When combined with the CYP3A4 substrate daclatasvir, the daclatasvir dosage should be reduced from 60 mg to 30 mg once daily. Recently, cobicistat was licensed as a CYP3A booster and used in combination with atazanavir.

Objective

We studied if the fixed-dose combination of atazanavir/cobicistat has an influence on daclatasvir pharmacokinetics comparable to that of as seen with the separate agent's atazanavir and ritonavir.

Methods

This was a prospective, open-label, two-period, randomized, cross-over trial was performed in 16 healthy subjects (NCT02565888). Treatment consisted of 300/100 mg of atazanavir/ritonavir plus 30 mg of daclatasvir once daily (reference) and a second period of 300/150 mg of 30 mg of atazanavir/cobicistat plus daclatasvir once daily (test). A 24-hour pharmacokinetic, steady-state curve was recorded for all drugs. Geometric mean ratios (GMRs) with 90% confidence interval (CI) were calculated for daclatasvir and atazanavir AUC_{tau} and C_{max} to compare the effect of both treatments (test versus reference). Laboratory safety and adverse events were evaluated throughout the trial.

Results

All 16 healthy subjects completed the study. Median (range) age and Body Mass Index (BMI) were 48.5 (21-55) years and 24.5 (19.0-29.2) kg/m^2, respectively. Pharmacokinetic parameters of ritonavir and cobicistat were comparable to those in the literature. The GMRs (90% CI) of daclatasvir AUC_{tau} and C_{max} (test versus reference) were 101% (92-111%) and 97% (89-106%), respectively. Atazanavir GMRs (90% CI) of AUC_{tau} and C_{max} were 82% (75-79%) and C_{max} 74% (68-81%), respectively. No serious adverse events were reported.

Conclusions

Atazanavir/cobicistat and atazanavir/ritonavir had a similar influence on daclatasvir pharmacokinetics in healthy volunteers. Daclatasvir at 30 mg once daily is the correct dose when combined with atazanavir/cobicistat.

INTRODUCTION

Combined treatment for infection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) is commonly applied in daily practice, because globally ~33% of HIV patients are co-infected with HCV[1, 2]. There are a number of combination regimens of specific antivirals available for both treatments.

Atazanavir is an HIV protease inhibitor (PI) that is mainly metabolized by cytochrome P450 (CYP) 3A4 in the liver. To improve the pharmacokinetics (PK) of atazanavir, the PI ritonavir is added as a 'booster'. Ritonavir is a strong inhibitor of CYP3A4 and therefore breakdown of atazanavir is inhibited. This extends the elimination half-life ($T_{1/2}$) of atazanavir (8.6 hour versus 6.5 hour[3]) and allows once daily dosing[3]. Apart from CYP3A4 inhibition, ritonavir inhibits CYP2D6 and P-glycoprotein (P-gp), and it induces e.g., CYP2C9, CYP1A2, and uridine 5'-diphospho-glucuronosyltransferase (UGT) 1A1[4].

Recently cobicistat became available as an alternative CYP3A booster and is approved to boost atazanavir, darunavir, and elvitegravir[5, 6]. Cobicistat is co-formulated with atazanavir (300 mg/150 mg). This combination is equally effective as atazanavir/ritonavir at 300 mg/100 mg[7]. Cobicistat is a specific inhibitor of CYP3A, CYP2D6, and P-gp, but it lacks the inductive capacity of ritonavir[8]. In addition, cobicistat has a more favorable toxicity profile than ritonavir. For example, cobicistat does not adversely affect the lipid profile.

Daclatasvir is part of a number of HCV regimens that target patients infected with genotypes 1, 3 and 4. Daclatasvir is a substrate of CYP3A4 and P-gp. This opens the door for a drug-drug interaction (DDI) with ritonavir and atazanavir. Atazanavir itself also inhibits drug-enzymes and transporters such as CYP3A4, CYP2D6, UGT1A1, and organic anion transporting polypeptides (OATPs).

According to the label, the dose of daclatasvir should be reduced from 60 mg to 30 mg once daily when it is administered concomitantly with atazanavir/ritonavir[9]. However, the impact of atazanavir/cobicistat on daclatasvir PK is unknown. Because these drugs will be used in combination in HIV/HCV co-infected patients, we sought to examine whether atazanavir/cobicistat has the same impact on daclatasvir PK as atazanavir/ritonavir. Our hypothesis is that 30 mg of daclatasvir is the sufficient dose when it is combined with atazanavir/cobicistat, because its CYP3A-inhibitory capacity is comparable to atazanavir/ritonavir.

PATIENTS & METHODS

This prospective, open-label, two-period, randomized, cross-over trial included 16 healthy subjects, aged 18-55 years. Main exclusion criteria were estimated glomerular filtration rate (eGFR) <60 mL/min, positive HIV, HCV or hepatitis B test, abnormal laboratory tests, and the use of medication or drugs (except acetaminophen [paracetamol] at ≤2 g/day).

Subjects were equally randomized into two treatment groups (AB and BA). Treatment A (reference) consisted of 10 days daclatasvir 30 mg once daily (Daklinza®, Bristol-Myers Squibb [BMS] Pharma EEIG) and the separate compounds atazanavir at 300 mg (Reyataz®, BMS) and ritonavir at 100 mg once daily (Norvir®, AbbVie Ltd). Treatment B (test) consisted of 10 days of daclatasvir at 30 mg once daily and the fixed-dose combination atazanavir/cobicistat at 300/150 mg once daily (Evotaz®, BMS). Between the treatments, a washout period of 11 days was included (Figure 1).

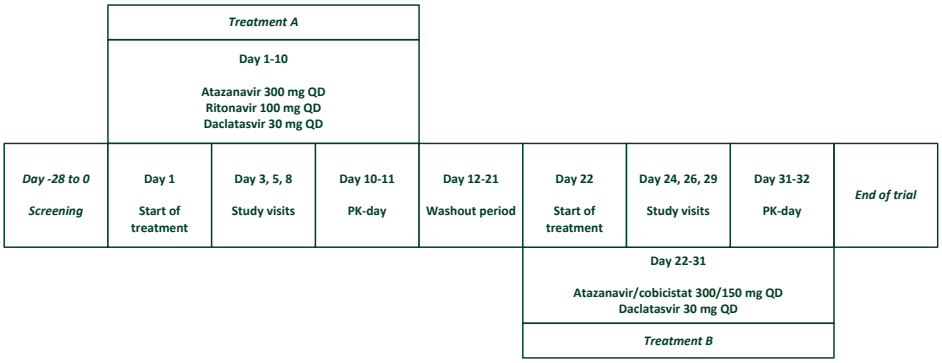


Figure 1: Overview of study design.
The shown design is treatment AB. Treatment BA has the same design and treatments, but the sequence was reversed.
QD: Once daily; PK: Pharmacokinetics.

On day 10 of both treatments (steady-state) the drugs were taken with a standardized breakfast consisting of a glass of milk and two slices of buttered wheat bread with Dutch 48+ cheese (48+ means ~25% of saturated fats) and cervelat (396 kCal). A 24-hour PK curve was recorded and blood samples were obtained at $t = 0$ (pre-dose) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours after intake of the study medication.

Atazanavir and ritonavir plasma concentrations were determined using a modification of a validated high performance liquid chromatography (HPLC) assay with ultraviolet (UV) detection[10]. Cobicistat was determined using a validated liquid chromatography

with tandem mass spectrometer (LC-MS/MS) method with a lower limit of quantification (LLOQ) of 0.031 mg/L. Daclatasvir was determined with a validated ultra performance liquid chromatography (UPLC) assay with UV detection (LLOQ: 0.03 mg/L).

PK parameters ($AUC_{0\text{-}tau}$, C_{max}, C_{last}, T_{max}, $T_{1/2}$) were calculated for all drugs using non-compartmental analysis (WinNonlin 6.4, Phoenix 64). To determine bioequivalence between treatments, geometric mean ratios (GMRs) with 90% CI of $AUC_{0\text{-}tau}$, C_{max}, and C_{last} were calculated comparing the effect of atazanavir/ritonavir with the effect of atazanavir/cobicistat on the PK of daclatasvir. Bioequivalence was demonstrated if the 90% CI of the GMR fell within 80-125%, which is in accordance with international guidelines[11, 12].

For sample size calculation we used power calculation in SAS® 9.2 (paired t-test for lognormal distribution for showing equivalence). We used the previously observed inter-subject variation of ~35% and intra-subject variation of ~10% for daclatasvir AUC in healthy subjects[13]. For 80% power a sample size of seven subjects needed to be included in the trial to detect bioequivalence for daclatasvir between treatments. To account for possible drop-outs and to follow the FDA guidelines (minimum of 12 subjects required), two groups of 8 subjects were included.

Secondarily, GMRs with 90% CI of atazanavir were calculated comparing the effects of cobicistat and ritonavir on atazanavir PK parameters.

During every visit, safety was assessed by serum biochemistry and hematology and subjects were asked about the presence of adverse events (AEs). Data were collected using Castor EDC (Castor Electronic Data Capture, Ciwit BV, Amsterdam, the Netherlands).

Ethics

The study was conducted in Radboud university medical center, Nijmegen, the Netherlands and approved by the local Ethical Committee. The trial was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. All participants signed informed consent prior to screening evaluations (NCT02565888).

RESULTS

All 16 healthy subjects (8 males, 16 Caucasian) completed the study. Median (range) age and BMI were 48.5 (21-55) years and 24.5 (19.0-29.2) kg/m^2, respectively.

Table 1 displays daclatasvir PK parameters with atazanavir/ritonavir versus atazanavir/cobicistat. Bioequivalence was established for daclatasvir between treatment groups, because GMRs 90% CI were all within the 80-125% range: $AUC_{0\text{-}tau}$ 101% (92-111%), C_{max} 97% (89-106%), and C_{last} 101% (89-115%). For atazanavir, exposure after boosting with cobicistat appeared to be lower than after boosting with ritonavir. The GMR values (B versus A) were: $AUC_{0\text{-}tau}$ 82% (75-79%), C_{max} 74% (68-81%), and C_{last} 86% (76-98%).

Figure 2 shows the PK curves of daclatasvir and atazanavir for both treatments. Figure 3 shows the PK curves of cobicistat and ritonavir with reference curves obtained from the literature.

Table 1: Steady-state pharmacokinetic (PK) parameters of daclatasvir, atazanavir, ritonavir, and cobicistat (*n* = 16).

Daclatasvir	Treatment A	Treatment B	GMR,% (90% CI)	Reference[9]	Ritonavir	Reference (GM)[14]
$AUC_{0\text{-}tau}$ (h·mg/L)	14.18 (33.72)	14.30 (36.73)	101 (92-111)	14.12	11.37 (37.48)	11.82
C_{max} (mg/L)	0.97 (32.26)	0.94 (33.32)	97 (88-106)	1.53	1.87 (32.60)	1.75
C_{last} (mg/L)	0.38 (52.22)	0.38 (46.23)	101 (89-115)	0.232	0.03 (78.47)	-
T_{max} (h)[a]	3.0 (1.5-4.0)	1.75 (0.5-4.0)	-	1-2	3.0 (1.0-4.0)	1.76
$T_{1/2}$ (h)[b]	19.56 (46.23)	19.08 (27.19)	-	12-15	4.04 (17.90)	3.29

Atazanavir	Treatment A	Treatment B	GMR,% (90% CI)	Reference[14, 15]c	Cobicistat	Reference (mean)[15]
$AUC_{0\text{-}tau}$ (h·mg/L)	55.56 (34.44)	45.48 (40.68)	82 (75-89)	ATV/r, mean: 46.72 ATV/c, GM: 34.84	11.32 (29.71)	8.91
C_{max} (mg/L)	5.46 (25.27)	4.05 (29.70)	74 (68 - 81)	ATV/r, mean: 4.90 ATV/c, GM: 4.10	1.46 (18.41)	1.35
C_{last} (mg/L)	1.04 (62.32)	0.90 (68.05)	86 (76 -98)	ATV/r, mean: 0.66 ATV/c, GM:0.45	0.04 (106.87)	-
T_{max} (h)[a]	3.0 (1.5-4.0)	1.75 (0.5-4.0)	-	ATV/r, mean: 1.80 ATV/c, GM: 2.5	2.0 (1.0-4.0)	2.5
$T_{1/2}$ (h)[b]	13.44 (52.57)	13.93 (60.90)	-	ATV/r, mean: 8.77 ATV/c, GM: 7.5	4.22 (30.04)	4.3

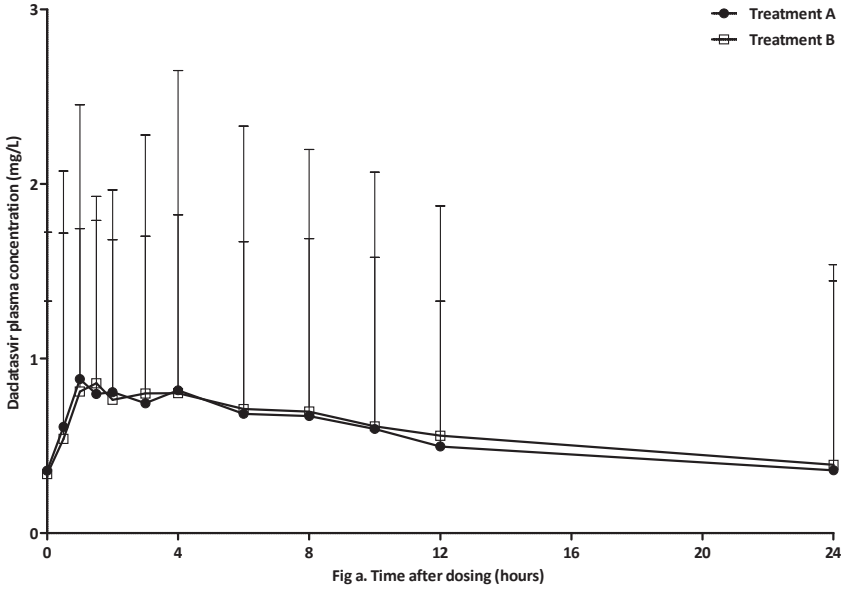
Geometric means are presented with coefficient of variation (CV%). Geometric mean ratios of treatment B (daclatasvir + atazanavir/cobicistat) versus treatment A (daclatasvir + atazanavir/ritonavir) are presented.

[a]Values presented are medians (range).

[b]Apparent elimination half-life ($T_{1/2}$).

[c]Reference data presented for atazanavir is without intake of food.

GMR: Geometric mean ratio; GM: Geometric mean; 90% CI: 90% Confidence interval; ATV/r: Atazanavir/ritonavir; ATV/c: Atazanavir/cobicistat.



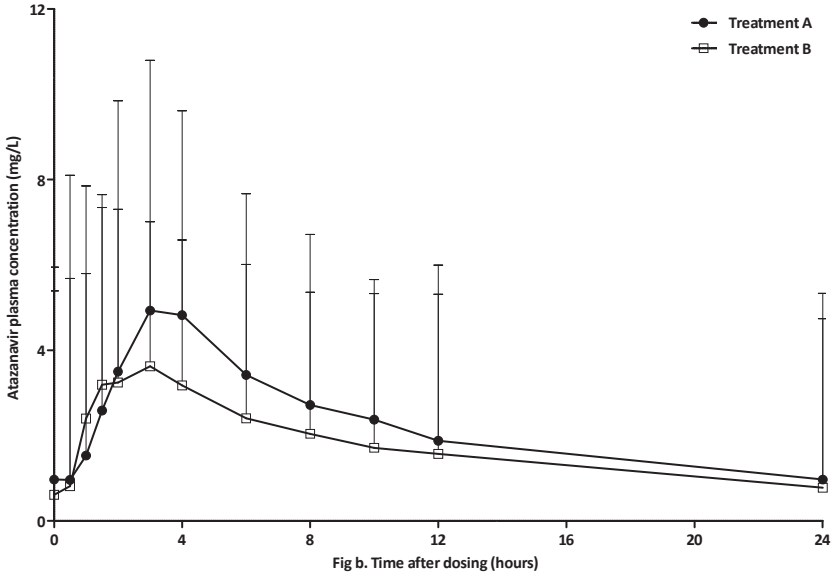


Figure 2: Daclatasvir (A) and atazanavir (B) plasma concentrations (mg/L) in 16 healthy volunteers.

Values are medians and upper limits.

A: Steady-state concentrations for 300 mg atazanavir once daily, 100 mg ritonavir once daily, and 30 mg daclatasvir once daily.

B: Steady-state concentrations 300/150 mg for atazanavir/cobicistat once daily and 30 mg daclatasvir once daily.

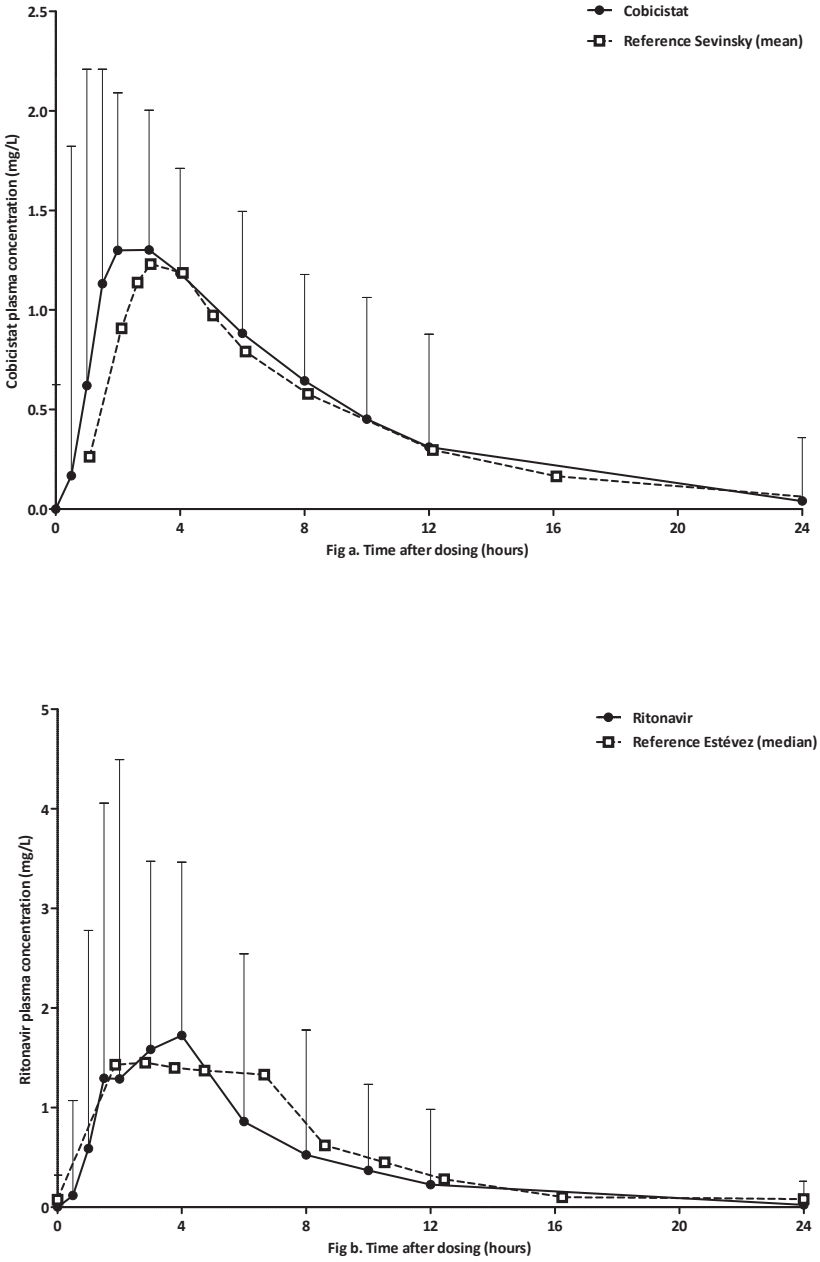


Figure 3: Cobicistat (A) and ritonavir (B) plasma concentrations (mg/L) in 16 healthy volunteers with reference curves obtained from literature.

Values are medians and upper limits.

A: Steady-state concentrations of cobicistat 150 mg once daily in combination with atazanavir 300 mg and daclatasvir 30 mg once daily. Reference curve for cobicistat is obtained from Sevinsky et al.

B: Steady-state concentrations of ritonavir 100 mg once daily in combination with atazanavir 300 mg and daclatasvir 30 mg once daily. Reference curve for ritonavir is obtained from Estévez et al.

No serious adverse events (SAEs) were reported in this trial. However, a total of 139 AEs occurred and 23% were classified as grade 3/4. AEs were comparable between treatment groups. AEs reported (≥5%) are given per treatment in Table 2.

All subjects had at least grade 1 hyperbilirubinemia (>1.0 mg/dL). Ten subjects developed jaundice, of whom six had jaundice during both treatments. Bilirubin concentrations normalized after cessation of atazanavir.

Table 2: Adverse events (AEs) reported during the trial, per treatment. Only AEs that are reported >5.0% are given.

	Patients, n	AEs, n	AEs, %	Grade 3/4, n	Patients, n	AEs, n	AEs, %	Grade 3-4, n
Asymptomatic hyperbilirubinemia	16	18	26.1	12	16	20	29.4	11
Jaundice	8	10	14.5	4	6	7	10.3	4
Headache	4	7	10.1	-	5	10	14.7	-
Fatigue / tired	5	6	8.7	-	4	5	7.4	1
Flatulence	4	4	5.8	-	3	5	7.4	-
Elevated amylase	3	4	5.8	-	1	1	1.5	-

Treatment A: Atazanavir 300 mg once daily, ritonavir 100 mg once daily, and daclatasvir 30 mg once daily.
Treatment B: Atazanavir/cobicistat 300/150 mg once daily and daclatasvir 30 mg once daily.
AEs: Adverse events.

Toxicity grades are judged by the trial physician and graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events ("DAIDS AE GradingTable"); version 1.0, December 2004, clarification August 2009.

DISCUSSION

The primary objective of our trial was to compare the influences of atazanavir/ritonavir and atazanavir/cobicistat on daclatasvir plasma concentrations. Our hypothesis was confirmed and daclatasvir plasma concentrations were comparable, because bioequivalence was established. Additionally, the daclatasvir plasma concentrations were in line with previously published data in healthy volunteers[9]. Therefore, we argue that 30 mg of daclatasvir is the right dose for HCV treatment when used in combination with atazanavir/cobicistat.

The GMR values with 90% CI of atazanavir/cobicistat versus atazanavir/ritonavir (secondary research question) fell outside the range of 80-125%. Apparently, cobicistat administered at a dose of 150 mg once daily dose is a weaker booster than steady-state

ritonavir, since atazanavir plasma concentrations were lower when given with cobicistat than with ritonavir. Phase-III studies have produced comparable efficacy and safety data for both treatments[16]. So, these mildly reduced atazanavir levels appear not to have clinical relevance. This is supported by the fact that atazanavir C_{last} in both study arms was >0.15 mg/L, which is the proposed minimal effective concentration[17]. The conclusion that 150 mg of cobicistat is a somewhat weaker CYP3A booster than 100 mg of ritonavir can also be found in a study that described the PK of darunavir/ritonavir and darunavir/cobicistat[18].

In both treatment groups, the PK parameters of atazanavir were increased compared with the reference values given in Table 1. We should notice, that the subjects in the study of Estévez et al[14] did not take atazanavir/ritonavir with food, which may explain this difference, as food intake increases the absorption of atazanavir[3]. The reference values of atazanavir/cobicistat where obtained from a study were the drugs were taken with a light meal (336 kCal), which is similar in our study[15].

Cobicistat and ritonavir exposures were both in line with the literature (Table 1). Reference values were obtained from studies in healthy volunteers treated with atazanavir/ritonavir or atazanavir/cobicistat[14, 15].

During the trial, unconjugated hyperbilirubinemia was seen in all subjects and jaundice was reported by 10 subjects. This was an expected AE, caused by UGT1A1 inhibition of atazanavir. UGT1A1 is responsible for the conjugation of bilirubin, so less enzyme is available for bilirubin conjugation. This results in a decreased elimination of bilirubin and thus hyperbilirubinemia (Gilbert's-like syndrome). After cessation of atazanavir bilirubin levels normalized in all subjects[3, 19].

CONCLUSIONS

Atazanavir/cobicistat and atazanavir/ritonavir had a similar influence on the PK of daclatasvir in healthy volunteers and are considered to be bioequivalent. The recommended dose of daclatasvir with atazanavir/ritonavir, 30 mg once daily, also applies cobicistat-boosted atazanavir. Since no unexpected side effects were reported in the trial, this combination can be used safely.

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CONFLICTS OF INTERESTS

Except the funding received for this study by Bristol-Myers Squibb Pharma EEIG, EJS, CTMMK, KVG, EPHC, and JPHD declare that they have no further conflict of interest that are directly relevant to the content of this manuscript. DMB joins advisory boards of AbbVie, Bristol-Myers Squibb, Gilead, Janssen, ViiV Healthcare, and Merck. He received sponsorship and research grants of Bristol-Myers Squibb, Janssen, ViiV Healthcare, and Merck. However, this did not influence the content of this manuscript.

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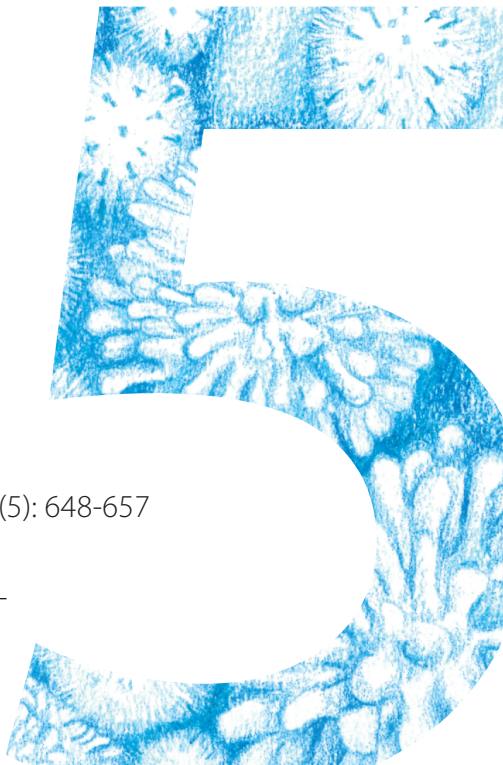
The majority of hepatitis C patients treated with direct-acting antivirals are at risk for relevant drug-drug interactions

EJ Smolders[±]
FAC Berden[±]
CTMM de Kanter
W Kievit
JPH Drenth
DM Burger

± Both authors contributed equally to this manuscript



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ABSTRACT

Background

Direct-acting antivirals have improved treatment of chronic hepatitis C virus infection significantly. Direct-acting antivirals inhibit/induce and can also be substrates of, drug-metabolizing enzymes and transporters. This increases the risk for drug-drug interactions.

Objective

The purpose of this study was to predict drug-drug interactions with co-medication used by hepatitis C virus- infected patients.

Methods

We assembled a nationwide cohort of hepatitis C patients and collected cross-sectional data on co-medication use. We compiled a list of currently available direct-acting antiviral regimens and cross-checked for potential drug-drug interactions with used co-medication.

Results

The cohort included 461 patients of which 77% used co-medication. We identified 260 drugs used as co-medication. Antidepressants (7.4%), proton pump inhibitors (7.1%), and benzodiazepines (7.1%) were most frequently used. Of the patients, 60% were at risk for a clinically relevant drug-drug interaction with at least one of the direct-acting antivirals regimens. Interactions were most common with paritaprevir/ritonavir, ombitasvir, and dasabuvir and least interactions were predicted with grazoprevir/elbasvir.

Conclusion

Co-medication use is rich in frequency and diversity in chronic hepatitis C patients. The majority of patients are at risk for drug-drug interactions which may affect efficacy or toxicity of direct-acting antivirals or co-medication. The most recently introduced direct-acting antivirals are associated with a lower risk of drug-drug interactions.

INTRODUCTION

Treatment of hepatitis C virus (HCV) infected patients has significantly improved with the introduction of direct-acting antivirals (DAAs). DAAs have the disadvantage that they can be involved in drug-drug interactions (DDIs) with patients' co-medication. DDIs might increase the risk for toxicity or result in poorer efficacy[1, 2]. The mechanism is twofold: DAAs can both be victim and/or perpetrator of DDIs. Drugs are victims of DDIs when their plasma concentration is affected by another drug. In contrast, drugs are perpetrators when they have the ability to influence plasma concentrations of drugs, for example by inhibiting or inducing metabolizing enzymes and/or drug-transporters[3, 4]. DAAs inhibit various cytochrome P450 (CYP) enzymes, responsible for drug-metabolism (Table 1). The clinical importance of DDIs was illustrated by the interaction between sofosbuvir-based DAA therapy and amiodarone, resulting in severe bradycardia[5, 6]. This report and other similar papers indicate that there is a genuine risk for relevant DDIs in patients treated with DAAs who use co-medication[5-11].

Table 1: Overview of enzymes and drug-transporters involved in the metabolism and transport of DAAs used for the treatment of hepatitis C.

Direct-acting antiviral	Victim (= substrate of)	Perpetrator	
		Inhibitor	**Inducer**
Daclatasvir	CYP3A4/5, P-gp	P-gp, OATP1B1	
Dasabuvir	CYP2C8, CYP3A4, P-gp, BCRP	UGT1A1, BCRP, P-gp	
Elbasvir	CYP3A, P-gp		
Grazoprevir	CYP3A, P-gp, OATP1B1/3	CYP3A (?)	
Ledipasvir	Pg-p, BCRP	P-gp, BCRP	
Ombitasvir	-	UGT1A1	
Paritaprevir/ ritonavir	CYP3A4/5, P-gp, OATP1B1/3, BCRP	CYP3A4/5, UGT1A1, CYP2D6(?),OATP1B1/3, OATP2B1, BCRP	CYP2C19
Simeprevir	CYP3A4/5	CYP3A4/5, CYP1A2, P-gp, OATP1B1/3	
Sofosbuvir	P-gp, BCRP		
Velpatasvir	P-gp, BCRP, CYP2B6, CYP2C8, CYP3A4	P-gp, BCRP, OATP1B1/2, OATP2B1	

? Unknown of the inhibition/induction in clinically relevant.
DAA: Direct-acting antiviral; CYP: Cytochrome P450; BCRP: Breast cancer resistance protein; P-gp: P-glycoprotein; UGT: Uridine 5'-diphospho-glucuronosyltransferase; OATP: Organic anion-transporting polypeptide.

The toxicity profiles of the currently used interferon-free DAA combinations, improved significantly relative to the DAAs combined with peg-interferon and ribavirin. Nowadays, more HCV patients with complex co-morbidities and thus co-medication receive anti-

viral treatment[12]. The combination of DAAs and many other drugs obviously increases the risk for DDIs. To date, limited data is available about the extent of co-medication use by HCV patients and the risk of DDIs as a consequence. Therefore, the aim of this study is to identify the co-medication use in a nationwide real-life HCV cohort in order to predict clinically relevant DDIs between co-medication and new DAA regimens.

METHODS

We performed this research in three steps: (1) we identified which co-medication were used by HCV-infected patients in a real-world cohort; (2) in order to predict DDIs we cross-checked the co-medication with DAAs in the database of the University of Liverpool (http://www.hep-druginteractions.org); and (3) we assessed the risk for DDIs per patient.

For this type of study (retrospective) formal consent was not required. Formal evaluation was waived by the institutional review board Arnhem-Nijmegen. Good Clinical Practice guidelines and the code of conduct for the use of data in health research were followed (http//www.federa.org).

Patients and use of co-medication

Data from a nationwide, real-life cohort were used[13]. This cohort included Dutch patients treated for a HCV genotype 1 mono-infection. Patients were identified based on local databases present in 45 hepatitis treatment centers in the Netherlands. Data collection was performed between January 2014 – July 2015. Baseline data were extracted from the patient's medical record and included patient characteristics, medical history, HCV genotype, and co-medication use prior to commencement of HCV treatment. Patients were excluded when data on co-medication use was missing and if patients had a co-infection with HIV or hepatitis B virus. In addition to prescribed medication, we included complementary and alternative medicine (CAM) when available in the medical record. Separate compounds of fixed-dose products were registered, except for CAMs, these were counted as one, even though they may have contained several chemical compounds. We did include drugs taken as part of a substance abuse disorder (e.g. methadone), although illicit drugs such as heroin or cocaine were not collected. We added Anatomical Therapeutic Chemical (ATC) codes to all co-medication reported in the patient's medical record, and grouped the drugs by therapeutic/pharmacological subgroups[14].

Predicted drug-drug interactions with direct-acting antivirals

The co-medication was cross-checked with currently approved DAA regimens in Europe and USA through the University of Liverpool database in an effort to predict DDIs (July 2016). The University of Liverpool database is a commonly used resource to check for DDIs[4, 15]. For cross-checking we included approved DAA regimens effective against HCV genotype 1: simeprevir plus sofosbuvir, daclatasvir plus sofosbuvir, ledipasvir plus sofosbuvir, paritaprevir/ritonavir, ombitasvir plus dasabuvir, grazoprevir plus elbasvir, and velpatasvir plus sofosbuvir. Ribavirin and first generation protease inhibitors were not taken into account. Ribavirin is considered not to cause any DDIs in this population as is not metabolized by or influencing any of the drug-metabolizing enzymes and the included patients do not use nucleoside reverse transcriptase inhibitors (NRTIs)[16]. The first generation DAAs are considered outdated.

We used four risk categories corresponding with the University of Liverpool database: 1) No clinically significant interaction; 2) Potential interaction - may require close monitoring, alternation of drug dosage or timing of administration; 3) Contra-indication, i.e. drugs should not be co-administered; 4) Unknown, as not available in the Liverpool database. For these unavailable drugs, the pharmacists (ES and DB) judged if there might be risk of a DDI. Pharmacokinetic parameters of these drugs were used (US FDA Prescribing Information and MicroMedex®) to evaluate these interactions. Overall, we defined category 2 and 3 as the clinically relevant DDIs[17].

Risk for drug-drug interactions per patient

To assess the number of patients at risk for a clinically relevant DDI, we counted the patients with at least one predicted DDI between co-medication and one of the DAA regimens. Further, we compared the risk for DDIs between subgroups of patients: (1) patients aged <65 years versus ≥65 years[17], and (2) in patients with versus without cirrhosis. We used Fib-4 index >3.25 to classify patients as cirrhotic[18].

Analyses

Descriptive analyses were performed with frequency counts and proportions. For the subgroup analyses we used chi-square tests. All analyses were performed in SPSS (IBM SPSS Statistics 20).

RESULTS

Patients and use of co-medication

This cohort included 467 patients; we excluded 6 patients from the analysis because data on co-medication was missing. There were 313 males and the mean age was 51 years (Table 2).

Table 2: Patient characteristics.

Characteristic	Overall (n = 461)
Age - mean (range), year	51 (19-77)
Age ≥65 years - n (%)	30 (7)
Male sex - n (%)	313 (68)
Caucasian - n (%)[a]	316 (90)
Treatment-naïve - n (%)[b]	269 (58)
Decompensated liver disease - n (%)	23 (5)
Fib-4 index >3.25 (cirrhosis) - n (%)[c]	115 (26)
Creatinine clearance <30 mL/min - n (%)[d]	3 (1)

[a]Race: available in 352 patients.
[b]Previous response: available in 448 patients.
[c]Fib-4 index: available in 437 patients.
[d]Creatinine clearance: available in 407 patients.

A total of 356 patients (77%) used co-medication at start of HCV therapy and 105 patients did not use any co-medication. The number of medications per patient ranged from 1-17 (median 2). Of the cohort, 12% used ≥6 medications at start of HCV therapy. Overall, the 356 patients had a total number of 1,329 prescriptions (including CAMs), which comprised 260 different drugs (Figure 1).

Most frequently used co-medication were antidepressants, proton pump inhibitors (PPIs), benzodiazepine derivatives, and drugs for opioid dependence (Table 3).

Table 3: Most frequently used (>2.0%) concomitant medications at start of hepatitis C treatment.

Drug class	ATC-code (4rd level)	Number (%)[a]
Antidepressants (both tricyclic antidepressants and selective serotonin reuptake inhibitors e.g. amitriptyline, sertraline)	N06AA, N06AB, N06AX	98 (7.4)
Proton pump inhibitors (e.g. omeprazole)	A02BC	94 (7.1)
Benzodiazepine derivatives (e.g. diazepam, flurazepam)	N05BA, N05CD	94 (7.1)
Drugs used in opioid dependence (e.g. methadone)	N07BC	74 (5.6)
Selective beta-2-adrenoreceptor agonists (respiratory systemic use and inhalants e.g. salbutamol)	R03AC, R03CC	55 (4.2)
Antipsychotics (e.g. olanzapine, risperidone)	N05AA, N05AB, N05AD, N05AF, N05AH, N05AL, N05AN, N05AX	46 (3.5)
Vitamin D and analogues (e.g. colecalciferol)	A11CC	38 (2.9)
Thiazides (e.g. hydrochlorothiazide)	C03AA	34 (2.6)
Selective beta blocking agents (e.g. metoprolol)	C07AB	32 (2.4)
ACE inhibitors (e.g. enalapril)	C09AA	32 (2.4)
Glucocorticoids (respiratory system e.g. beclomethasone)	R03BA	32 (2.4)
Biguanides (e.g. metformin)	A10BA	27 (2.0)
Platelet aggregation inhibitors excl. heparin (e.g. acetylsalicylic acid)	B01AC	26 (2.0)
Dihydropyridine derivatives (calcium channel blockers e.g. amlodipine)	C08CA	26 (2.0)

[a]Percentage is calculated using the total number of prescriptions in this cohort (n = 1,329)
ACE: Angiotensin I converting enzyme; ATC: Anatomical Therapeutic Chemical.

Predicted drug-drug interactions with direct-acting antivirals

We used our cohort to predict DDIs between co-medication and DAA regimens. Figure 1 presents the distribution of the DDI categories per DAA regimen for 260 different drugs. The combination of grazoprevir plus elbasvir and velpatasvir plus sofosbuvir had the lowest number of predicted DDIs in our mono-infected cohort. Grazoprevir plus elbasvir and daclatasvir plus sofosbuvir had no contra-indicated drugs (category 3) and no clinical significant interactions were predicted with 72% and 63%, respectively, of the concomitantly used drugs (category 1).

The combination of paritaprevir/ritonavir, ombitasvir plus dasabuvir had the most contra-indications (4%), followed by simeprevir (2%), and velpatasvir (1%). Category 2 interactions were also mainly predicted with the regimen containing paritaprevir/ritonavir, ombitasvir plus dasabuvir (33%) and simeprevir plus sofosbuvir (26%). Interestingly, ~90% of these category 2 DDIs have not been studied *in vivo*. These potential interactions were predicted by the pharmacologist of the University of Liverpool database, based on the characteristics of the drugs. The top 5 medications which can cause clinically relevant DDIs with at least one of the antiviral regimens are given in Table 4.

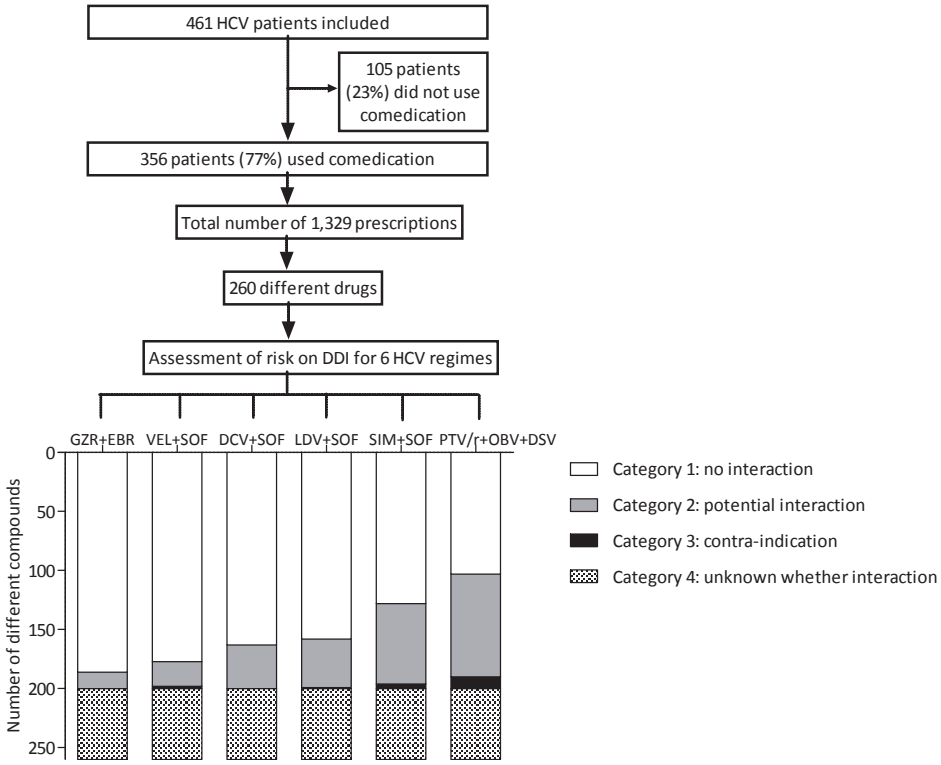

Figure 1: Overview of concomitant medication and expected number of drug-drug interactions between the direct-acting antiviral combinations of regimens and 260 different compounds.

Sofosbuvir (SOF), simeprevir (SIM), and daclatasvir (DCV) are licensed as separate compounds for hepatitis C virus (HCV) infected patients. These drugs are separately available in the Liverpool database. However, we present these regimes together, because in clinical practice these drugs are used in combination. Drug-drug interactions (DDI) with PTV/r, OBV plus DSV (paritaprevir/ritonavir, ombitasvir plus dasabuvir), ledipasvir (LDV) plus SOF, velpatasvir (VEL) plus SOF, and grazoprevir (GZR) plus elbasvir (EBR) were available per combination in the Liverpool database.

Table 4: Top 5 concomitant medication causing clinically relevant interactions with at least one of the antiviral regimens.

	Drug class	ATC code	Number
DDI category 2	1. Benzodiazepines	N05BA	61
	2. Antidepressants	N06A	43
	3. Proton pump inhibitors (such as omeprazole)	A02BC	42
	4. Glucocorticoids airway	R03BA	30
	5. Selective beta-blocking agents	C07AB	29
DDI category 3	1. Proton pump inhibitors (such as esomeprazole, pantoprazole)	A02BC	52
	2. HMG CoA reductase inhibitors (statins)	C10AA	19
	3. Antipsychotics	N05A	13
	4. Selective beta-2-adrenoreceptor agonists respiratory system	R03AC/ R03CC	12
	5. CAM	no ATC	2

CAM: Complementary and alternative medicine; DDI: Drug-drug interaction; ATC: Anatomical Therapeutic Chemical.

The risk of DDIs could not be assessed in 60 of the 260 different drugs (category 4), because the drugs were not listed in the University of Liverpool database (July 2016). The top three of therapeutic subgroup (2nd ATC level) in Category 4 were antihemorrhagics (B02; e.g. coagulation factors), vitamins (A11; e.g. colecalciferol), and psycholeptics (N05; e.g. flunitrazepam) which were used by a total of 17, 33, and 13 patients, respectively.

The pharmacists (ES and DB) judged if there were potential interactions with these 60 drugs and DAAs. We predicted that 11 drugs had a potential interaction, 30 drugs would not cause interactions, and for 19 drugs it is unknown if there is a potential interaction (for example: metabolism not known of the co-medication), data is given in Table 5.

Table 5: Predicted drug-drug interactions and recommendations of drugs not available in the Liverpool database.

Generic Name	ATC	Metabolism	Proposed interaction mechanism	Recommendation	References
Barnidipine	C08CA12	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Barnidipine levels may increase, monitor for adverse reactions or monitor blood pressure.	[19]
Calcitriol	A11CC04	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Calcitriol levels may increase, monitor for adverse reactions.	[a]
Chlordiazepoxide	N05BA02	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Chlordiazepoxide levels may increase, monitor adverse reactions.	[a]
Deferasirox	V03AC03	UGT1A1 and UGT1A3 substrate (CYP450-catalyzed metabolism: minor (8%). MRP2, BCRP substrate	UGT1A1 inhibition by ombitasvir, paritaprevir/ritonavir. BCRP inhibition by ledipasvir, paritaprevir/ritonavir, velpatasvir.	Adverse reactions of deferasirox are dose dependent and frequently reported, therefore we advise to minimize the use of deferasirox during DAA therapy as increased plasma levels of deferasirox might occur.	[20]
		CYP3A4, CYP2C8, CYP1A2 inhibitor	Daclatasvir, dasabuvir, elbasvir, grazoprevir, paritaprevir/ritonavir, simeprevir, velpatasvir are substrate of CYP3A4. Dasabuvir is a substrate of CYP2C8.	Deferasirox inhibits CYP3A4 and CYP2C8 which might increase DAA levels, monitor adverse events.	
Phenprocoumon	B01AA04	CYP2C9, CYP2C19, CYP1A2, and CYP3A4 substrate	CYP2C19 inhibition by paritaprevir/ritonavir. CYP1A2 inhibition by simeprevir. CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Inhibition of the various CYP enzymes may increase the phenprocoumon concentration, therefore monitor INR more frequently.	[a]
Isosorbide mononitrate	C01DA14	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Isosorbide mononitrate levels may increase, monitor for adverse reactions.	[a]
Levonorgestrel	G02BA03	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Levonorgestrel levels may increase, monitor for adverse reactions.	[a]

Table 5: Predicted drug-drug interactions and recommendations of drugs not available in the Liverpool database. (*continued*)

Generic Name	ATC	Metabolism	Proposed interaction mechanism	Recommendation	References
Medroxyprogesterone	G03AC06	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Medroxyprogesterone levels may increase, monitor for adverse reactions.	[a]
Misoprostol	A02BB01	Unknown	Pharmacodynamic interaction with ledipasvir. Misoprostol decreases gastrointestinal pH.	Based on studies with antacida: separate misoprostol and ledipasvir/sofosbuvir intake by 4 hours.	[a]
Oral contraceptives	G03A	Dependent on compounds. CYP3A4 metabolizes e.g., levonorgestrel and cyproteron	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Dependent on the oral contraceptive agent there may be an interaction. Due to CYP inhibition of the DAAs the plasma levels of the contraceptive agent may increase with risk on toxicity. Switch to non-hormonal contraception during HCV therapy.	[a]
Solifenacin	G04BD08	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Adverse reactions of solifenacin are dose dependent and solifenacin levels may increase when combined with CYP3A4 inhibitors. Adverse reactions are mostly anticholinergic. Preferably do not use during with CYP3A4 inhibitors.	[a]

[a]The product label and other sources such as Micromedex® Solutions and Lexicomp database (available via http://www.uptodate.com) were used to determine the pharmacokinetic profile and metabolism of the drugs. This information is used to predict theoretical interactions between the concomitant medication and DAAs (expert opinion ES and DB).

? Unknown whether the interaction is clinically relevant.

HCV: Hepatitis C virus; CYP: Cytochrome P450; INR: International normalized ratio; DAA: Direct-acting antiviral; ATC: Anatomical therapeutic chemical; UGT: Uridine 5'-diphosphoglucuronosyltransferase; MRP: Multidrug resistance-associated protein; BCRP: Breast cancer resistance protein

Risk for drug-drug interaction per patient

The majority of the patients in our cohort (60%) was at risk for a clinically relevant DDI with at least one of the DAA regimens: 93 patients (20%) used a drug that would be contra-indicated (category 3) and 184 patients (40%) had co-medication leading to a possible interaction (category 2), which would require close monitoring, alternation of drug dosage or timing of administration. Figure 2 shows the risk of a DDI per DAA regimen per patient. The risk for DDIs per patient did not differ in patients aged below or above 65 years (60 versus 67%, $p = 0.45$), nor between patients without cirrhosis and with cirrhosis (60 versus 64%, $p = 0.50$).

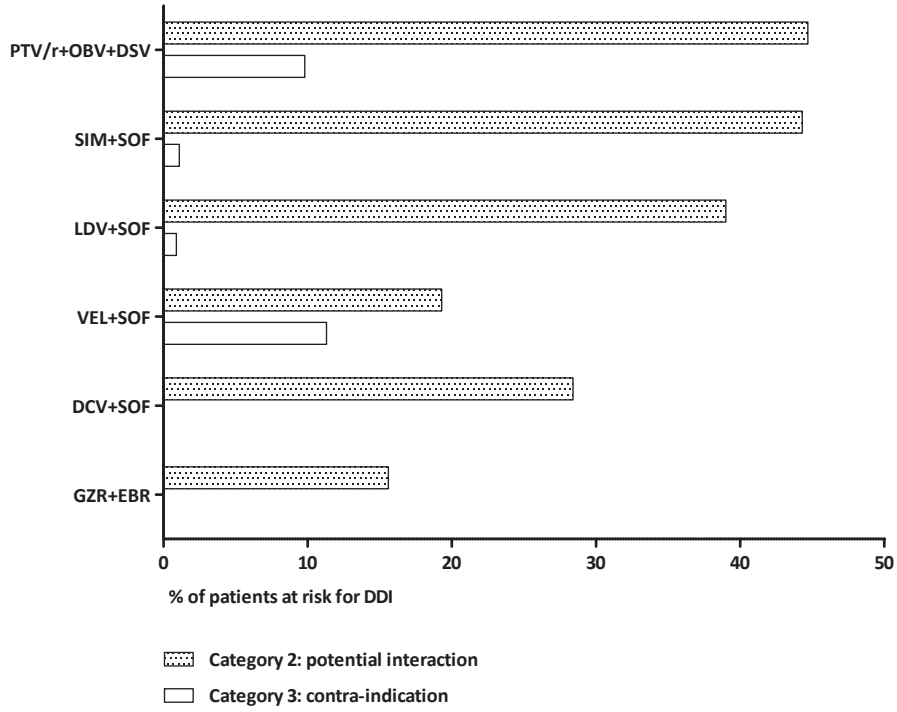

Figure 2: Risk on a clinically relevant drug-drug interaction per patient, grouped per direct-acting antiviral regimen (*n* = 461).

DDI: Drug-drug interaction; SIM: Simeprevir; SOF: Sofosbuvir; PTV/r: Paritaprevir/ritonavir; OBV: Ombitasvir; LDV: Ledipasvir; DCV: Daclatasvir; DSV: Dasabuvir; GZR: Grazoprevir; EBR: Elbasvir; VEL: Velpatasvir.

DISCUSSION

In this nationwide, real-life cohort study, we show that the majority of HCV-infected patients is at risk for having a clinically relevant DDI with new DAAs. This can have a negative influence on treatment outcomes and could potentially harm the patient[1]. In this cohort, patients with cirrhosis or ≥65 years old did not have a higher risk for a DDI when compared with patients <65 years old or without cirrhosis. This contrasts with a recently published study[17] and might be explained due to low number of elderly patients in our cohort and the lower mean age of patients ≥65 years (68 years, standard deviation [SD] 3). This shows that not only the elderly are at risk for a DDI. The psychoactive agents such as antidepressants (7.4%) and benzodiazepines (7.1%) were the most frequently used drugs in our cohort, as well as in the literature[3]. This is relevant because these drugs increase the risk for DDIs: antidepressants and benzodiazepines are extensively metabolized through CYP enzymes, which can be inhibited by DAAs[21, 22]. This causes increased plasma concentrations of psychoactive agents increasing the likelihood of toxicity.

PPIs were also responsible for many clinically relevant DDIs in our cohort, both as victim and perpetrator[15]. Omeprazole is a victim of paritaprevir/ritonavir, ombitasvir plus dasabuvir due to CYP2C19 induction of ritonavir, decreasing omeprazole exposure with 40-50%[23]. In contrast, PPIs are the perpetrators of a DDI with ledipasvir and velpatasvir. PPIs increase gastric pH, which decreases exposure to DAAs due to its insolubility at higher pH ranges[24, 25]. The clinical relevance for DDIs between PPIs and ledipasvir is under debate[26, 27]. For velpatasvir, the product label states that co-administration of omeprazole or other PPIs is not recommended, and that esomeprazole and pantoprazole are contra-indicated[25].

Most frequently predicted DDIs were found with paritaprevir/ritonavir, ombitasvir plus dasabuvir, which fits with data from the published literature[11]. These interactions are predominantly caused by ritonavir, which strongly inhibits the most important drug-metabolizing enzyme (CYP3A4) and various other enzymes and drug-transporters are influenced (e.g., CYP2D6, P-glycoprotein [P-gp])[28, 29]. The fewest interactions were seen with the newest regimens: velpatasvir plus sofosbuvir and grazoprevir plus elbasvir. Grazoprevir and elbasvir are substrates of P-gp and CYP3A and only strong CYP3A inhibitors or inducers lead to clinically relevant DDIs. Grazoprevir is also a (weak) CYP3A inhibitor, but no DDIs between this combination and CYP3A substrates are listed[30]. However, we recommend caution when combining elbasvir and grazoprevir with CYP3A substrates with a narrow therapeutic range, such as tacrolimus[31].

The contra-indicated drugs count for up to 4% of the predicted interactions. This is a very clear signal to the physician: do not combine the co-medication with this DAA regimen. The dilemma is mostly present in the drugs categories in 2 and 4. In our study, ~90% of category 2 DDIs have not been studied, but were predicted by the University of Liverpool group. However, some DDIs cannot be predicted on theoretical grounds but do occur in clinical practice. For example, the unexpected severe bradycardia that occurred in nine patients who were on amiodarone treatment and received a sofosbuvir-containing regimen. The mechanism of this DDI and the role of sofosbuvir is still unclear[6, 32-34]. Further, 23% (n = 60) of drugs used by patients from our cohort were not listed in the University of Liverpool database (category 4). We judged that 11 of these drugs might cause an enzymatic interaction with the currently used DAAs. Prescribers should be aware that when the drug is not mentioned in the database, it does not mean there is no interaction.

A strength of this study is that it is a nationwide multicenter cohort with a large number of patients. This cohort provides a representative overview of co-medication use in the Dutch HCV genotype 1 population with a treatment indication. Genotype 1 is globally the main genotype (60%) and we expect that the patients of the cohort reflect the patients who will be subjected to therapy[35, 36]. Further, we provide a risk assessment for drugs not available in the University of Liverpool database. Limitations of our study are the retrospective design and that our study describes predicted DDIs and not observed DDIs. Finally, the research question that led to this study was not the primary objective of data collection.

In conclusion, co-medication use is rich in both frequency and diversity in chronic HCV-infected patients. DDIs may result in subtherapeutic or increased drug concentrations of DAAs or co-medication, and can cause treatment failure or toxicity. Physicians should be aware that the majority of patients are at risk for clinically relevant DDIs. In that case, co-medication can be adjusted prior to DAA therapy or DAA treatment can be aligned with co-medication use.

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CONFLICTS OF INTERESTS

EJS, FACB CTMMK, and WK declare no conflicts of interest that are directly relevant to the content of this manuscript. JPHD joins advisory boards of Abbvie, BMS, Gilead, Janssen, and Merck and received sponsorship/research grants from Abbvie and Janssen. DMB joins advisory boards of Abbvie, BMS, Gilead, Janssen, Merck, and ViiV, and

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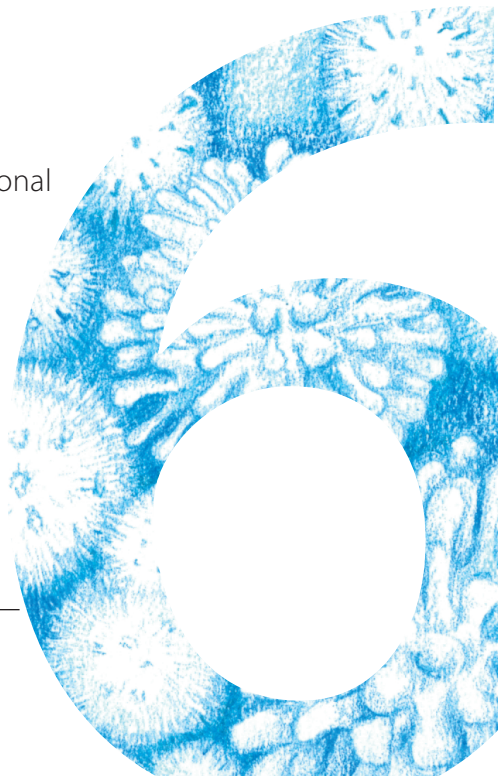
High need to switch cART or co-medication with the initiation of direct-acting antivirals in elderly HIV/HCV co-infected patients

EJ Smolders
C Smit
CTMM de Kanter
ASM Dofferhoff
JE Arends
K Brinkman
B Rijnders
M van der Valk
P Reis
DM Burger

on behalf the ATHENA national HIV observational cohort



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ABSTRACT

Background

To describe the use of non-antiretroviral co-medication and combination antiretroviral therapy (cART) in patients co-infected with HIV/hepatitis C virus (HCV), and to predict the potential for drug-drug interactions (DDIs) with direct-acting antivirals (DAAs) against HCV.

Methods

This is a retrospective, cross-sectional study, using the Dutch nationwide ATHENA observational HIV cohort database. All patients with a known HIV/HCV co-infection on 1 January 2015 were included. Co-medication and cART registered in the database were listed. The potential for DDIs between DAAs and co-medication/cART were predicted using http://www.hep-druginteractions.org. DDIs were categorized as: (1) no clinically relevant DDI; (2) possible DDI; (3) contra-indication; or (4) no information available.

Results

We included 777 patients of whom 488 (63%) used non-antiretroviral co-medication. At risk for a category 2/3 DDI with non-antiretroviral co-medications were 299 patients (38%). Most DDIs were predicted with paritaprevir/ritonavir, ombitasvir ± dasabuvir (47% of the drugs) and least with grazoprevir/elbasvir (11% of the drugs).

Concerning cART, daclatasvir/sofosbuvir is the most favorable combination as no cART is contra-indicated with this combination. In genotype 1/4 patients, grazoprevir/elbasvir is least favorable, as 75% of the patients must alter their cART.

Conclusions

This study showed that co-medication use in the aging HIV/HCV population is frequent and diverse. There is a high potential for DDIs between DAAs and co-medication/cART.

INTRODUCTION

Because of shared routes of transmission and overlapping at-risk populations, patients with HIV are commonly co-infected with hepatitis C virus (HCV). It is estimated that, worldwide, 2.3 million people live with an HIV/HCV co-infection[1]. In the Netherlands, 12% of the HIV-infected patients tested were positive for HCV antibody or HCV RNA. Most of these patients are men who have sex with men (46%) or current or former drug users (31%)[2].

Both HIV (combination antiretroviral therapy [cART]) and HCV (direct-acting antivirals [DAAs]) treatments can be victims (substrates) and/or perpetrators (cause) of drug-drug interactions (DDIs)[3]. For example, nevirapine is a strong inducer of cytochrome P450 (CYP) 3A4, and therefore interacts with velpatasvir (CYP3A4 substrate)[4]. On the other hand, the combination of paritaprevir/ritonavir, ombitasvir, with dasabuvir (PrOD), strongly inhibits CYP3A4, causing increased rilpivirine (CYP3A4 substrate) levels[5].

These examples demonstrate that DDIs could be a potential problem in HIV/HCV co-infected patients. So far, this has been studied mainly focusing on cART/DAA interactions[6-9]. However, treatment of co-infected patients is complicated in the aging HIV population, because these patients often have somatic or psychiatric co-morbidities for which co-medication is prescribed. Thus, besides cART, management of DDIs in HIV/HCV co-infected patients should also focus on interactions between DAAs and these co-medications. Furthermore, earlier publications in general did not include evaluations of the most modern DAAs, such as velpatasvir and grazoprevir/elbasvir, which are now recommended first-line agents.

We aimed to identify the use of co-medication and cART and predicted DDIs of these medications with all currently available DAAs in a Dutch nationwide HIV/HCV co-infected cohort.

METHODS

This retrospective, cross-sectional study used the ATHENA database managed by the HIV monitoring Foundation (http://www.hiv-monitoring.nl). In this Dutch, nationwide registry all HIV-infected patients in care who did not opt out are registered. All patients with a known HIV/HCV co-infection on 1 January 2015 were included (HCV RNA positive). These patients were not treated with DAAs before, as these drugs became available in the Netherlands on 1 January 2015. The included patients represent the total population of patients who could potentially be treated with DAAs and co-medication

and cART were thus not altered because of DDIs with DAAs. The reported co-medication and cART was used to predict DDIs using the database of the University of Liverpool (http://www.hep-druginteractions.org; September 2016).

This analysis was done in four steps: 1) identification of co-medication used in the cohort; 2) prediction of DDIs between co-medication and DAAs; 3) identification of cART used in the cohort; and 4) prediction of DDIs between cART and DAAs.

Identification of co-medication

All non-antiretroviral co-medication was extracted from the database, from which a list was compiled of all unique co-medications.

Prediction of drug-drug interactions between co-medication and direct-acting antivirals

The extracted list of co-medications was used for the prediction of DDIs. Each drug was cross-checked if DDIs exist with one of the DAA regimens. We included all DAA regimens recommended in Dutch guidelines in November 2016[10]. DDIs were categorized as: (1) no clinically relevant DDI expected; (2) possible DDI expected, i.e. monitor the patient or alter drug dosage/timing; (3) contra-indication, do not co-administer; or (4) no information available in the Liverpool database. Category 2 and 3 DDIs were defined as clinically relevant. We reported per DAA regimen the number of co-medications with a potential DDI.

After determination of the DDIs between the unique co-medications and DAA regimens, we assessed the number of patients, per genotype, at risk for a clinically relevant DDI. We counted the patients who had at least one predicted DDI with any of the DAA regimens. Dutch recommendations of November 2016 were used to determine which DAA regimen can be used per genotype[10]. Patients with an unknown HCV genotype were analyzed with pan-genotypic regimens: daclatasvir + sofosbuvir and velpatasvir + sofosbuvir. We reported per genotype, the frequency of patients at risk for a DDI.

In addition, patients with DDIs were counted for those (1) with or without cirrhosis, and those (2) <60 or ≥60 years. Cirrhosis (METAVIR F3/F4) was defined using a pathology or Fibroscan report (stiffness >9.5 kPa).

Identification of cART

Antiretroviral drugs registered in the database were extracted and a list of antiretroviral drugs per patient was compiled.

Prediction of drug-drug interactions between cART and direct-acting antivirals

The compiled list of antiretroviral drugs was used for the prediction of DDIs. To simplify the analysis, only patients with a double nucleoside reverse transcriptase inhibitors (NRTI) backbone and 1 additional drug were included. These additional drugs can be a (boosted) protease inhibitor (PI), (boosted) integrase inhibitor (INSTI), or a non-nucleoside reverse transcriptase inhibitor (NNRTI). These additional drugs usually cause DDIs and therefore used in this analysis. Patients with other regimens were excluded. Per genotype and DAA regimen, the number of patients at risk for a DDI was reported.

Finally, patients using tenofovir disoproxil fumarate (TDF) and boosted PIs were identified. This combination interacts with ledipasvir and velpatasvir, causing possible renal toxicity. It is therefore recommended to discontinue TDF or the boosted PI before ledipasvir and velpatasvir therapy (category 2).
Analyses were performed using IBM SPSS Statistics 20.

RESULTS

The ATHENA database contained data on 777 HIV/HCV co-infected patients known to be in care on 1 January 2015. The majority of these patients were male (666; 86%). Median (range) age was 49.3 (23-80) years; 689 patients were <60 years and 88 were ≥60 years. A METAVIR score F0/F1/F2 was reported for 438 (56%) patients and F3/F4 for 181 (23%) patients (158 unknown). Genotype 1 and 4 were most prevalent, in 495 (64%) and 139 (18%) patients, respectively (Table 1).

Table 1: Baseline patient characteristics.

Characteristics	Number
Total HIV/HCV-infected patients with positive HCV RNA in care[a]	777
Patients on cART	762
Patients using co-medication	488
Age, median (range)	49.3 (23-80)
Sex	
Male	666
Female	111
METAVIR score	
F0, F1, F2	438
F3,F4	181
Unknown	158
Genotype	
1	495
2	26
3	68
4	139
Unknown	49

[a]Cohort in 2016, 7%, HCV RNA data were not documented (see http://www.hivmonitoring.nl).
HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; cART: combination antiretroviral therapy.

Identification of co-medication

An overview of co-medication use is presented in Figure 1, showing that 488 patients used 156 unique non-antiretroviral co-medications. Medication use varied from 1 to 14 prescriptions per patient (excluding cART), in total 1,245 prescriptions were reported. Most frequently used medications were drugs for opioid dependence (138; 11%), proton pump inhibitors (110; 9%), calcium supplements (77; 6%), selective serotonin reuptake inhibitors (56; 4%), platelet aggregation inhibitors (53; 4%), vitamin D (46; 4%), and statins (45; 4%). In Table 2 these drug classes are broken down to the drugs that were prescribed at least to 10 patients (single molecules).

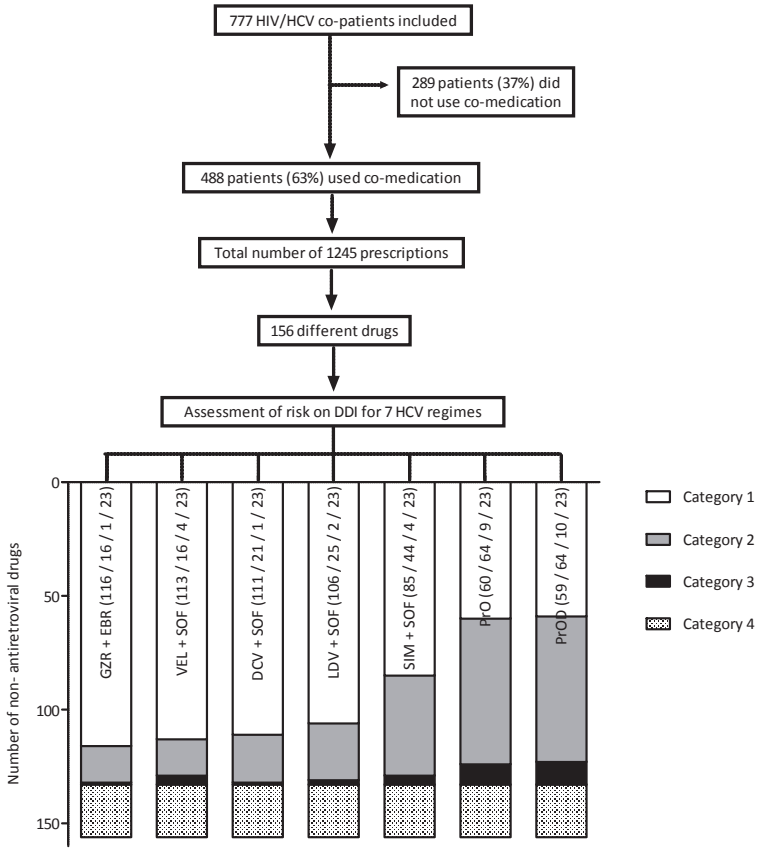

Figure 1: Flowchart of the study including the number of predicted drug-drug interactions with various DAA regimens and co-medication. A total of 1,245 prescriptions were available for 488 patients. These prescriptions contained 156 unique drugs, which were used for the analysis.

The number of drugs for each category (1-4) are shown in parentheses.

DDI: Drug-drug interaction; HCV: Hepatitis C virus; PrOD: Paritaprevir/ritonavir, ombitasvir with dasabuvir; PrO: Paritaprevir/ritonavir, ombitasvir; SIM + SOF: Simeprevir and sofosbuvir; LDV + SOF: Ledipasvir and sofosbuvir; DCV + SOF: Daclatasvir and sofosbuvir; VEL+SOF: Velpatasvir and sofosbuvir: GZR + EBR: Grazoprevir and elbasvir.

Table 2: Overview of drugs used by at least 10 HIV/HCV co-infected patients.

Drug name	Number of patients
Methadone	138
Calcium carbonate/colecalciferol	77
Omeprazole	50
Pantoprazole	48
Colecalciferol	43
Co-Trimoxazole	35
Valaciclovir	30
Diazepam	27
Metoprolol	27
Citalopram	24
Lisinopril	24
Carbasalate calcium	23
Pravastatin	23
Acetylsalicylic acid	22
Testosterone	22
Mirtazapine	21
Sildenafil	19
Alendronic acid	17
Olanzapine	17
Spironolactone	15
Escitalopram	14
Furosemide	14
Ranitidine	14
Metformin	13
Pregabaline	13
Quetiapine	13
Salmeterol/fluticasone	12
Tramadol	12
Clonazepam	11
Promethazine	11
Venlafaxine	10

Prediction of drug-drug interactions between co-medications and direct-acting antivirals

Grazoprevir/elbasvir and velpatasvir/sofosbuvir had the lowest number of predicted DDIs with the 156 co-medications. PrOD and simeprevir/sofosbuvir account for the highest number of predicted category 2 and 3 interactions with the used co-medication. Overall, the number of truly contra-indicated drugs is low, with a maximum of 10 drugs for PrOD. We were not able to predict potential DDIs of 23 drugs (category 4), as these drugs were unavailable in the Liverpool database (Figure 1).

Converting the number of drugs (156) to the number of patients with a category 2 or 3 DDI with any of the DAAs, we found that 299 patients were at risk. This concerns 205 (41%) genotype 1, 34 (36%) genotype 2/3, 54 (39%) genotype 4, and 6 (12%) patients with an unknown genotype. Furthermore, 269 (40%) patients <60 years and 55 (77%) patients ≥60 years were at risk for a category 2 or 3 DDI with any of the DAA regimens. Similarly, 147 (34%) and 100 (55%) patients without and with cirrhosis, respectively, were at risk for a DDI.

Identification of cART

A total of 762 (98%) patients were treated with cART. The NRTI backbone containing TDF + emtricitabine was used by 536 (70%) of patients, and 103 (14%) patients used abacavir + lamivudine.

Most patients used 1 additional (e.g., PI, INSTI, NNRTI) antiretroviral drug (670; 88%) and 40 (5%) patients used more than 1 additional antiretroviral. Most frequently used additional drugs were NNRTIs (307; 46%), followed by the boosted PIs (247; 37%), and INSTIs (116; 17%). It should be noted that on the date of evaluation, 1 January 2015, dolutegravir had only been available for 2 months (Table 3).

Prediction of drug-drug interactions between cART and direct-acting antivirals

Per genotype, the predicted DDIs per patients are shown in Figure 2 (n = 669). None of the genotypes 1 and 4 patients would have to change their cART when treated with daclatasvir/sofosbuvir. However, the dosage of daclatasvir should be altered depending on some specific cART regimens. Ledipasvir and velpatasvir in combination with sofosbuvir can be safely used with all third additional drugs. However, 199 (31%) patients with genotype 1 or 4 used TDF with boosted PIs, which makes it necessary to switch either TDF or the PI. Comparably, in combination with velpatasvir, patients infected with all genotypes using TDF with a boosted PI (n = 231; 29%), are recommended to switch either TDF or the PI.

Table 3: Overview of antiretroviral drugs used by the patients in the cohort and the interactions between these antiretrovirals and direct-acting antivirals.

Antiretroviral drug	Total number	GT1	GT2	GT3	GT4	GT6	Unknown GT	DCV + SOF	GZR + EBR	LDV + SOF	PrO	PrOD	SIM + SOF	VEL + SOF
Atazanavir/r	81	59	2	6	11		3	Possible DDI	CI	No DDI	Possible DDI	Possible DDI	CI	No DDI
Darunavir/r	110	73	4	12	17	1	3	No DDI	CI	No DDI	Possible DDI	Possible DDI	CI	No DDI
Lopinavir/r	49	30	2	6	8		3	No DDI	CI	No DDI	CI	CI	CI	No DDI
Fosamprenavir/r	2	2	0	0	0			Possible DDI	CI	No DDI	Possible DDI	Possible DDI	CI	No DDI
Saquinavir/r	4	3	0	1	0			Possible DDI	CI	No DDI	CI	CI	CI	No DDI
Indinavir/r	1	0	0	0	1			Possible DDI	CI	No DDI	CI	CI	CI	No DDI
Efavirenz	130	85	3	13	22		7	Possible DDI	CI	Possible DDI	CI	CI	CI	CI
Etravirine	3	2	0	0	1			Possible DDI	CI	No DDI	CI	CI	CI	CI
Nevirapine	112	63	7	9	29		4	Possible DDI	CI	No DDI	CI	CI	CI	CI
Rilpivirine	62	37	2	4	15		4	No DDI	No DDI	No DDI	Possible DDI	Possible DDI	No DDI	No DDI
Dolutegravir	35	21	0	0	12		2	No DDI	No DDI	No DDI	No DDI	No DDI	No DDI	No DDI
Elvitegravir/ cobicistat	22	17	1	0	3		1	Possible DDI	CI	Possible DDI	CI	CI	CI	No DDI
Raltegravir	59	39	4	4	8		4	No DDI	No DDI	No DDI	No DDI	No DDI	No DDI	No DDI

CI: Contra-indication; GT: Genotype; /r: ritonavir; PrOD: Paritaprevir/ritonavir, ombitasvir with dasabuvir; PrO: Paritaprevir/ritonavir, ombitasvir; SIM + SOF: Simeprevir and sofosbuvir; LDV + SOF: Ledipasvir and sofosbuvir; DCV + SOF: Daclatasvir and sofosbuvir; VEL+SOF: Velpatasvir and sofosbuvir: GZR + EBR: Grazoprevir and elbasvir.

Grazoprevir/elbasvir causes the most category 3 DDIs, necessitating a change in DAA or cART regimen. Other regimens with category 3 interactions were sofosbuvir with velpatasvir or simeprevir and PrOD. For patients with genotype 2/3 or an unknown genotype, it is shown that sofosbuvir/daclatasvir can be used without switching the cART.

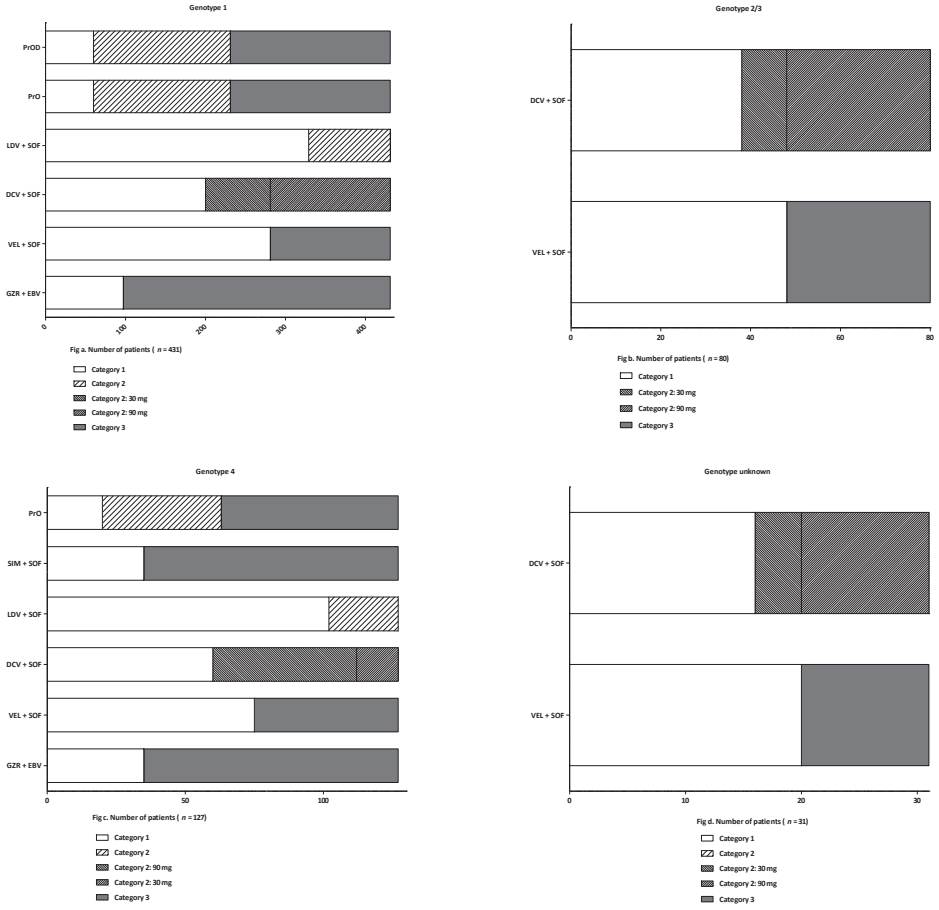

Figure 2: The number of patients predicted to have a drug-interaction between cART and the various combinations of direct-acting antivirals shown per genotype.

Only patients with one additional (third) drug are included in this analysis ($n = 670$).

Genotype 6 is excluded from this analysis, as only one patient was listed with genotype 6 ($n = 669$).

Category 2 30 mg: reduce the daclatasvir dose to 30 mg.

Category 2 90 mg: increase the daclatasvir dose to 90 mg.

PrOD: Paritaprevir/ritonavir, ombitasvir with dasabuvir; PrO: Paritaprevir/ritonavir, ombitasvir; SIM + SOF: Simeprevir and sofosbuvir; LDV + SOF: Ledipasvir and sofosbuvir; DCV + SOF: Daclatasvir and sofosbuvir; VEL+SOF: Velpatasvir and sofosbuvir: GZR + EBR: Grazoprevir and elbasvir.

DISCUSSION

This cohort represents all Dutch HIV/HCV co-infected patients in care in the Netherlands who might be treated with the novel DAAs. Most commonly used co-medications reflect the characteristics of the HIV/HCV patient population, such as the drugs used for opioid dependence[2]. Other drug classes in the top 5 are comparable with HCV mono-infected patients in the Netherlands[11] and represent the aging HIV population with an increasing number of co-morbidities. This is supported by our subgroup analysis in which patients aged ≥60 years had a higher risk of DDIs than patients <60 aged years. Similarly, patients with cirrhosis had a higher predicted risk of DDIs than patients without cirrhosis. This is comparable to the findings in HCV mono-infected patients[12].

PrOD and sofosbuvir/simeprevir have the highest number of predicted DDIs with non-antiretroviral co-medication, which is in line with previous studies[6, 8, 9]. Both combinations contain inhibitors of CYP3A4 (i.e. ritonavir, simeprevir), which is the main drug-metabolizing enzyme[5, 13]. However, it should be mentioned that, in daily practice, these regimens are infrequently used, because side effects and DDIs caused by the protease inhibitors.

Grazoprevir/elbasvir had the lowest number of DDIs with co-medication because they have minimal influence on drug-enzymes and transporters[14]. One should notice that grazoprevir is a mild inhibitor of CYP3A4. Therefore, we recommend being careful with CYP3A4 substrates with a narrow therapeutic range. However, it remains unclear whether these DDIs are clinically relevant.

Daclatasvir/sofosbuvir can be easily combined with cART, because of the possibility of a dose adaptation and no contra-indicated cART regimens. Although ledipasvir has only category 2 DDIs, it is less favorable, because ledipasvir is not recommended with the combination of a boosted PI and TDF, an issue that would require a switch in cART in 31% of patients. This interaction, as well as the interaction with velpatasvir (29%), can also be avoided when switching from TDF to tenofovir alafenamide. Plasma concentrations of tenofovir alafenamide are not affected by ledipasvir[15]. In most countries, the separate agents, daclatasvir and sofosbuvir are in general a more expensive DAA regimen compared with the fixed-dose combinations with velpatasvir and ledipasvir and therefore prescribed in a lesser extent. In the Netherlands, the prices of DAAs are unknown and therefore not a criterion for selecting a DAA regimen[10].

It is striking that grazoprevir/elbasvir has the lowest number of interactions with non-antiretroviral co-medication, but this combination has the highest number of DDIs

with cART. Grazoprevir/elbasvir (and simeprevir) is contra-indicated with all boosted PIs, NNRTIs (except rilpivirine), and elvitegravir/cobicistat; this makes it an unfavorable combination in this co-infected population because almost all patients would need to alter their cART regimen, if they are not already on raltegravir or dolutegravir. NNRTIs and PIs are most frequently used in our cohort, but with the introduction of dolutegravir, the use of NNRTIs and PIs decreased[2].

A limitation of the analysis is that patients with the most complicated cART regimens (e.g., >1 additional drug, no NNRTI backbone) were excluded from the analysis presented in Figure 2. These patients are probably the most difficult-to-treat HIV patients, because they have deviating cART regimens, and therefore, switching cART is probably not an option in these cases (e.g., resistance, toxicity). For these patients, the treatment strategy is to use a DAA regimen with a least number of (possible) drug-interactions.

Finally, we must comment that the majority of the DDIs, which are discussed in this article, only are studied in healthy volunteers and not in HIV/HCV co-infected patients. These drug-interaction studies in healthy volunteers give a good indication of the direction of the DDI; however, as healthy volunteers substantially differ from HIV/HCV co-infected patients, the magnitude of the DDIs could also differ, for example, the exposure to DAAs and antiretroviral drugs is probably different in healthy volunteers and HIV/HCV patients[16].

Concluding, this study showed that co-medication use in the aging HIV/HCV population is frequent and diverse and that there is a high potential of DDIs between DAAs plus co-medication/cART. Combining the results from our analysis, from the perspective of potential DDIs with co-medication and/or cART, the most favorable regimen seems to be daclatasvir/sofosbuvir.

ETHICS STATEMENT

At initiation, the ATHENA observational cohort was approved by the institutional review board of all participating centers. It has subsequently become integral part of HIV care and includes pseudonymized data and stored plasma samples from HIV-infected patients living in the Netherlands and receiving care in one of the designated HIV treatment centers. Patients can opt out after being informed by their treating physician of the purpose of collection of data and samples. Data from patients who opt out are not included in the ATHENA database. Pseudonymized data may be used for scientific purposes without further review. Patients are informed that in case of future requests for use of stored plasma samples for scientific research, they will be asked for prior consent by their treating HIV physician. Data are pseudonymized before being provided to investigators. For the purpose of our analysis, only existing data have been used, and therefore no additional review or consent has been necessary.

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CLINICAL CENTERS

** denotes site coordinating physician*

Academic Medical Center of the University of Amsterdam: *HIV treating physicians:* J.M. Prins*, T.W. Kuijpers, H.J. Scherpbier, J.T.M. van der Meer, F.W.M.N. Wit, M.H. Godfried, P. Reiss, T. van der Poll, F.J.B. Nellen, S.E. Geerlings, M. van Vugt, D. Pajkrt, W.J. Wiersinga, M. van der Valk, A. Goorhuis, J.W. Hovius. *HIV nurse consultants:* M.A.H. Bijsterveld, J. van Eden, A.M.H. van Hes, M. Mutschelknauss, H.E. Nobel, F.J.J. Pijnappel, A.M. Weijsenfeld. *HIV clinical virologists/chemists:* S. Jurriaans, N.K.T. Back, H.L. Zaaijer, B. Berkhout, M.T.E. Cornelissen, C.J. Schinkel, X.V. Thomas. **Admiraal De Ruyter Ziekenhuis, Goes:** *HIV treating physicians:* M. van den Berge, A. Stegeman. *HIV nurse consultants:* S. Baas, L. Hage de Looff. *HIV clinical virologists/chemists:* B Wintermans, J Veenemans. **Catharina Ziekenhuis, Eindhoven:** *HIV treating physicians:* M.J.H. Pronk*, H.S.M. Ammerlaan. *HIV nurse consultants:* E.S. de Munnik, E. van Beek. *HIV clinical virologists/chemists:* A.R. Jansz, J. Tjhie, M.C.A. Wegdam, B. Deiman, V. Scharnhorst. **Elisabeth-TweeSteden Ziekenhuis, Tilburg:** *HIV treating physicians:* M.E.E. van Kasteren*, A.E. Brouwer. *HIV nurse consultants:* R. van Erve, B.A.F.M. de Kruijf-van de Wiel, S.Keelan-Pfaf, B. van der Ven.

Data collection: B.A.F.M. de Kruijf-van de Wiel, B. van der Ven. *HIV clinical virologists/chemists:* A.G.M. Buiting, P.J. Kabel, D.Versteeg. **Emma Kinderziekenhuis, Amsterdam:** *HIV nurse consultants:* A. van der Plas, A.M. Weijsenfeld. **Erasmus MC, Rotterdam:** *HIV treating physicians:* M.E. van der Ende*, H.I. Bax, E.C.M. van Gorp, J.L. Nouwen, B.J.A. Rijnders, C.A.M. Schurink, A. Verbon, T.E.M.S. de Vries-Sluijs. *HIV nurse consultants:* N. Bassant, J.E.A. van Beek, M. Vriesde, L.M. van Zonneveld. *Data collection:* H.J. van den Berg-Cameron, F.B. Bruinsma-Broekman, J. de Groot, M. de Zeeuw-de Man. *HIV clinical virologists/chemists:* C.A.B. Boucher, M.P.G Koopmans, J.J.A van Kampen, S.D. Pas. **Erasmus MC-Sophia, Rotterdam:** *HIV treating physicians:* G.J.A. Driessen, A.M.C. van Rossum. *HIV nurse consultants:* L.C. van der Knaap, E. Visser. **Flevoziekenhuis, Almere:** *HIV treating physicians:* J. Branger*, A. Rijkeboer-Mes. *HIV nurse consultant and data collection:* C.J.H.M. Duijf-van de Ven. **HagaZiekenhuis, Den Haag:** *HIV treating physicians:* E.F. Schippers*, C. van Nieuwkoop. *HIV nurse consultants:* J.M. van IJperen, J. Geilings. *Data collection:* G. van der Hut. *HIV clinical virologist/chemist:* P.F.H. Franck. **HIV Focus Centrum (DC Klinieken), Amsterdam:** *HIV treating physicians:* A. van Eeden*. *HIV nurse consultants:* W. Brokking, M. Groot, L.J.M. Elsenburg. *HIV clinical virologists/chemists:* M. Damen, I.S. Kwa. **Isala, Zwolle:** *HIV treating physicians:* P.H.P. Groeneveld*, J.W. Bouwhuis. *HIV nurse consultants:* J.F. van den Berg, A.G.W. van Hulzen. *Data collection:* G.L. van der Bliek, P.C.J. Bor. *HIV clinical virologists/chemists:* P. Bloembergen, M.J.H.M. Wolfhagen, G.J.H.M. Ruijs. **Leids Universitair Medisch Centrum, Leiden:** *HIV treating physicians:* F.P. Kroon*, M.G.J. de Boer, H. Jolink, A.M. Vollaard. *HIV nurse consultants:* W. Dorama, N. van Holten. *HIV clinical virologists/chemists:* E.C.J. Claas, E. Wessels. **Maasstad Ziekenhuis, Rotterdam:** *HIV treating physicians:* J.G. den Hollander*, K. Pogany, A. Roukens. *HIV nurse consultants:* M. Kastelijns, J.V. Smit, E. Smit, D. Struik-Kalkman, C. Tearno. *Data collection:* M. Bezemer, T. van Niekerk. *HIV clinical virologists/chemists:* O. Pontesilli. **Maastricht UMC+, Maastricht:** *HIV treating physicians:* S.H. Lowe*, A.M.L. Oude Lashof, D. Posthouwer. *HIV nurse consultants:* R.P. Ackens, J. Schippers, R. Vergoossen. *Data collection:* B. Weijenberg-Maes. *HIV clinical virologists/chemists:* I.H.M. van Loo, T.R.A. Havenith. **MCH-Bronovo, Den Haag:** *HIV treating physicians:* E.M.S. Leyten*, L.B.S. Gelinck. *HIV nurse consultants:* A.Y. van Hartingsveld, C. Meerkerk, G.S. Wildenbeest. *HIV clinical virologists/chemists:* J.A.E.M. Mutsaers, S.Q. van Veen. **MC Slotervaart, Amsterdam:** *HIV treating physicians:* J.W. Mulder*, S.M.E. Vrouenraets, F.N. Lauw. *HIV nurse consultants:* M.C. van Broekhuizen, H. Paap, D.J. Vlasblom. *HIV clinical virologists/chemists:* P.H.M. Smits. **MC Zuiderzee, Lelystad:** *HIV treating physicians:* S. Weijer*, R. El Moussaoui. *HIV nurse consultant:* A.S. Bosma. **Medisch Centrum Leeuwarden, Leeuwarden:** *HIV treating physicians:* M.G.A.van Vonderen*, D.P.F. van Houte, L.M. Kampschreur. *HIV nurse consultants:* K. Dijkstra, S. Faber. *HIV clinical virologists/chemists:* J Weel. **Medisch Spectrum Twente, Enschede:** *HIV treating physicians:* G.J. Kootstra*, C.E. Delsing. *HIV nurse consultants:* M. van der Burg-van de Plas, H. Heins. *Data collection:* E. Lucas. **Noordwest**

Ziekenhuisgroep, Alkmaar: *HIV treating physicians:* W. Kortmann*, G. van Twillert*, J.W.T. Cohen Stuart, B.M.W. Diederen, R. Renckens. *HIV nurse consultant and data collection:* D. Ruiter-Pronk, F.A. van Truijen-Oud. *HIV clinical virologists/chemists:* W. A. van der Reijden, R. Jansen. **OLVG, Amsterdam:** *HIV treating physicians:* K. Brinkman*, G.E.L. van den Berk, W.L. Blok, P.H.J. Frissen, K.D. Lettinga W.E.M. Schouten, J. Veenstra. *HIV nurse consultants:* C.J. Brouwer, G.F. Geerders, K. Hoeksema, M.J. Kleene, I.B. van der Meché, M. Spelbrink, H. Sulman, A.J.M. Toonen, S. Wijnands. *HIV clinical virologists:* M. Damen, D. Kwa. *Data collection:* E. Witte. **Radboudumc, Nijmegen:** *HIV treating physicians:* R. van Crevel*, M. Keuter, A.J.A.M. van der Ven, H.J.M. ter Hofstede, A.S.M. Dofferhoff. *HIV nurse consultants:* M. Albers, K.J.T. Grintjes-Huisman, M. Marneef, A. Hairwassers. *HIV clinical virologists/chemists:* J. Rahamat-Langendoen. *HIV clinical pharmacology consultant:* D. Burger. **Rijnstate, Arnhem:** *HIV treating physicians:* E.H. Gisolf*, R.J. Hassing, M. Claassen. *HIV nurse consultants:* G. ter Beest, P.H.M. van Bentum, N. Langebeek. *HIV clinical virologists/chemists:* R. Tiemessen, C.M.A. Swanink. **Spaarne Gasthuis, Haarlem:** *HIV treating physicians:* S.F.L. van Lelyveld*, R. Soetekouw. *HIV nurse consultants:* L.M.M. van der Prijt, J. van der Swaluw. *Data collection:* N. Bermon. *HIV clinical virologists/chemists:* W.A. van der Reijden, R. Jansen, B.L. Herpers, D.Veenendaal. **Medisch Centrum Jan van Goyen, Amsterdam:** *HIV treating physicians:* D.W.M. Verhagen. *HIV nurse consultants:* M. van Wijk. **Universitair Medisch Centrum Groningen, Groningen:** *HIV treating physicians:* W.F.W. Bierman*, M. Bakker, J. Kleinnijenhuis, E. Kloeze, H. Scholvinck, Y. Stienstra, C.L. Vermont, K.R. Wilting. *HIV nurse consultants:* A. Boonstra, H. de Groot-de Jonge, P.A. van der Meulen, D.A. de Weerd. *HIV clinical virologists/chemists:* H.G.M. Niesters, C.C. van Leer-Buter, M. Knoester. **Universitair Medisch Centrum Utrecht, Utrecht:** *HIV treating physicians:* A.I.M. Hoepelman*, J.E. Arends, R.E. Barth, A.H.W. Bruns, P.M. Ellerbroek, T. Mudrikova, J.J. Oosterheert, E.M. Schadd, M.W.M. Wassenberg, M.A.D. van Zoelen. *HIV nurse consultants:* K. Aarsman, D.H.M. van Elst-Laurijssen, E.E.B. van Oers-Hazelzet. *Data collection:* M. van Berkel. *HIV clinical virologists/chemists:* R. Schuurman, F. Verduyn-Lunel, A.M.J. Wensing. **VUmc, Amsterdam:** *HIV treating physicians:* E.J.G. Peters*, M.A. van Agtmael, M. Bomers, J. de Vocht. *HIV nurse consultants:* M. Heitmuller, L.M. Laan. *HIV clinical virologists/chemists:* C.W. Ang, R. van Houdt, A.M. Pettersson, C.M.J.E. Vandenbroucke-Grauls. **Wilhelmina Kinderziekenhuis, UMCU, Utrecht:** *HIV treating physicians:* S.P.M. Geelen, T.F.W. Wolfs, L.J. Bont. *HIV nurse consultants:* N. Nauta.

COORDINATING CENTER

Director: P. Reiss. *Data analysis:* D.O. Bezemer, A.I. van Sighem, C. Smit, F.W.M.N. Wit, T.S. Boender. *Data management and quality control:* S. Zaheri, M. Hillebregt, A. de Jong. *Data monitoring:* D. Bergsma, A. de Lang, S. Grivell, A. Jansen, M.J. Rademaker, M. Raethke, R. Meijering, S. Schnörr. *Data collection:* L. de Groot, M. van den Akker, Y. Bakker, E. Claessen,

A. El Berkaoui, J. Koops, E. Kruijne, C. Lodewijk, L. Munjishvili, B. Peeck, C. Ree, R. Regtop, Y. Ruijs, T. Rutkens, L. van de Sande, M. Schoorl, A. Timmerman, E. Tuijn, L. Veenenberg, S. van der Vliet, A. Wisse, T. Woudstra. *Patient registration:* B. Tuk.

CONFLICTS OF INTEREST

EJS, CS, CTMMK and ASMD declare that they have no conflicts of interest that are directly relevant to the content of this manuscript. JEA joins advisory boards of Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, ViiV Healthcare, and Merck. He received sponsorship of Bristol-Myers Squibb, ViiV Healthcare, Abbvie, and Merck. KB joins advisory board of Gilead Sciences, Bristol-Myers Squibb, Janssen, Abbvie, ViiV Healthcare, and Roche. BR received research grants from MSD and Gilead Sciences, travel grants from ViiV Healthcare, MSD, Bristol-Myers Squibb, Gilead Sciences, and Janssen-Cilag. He received speakers fees from Bristol-Myers Squibb, Gilead Sciences, and Janssen-Cilag and personal fees from Bristol-Myers Squibb, Gilead Sciences, and Janssen-Cilag. MV joins advisory boards of Abbvie, Bristol-Myers Squibb, Gilead, Janssen, ViiV Healthcare and Merck. He received sponsorship and research grants of Gilead, Janssen, and Merck. PR through his institution received independent scientific grant support, unrelated to the content of this manuscript, from Gilead Sciences Sciences, Janssen Pharmaceuticals Inc, Merck, Bristol-Myers Squibb and ViiV Healthcare; he has served on a scientific advisory board for Gilead Sciences and a data safety monitoring committee for Janssen Pharmaceuticals Inc; he chaired a scientific symposium by ViiV Healthcare, for which his institution has received remuneration. DMB joins advisory boards of Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, ViiV Healthcare, and Merck. He received sponsorship and research grants of Bristol-Myers Squibb, Janssen Pharmaceuticals, ViiV Healthcare, and Merck.

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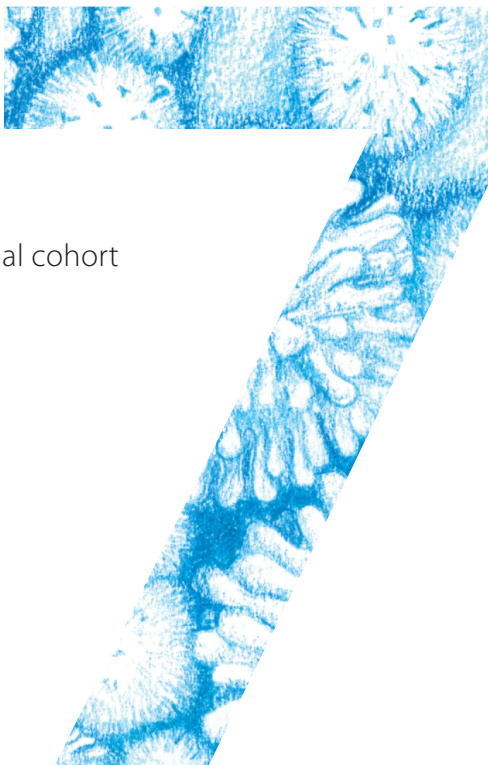
Management of drug-interactions with direct-acting antivirals in Dutch HIV/HCV co-infected patients: adequate but not perfect

EJ Smolders
C Smit
CTMM de Kanter
ASM Dofferhoff
JE Arends
K Brinkman
B Rijnders
M van der Valk
P Reiss
DM Burger

on behalf the ATHENA national HIV observational cohort



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ABSTRACT

Objective

Direct-acting antivirals (DAAs) for chronic hepatitis C virus (HCV) infection treatment can cause drug-interactions (DDIs), with combination antiretroviral therapy (cART) and non-antiretroviral co-medication. We mapped how physicians manage DDIs between DAAs and co-medication and analyzed treatment outcomes.

Methods

Data was prospectively collected as part of the ATHENA HIV observational cohort and retrospectively analyzed. Dutch patients with HIV/HCV co-infection who initiated treatment with DAAs between January 2015 and May 2016 were included. Co-medication 3 months prior to and during DAA therapy was identified. Potential DDIs with the DAAs were checked using http://www.hep-druginteractions.org. DDIs were categorized as: (1) no interaction expected; (2) potential interaction; (3) contra-indication; (4) no recommendation. This was used to determine which patients switched or had a DDI during DAA therapy with co-medication.

Results

423 patients were treated with DAAs, of whom 418 (99%) used cART and 251 (59%) used non-antiretroviral co-medication. Before commencing DAA treatment, in 17/84 (20%) patients the non-antiretroviral co-medication which could result in a category 2 or 3 DDI, were discontinued before DAA initiation, including 2/6 (33%) prescriptions of category 3 drugs.

196/418 (47%) patients had a category 2 or 3 DDI between their DAA regimen and cART. Category 2 or 3 DDIs were prevented by switching cART in 78/147 (53%) and 47/49 (96%) patients. 367/423 (87%) patients have reached SVR (33 in follow-up).

Conclusions

Prescription patterns suggest physicians to be aware of potential DDIs between co-medication and DAAs, in particular where it concerns DDIs with cART. Awareness is needed for category 3 interactions between non-antiretroviral co-medication and DAAs.

INTRODUCTION

Several drug classes used for the treatment of HIV (combination antiretroviral therapy [cART]) may cause drug-drug interactions (DDIs), especially the boosted protease inhibitors (PIs) and most of the non-nucleoside reverse transcriptase inhibitors (NNRTIs). Both classes influence the activity of drug-metabolizing enzymes and/or drug-transporters and, in addition, they can also be a substrate of these enzymes and transporters[1]. Simultaneous treatment of both HIV and hepatitis C virus (HCV) infection increases the risk of DDIs, because the direct-acting antivirals (DAAs) against HCV are metabolized by the enzymes that are influenced by cART, and vice versa[2].

Earlier studies predicted a high risk of clinically relevant DDIs when patients are simultaneously treated for HIV and HCV[3-5]. For instance, it was previously predicted that 51% of HIV/HCV co-infected patients had a cART regimen that would be contra-indicated with simeprevir/sofosbuvir or grazoprevir/elbasvir[5]. Co-medication other than cART can also be involved in DDIs[6]. This has already been shown for HCV mono-infected patients[4, 7-10]. Vermehren et al predicted that 60% of HCV mono-infected patients using non-antiretroviral co-medication had a DDI with at least one DAA regimen and that patients ≥65 years had an increased risk for DDIs[9].

Therefore, when DAAs are co-administered with cART and non-antiretroviral medications there is a risk of clinically relevant DDIs. Various tools are available that provide information for clinical decision making and preventing DDIs, such as electronic medication warnings during the process of drug prescription or the website of the University of Liverpool (http://www.hep-druginteractions.org)[11]. However, two important questions have remained largely unanswered so far: how do physicians handle these potential DDIs in clinical practice, and does this affect the outcome of HCV treatment? Modifying cART to prevent a DDI may not be without risk, and could result in new drug toxicity or HIV virological failure. Therefore, this analysis was conducted to analyze how physicians managed potential DDIs between DAAs and either non-antiretroviral co-medication or cART co-medication and how this affected subsequent HIV and HCV virological treatment outcomes in a nationwide cohort of HIV/HCV co-infected patients in the Netherlands.

METHODS

Data were used from the ATHENA database managed by the HIV monitoring Foundation (http://www.hiv-monitoring.nl). This is a registry of all Dutch HIV patients in care, except a small minority who opt out of having their data registered (2%).

All HIV/HCV co-infected patients initiating treatment with DAAs between January 2015 and May 2016 were included. DAAs available during that time in the Netherlands were simeprevir, daclatasvir, sofosbuvir, ledipasvir/sofosbuvir, and the combination of paritaprevir/ritonavir, ombitasvir and dasabuvir.

Patient characteristics were extracted from the database three months before DAA treatment was started. We identified which non-antiretroviral and cART co-medication was used by the patients three months before and during DAA therapy.

Potential DDIs between cART and other co-medication and the selected DAA regimen were checked using the website of the University of Liverpool (http://www.hep-druginteractions.org; September 2016). DDIs were categorized as defined by the Liverpool database: (1) no interaction expected (green); (2) potential interaction, i.e. monitor the patient or alter drug dosage/timing (amber); (3) contra-indication, do not co-administer (red); or (4) no clear data, so no recommendation is given. We defined category 2 and 3 as clinically relevant, given that according to the website, action is needed when the drugs are combined. For patients with a category 2 or 3 DDI between DAA and non-antiretroviral co-medication and DAAs and cART, the information about DDIs before commencing DAA therapy was evaluated. Information about any modification of the cART regimen both before starting and during DAA treatment was available in the database. However, for non-antiretroviral co-medications information was only available concerning whether therapy was stopped or continued.

Data on sustained virological response 12 weeks after the end of HCV therapy (SVR12) and HIV viral load were also extracted; (HIV RNA <100 copies/mL was defined as undetectable to exclude blips). We performed descriptive analyses (frequency counts, proportions) using SPSS (IBM SPSS Statistics 20).

RESULTS

Data from 423 HIV/HCV co-infected patients were analyzed. The median (interquartile range [IQR]) age at time of DAA initiation was 50 (45-55) years, 377 (89%) patients were male, 118 (28%) patients had METAVIR score ≥F3 (liver stiffness ≥9.5 kPa). The majority of patients were infected with HCV genotype 1 (288; 68%).

Most frequently prescribed regimens were ledipasvir/sofosbuvir (213; 50%), daclatasvir/sofosbuvir ± ribavirin (115; 27%) and simeprevir/sofosbuvir ± ribavirin (59; 14%). In total, 418 (99%) patients were being treated for their HIV infection with cART, of whom 405/418 (96%) had an undetectable plasma HIV-1 RNA level at start of DAA treatment.

The majority of patients were treated with a nucleoside reverse transcriptase inhibitor (NRTI) backbone consisting of tenofovir disoproxil fumarate (TDF) plus emtricitabine (298/418; 70%) or lamivudine plus abacavir (63/418; 15%). Boosted PIs, NNRTIs, and integrase inhibitors (INSTIs) were used by 125/418 (30%), 175/418 (41%), and 76/418 (18%) patients, respectively.

In conjunction with starting DAA treatment, 166/418 (40%) patients switched their boosted PI, NNRTI, or INSTI and 57/418 (14%) of the patients switched their NRTI backbone. The majority of these patients switched from a boosted PI or NNRTI to INSTIs such as raltegravir or dolutegravir (Table 1). Supplementary Table 1 shows that switching cART did not influence HIV suppression.

Table 1: Baseline patient characteristics (*n* = 423).

Characteristic	**Number**	
Male, n (%)	377 (89)	
Age, median (IQR)	50 (45-55)	
Genotype, n (%)		
1	57 (14)	
1a	202 (48)	
1b	29 (7)	
2	13 (3)	
3	30 (7)	
4	76 (18)	
6	1 (0)	
Missing	15 (4)	
METAVIR score, n (%)		
F0, F1, F2 (liver stiffness <9.5 kPa)	255 (60)	
F3, F4 (liver stiffness ≥9.5 kPa)	118 (28)	
Missing	50 (12)	
Creatinine µmol/L, median (IQR)	87 (24)	
DAA regimen, n (%)		
PrOD	17 (4)	
Daclatasvir containing[a]	116 (27)	
Simeprevir containing[b]	67 (16)	
Ledipasvir/sofosbuvir	213 (50)	
Sofosbuvir, peg-interferon alfa, ribavirin	10 (2)	
cART, n (%)	**Before DAA treatment**	**After DAA treatment**
Tenofovir disoproxil fumarate + emtricitabine	298 (70)	271(64)
Lamivudine + abacavir	63 (15)	85 (20)
Lamivudine + zidovudine	8 (2)	2 (1)
Lamivudine + zidovudine + abacavir	0 (0)	3 (1)
Other NRTI	30 (7)	33 (8)
No NRTI backbone	19 (5)	27 (6)

Table 1: Baseline patient characteristics (*n* = 423). (*continued*)

Characteristic	Number	
Boosted PI	125 (30)	66 (16)
NNRTI	175 (41)	103 (24)
INSTI	76 (18)	204 (48)
Other	35 (8)	40 (10)
No PI, NNRT, INSTI	7 (2)	5 (1)
No cART	5 (1)	2 (1)
HIV RNA, n (%)		
Undetectable (<100 IU/mL)	405 (96)	379 (90)
Detectable (>100 IU/mL)	18 (4)	13 (3)
Missing		31 (7)

[a]Regimens containing daclatasvir: daclatasvir + sofosbuvir ± ribavirin or daclatasvir + peg-interferon ± ribavirin.
[b]Regimens containing simeprevir: simeprevir + sofosbuvir ± ribavirin, simeprevir + daclatasvir, or simeprevir + peg-interferon ± ribavirin.
DAA: Direct-acting antiviral; PrOD: Paritaprevir/ritonavir, ombitasvir, dasabuvir; cART: combination antiretroviral therapy; NRTI; Nucleoside reverse transcriptase inhibitor; NNRTI: Non-nucleoside reverse transcriptase inhibitor; PI: Protease inhibitor; INSTI: Integrase inhibitor.

At least one non-antiretroviral co-medication was used by 251 (59%) patients (range: 1-11 drugs per patient). In total 570 unique prescriptions were used by these 251 patients. Most frequently used non-antiretroviral co-medication was methadone (39 prescriptions), followed by the fixed-dose combination of calcium carbonate/colecalciferol (38 prescriptions), colecalciferol (26 prescriptions), omeprazole (24 prescriptions), and pantoprazole (22 prescriptions). The top 20 of the most frequently prescribed non-antiretroviral co-medications are presented in Table 2.

Drug-drug interactions

In Figure 1 an overview is given of the category 2 and category 3 interactions before and during DAA treatment. This figure includes all co-medication: non-antiretroviral co-medication as well as cART. Three months before DAA treatment, 199/423 (47%) patients were at risk for a category 2 DDI of which 55/199 (28%) patients would have had a DDI with non-antiretroviral co-medication, 121/199 (61%) with cART, and 23/199 (12%) with both non-antiretroviral and cART co-medication. This number was reduced to 125/423 (30%) during DAA treatment, mainly as a result of the fact that 53/199 (27%) patients had modified their cART regimen prior to starting DAAs.

Comparably, 47/423 (11%) patients were at risk for a category 3 DDI prior to DAA treatment, the majority was at risk for an interaction between their DAA regimen and cART. Only six (1%) patients continued their category 3 drug during DAA treatment, of which 4/6 (1%) patients continued contra-indicated non-antiretroviral co-medications. Two out of these six patients had both a category 2 and category 3 DDI.

Table 2: Top 20 prescriptions co-medication (total number of prescriptions is 570) used by 251 patients.

Drug	Number prescriptions
Methadone	39
Calcium carbonate /colecalciferol	38
Colecalciferol	26
Omeprazole	24
Pantoprazole	22
Lisinopril	18
Valaciclovir	18
Pravastatin	14
Testosterone	14
Co-Trimoxazole	11
Metoprolol	11
Acetylsalicylic acid	10
Citalopram	10
Sildenafil	10
Carbasalate calcium	9
Metformin	9
Ranitidine	8
Escitalopram	7
Hydrochlorothiazide	7
Alendronic acid	6

Drug-drug interactions between direct-acting antivirals and non-antiretroviral co-medication

Table 3 provides an overview of all category 2 and 3 DDIs between non-antiretroviral co-medication and the DAA regimens which were used.

Before commencing DAA treatment, 78/423 (18%) patients were at risk for a category 2 DDI between their DAA regimen and non-antiretroviral co-medication (90 prescriptions). These category 2 DDIs were mostly caused by proton pump inhibitors (PPIs; omeprazole, pantoprazole) and statins (atorvastatin, pravastatin). Of the 90 interacting prescriptions, 21/90 (23%) were discontinued before DAA initiation. Ledipasvir/sofosbuvir had most category 2 DDIs with non-antiretroviral co-medications: 45/78 (58%) patients accounting for 57 prescriptions, of which 27 prescriptions included acid-reducing agents such as PPIs and H_2-antagonists (e.g. ranitidine). Seventeen patients continued their acid-reducing agent during HCV therapy with ledipasvir/sofosbuvir; 14/17 (82%) achieved SVR12; 1/17 patient did not achieve SVR, and 2/17 are still in follow-up for their SVR (Figure 2).

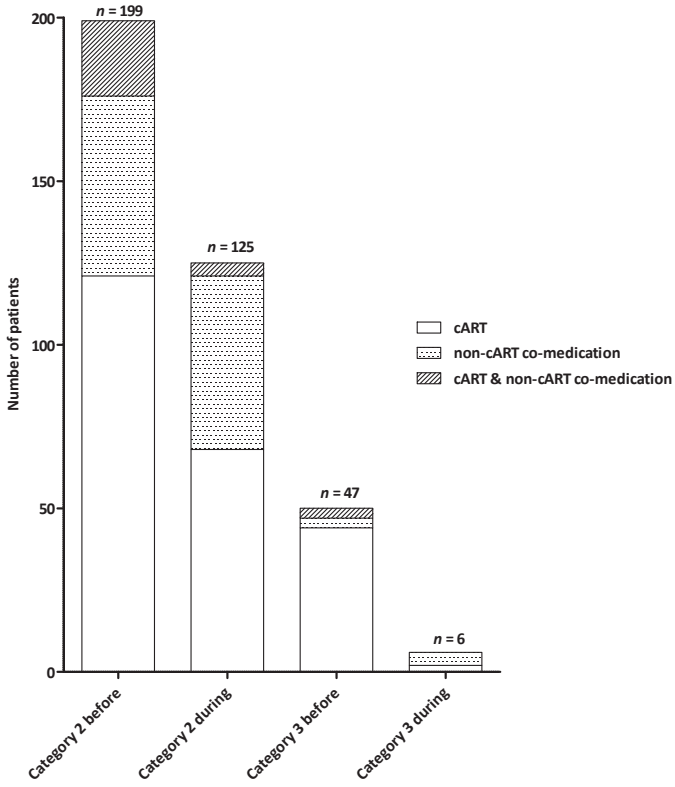

Figure 1: Number of patients that had an interaction between the used direct-acting antivirals (DAAs) and non-antiretroviral and cART co-medication before and during DAA treatment.

Number represents the number of patients that have a category 2 or 3 interaction.
Before: drug-drug interactions before DAA treatment.
During: drug-drug interactions during DAA treatment.
cART: combination antiretroviral therapy.

In 2 out of 6 (33%) patients who used non-antiretroviral co-medication that had a category 3 DDI with ledipasvir and paritaprevir/ritonavir, ombitasvir plus dasabuvir, the interacting drug was discontinued before DAA initiation. The other four (67%) patients who continued the interacting drug were able to complete the DAA therapy and all reached SVR.

In September 2016, 23 non-antiretroviral drugs were not included on the Liverpool website. These drugs were used by 91 patients, varying from one to three drugs per patient (105 prescriptions). None of these drugs were discontinued before HCV treatment. The most common of these drugs were calcium carbonate/colecalciferol (38

prescriptions), colecalciferol (26 prescriptions), and testosterone (14 prescriptions) for which no interactions were expected with any of the DAAs which were prescribed.

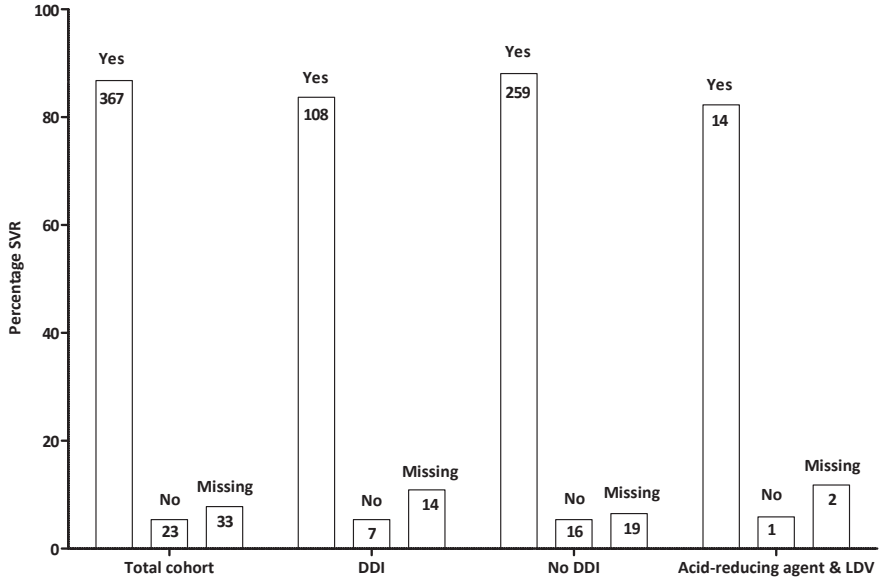

Figure 2: Sustained virological response (SVR) rates shown for the total cohort, patients with and without a drug-drug interaction, and patients treated with an acid-reducing agent and ledipasvir.

SVR: sustained virological response rate of the total cohort.

DDI: SVR-rate for patients with a DDI during treatment.

No DDI: SVR-rate for patients without a DDI during treatment.

Acid-reducing agent & LDV: SVR-rate for patients simultaneously treated with an acid-reducing agent and ledipasvir.

Drug-drug interactions between direct-acting antivirals and cART

In Figure 3, the interacting cART regimens per DAA regimen are presented. In the figure only the boosted PI, NNRTI, and INSTI are shown, as there are no DDIs with the NRTI backbone. The exception is the potential interaction when ledipasvir is combined with TDF and a boosted PI or boosted elvitegravir which is presented separately in Figure 3.

In total, 196 (47%) of 418 patients had a category 2 (147/418; 35%) or 3 (49/418; 12%) potential DDI between their prescribed DAA regimen and cART prior to initiation of DAA treatment.

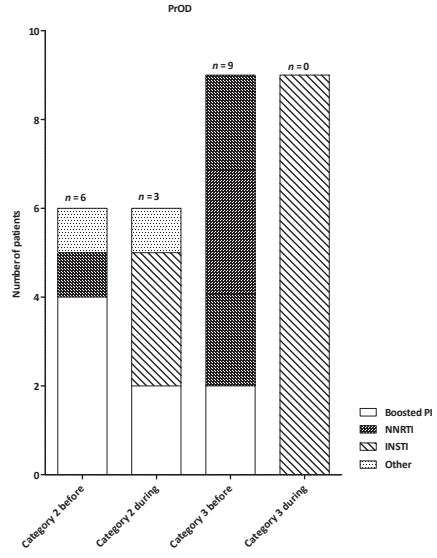

Fig a.

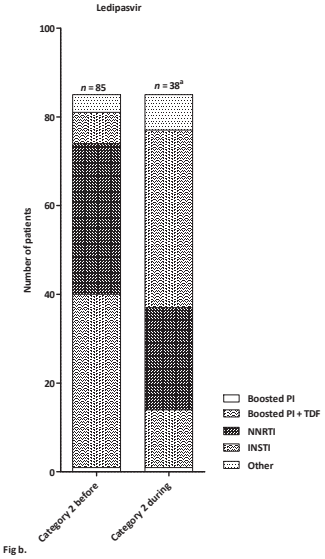

Fig b.

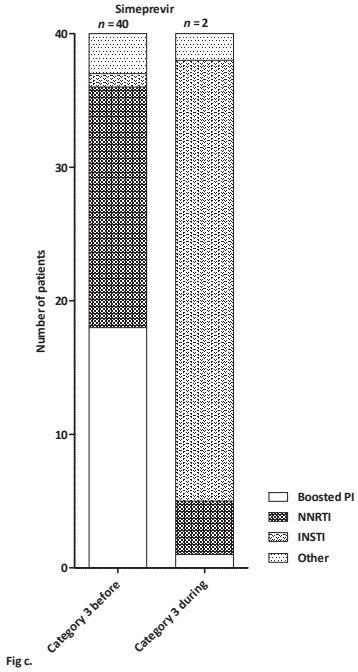

Fig c.

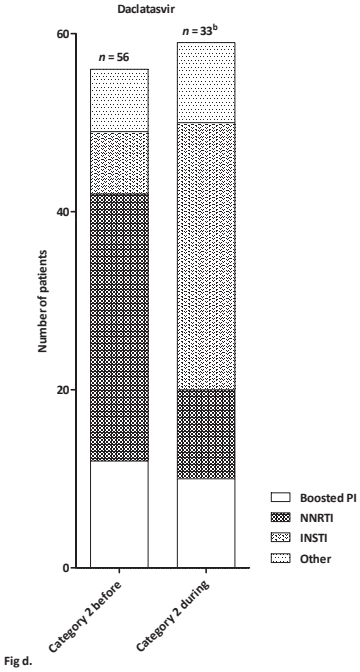

Fig d.

Figure 3: Overview of cART regimens used (third drugs) that caused category 2 and 3 drug-drug interactions 3 months prior to treatment.

Figure 3: Overview of cART regimens used (third drugs) that caused category 2 and 3 drug-drug interactions 3 months prior to treatment. *(continued)*

[a]Two patients did not have an interacting cART regimen before DAA treatment but switched during DAA treatment to an interacting regimen.

[b]Three patients did not have an interacting cART regimen before DAA treatment but switched during DAA treatment to an interacting regimen.

Before: drug-drug interactions before DAA treatment.

During: drug-drug interactions during DAA treatment.

PI: Protease inhibitor; INSTI: Integrase inhibitor; NNRTI: Non-nucleoside reverse transcriptase inhibitor; TDF: Tenofovir disoproxil fumarate.

NNRTIs (e.g., efavirenz, etravirine, and nevirapine) and boosted PIs (atazanavir and darunavir boosted with ritonavir) were the most commonly used third drugs which were also responsible for the majority of the category 2 DDIs (91 [22%] patients). Of the 147 patients with a category 2 DDI, 78 (51%) switched to a non-interacting cART regimen. The majority of these patients switched to a regimen with an INSTI (75/78; 96%), as raltegravir and dolutegravir have no DDIs with the DAAs. At start of DAA therapy 39/213 (18%) patients treated with ledipasvir used a boosted PI and TDF, while during DAA treatment this number was reduced to 13/213 (7%). The creatinine clearance of the patients that continued TDF and the boosted PI in combination with ledipasvir, remained stable during DAA therapy (supplementary Table 1).

The patients that did not switch cART, usually continued their potentially interacting regimen with a NNRTI or a boosted PI. However, we must comment that the daclatasvir dose can be easily adapted to 30 mg with enzyme inhibitors (atazanavir/ritonavir) or to 90 mg with enzyme inducers (e.g., efavirenz, etravirine). Information concerning such dose alterations were however not available in the database.

Another interesting but unexpected finding was that five patients who used a non-interacting cART regimen before starting DAA treatment, were switched during treatment to a potentially interacting cART regimen (Figure 3).

Category 3 cART regimens were used by 49/418 (12%) of the patients. Only 2/49 (4%) patients continued these interacting regimens during DAA treatment.

Efficacy of direct-acting antiviral treatment

Figure 2 shows the SVR12 rate of the cohort. At the time of our analysis, 367/423 (87%) patients had reached SVR (33 patients were still in follow-up). There was no difference in SVR-rates between patients with and without a potential DDI (84% and 88%, respectively). The SVR-rate of the patients that continued their PPI during ledipasvir treatment was 82%.

Table 3: Overview of category 2 and 3 interactions with co-medication.

Ledipasvir (n = 213)		Daclatasvir (n = 116)		Simeprevir (n = 67)		PrOD (n = 17)	
Category 2		Category 2		Category 2		Category 2	
Before DAA (p = 57 ; n = 45)	**After DAA (p = 42; n = 35)**	**Before DAA (p = 18; n = 18)**	**After DAA (p = 17; n = 17)**	**Before DAA (p = 14; n = 13)**	**After DAA (p = 10; n = 10)**	**Before DAA (p = 2; n = 2)**	**After DAA (p = 1; n = 1)**
Omeprazole (11)	Pravastatin (9)	Atorvastatin (5)	Atorvastatin (5)	Formoterol/ Budesonide (2)	Formoterol/ Budesonide (2)	Omeprazole (1)	Omeprazole (1)
Pravastatin (11)	Omeprazole (8)	Alendronic acid (2)	Alendronic acid (2)	Nifedipine (2)	Nifedipine (2)	Tramadol (1)	
Pantoprazole (9)	Pantoprazole (5)	Miconazole (2)	Miconazole (2)	Pravastatin (2)	Amlodipine (1)		
Alendronic acid (4)	Amlodipine (4)	Rosuvastatin (2)	Rosuvastatin (2)	Amlodipine (1)	Clonazepam (1)		
Amlodipine (4)	Alendronic acid (3)	Diltiazem (1)	Diltiazem (1)	Clonazepam (1)	Diazepam (1)		
Lansoprazole(3)	Lansoprazole (2)	Fluvastatin (1)	Fluvastatin (1)	Diazepam (1)	Doxazosin (1)		
Metformin (3)	Metformin (2)	Ketoconazole (1)	Ketoconazole (1)	Doxazosin (1)	Quetiapine (1)		
Calcium carbonate (2)	Buprenorphine (1)	Levothyroxine (1)	Levothyroxine (1)	Midazolam (1)	Salmeterol/ Fluticasone (1)		
Ranitidine (2)	Calcium carbonate (1)	Nifedipine (1)	Nifedipine (1)	Quetiapine (1)			
Buprenorphine (1)	Diltiazem (1)	Pravastatin (1)	Simvastatin (1)	Rosuvastatin (1)			
Diltiazem (1)	Esomeprazole (1)	Simvastatin (1)		Salmeterol/ Fluticasone (1)			
Esomeprazole (1)	Irbesartan (1)						
Irbesartan (1)	Loperamide (1)						
Loperamide (1)	Miconazole/ Hydrocortisone (1)						
Miconazole/ Hydrocortisone (1)	Olmesartan/ Amlodipine (1)						
Olmesartan /amlodipine (1)	Rabeprazol (1)						
Rabeprazol (1)							
Category 3 (p = 2; n = 2)	**Category 3 (p = 1; n = 1)**			**Category 3 (p = 2; n = 2)**	**Category 3 (p = 2; n = 2)**	**Category 3 (p = 2; n = 2)**	**Category 3 (p = 1; n = 1)**
Rosuvastatin (2)	Rosuvastatin (1)			Ketoconazole (1) Pimozide (1)	Ketoconazole (1) Pimozide (1)	Ketoconazole (2)	Ketoconazole (1)

Between brackets the number of prescriptions (p) and the number of patients (n) is presented.
DAA: Direct-acting antiviral; PrOD: Paritaprevir/ritonavir, ombitasvir, dasabuvir.

DISCUSSION

This is the first study describing the outcome of clinicians managing possible DDIs with DAAs in a nationwide cohort of HIV/HCV co-infected patients. Not surprisingly, we showed that a high number of HIV/HCV co-infected patients were at risk for a clinically relevant DDI with both non-antiretroviral and cART co-medication before initiating HCV therapy. Analysis of the prescribing patterns of physicians showed that Dutch physicians are well aware of DDIs and are keen on preventing them. Especially, interactions between DAAs and cART are managed accurately.

Interacting non-antiretroviral co-medications were less often appropriately discontinued before HCV treatment was started (Table 2). Drugs with a category 3 DDI are contraindicated and must be substituted; however, 4/6 of these drugs were not discontinued. Therefore, it can be stated that management of category 3 DDIs is suboptimal for non-antiretroviral co-medications.

Many category 2 DDIs between DAAs and non-antiretroviral co-medication can be handled by monitoring the patient, separating drug intake, or adjusting the drug dose. However, for some of these category 2 DDIs in daily practice, we strongly recommend to discontinue the non-antiretroviral co-medication during DAA therapy. For example, when statins are combined with several DAAs such as simeprevir, daclatasvir, and ledipasvir[12-14]. DAAs inhibit a number of uptake transporters, such as organic anion-transporting polypeptide 1B1/3 at the surface of hepatocytes, which can lead to increased plasma concentrations of the statins. Elevated plasma concentrations of statins might result in severe myopathy or even rhabdomyolysis[12-15].

Another example are PPIs and other acid-reducing agents. Due to low solubility of ledipasvir at higher pH, acid-reducing agents are not recommended to be combined with ledipasvir[14]. There is an ongoing debate whether the use of acid-reducing agents affects SVR[16-18]. This uncertainty is reflected in our cohort showing that PPIs were either continued or discontinued before ledipasvir was started. SVR was achieved in 82% of the patients (6% no SVR, 12% missing SVR data) who continued an acid-reducing agent in combination with ledipasvir.

Noticeable was that for category 2 interactions between cART and DAAs, 51% of patients switched their cART regimen. These interactions included the interactions with daclatasvir and ledipasvir which can be handled by adjusting the daclatasvir dose or monitoring renal function during ledipasvir therapy with TDF and a boosted PI or elvitegravir/cobicistat. Also, all interactions with the combination of paritaprevir/ritonavir, ombitasvir

and dasabuvir were avoided. It seems that physicians are more willing to switch cART to avoid interactions with the DAAs. This has become rather simple now that the integrase inhibitor dolutegravir and the NNRTI rilpivirine, which in contrast to older NNRTIs, has no cytochrome P450 (CYP) induction potential, are available in once daily regimens[19-22]. Of note however, dolutegravir, raltegravir, and rilpivirine do have a limited number of potential drug-interactions with non-antiretroviral co-medication. Switching cART did not affect the short-term viral suppression of HIV. Still, some physicians were cautious with switching cART because not all patients can switch, due to HIV drug resistance, adherence problems, or adverse events. DAA regimens which do not require modifying a patient's cART regimen would represent an additional future advance.

Strength of this study is that it was conducted within the framework of a nationwide cohort which is very much representative of all HIV/HCV-infected patients treated with DAAs in the Netherlands. We were able to identify possible DDIs at the beginning of DAA treatment and how the DDIs were managed. A limitation is that we were not able to identify toxicities or inefficacy of non-antiretroviral co-medications as a result of potential interactions, in case these did not result in discontinuation of such co-medications. In addition, we were not able to detect whether category 2 interactions were monitored or dosages adjusted, as we could only identify discontinued drugs. Lastly, the website of the University of Liverpool is recently updated with a yellow category. This category represents an interaction of weak intensity for which additional action/monitoring or dosage adjustment is unlikely to be required. At the time of prescribing this category was not yet available and therefore could not be used for the present data analysis.

In conclusion, DDIs are a significant potential problem when combining treatment for HIV and HCV. Despite the high risk of DDIs, Dutch HIV physicians seem well aware of possible DDIs and manage to prevent the large majority of these. Especially, cART regimens are appropriately switched to non-interacting regimens before DAA treatment in case of both category 2 and 3 DDIs. There is however room for improvement where it concerns modifying potentially interacting non-antiretroviral co-medication. Finally, the limited number of patients who continue category 3 contra-indicated medications deserves further study.

ETHICS STATEMENT

At initiation, the ATHENA observational cohort was approved by the institutional review board of all participating centers. It has subsequently become integral part of HIV care and includes pseudonymised data and stored plasma samples from HIV-infected patients living in the Netherlands and receiving care in one of the designated HIV treatment centers. Patients can opt out after being informed by their treating physician of the purpose of collection of data and samples. Data from patients who opt out are not included in the ATHENA database. Pseudonymised data may be used for scientific purposes without further review. Patients are informed that in case of future requests for use of stored plasma samples for scientific research they will be asked for prior consent by their treating HIV physician. Data are pseudonymised before being provided to investigators. For the purpose of our analysis only existing data have been used and therefore no additional review or consent has been necessary.

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CLINICAL CENTERS

** denotes site coordinating physician*

Academic Medical Center of the University of Amsterdam: *HIV treating physicians:* J.M. Prins*, T.W. Kuijpers, H.J. Scherpbier, J.T.M. van der Meer, F.W.M.N. Wit, M.H. Godfried, P. Reiss, T. van der Poll, F.J.B. Nellen, S.E. Geerlings, M. van Vugt, D. Pajkrt, W.J. Wiersinga, M. van der Valk, A. Goorhuis, J.W. Hovius. *HIV nurse consultants:* M.A.H. Bijsterveld, J. van Eden, A.M.H. van Hes, M. Mutschelknauss, H.E. Nobel, F.J.J. Pijnappel, A.M. Weijsenfeld. *HIV clinical virologists/chemists:* S. Jurriaans, N.K.T. Back, H.L. Zaaijer, B. Berkhout, M.T.E. Cornelissen, C.J. Schinkel, X.V. Thomas. **Admiraal De Ruyter Ziekenhuis, Goes:** *HIV treating physicians:* M. van den Berge, A. Stegeman. *HIV nurse consultants:* S. Baas, L. Hage de Looff. *HIV clinical virologists/chemists:* B Wintermans, J Veenemans. **Catharina Ziekenhuis, Eindhoven:** *HIV treating physicians:* M.J.H. Pronk*, H.S.M. Ammerlaan. *HIV nurse consultants:* E.S. de Munnik, E. van Beek. *HIV clinical virologists/chemists:* A.R. Jansz, J. Tjhie, M.C.A. Wegdam, B. Deiman, V. Scharnhorst. **Elisabeth-TweeSteden Ziekenhuis, Tilburg:** *HIV treating physicians:* M.E.E. van Kasteren*, A.E. Brouwer. *HIV nurse consultants:* R. van Erve, B.A.F.M. de Kruijf-van de Wiel, S.Keelan-Pfaf, B. van der Ven.

Data collection: B.A.F.M. de Kruijf-van de Wiel, B. van der Ven. *HIV clinical virologists/chemists:* A.G.M. Buiting, P.J. Kabel, D.Versteeg. **Emma Kinderziekenhuis, Amsterdam:** *HIV nurse consultants:* A. van der Plas, A.M. Weijsenfeld. **Erasmus MC, Rotterdam:** *HIV treating physicians:* M.E. van der Ende*, H.I. Bax, E.C.M. van Gorp, J.L. Nouwen, B.J.A. Rijnders, C.A.M. Schurink, A. Verbon, T.E.M.S. de Vries-Sluijs. *HIV nurse consultants:* N. Bassant, J.E.A. van Beek, M. Vriesde, L.M. van Zonneveld. *Data collection:* H.J. van den Berg-Cameron, F.B. Bruinsma-Broekman, J. de Groot, M. de Zeeuw-de Man. *HIV clinical virologists/chemists:* C.A.B. Boucher, M.P.G Koopmans, J.J.A van Kampen, S.D. Pas. **Erasmus MC-Sophia, Rotterdam:** *HIV treating physicians:* G.J.A. Driessen, A.M.C. van Rossum. *HIV nurse consultants:* L.C. van der Knaap, E. Visser. **Flevoziekenhuis, Almere:** *HIV treating physicians:* J. Branger*, A. Rijkeboer-Mes. *HIV nurse consultant and data collection:* C.J.H.M. Duijf-van de Ven. **HagaZiekenhuis, Den Haag:** *HIV treating physicians:* E.F. Schippers*, C. van Nieuwkoop. *HIV nurse consultants:* J.M. van IJperen, J. Geilings. *Data collection:* G. van der Hut. *HIV clinical virologist/chemist:* P.F.H. Franck. **HIV Focus Centrum (DC Klinieken), Amsterdam:** *HIV treating physicians:* A. van Eeden*. *HIV nurse consultants:* W. Brokking, M. Groot, L.J.M. Elsenburg. *HIV clinical virologists/chemists:* M. Damen, I.S. Kwa. **Isala, Zwolle:** *HIV treating physicians:* P.H.P. Groeneveld*, J.W. Bouwhuis. *HIV nurse consultants:* J.F. van den Berg, A.G.W. van Hulzen. *Data collection:* G.L. van der Bliek, P.C.J. Bor. *HIV clinical virologists/chemists:* P. Bloembergen, M.J.H.M. Wolfhagen, G.J.H.M. Ruijs. **Leids Universitair Medisch Centrum, Leiden:** *HIV treating physicians:* F.P. Kroon*, M.G.J. de Boer, H. Jolink, A.M. Vollaard. *HIV nurse consultants:* W. Dorama, N. van Holten. *HIV clinical virologists/chemists:* E.C.J. Claas, E. Wessels. **Maasstad Ziekenhuis, Rotterdam:** *HIV treating physicians:* J.G. den Hollander*, K. Pogany, A. Roukens. *HIV nurse consultants:* M. Kastelijns, J.V. Smit, E. Smit, D. Struik-Kalkman, C. Tearno. *Data collection:* M. Bezemer, T. van Niekerk. *HIV clinical virologists/chemists:* O. Pontesilli. **Maastricht UMC+, Maastricht:** *HIV treating physicians:* S.H. Lowe*, A.M.L. Oude Lashof, D. Posthouwer. *HIV nurse consultants:* R.P. Ackens, J. Schippers, R. Vergoossen. *Data collection:* B. Weijenberg-Maes. *HIV clinical virologists/chemists:* I.H.M. van Loo, T.R.A. Havenith. **MCH-Bronovo, Den Haag:** *HIV treating physicians:* E.M.S. Leyten*, L.B.S. Gelinck. *HIV nurse consultants:* A.Y. van Hartingsveld, C. Meerkerk, G.S. Wildenbeest. *HIV clinical virologists/chemists:* J.A.E.M. Mutsaers, S.Q. van Veen. **MC Slotervaart, Amsterdam:** *HIV treating physicians:* J.W. Mulder*, S.M.E. Vrouenraets, F.N. Lauw. *HIV nurse consultants:* M.C. van Broekhuizen, H. Paap, D.J. Vlasblom. *HIV clinical virologists/chemists:* P.H.M. Smits. **MC Zuiderzee, Lelystad:** *HIV treating physicians:* S. Weijer*, R. El Moussaoui. *HIV nurse consultant:* A.S. Bosma. **Medisch Centrum Leeuwarden, Leeuwarden:** *HIV treating physicians:* M.G.A.van Vonderen*, D.P.F. van Houte, L.M. Kampschreur. *HIV nurse consultants:* K. Dijkstra, S. Faber. *HIV clinical virologists/chemists:* J Weel. **Medisch Spectrum Twente, Enschede:** *HIV treating physicians:* G.J. Kootstra*, C.E. Delsing. *HIV nurse consultants:* M. van der Burg-van de Plas, H. Heins. *Data collection:* E. Lucas. **Noordwest**

Ziekenhuisgroep, Alkmaar: *HIV treating physicians:* W. Kortmann*, G. van Twillert*, J.W.T. Cohen Stuart, B.M.W. Diederen, R. Renckens. *HIV nurse consultant and data collection:* D. Ruiter-Pronk, F.A. van Truijen-Oud. *HIV clinical virologists/chemists:* W. A. van der Reijden, R. Jansen. **OLVG, Amsterdam:** *HIV treating physicians:* K. Brinkman*, G.E.L. van den Berk, W.L. Blok, P.H.J. Frissen, K.D. Lettinga W.E.M. Schouten, J. Veenstra. *HIV nurse consultants:* C.J. Brouwer, G.F. Geerders, K. Hoeksema, M.J. Kleene, I.B. van der Meché, M. Spelbrink, H. Sulman, A.J.M. Toonen, S. Wijnands. *HIV clinical virologists:* M. Damen, D. Kwa. *Data collection:* E. Witte. **Radboudumc, Nijmegen:** *HIV treating physicians:* R. van Crevel*, M. Keuter, A.J.A.M. van der Ven, H.J.M. ter Hofstede, A.S.M. Dofferhoff. *HIV nurse consultants:* M. Albers, K.J.T. Grintjes-Huisman, M. Marneef, A. Hairwassers. *HIV clinical virologists/chemists:* J. Rahamat-Langendoen. *HIV clinical pharmacology consultant:* D. Burger. **Rijnstate, Arnhem:** *HIV treating physicians:* E.H. Gisolf*, R.J. Hassing, M. Claassen. *HIV nurse consultants:* G. ter Beest, P.H.M. van Bentum, N. Langebeek. *HIV clinical virologists/chemists:* R. Tiemessen, C.M.A. Swanink. **Spaarne Gasthuis, Haarlem:** *HIV treating physicians:* S.F.L. van Lelyveld*, R. Soetekouw. *HIV nurse consultants:* L.M.M. van der Prijt, J. van der Swaluw. *Data collection:* N. Bermon. *HIV clinical virologists/chemists:* W.A. van der Reijden, R. Jansen, B.L. Herpers, D.Veenendaal. **Medisch Centrum Jan van Goyen, Amsterdam:** *HIV treating physicians:* D.W.M. Verhagen. *HIV nurse consultants:* M. van Wijk. **Universitair Medisch Centrum Groningen, Groningen:** *HIV treating physicians:* W.F.W. Bierman*, M. Bakker, J. Kleinnijenhuis, E. Kloeze, H. Scholvinck, Y. Stienstra, C.L. Vermont, K.R. Wilting. *HIV nurse consultants:* A. Boonstra, H. de Groot-de Jonge, P.A. van der Meulen, D.A. de Weerd. *HIV clinical virologists/chemists:* H.G.M. Niesters, C.C. van Leer-Buter, M. Knoester. **Universitair Medisch Centrum Utrecht, Utrecht:** *HIV treating physicians:* A.I.M. Hoepelman*, J.E. Arends, R.E. Barth, A.H.W. Bruns, P.M. Ellerbroek, T. Mudrikova, J.J. Oosterheert, E.M. Schadd, M.W.M. Wassenberg, M.A.D. van Zoelen. *HIV nurse consultants:* K. Aarsman, D.H.M. van Elst-Laurijssen, E.E.B. van Oers-Hazelzet. *Data collection:* M. van Berkel. *HIV clinical virologists/chemists:* R. Schuurman, F. Verduyn-Lunel, A.M.J. Wensing. **VUmc, Amsterdam:** *HIV treating physicians:* E.J.G. Peters*, M.A. van Agtmael, M. Bomers, J. de Vocht. *HIV nurse consultants:* M. Heitmuller, L.M. Laan. *HIV clinical virologists/chemists:* C.W. Ang, R. van Houdt, A.M. Pettersson, C.M.J.E. Vandenbroucke-Grauls. **Wilhelmina Kinderziekenhuis, UMCU, Utrecht:** *HIV treating physicians:* S.P.M. Geelen, T.F.W. Wolfs, L.J. Bont. *HIV nurse consultants:* N. Nauta.

COORDINATING CENTER

Director: P. Reiss. *Data analysis:* D.O. Bezemer, A.I. van Sighem, C. Smit, F.W.M.N. Wit, T.S. Boender. *Data management and quality control:* S. Zaheri, M. Hillebregt, A. de Jong. *Data monitoring:* D. Bergsma, A. de Lang, S. Grivell, A. Jansen, M.J. Rademaker, M. Raethke, R. Meijering, S. Schnörr. *Data collection:* L. de Groot, M. van den Akker, Y. Bakker, E. Claessen,

A. El Berkaoui, J. Koops, E. Kruijne, C. Lodewijk, L. Munjishvili, B. Peeck, C. Ree, R. Regtop, Y. Ruijs, T. Rutkens, L. van de Sande, M. Schoorl, A. Timmerman, E. Tuijn, L. Veenenberg, S. van der Vliet, A. Wisse, T. Woudstra. *Patient registration:* B. Tuk.

CONFLICTS OF INTEREST

EJS, CS, CTMMK and ASMD declare that they have no conflicts of interest that are directly relevant to the content of this manuscript. JEA joins advisory boards of Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, ViiV Healthcare and Merck. He received sponsorship of Bristol-Myers Squibb, ViiV Healthcare, Abbvie, and Merck. KB joins advisory board of Gilead Sciences, Bristol-Myers Squibb, Janssen, Abbvie, ViiV Healthcare, and Roche. BR received research grants from MSD and Gilead Sciences, travel grants from ViiV Healthcare, MSD, Bristol-Myers Squibb, Gilead Sciences, and Janssen-Cilag. He received speakers fee from Bristol-Myers Squibb, Gilead Sciences, and Janssen-Cilag and personal fees from Bristol-Myers Squibb, Gilead Sciences, and Janssen-Cilag. MV joins advisory boards of Abbvie, Bristol-Myers Squibb, Gilead, Janssen, ViiV Healthcare and Merck. He received sponsorship and research grants of Gilead, Janssen and Merck. PR through his institution received independent scientific grant support, unrelated to the content of this manuscript, from Gilead Sciences Sciences, Janssen Pharmaceuticals Inc, Merck & Co, Bristol-Myers Squibb and ViiV Healthcare; he has served on a scientific advisory board for Gilead Sciences and a data safety monitoring committee for Janssen Pharmaceuticals Inc; he chaired a scientific symposium by ViiV Healthcare, for which his institution has received remuneration. DMB joins advisory boards of Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, ViiV Healthcare and Merck. He received sponsorship and research grants of Bristol-Myers Squibb, Janssen Pharmaceuticals, ViiV Healthcare and Merck.

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Supplementary table 1: Creatinine levels for patients that were treated with ledipasvir, tenofovir disoproxil fumarate, and a protease inhibitor and HIV RNA levels for patients that switched cART.

	Ledipasvir with TDF and boosted PI (n = 13)		**Ledipasvir without TDF and boosted PI (n = 198[a])**	
	Before	During	Before	During
Creatinine µmol/L, median (IQR)	82 (12)	85 (28)	90 (23)	93 (24)
	Switch cART (n = 166)		**No switch cART (n = 257)**	
	Before	During	Before	During
HIV RNA undetectable	159	152	246	227
HIV RNA detectable	7	6[b]	11	7[c]

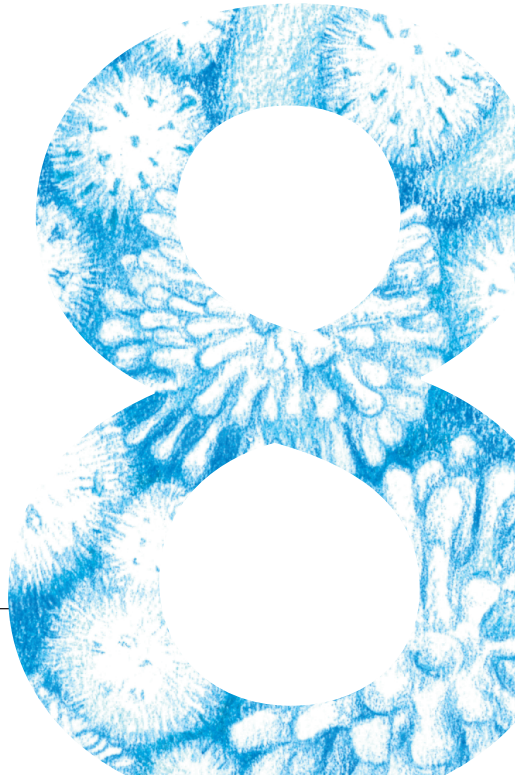
[a]Creatinine levels were missing for 2 patients.
[b]Follow up HIV RNA was missing for 8 patients.
[c]Follow up HIV RNA was missing for 23 patients.
TDF: Tenofovir disoproxil fumarate; PI: Protease inhibitor; cART: combination antiretroviral therapy.

Part 2

Ribavirin pharmacokinetics and therapeutic drug monitoring

Measuring plasma concentrations of ribavirin: first report from a quality control program

EJ Smolders
R Kan
CTMM de Kanter
M van Luin
RE Aarnoutse
DJ Touw
DM Burger



Ther Drug Monit. 2016 Oct; 38 (5): 646-647

To the editor,

A chronic hepatitis C virus (HCV) infection is nowadays treated with combination therapy of novel direct-acting antivirals. These drugs are very effective, and high sustained virological response rates are achieved in patients without cirrhosis (>90%)[1]. Cirrhotic patients are more difficult-to-treat, which could be due to by physiological changes caused by scarring of the liver. Therefore, ribavirin is added to direct-acting antiviral therapy, which improves HCV treatment response and gives an opportunity to shorten treatment duration from 24 to 12 weeks[1].

Ribavirin has a strong concentration-effect relationship, and therapeutic drug monitoring (TDM) can be used to individualize the dose of ribavirin[2]. Therefore, several laboratories developed ribavirin assays. These methods are generally validated internally for validation parameters such as accuracy, precision, selectivity, sensitivity, reproducibility, and stability according to international guidelines[3, 4]. To ensure the accuracy of these bioanalytical methods and to alert laboratories to previously undetected problems, we developed an international external quality control (QC) or proficiency testing (PT) program for measurement of ribavirin.

The aim of this report was to describe the results of the first year of this ribavirin PT program.

In 2015, we dispatched two samples per round (two rounds in total) to the participating laboratories. For these samples, bovine serum was spiked with low and high concentrations of ribavirin. These samples were freeze dried and were shipped by regular mail. For two participants, the samples were shipped on dry ice because they participated in another program requiring shipment on dry ice. The concentrations chosen resembled the range of concentrations measured in patients treated with a normal dose of ribavirin (patient weight <75 kg = 1,000 mg/day and ≥75 kg = 1,200 mg/day)[5]. The target range for ribavirin plasma concentrations at steady-state is 2.2-3.6 mg/L[2].

Details of similar programs have been described previously[6-8]. Participants were informed about their performance in measuring ribavirin concentrations. Accuracy was considered to be acceptable if measurements were within the 80-120% limits of the spiked (weighed-in) 'expert' concentrations. This 20% threshold was based on guidelines for method validation for bioanalysis of drugs used as fixed criterion for inaccuracy at the lowest level of quantification and on maximum allowable error specifications for drug measurements according to the US Clinical Laboratory Improvement Amendments (CLIA) of 1988[9-11].

Eight laboratories participated in the program, of which two participants completed one round. Most participants (n = 7) used liquid chromatography with mass spectrometry detection to determine ribavirin concentrations. One center used high performance liquid chromatography with an ultraviolet detector.

In round 1, 81% of the samples (i.e., 13 out of 16 samples) were determined accurately, and the variation in accuracy of samples with low concentrations was 86-336%. The samples spiked with high ribavirin concentrations varied from 55 to 160% inaccuracy (Table 1, Figure 1).

Table 1: Overall performance of the 8 laboratories.

Round, Concentration	# samples	Expert concentration, (mg/L)	Measured concentration, median mg/L (range mg/L)	Median inaccuracy, median % (range %)[a]	Number and percentage of measurements with acceptable accuracy, n (%)
1, High	8	3.64	3.66 (2.00-5.84)	8.2 (0.8-60.4)	6/8 (75)
2, High	6	2.18	2.25 (2.12-3.22)	4.1 (0.9-47.7)	4/6 (67)
1, Low	8	0.82	0.80 (0.70-2.74)	6.4 (1.8-236.2)	7/8 (88)
2, Low	6	0.51	0.55 (0.49-1.54)	7.5 (1.6-203.2)	5/6 (83)

[a]Inaccuracy is percentage bias from the true concentration.
Measured/expert concentration >100%: inaccuracy = (100 x measured concentration / true concentration) – 100%.
Measured/expert concentration <100%: inaccuracy = 100% - (100 x measured concentration / true concentration).

In round 2, a total of 75% samples (i.e., 9 out of 12) were determined accurately within 80-120% of the weighed-in concentrations. Accuracy for samples with low and high concentrations varied from 97 to 303% and from 97 to 148%, respectively.

The median inaccuracy for all measurements was 6.1% (range: 0.8-236.2%). It was 6.7% (range: 1.6-236.2%) and 6.1% (range: 0.8-60.4%) for low and high ribavirin concentrations, respectively.

Of the eight participating laboratories, five had all samples analyzed within 80-120% limits of the weighed-in concentrations, three reported at least one inaccurate result, of which one participant reported all four samples inaccurately (>120%).

The aim of a PT program is to provide external validation of bioanalytical assays to assure and improve quality. Participating laboratories were informed about their performance and their ability to measure the correct analyte concentrations. This may help them to improve their laboratory performance. The analysis of this small sample set showed that 6/28 of the ribavirin samples were measured inaccurately, which was in line with

first rounds of similar QC programs[6-8]. The laboratories with a poor performance should improve their analyses. In the specific case of the laboratory that inaccurately reported all four concentrations, intra laboratory validation was possibly incorrect, or other errors might have been involved, such as methodological, clerical, or technical errors.

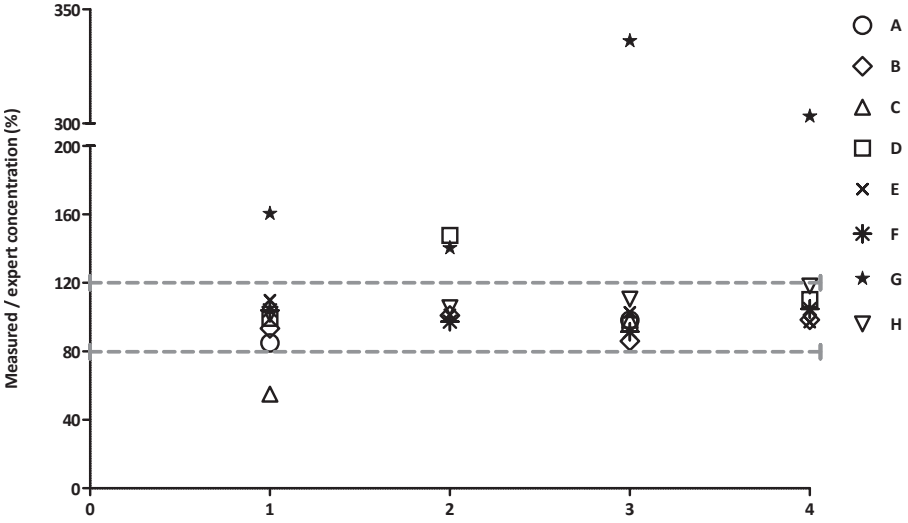


Figure 1: Measured concentration relative to expert value, 80-120% ranges.
Each symbol represents a participating laboratory (A-H) and each point represents a single measured ribavirin concentration, shown as percentage of the ribavirin expert (weighed-in) concentration. Accuracy was acceptable if measurements fell within 80-120% limits of the expert (weighed-in) concentrations (red lines).
1 = round 1, high spiked concentration.
2 = round 2, high spiked concentration.
3 = round 1, low spiked concentration.
4 = round 2, low spiked concentration.

The impact of reporting inaccurate high ribavirin plasma concentrations is that dosages might be reduced unnecessarily, possibly leading to subtherapeutic ribavirin concentrations and potentially causing virologic failure. For concentrations reported with an accuracy <80%, this might lead to increased dosages, causing unnecessary adverse advents such as anemia.

Ribavirin is important for HCV treatment in cirrhotic patients. To ensure safety and efficacy, TDM may be used in daily practice. TDM is especially relevant in specific patient populations such as patients with renal failure or patients receiving hemodialysis. Thus, for treatment of HCV patients it is critical that laboratories are able to measure the correct plasma concentrations, which is ensured by our external quality control program. Laboratories measuring ribavirin are encouraged to participate in our PT program (http://www.kkgt.nl).

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CONFLICTS OF INTEREST

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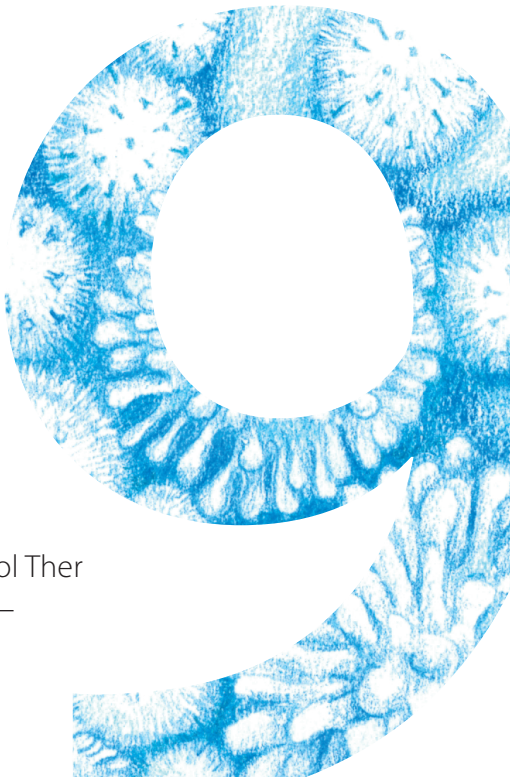
Ribavirin steady-state plasma level is a predictor of sustained virological response in hepatitis C infected patients treated with direct-acting antivirals

M van Tilborg[±]
FI Lieveld[±]
EJ Smolders
KJ van Erpecum
CTMM de Kanter
R Maan
M van der Valk
JE Arends
ASM Dofferhoff
H Blokzijl
M Bijmolen
JPH Drenth
RJ de Knegt
DM Burger

on behalf of the HepNed study group
± Authors share first co-authorship



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ABSTRACT

Background

In the era of highly effective direct-acting antivirals (DAAs) for treatment of patients with chronic hepatitis C virus (HCV) infection, ribavirin is still considered beneficial in certain patients.

Aim

To assess the association between ribavirin steady-state plasma levels and sustained virological response (SVR).

Methods

Consecutive HCV-infected patients treated with DAAs plus ribavirin from four Dutch academic medical centers were enrolled. Ribavirin steady-state plasma levels were prospectively measured at treatment week 8 using validated assays. Logistic regression analyses were performed to assess influence of ribavirin steady-state plasma level on SVR, and ribavirin therapeutic range was explored using Area Under the ROC curve analyses.

Results

A total of 183 patients were included, of whom 85% had one or more difficult-to-cure characteristics (i.e. treatment-experienced, HCV genotype 3, cirrhosis). The majority was treated with a sofosbuvir-based regimen and 163 (89%) patients achieved SVR. Median ribavirin dose was 12.9 (interquartile range 11.2-14.7) mg/kg/day and median ribavirin steady-state plasma level was 2.66 (1.95-3.60) mg/L. In multivariable analyses, higher ribavirin steady-state plasma level (adjusted Odds Ratio 1.79 [95% CI 1.09-2.93]) was an independent predictor of SVR. With regard to the optimal ribavirin therapeutic range, 2.28 mg/L was the optimal lower cut-off for achieving SVR and 3.61 mg/L was the upper cut-off for preventing significant anemia (hemoglobin <10 g/dL).

Conclusion

In this cohort of mainly difficult-to-cure patients treated with DAAs plus ribavirin, higher ribavirin steady-state plasma level was an independent predictor of SVR.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major global health problem with an estimated 130-150 million infected individuals worldwide[1]. Until 2013, peg-interferon alfa (Peg-IFN) based treatment was the standard of care for HCV infection. Ribavirin, a guanosine analogue, was considered an important additive to increase the efficacy of Peg-IFN. Studies performed with Peg-IFN and ribavirin found a clear association between virological relapse and ribavirin dose reduction or discontinuation[2, 3]. The introduction of direct-acting antivirals (DAAs) has changed the HCV therapeutic landscape completely, and currently, in more than 90% of patients, viral clearance is achieved with these highly effective agents[4-7].

Despite this development, and in contrast to Peg-IFN, ribavirin has not left the therapeutic arena and is still used as an additive under certain circumstances such as presence of cirrhosis, genotype 3 infection, presence of resistance-associated substitutions, previous antiviral treatment failure, and/or in specific DAA regimens to increase treatment efficacy[8]. Unfortunately, ribavirin is also associated with serious side effects, of which the most concerning is hemolytic anemia[9]. Moreover, a high ribavirin plasma level is known to be associated with anemia[10]. Despite weight-based dosing of ribavirin, the measured plasma levels vary widely between individuals[11], whereas variability within an individual is minimal[12].

Studies investigating the association between ribavirin steady-state plasma level and sustained virological response (SVR) in HCV mono- and HIV/HCV co-infected patients show contradictory results, possibly due to their small sample sizes and retrospective nature[13-16]. Moreover, studies on ribavirin steady-state plasma levels in DAA-treated patients are scarce. Therefore, in a large cohort of HCV-infected patients treated with DAAs in combination with ribavirin, we assessed if there is an association between ribavirin steady-state plasma levels and SVR.

METHODS

Study design and participants

This prospective cohort study was conducted in four Dutch academic medical centers (Erasmus MC University Medical Center Rotterdam, University Medical Center Utrecht, Radboud university medical center, and University Medical Center Groningen). Consecutive adult patients with chronic HCV infection (i.e. positive HCV RNA >6 months), in whom interferon-free treatment with DAAs (except telaprevir and boceprevir) and ribavirin was initiated from January 2015 until May 2016, were included.

Selection of DAA regimen and treatment duration were at the discretion of the physician and according to international and national guidelines[17-19]. Ribavirin dosages were weight-based (<75 kg: 1,000 mg/day and ≥75 kg 1,200 mg/day) and administered twice daily with meals. There were no strict rules of action following therapeutic drug monitoring at week 8 and ribavirin dose adjustment or discontinuation was at the decision of the physician. Ribavirin plasma samples were obtained and analyzed at treatment week 8 or later. Clinical and demographic data were prospectively collected. Furthermore, patient-reported adherence and adverse events (AEs) were collected from the electronic patient file.

The study protocol was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the local ethics review boards.

Definitions

SVR was defined as the absence of HCV RNA ≥12 weeks after completing antiviral treatment. Ribavirin steady-state plasma level was defined as ribavirin plasma level ≥ treatment week 8. The lower cut-off for the ribavirin therapeutic range was determined by the optimal value for predicting SVR, and the upper cut-off was the optimal value for preventing significant anemia (i.e. plasma hemoglobin <10 g/dL)[20]. The presence of either cirrhosis, being treatment-experienced (all genotypes), and/or having HCV genotype 3 infection were considered difficult-to-cure characteristics. Cirrhosis was assessed by liver histology (F4 according to METAVIR) or transient elastography (Fibroscan®; cut-off value ≥12.5 kPa[21]). A Child-Pugh score ≥7 was considered decompensated cirrhosis. Estimated glomerular filtration rate (eGFR) was based on the Modification of Diet in Renal Disease equation: 186 x (serum creatinine [µmol/L] / 88.4) – 1.154 x age (years) – 0.203 (x 0.742 if female). Renal function was classified according to international guidelines: eGFR >90 normal, 50–89 mild to moderate, and <50 mL/min/1.73 m^2 moderate to severe renal impairment[22].

Measurement of ribavirin

Plasma ribavirin levels were measured by the pharmacy laboratories of the four participating centers. While four different analytical methods were used, all were validated using international standards[23, 24]. Moreover, all four centers participate in the Dutch external quality control proficiency testing program for measurement of ribavirin, to ensure accuracy and comparability of these bioanalytical methods[25, 26]. All assays were able to measure ribavirin steady-state plasma levels within a 90-110% limit of the spiked (weighed-in) 'expert' concentration. This is within the 20% threshold that is frequently used to describe accuracy in international external quality control or proficiency testing programs[27, 28]. Used analytical methods were: 1) high pressure liquid chromatography

with ultraviolet detection (detection limit: 0.03-12.0 mg/L)[23]; 2) liquid chromatography (LC) with tandem mass spectrometry detection (MS/MS) (detection limit: 0.2-30 mg/L) [24]; 3) LC-MS/MS (detection limit: 0.1-10 mg/L), and 4) LC-MS/MS (detection limit: 0.5-7.5 mg/L)[23].

Statistical analysis

Differences in characteristics were described with the chi-square test or Fisher's exact test for qualitative data and the Mann–Whitney *U* test or Kruskal Wallis test for quantitative data. Correlations were assessed by Spearman's rank correlation coefficient. Predictors of SVR were analyzed using binary univariable logistic regression analyses, with subsequent multivariable logistic regression analyses for all covariates with a p-value <0.10. Multicollinearity analyses with all covariates were performed to determine whether confounding was present. Included covariates were: ribavirin steady-state plasma level, age, gender, ethnic descent, medical center, baseline creatinine, treatment experience, cirrhosis, average ribavirin dose, hepatitis B and HIV co-infection. Receiver Operating Characteristic (ROC) plots were created to determine optimal cut-off values of ribavirin steady-state plasma level, for prediction of SVR and significant anemia. One-way ANOVA analyses were done to compare geometric means of ribavirin plasma levels between eGFR groups. Finally, the coefficient of variance was calculated for ribavirin steady-state plasma level. A two-sided p-value <0.05 was considered statistically significant. SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), SPSS version 24 (SPSS Inc., Chicago, IL, USA), and GraphPad Prism version 7.02 (GraphPad Software, La Jolla, CA, USA) were used for statistical analyses.

RESULTS

Study population characteristics

From January 2015 until May 2016, a total of 185 chronic HCV-infected patients started interferon-free treatment with a combination of DAAs and ribavirin. In two patients, SVR could not be determined due to lost to follow-up and they were therefore excluded (Figure 1). The characteristics of the 183 included patients are given in Table 1. Median age in the study population was 57 (interquartile range [IQR] 52-64) years, 79% was male, and the majority was of Caucasian (74%) or North African/Middle Eastern (12%) descent (Table 1). The majority of patients had cirrhosis (60%), 6% of these patients had decompensated cirrhosis (all Child-Pugh B), and 24 (13%) were liver transplant recipients. HCV genotypes 1a (38%), 1b (21%), and 3 (23%), were the most prevalent, and median baseline HCV RNA was 6.20 (5.82-6.54) $\log_{10}$ IU/mL.

Table 1: Baseline characteristics of the study population according to treatment response.

Number	Non-SVR (n = 20)	SVR (n = 163)	p-value
Age (years)	56 (53-59)	57 (52-64)	0.38
Male gender	17 (85%)	128 (79%)	0.50
Body Mass Index (kg/m^2)	28 (22-32)	26 (23-29)	0.52
Ethnic descent			
Caucasian	14 (70%)	120 (74%)	0.90
Asian	0	4 (3%)	0.48
Sub-Sahara African	0	2 (1%)	0.62
North African/Middle Eastern	2 (10%)	20 (12%)	0.77
Other	4 (20%)	17 (10%)	0.54
HCV genotype			
1a	5 (25%)	65 (40%)	0.20
1b	1 (5%)	38 (23%)	0.06
2	1 (5%)	10 (6%)	0.84
3	12 (60%)	30 (18%)	<0.001
4	1 (5%)	19 (12%)	0.37
6	0	1 (1%)	0.73
HCV RNA (log_{10} IU/mL)	6.17 (5.8-6.7)	6.2 (5.8-6.5)	0.89
Treatment-experienced	13 (65%)	96 (60%)	0.62
PR	12 (92%)	76 (79%)	0.26
PR + 1st generation NS3/4A PI	1 (8%)	8 (5%)	0.99
Other	0	12 (13%)	0.21
Cirrhosis	16 (80%)	95 (58%)	0.06
Decompensated cirrhosis[a]	3 (15%)	8 (5%)	0.07
Liver transplantation	1 (5%)	23 (14%)	0.26
Liver biochemistry			
Total bilirubin (µmol/L)	17 (14-23)	12 (9-17)	0.01
ALT (U/L)	89 (42-157)	75 (45-130)	0.81
Albumin (g/L)	39 (30-42)	41 (35-45)	0.17
Platelet count (x10/L)	104 (75-213)	169 (111-203)	0.14
Creatinine (µmol/L)	69 (60-85)	70 (59-83)	0.84
HIV co-infection	2 (10%)	8 (5%)	0.34
HBV co-infection	1 (5%)	3 (2%)	0.36
DAA regimen[b]			
DCV/SOF	4 (2%)	74 (40%)	0.03
SIM/SOF	4 (2%)	38 (21%)	0.74
LDV/SOF	1 (0.5%)	24 (13%)	0.23
PTV/r/OBV	0	3 (2%)	0.54
PTV/r/OBV/DSV	0	5 (3%)	0.43
SOF	11 (6%)	19 (10%)	<0.001

Data are expressed as median (IQR) or n (%).

[a]Defined as Child-Pugh score ≥7.

[b]All regimens include weight-based ribavirin.

HCV; Hepatitis C virus; PR: Peg-interferon/ribavirin; PI: Protease inhibitor; ALT; Alanine aminotransferase; HIV; Human immunodeficiency virus; HBV: Hepatitis B virus; DAA: Direct-acting antiviral; SOF: Sofosbuvir; DCV: Daclatasvir; SIM: Simeprevir; LDV: Ledipasvir; OBV: Ombitasvir; PTV/r: Paritaprevir/ritonavir; DSV: Dasabuvir.

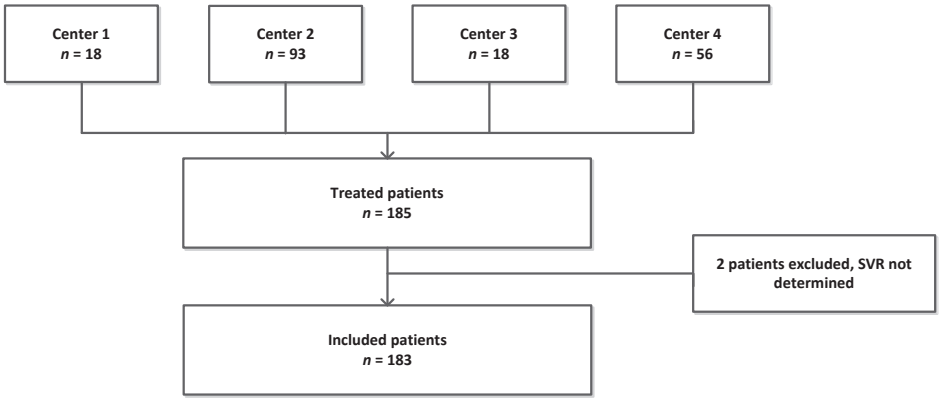

Figure 1: Flowchart of the study population consisting of hepatitis C patients treated with direct-acting antivirals plus ribavirin in four tertiary centers in the Netherlands.
SVR: Sustained virological response.

Treatment characteristics

Distribution of applied DAA regimens is given in Table 2. The majority of patients had either one (34%) or more (51%) difficult-to-cure characteristics, with only 15% having none of these characteristics (Table 2). In 157 (86%) patients, adherence to ribavirin was reported. Median patient-reported adherence was 100% (range 75-100%), with only one patient reporting adherence <80%. Median ribavirin start dose was 13.6 (IQR 12.1-15.2) mg/kg/day, 13.1 (11.6-14.7) mg/kg/day at week 8, and 13.0 (11.2-14.7) mg/kg/day at end-of-treatment, as a result of ribavirin dose reductions and/or discontinuation. In 50 (27%) patients, ribavirin dose was adjusted, and in 16 (9%), ribavirin was discontinued. Main reasons for dose reduction were anemia (50%), other AEs (16%), or therapeutic drug monitoring (32%).

Regarding ribavirin start dose and dose reductions/discontinuations there was no statistically significant difference between non-cirrhotic patients and patients with compensated or decompensated cirrhosis ($p = 0.50$; $p = 0.29$; $p = 0.53$, respectively) (Supplementary table 1).

A total of 32 patients had anemia (hemoglobin <10 g/dL) of which 9 had a hemoglobin <8 g/dL.

Median ribavirin steady-state plasma level in the full cohort was 2.66 (IQR 1.95-3.60) mg/L, and the inter-patient coefficient of variation (CV) for ribavirin steady-state plasma level was 50.5%. There was no significant correlation between average ribavirin dose and ribavirin steady-state plasma level (R = 0.03, $p = 0.706$).

Table 2: Antiviral treatment characteristics of the study population.

Number	183
Difficult-to-cure characteristics[a]	
0	27 (15%)
1	63 (34%)
2	81 (44%)
3	12 (7%)
DAA regimen[b]	
DCV/SOF	78 (43%)
SIM/SOF	42 (23%)
LDV/SOF	25 (14%)
PTV/r/OBV	3 (2%)
PTV/r/OBV/DSV	5 (3%)
SOF	30 (16%)
RBV start dose (mg/kg/day)	13.6 (12.1-15.2)
Mean RBV dose TW8 (mg/kg/day)	13.1 (11.6-14.7)
Mean RBV dose EOT (mg/kg/day)	12.9 (11.2-14.7)
RBV steady-state plasma level (mg/L)	2.66 (1.95-3.60)
SVR	163 (89%)

Data are expressed as median (IQR) or n (%).

[a]Presence of cirrhosis, being treatment-experienced, and hepatitis C genotype 3: score from '0' to '3', where every factor is scored as 1 point, and presence of none of the factors is scored as 0 points.

[b]All regimens include weight-based ribavirin.

DAA: Direct-acting antiviral; SOF: Sofosbuvir; DCV: Daclatasvir; SIM: Simeprevir; LDV: Ledipasvir; OBV: Ombitasvir; PTV/r: Paritaprevir/ritonavir; DSV: Dasabuvir; RBV: Ribavirin; TW: Treatment week; EOT: End-of-treatment; SVR: Sustained virological response 12 weeks after treatment completion.

Factors associated with sustained virological response

In total, 163 (89%) patients achieved SVR. Patients with adherence <100% and those that experienced ribavirin dose adjustments had similar SVR-rates of 92% and 89%, respectively. In univariable logistic regression analyses, there was no association between SVR and gender, ethnic descent, medical center, cirrhosis status, mean ribavirin dose, treatment experience, serum creatinine, and hepatitis B and HIV co-infection status. On the contrary, ribavirin steady-state plasma level (unadjusted Odds Ratio (OR) 1.86 [95% confidence interval (CI) 1.14-3.04]), was significantly associated with SVR (Table 3). Moreover, in multivariable logistic regression analyses, higher ribavirin steady-state plasma level (adjusted OR 1.79 [1.09-2.93], $p = 0.021$) remained an independent predictor of SVR.

Table 3: Unadjusted and adjusted odds ratios for sustained virological response (*n* = 183).

Covariate	Unadjusted Odds Ratio (95%CI)	p-value	Adjusted Odds Ratio (95% CI)	p-value
RBV steady-state plasma level (mg/L)	1.86 (1.14-3.04)	0.013	1.79 (1.09-2.93)	0.021
Cirrhosis, vs. no	0.35 (0.11-1.09)	0.070	0.33 (0.10-1.10)	0.072
Mean RBV dose (mg/kg/day)	1.16 (0.98-1.36)	0.084	1.19 (0.99-1.42)	0.059
Male gender, vs. female	1.55 (0.43-5.59)	0.504		
Ethnic descent, vs. Caucasian		1.0		
Asian	-	-		
Sub-Sahara African	-	-		
North African/Middle Eastern	1.02 (0.32-3.26)	0.978		
Caribbean/Hispanic	-	-		
Medical center, vs. A		0.345		
B	2.13 (0.33-13.86)	0.431		
C	-	-		
D	0.65 (0.14-3.13)	0.591		
Treatment-experienced, vs. naïve	0.78 (0.30-2.07)	0.622		
Creatinine (µmol/L)	1.00 (0.99-1.01)	0.533		
HIV co-infection, vs. no	2.15 (0.42-10.93)	0.355		
HBV co-infection, vs. no	2.81 (0.28-28.35)	0.382		

[a]Adjusted for HCV genotype, DAA regimen, and cirrhosis.

[b]All regimens include ribavirin.

OR: Odds Ratio; RBV: Ribavirin; HCV: Hepatitis C virus; DAA: Direct-acting antiviral; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus.

Therapeutic range for ribavirin steady-state plasma levels

Ribavirin steady-state plasma levels were significantly higher in patients who achieved SVR (*n* = 163) than in those without SVR (*n* = 20): geometric mean 2.69 (95% CI 2.80-2.91) versus 2.02 (1.66-2.47) mg/L, *p* = 0.019 (Figure 2). The optimal ribavirin steady-state plasma level cut-off value for predicting SVR was ≥2.28 mg/L (Figure 3a: AUC 0.70, *p* = 0.004), with a sensitivity of 70% (95% CI 63-76%), a specificity of 60% (38-81%), a positive predictive value (PPV) of 93% (89-96%), and a negative predictive value (NPV) of 19% (14-26%).

The geometric mean of ribavirin steady-state plasma level was not significantly higher in the 32 patients with significant anemia than in the 151 patients without (3.04 [95% CI 2.49-3.70] versus 2.52 [2.33-2.73] mg/L, *p* = 0.061). The optimal ribavirin steady-state plasma level cut-off to prevent significant anemia was ≤3.61 mg/L (Figure 3b: AUC 0.61, *p* = 0.042), with a sensitivity of 44% (95% CI 27-62%), specificity of 81% (74-87%), PPV of 10% (23-46%), and NPV of 61% (84-90%). The ribavirin therapeutic range was, thus, defined as a ribavirin steady-state plasma level between 2.28-3.61 mg/L.

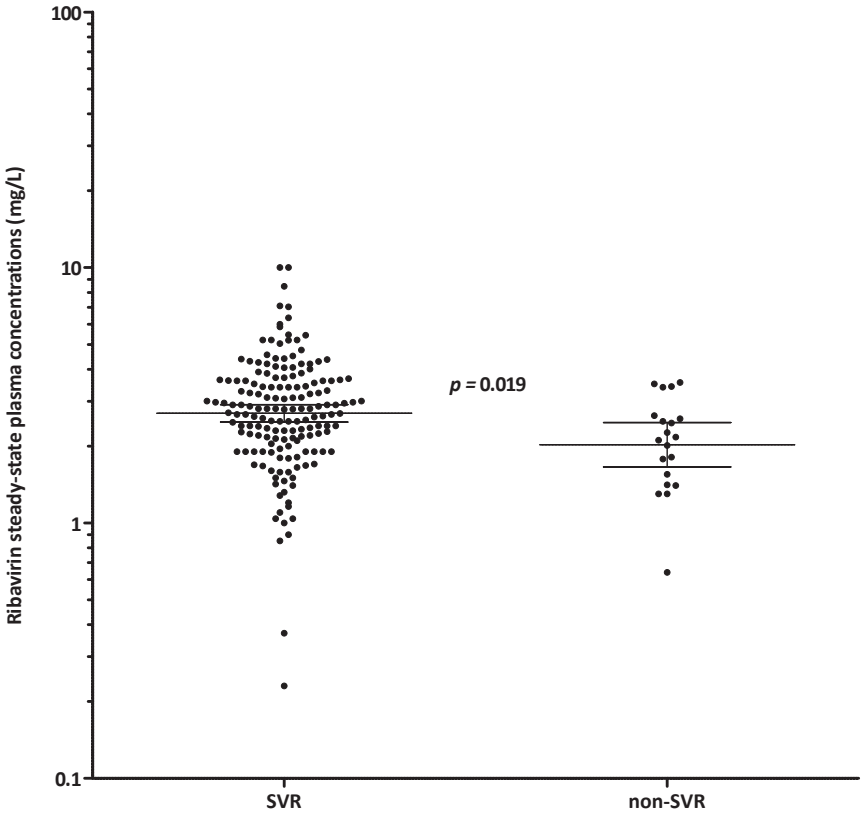

Figure 2: Ribavirin steady-state plasma levels (geometric mean and 95% confidence interval) stratified by treatment response in hepatitis C patients treated with direct-acting antivirals plus ribavirin.

RBV: Ribavirin; SVR: Sustained virological response.

A total of 119 (65%) patients had a ribavirin steady-state plasma level ≥2.28 mg/L of which 42 (35%) had a ribavirin steady-state plasma level >3.61 mg/L. This translates into 77 (42%) patients being in the therapeutic range. Of these patients, 69 (90%) achieved SVR and 9 (12%) developed significant anemia. Of the 64 patients with a ribavirin steady-state plasma level below the therapeutic range, 52 (81%) achieved SVR and 9 (14%) developed significant anemia. Lastly, in the 42 patients with a ribavirin steady-state plasma level above the therapeutic range, all (100%) achieved SVR but 14 (33%) developed significant anemia (Figure 4).

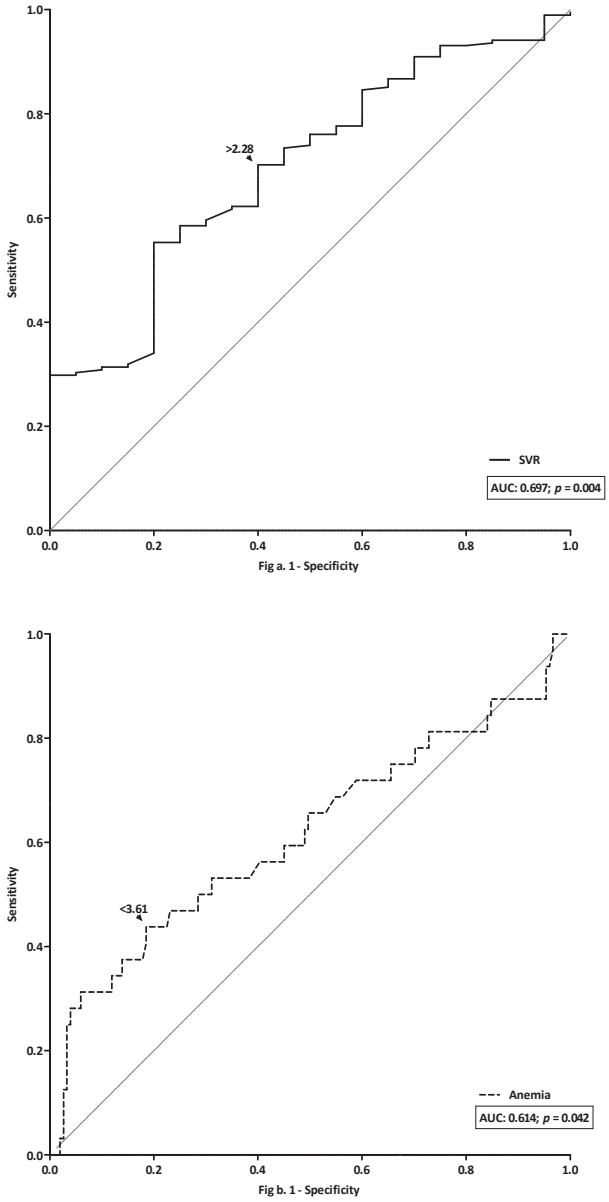

Figure 3: Receiver Operating Characteristics (ROC) curves for ribavirin steady-state plasma levels as predictors of sustained virological response (A) and significant anemia (B) in hepatitis C infected patients treated with direct-acting antivirals plus ribavirin. The arrow represents cut-off point.

Significant anemia (hemoglobin <10 g/dL)

AUC: Area under the curve.

Renal function and ribavirin levels

Baseline eGFR was available in 177 patients (97%), of whom 103 had an eGFR ≥90, 64 eGFR 50-89, 9 eGFR <50, and only one patient had an eGFR <30 mL/min/1.73m^2. Remarkably, no significant difference was seen in ribavirin start dose between the groups. At treatment week 8, patients with an eGFR <50 had significantly more ribavirin dose reductions (Table 4: 20% vs. 14% vs. 3%, p <0.0001). Nevertheless, ribavirin steady-state plasma level geometric means were 2.13, 2.95, and 3.47 mg/L for eGFR ≥90, 50-89, and <50 mL/min/1.73m^2 respectively (p <0.0001) (Figure 5). This went parallel to lower hemoglobin levels at treatment week 8, with a significant higher proportion in the eGFR <50 group that developed significant anemia (80% vs. 31% vs. 4%, p <0.0001).

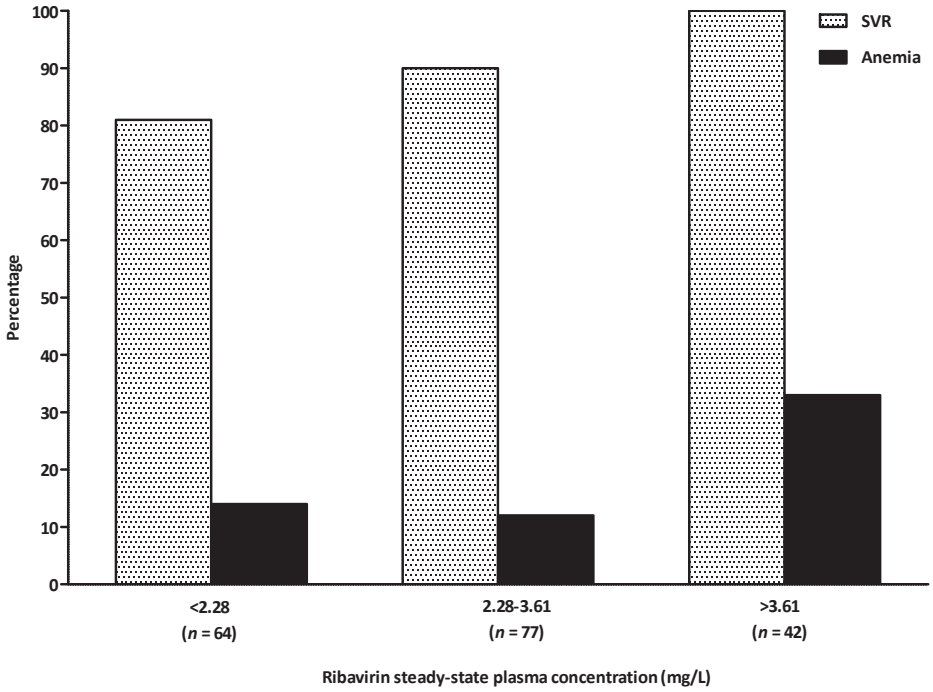

Figure 4: Bar charts showing proportions (%) of sustained virological response and anemia in hepatitis C infected patients treated with direct-acting antivirals plus ribavirin and having ribavirin steady-state plasma levels below, in, and above therapeutic range (2.28-3.61 mg/L).

RBV: Ribavirin; SVR: Sustained virological response.

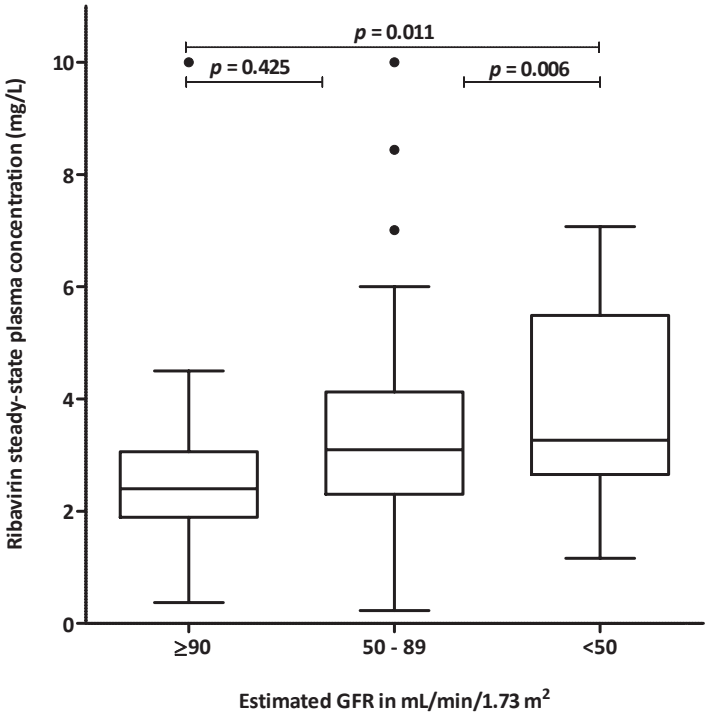

Figure 5: Boxplots showing the distribution (geometric mean and 95% confidence interval) of ribavirin steady-state plasma levels stratified by renal function in hepatitis C patients treated with direct-acting antivirals plus ribavirin.

RBV: Ribavirin; eGFR: estimated glomerular filtration rate.

Table 4: Treatment characteristics in patients stratified by renal function.

	eGFR (mL/min/1.73m^2)			
	<50 (n = 10)	**50-89** (n = 64)	**≥90** (n = 103)	**p-value**
RBV start dose (mg/kg/day)	12.3 (11.4-17.4)	13.5 (12.0-15.4)	13.6 (12.3-15.0)	0.696
RBV TW8 dose (mg/kg/day)	9.9 (8.1-12.4)	12.0 (9.0-14.0)	13.5 (12.2-14.9)	<0.0001
RBV dose reduction	2 (20%)	9 (14%)	3 (3%)	0.011
RBV steady-state plasma level (mg/L)	3.47 (2.81-5.25)	2.95 (2.33-4.07)	2.13 (1.90-3.01)	<0.0001
Baseline hemoglobin (g/dL)	12.5 (11.8-13.5)	14.3 (13.1-15.5)	15.1 (13.9-16.0)	<0.0001
TW8 hemoglobin (g/dL)	10.2 (8.5-10.5)	11.4 (10.2-12.7)	12.7 (11.8-14.2)	<0.0001
Hemoglobin <10 g/dL	8 (80%)	20 (31%)	4 (4%)	<0.0001

Data are expressed as geometric mean (IQR), median (IQR) or n (%).

eGFR: estimated glomerular filtration rate; RBV: Ribavirin; TW: Treatment week.

DISCUSSION

In this prospective study, the influence of ribavirin steady-state plasma level on achieving SVR in HCV-infected patients treated with a combination of DAAs and ribavirin was determined. The main finding of this study is that a higher ribavirin steady-state plasma level is an independent predictor of achieving SVR. In addition, the optimal ribavirin steady-state plasma therapeutic range that balances SVR with risk of anemia was found to be 2.28-3.61 mg/L.

Despite the great successes of DAAs, some patients remain difficult-to-cure[29]. Adding ribavirin to DAAs in these patients can increase SVR-rates[8, 30]. Studies in the interferon-era established that higher ribavirin steady-state plasma levels were positively correlated with SVR[12, 23, 31, 32]. We similarly found that higher ribavirin steady-state plasma levels led to a 1.8-fold increase in SVR in DAA-treated patients. This demonstrates that higher ribavirin plasma levels are effective to improve response rates, particularly in difficult-to-cure patients with a lower *a priori* chance of achieving SVR. Possibly, therapeutic drug monitoring can help achieving these high ribavirin plasma levels.

A recent study including patients treated with sofosbuvir/ribavirin found no association between ribavirin steady-state plasma levels and SVR[13]. However, this study had a small sample size of 47 treatment-naïve, predominantly genotype 1-infected, non-cirrhotic patients, and retrospectively analyzed ribavirin plasma samples. Moreover, SVR-rates were low (55%), probably because sofosbuvir/ribavirin is a suboptimal treatment for genotype 1[18, 17] and these results should, therefore, be carefully interpreted. Our study, however, represents a large real-world cohort of mainly difficult-to-cure patients on various DAA combinations with prospectively analyzed plasma samples and SVR-rates similar to clinical trials and real-world cohorts[17, 18].

In case the addition of ribavirin is considered beneficial and is administered, it is important to balance efficacy and side effects. Although side effects of ribavirin in combination with DAAs are generally considered very mild, almost one in five of our patients developed significant anemia. This study found an optimal ribavirin steady-state plasma level therapeutic range of 2.28-3.61 mg/L. Interestingly, this study as well as studies with triple-therapy including a first-generation DAA[32, 33], found somewhat higher therapeutic ranges compared with studies done with Peg-IFN/ribavirin alone[23, 34, 35]. The most likely explanation for this is that Peg-IFN contributes to anemia through bone marrow suppression and as such augments the toxicity of ribavirin at any given dose/concentration. Another explanation for this could be that with the excellent safety profile of DAAs, sicker patients than those in the Peg-IFN/ribavirin era can now be

treated[36]. A significant proportion of our patients had (decompensated) cirrhosis, were liver transplant recipients and/or had renal impairment, which could result in higher ribavirin levels. Finally, to significantly increase the likelihood of achieving SVR in the context of the already highly effective DAAs, a more pronounced increase in ribavirin exposure is necessary.

Still, the question remains if and how ribavirin therapeutic drug monitoring should be implemented in the treatment of patients without difficult-to-cure characteristics. With current DAA regimens lasting only 12 weeks[18, 17], when reaching ribavirin steady-state plasma level, only 4 weeks of treatment are left to adjust ribavirin dosage. Thus, for most patients, ribavirin exposure could perhaps best be monitored based on toxicity instead of ribavirin steady-state plasma levels. However, re-treatment of patients who fail therapy is considerably more expensive than therapeutic drug monitoring, and reaching adequate ribavirin plasma levels is not a certainty when relying on toxicity alone. Co-morbidities, hepatic and/or renal dysfunction, and ITPA gene polymorphism[37, 38] can affect ribavirin pharmacokinetics and thus ribavirin steady-state plasma levels without evident toxicity[39]. For example, if a patient has a stable hemoglobin level, it is uncertain whether ribavirin steady-state plasma level will be in or below the therapeutic range. Assessment of ribavirin plasma level at week 8 of treatment (ribavirin steady-state plasma level) may be too late for intervention. An option for therapeutic drug monitoring and intervention at an earlier stage is measuring ribavirin plasma levels at treatment week 2. Van Vlerken and colleagues reported that a week 2 ribavirin level of ≥1.29 mg/L predicted adequate ribavirin steady-state plasma levels at week 8 in patients treated with Peg-IFN/ribavirin[23]. Future studies are needed to determine if this also applies to patients treated with DAAs.

Especially in patients with impaired renal function, therapeutic drug monitoring is important. This study found that despite the Food and Drug Administration and European Medicines Agency recommending ribavirin dose reduction in patients with eGFR <50 mL/min[40, 41], they still received usual weight-based start dosages. As a result, these patients required more dose adjustments, resulting in lower average ribavirin doses, but still higher ribavirin steady-state plasma levels and high anemia rates. This study indicates that patients with a renal function <90 mL/min (especially those ≤50 mL/min) should be monitored closely during treatment, so that ribavirin can be timely adjusted when necessary.

To the best of our knowledge, this is the largest prospective study analyzing the association between ribavirin steady-state plasma level and SVR in the current DAA era. Nevertheless, some limitations are present. First, all four participating centers used

different assays to measure plasma ribavirin. However, we expect the influence of the different assays to be very limited, since all assays are validated according to international standards and have undergone strict evaluation and comparison in the Dutch quality control program. In addition, logistic regression analyses did not show a center effect. Second, due to the real-life nature of the study, ribavirin dose adjustment and selection of DAA regimen were at the discretion of the treating physician, thus leading to variation. Nonetheless, DAA regimens were selected according to international and national guidelines. In hindsight, while previously recommended by guidelines[42], sofosbuvir/ribavirin is now considered a suboptimal treatment for genotype 3 patients with cirrhosis, which has led to a high proportion of relapsers within this population. Therefore, DAA regimen and HCV genotype were excluded in our logistic regression model. However, even in sensitivity analyses where DAA regimen, HCV genotype (as a combined variable or as separate variables), or both variables were added to the model, ribavirin steady-state plasma level still remained an independent predictor of SVR. For dose adjustments and other factors that could have influenced ribavirin exposure, we tried to account by adjusting for average ribavirin dose during the entire treatment, presence of cirrhosis, and creatinine levels in our logistic regression models. Lastly, influence of ITPA gene polymorphism was not assessed.

In conclusion, a higher ribavirin steady-state plasma level is an independent predictor of SVR in patients treated with DAAs.

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CONFLICTS OF INTERESTS

MT, FIL, CTMMK, RM, MvdV, ASMD, HB, MB, JPHD declare that they do not have any conflicts of interests regarding this manuscript. DMB has served as an advisory board member of Merck, BMS, Janssen/Tibotec, Gilead, ViiV, and Abbvie. KE participated in advisory boards of Janssen-Cilag, BMS, Gilead, Abbvie, and Intercept; and received support from Janssen-Cilag, Gilead, and Abbvie for HCV patient care path. JEA has served as an advisory board member of MSD, Abbvie, BMS, Janssen, Gilead, and Viiv; (Research) grants from Abbvie, BMS, MSD, and ViiV. RJK has served as an advisory board member of AbbVie, BMS, Gilead, Merck, and Janssen; and received support from Roche. EJS received travel grants from Gilead and Abbvie.

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Supplementary table 1: Treatment characteristics in patients stratified by Child-Pugh class.

	Cirrhosis severity			
	No cirrhosis (n = 72)	**CP-A** (n = 100)	**CP-B** (n = 11)	**p-value**
RBV start dose (mg/kg/day)	13.9 (12.4-15.3)	13.3 (12.2-15.0)	12.9 (9.1-14.9)	0.50
RBV TW8 dose (mg/kg/day)	13.2 (10.7-14.8)	13.1 (12.0-14.6)	12.3 (8.9-14.7)	0.51
RBV dose reduction	21 (29%)	24 (24%)	5 (46%)	0.29
RBV discontinuation	6 (8%)	10 (10%)	0 (0%)	0.53
RBV steady-state plasma level (mg/L)	2.8 (2.2-3.7)	2.5 (1.9-3.4)	2.5 (2.1-4.3)	0.48

Data are expressed as median (IQR) or n (%).
CP-A: Child-Pugh class A; CP-B: Child-Pugh class B; RBV: Ribavirin; TW: Treatment week.

Peg-interferon and ribavirin treatment in HIV/HCV co-infected patients in Thailand: efficacy, safety, and pharmacokinetics (HIV-NAT 202)

EJ Smolders
N Thammajaruk
CTMM de Kanter
EPH Colbers
P Chaiyahong
T Cuprasitrut
S Chittmittraprap
T Apornpong
S Khemnark
P Tangkijvanich
DM Burger
A Avihingsanon

on behalf of the HIV-NAT 174/202 study team

Submitted

ABSTRACT

Aim

In Thailand, 7.2% of HIV patients are co-infected with the hepatitis C virus (HCV) and these patients are treated with peg-interferon + ribavirin (PR) for their HCV-infection. This study evaluates efficacy and safety of PR treatment and pharmacokinetics of ribavirin in this population.

Methods

HIV/HCV co-infected Thai patients were treated with PR for 24 or 48 weeks. Ribavirin plasma concentrations were measured during treatment. Sustained virological response 24 weeks after end-of-treatment (SVR24) was used to describe efficacy. (Laboratory) safety and ribavirin concentrations were evaluated during study visits. Ribavirin concentrations were compared for patients with and without anemia (hemoglobin <10 g/dL) and SVR24.

Results

101 HIV/HCV co-infected patients were included. The majority of patients were male (n = 88) and infected with genotype 3 (n = 46). The median ribavirin start dose was 14.28 mg/kg/day. SVR24-rate was 56%. All patients reported at least 1 (serious) adverse event, of which 28% of patients developed anemia. Seven patients discontinued treatment due to toxicity issues. Geometric mean ribavirin concentration was 1.81 mg/L at week 8 of treatment. At week 8, patients with anemia had higher ribavirin levels (2.29 versus 1.63 mg/L; p = 0.002) while patients without SVR had somewhat lower levels (1.74 versus 1.91 mg/L; p = 0.378).

Conclusions

PR treatment has comparable response rates and toxicity profile in Thai HIV/HCV co-infected patients as in Western HIV/HCV patients. Ribavirin plasma concentrations were lower than reported in previously published studies in HCV mono-infected patients. Patients without SVR tend to have lower ribavirin plasma concentrations.

INTRODUCTION

Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are both transmitted through blood-blood contact, explaining why it is estimated that globally 2.3 million people live with a co-infection[1]. In Thailand, it has been estimated that 7.2% of HIV patients have a co-infection with HCV (estimated number inhabitants 68 million). The HIV infection of these patients originated mostly from heterosexual contact, homosexual contact, or intravenous drug use[2].

In Thai patients, HCV genotype 3 is most prevalent (43%), followed by genotype 1b (13%)[3]. Genotype 3 demonstrates a better response to treatment with peg-interferon plus ribavirin (PR) therapy compared with genotype 1[4, 5]. Contradictory, when HCV is treated with all oral therapy consisting of direct-acting antivirals (DAAs), genotype 3 is the less favorable genotype.

In HIV/HCV co-infected patients, HIV has a negative influence on the prognosis of HCV infection, because these patients develop cirrhosis more rapidly than mono-infected patients[6]. This is especially true when they have a detectable HIV viral load[7]. In addition, co-infected patients have lower sustained virological response (SVR) rates when treated with PR. For genotype 1 and 4 SVR-rates of 14-29% and 44-73% for genotypes 2 and 3[4] are previously reported for HIV/HCV co-infect patients in literature. Compared with SVR-rates of 42-56% for genotype 1 and 75-84% for genotype 3 in mono-infected patients[4]. With DAA therapy, HIV/HCV co-infected patients have similar response rates as mono-infected patients.

One explanation of lower response rates in HIV/HCV co-infected patients on PR treatment could be that these patients have lower and suboptimal ribavirin plasma concentrations when compared to mono-infected patients. This has been described by Deenen et al, who reported that the mean ribavirin plasma concentrations at week 12 of treatment were 2.62 mg/L and 2.14 mg/L in mono- and co-infected Western patients, respectively[8].

Despite the advantages of DAA treatment in co-infected patients, even for genotype 3, Thai patients are still treated with PR, as DAAs remain to be expensive and are currently not widely accessible in Thailand.

The current study was conducted to develop an appropriate HCV treatment program to improve the quality of life for HIV/HCV co-infected patients in Thailand. This was a cooperation between the National Health Security Office and the Thai AIDS society.

This is the first report describing the efficacy and safety of HCV treatment with PR in Thai patients along with the pharmacokinetics of ribavirin.

METHODS

Study design and participants

This was an open-label, prospective, multi-center study performed in three centers in Thailand. HIV/HCV co-infected patients were included from April 2014 to August 2015. Patients were included and treated with PR, regardless of HCV genotype. Patients with HCV genotype 2 or 3, a METAVIR score <F3, and a negative HCV RNA at week 4 of treatment were treated for 24 weeks. Genotype 2 and 3 patients with a METAVIR score >F3 or any other genotype were treated for 48 weeks. Patients with a positive HCV RNA at week 4, needed a drop in HCV RNA of at least 2 $\log_{10}$ at week 12, otherwise treatment was discontinued. Patients who continued PR and had an undetectable HCV RNA at week 24, received 48 weeks of treatment. If the HCV RNA load was detectable at week 24 the treatment was discontinued. The study treatment algorithm is shown in Figure 1. All patients continued antiretroviral treatment for HIV during HCV therapy.

Patients had to be at least 18 years of age and had a CD4 count >350 cells/μL. Cirrhotic patients were included with a Child-Pugh score ≤6, without ascites, hepatic encephalopathy, or bleeding varices. Both males and females had to use adequate contraception during treatment and up to 24 weeks after treatment. Patients were excluded from this study if they had severe depression or any other psychiatric illness, significant liver disease other than cirrhosis, hemoglobin <11 g/dL for women or <12 g/dL for men, ALT and AST >10 times the Upper Limit of Normal (ULN), creatinine >1.5 times the normal ULN, neutrophil count <1,500 cells/mm^3, platelet count <90,000 cells/mm^3, thyroid dysfunction, use of didanosine, or evidence of severe retinopathy or any other severe illness not related to HIV/HCV co-infection (judged by the physician). In addition, it was judged by the study physician if active drugs use or alcohol consumption potentially compromised treatment safety. Lastly, pregnant women or women breastfeeding were also excluded (confirmed by pregnancy test at screening).

Before enrolment, written informed consent was obtained from all patients. The study was approved by a local ethics committee and executed in accordance with the principals of the Declaration of Helsinki and Good Clinical Practice (BIDI IRB P005h/57 and HIV-NAT/Chulalongkorn Hospital IRB 478/56).

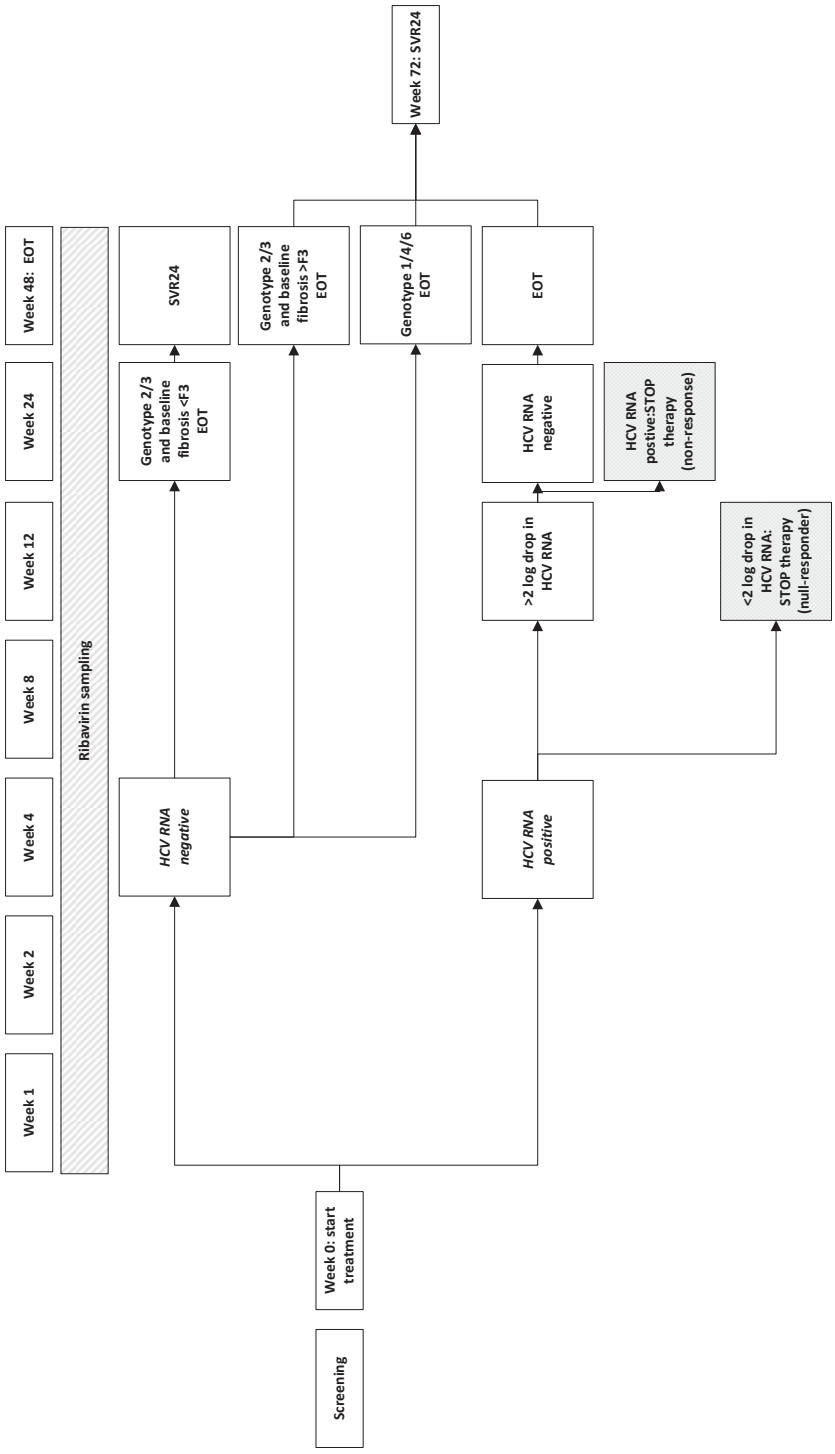

Figure 1: Study treatment algorithm.

During every visit adverse events and laboratory safety was assessed. Additional visits (not shown in study algorithm) at week 16, 20, 30, 36, and 42.
SVR24: Sustained virological response 24 weeks after end-of-treatment; EOT: End-of-treatment; F3: METAVIR score F3.

Treatment and study procedures

At screening, baseline patient characteristics such as age, weight, and several laboratory parameters were determined (Table 1). A Fibroscan® was performed to determine the liver stiffness (kPa) and a METAVIR score[9, 10].

After inclusion, the patients were treated with peg-interferon alfa 2a (Pegasys®, Hoffmann- La Roche Inc, South San Francisco, CA) 180 mcg per week or peg-interferon alfa 2b (PegIntron®, Merck Sharp & Dome Corp, Whitehouse Station, NJ), which was dosed weight-based: 1.5 mcg/kg/week. It was at the discretion of the physician whether peg-interferon alfa 2a or 2b was used. The dose of peg-interferon alfa 2a was adjusted based on absolute neutrophil count (ANC) and platelet count according to the drug label.

Ribavirin (Rebetol®, Merck Sharp & Dome Corp, Whitehouse Station, NJ) was taken twice daily with food and also dosed weight-based according to the label: <65 kg: 800 mg/day, 65-80 kg: 1,000 mg/day, 81-105 kg: 1,200 mg/day, and >105 kg: 1,400 mg/day. The ribavirin dose was adjusted based on side effects (anemia [hemoglobin <10 g/dL]) and ribavirin plasma concentrations (therapeutic drug monitoring [TDM]).

There were study visits at baseline and week 1 (optional), 2, 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, and week 72. Twenty-four weeks after end-of-treatment (week 48 or 72) the patients came to the study center for determination of HCV RNA. SVR was defined as undetectable HCV RNA 24 weeks post treatment (SVR24). Week 24 or 48 were both end-of-treatment visits. Patients that were treated for 24 weeks were dismissed for further visits, except the week 48 visit for determination of SVR. Safety (adverse events) and laboratory safety were evaluated during all study visits.

Laboratory safety included hemoglobin, lymphocytes, neutrophils, platelets, white blood cell count (WBC), hematocrit, creatinine, ALT, and AST. Creatinine was measured as ribavirin is mainly renally cleared and when the renal function alters this is reflected in the ribavirin plasma concentrations. HCV RNA was measured (RealTime HCV, Abbott Molecular) at week 0, 4, 12, 24, and 48 of treatment.

If the patients gave consent, additional blood was drawn to determine the ribavirin plasma concentrations at week 1, 2, 4, 8, 12, 24, and 48. Preferably, trough samples were taken (C_{12}), however, considering the very long elimination half-life of ribavirin (300 hours), this was not mandatory and other sampling times were not excluded from the analysis.

Blood samples were sent to laboratory of the HIV-NAT research collaboration immediately. The samples were centrifuged at 3,220 g for 10 minutes at 20°C. Plasma was divided and transferred to labeled polypropylene tubes and stored at -20°C until analysis. Total sample processing was performed within 2 hours after collection. Ribavirin plasma concentrations were determined by a validated high performance liquid chromatography (HPLC) with ultraviolet detection (UV) method[11], with a lower limit of quantification of 0.300 mg/L. The ribavirin calibration curve was linear over the concentration range of 0.300 to 12.000 mg/L. The within-run and between-run variation (precision) was less than 5% and the accuracy results were between 95-105%.

Statistical analysis

First, patient characteristics, laboratory parameters, and ribavirin plasma concentrations were analyzed using descriptive statistics (medians with interquartile range [IQR]). The total population that completed follow up was included in the percentage of patients with SVR. Non-responders or treatment failures were dismissed from treatment visits, but were included in the efficacy analysis. Dose adjustments of peg-interferon and ribavirin were reported. All biological parameters were log-transformed (e.g., hemoglobin, and ribavirin plasma concentrations) and geometric means or medians were reported.

Geometric mean ribavirin plasma concentrations were calculated for week 1, 2, 4, 8, 12, 24, and, 48 of treatment. Intra- and inter-subject variation of ribavirin plasma concentrations were expressed as coefficient of variation (CV%). Second, independent samples t-tests were performed to evaluate whether patients with and without SVR24 or anemia had different ribavirin plasma concentrations (all weeks).

All statistical analyses were performed in IBM SPSS Statistics, version 22.

RESULTS

A total of 106 patients were screened of whom 101 were eligible for inclusion (Figure 2).

In Table 1 the baseline characteristics of the cohort are presented. The majority of patients were male (*n* = 88; 87%) and most patients were infected with HCV genotype 3 (*n* = 46; 46%) or 1 (*n* = 38; 38%). Median (IQR) age and weight were 43 (38-50) years and 60 (53-70) kg, respectively. Median (IQR) baseline HCV viral load was $\log_{10}$ 6.13 (5.42-6.58) IU/mL. Before treatment, 68 patients had an undetectable HIV RNA load (of 25 patients HIV RNA was missing), the median (IQR) liver stiffness obtained with Fibroscan® was 11.9 (8.75-24.8) kPa and ALT and AST were 77 (49-115) and 64 (42.5-100.5) U/L, respectively.

Table 1: Baseline characteristics of the study population (*n* = 101).

Characteristic	Baseline, median (IQR)	Week 48, median (IQR)
Patients[a]	101	74
Age, years	43 (38-50)	44 (38-50)
Gender, male[a]	88 (87%)	66 (89%)
Weight, kg	60.3 (52.9-69.95)	58.20 (50.83-68.01)
Hemoglobin, g/dL	14.7 (13.4-16.0)	12.2 (11.13-12.9)
Creatinine, mg/dL	0.86 (0.78-1.00)	0.79 (0.74-0.89)
Platelet count, 10^3/uL	202 (154-246)	176 (132-213)
Neutrophil count, 10^3/uL	3.02 (2.27-3.75)	1.55 (1.09-2.46)
HIV RNA undetectable[a]	70 (69%)[b]	-
CD4 count, cells/µL	595 (435-732)	-
Log_{10} HCV RNA, IU/mL	6.13 (5.42-6.58)	-
HCV genotype[a]		
1	38 (38%)	27 (36%)
3	46 (49%)	35 (47%)
6	14 (14%)	10 (14%)
Mixed genotype[d]	3 (3%)	2 (3%)
Liver stiffness (Fibroscan®), kPa	11.9 (8.75-19.1)	8.25 (5.7-21.1)
ALT, U/L	77 (49-115)	31 (23-46)
AST, U/L	64 (42.5-100.5)	32 (23-45)

[a]Number of patients is given.

[b]25 patients missing, HIV RNA undetecable <50 copies/mL.

[c]1 woman and 1 man had a hemoglobin <11 and 12 g/dL, respectively.

[d]Mixed genotypes include: 1,3; 1,3,4; 1,4.

IQR: Interquartile range; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

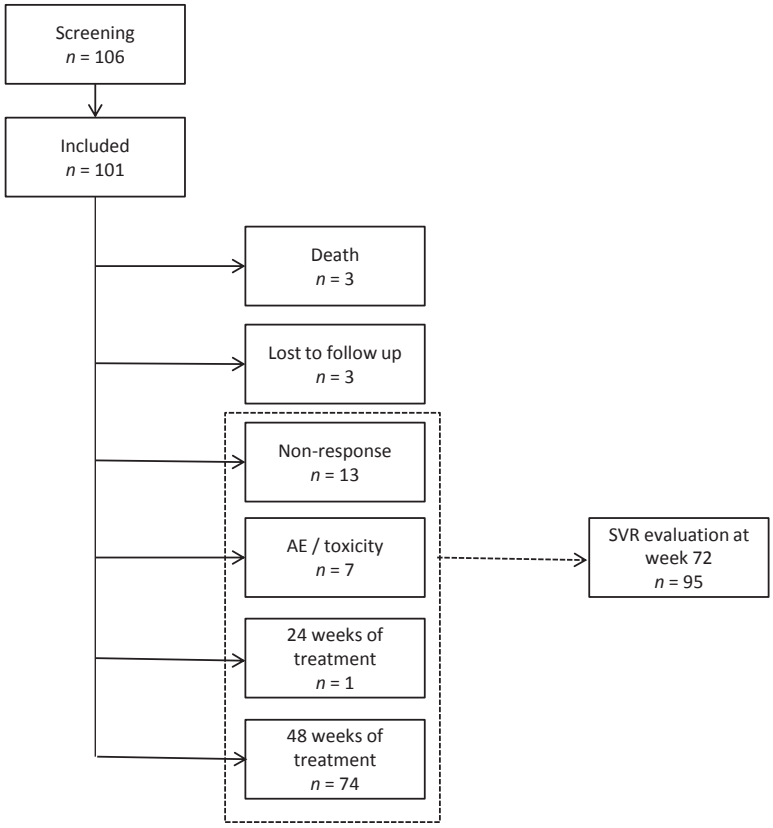

Figure 2: Overview study participation.
AE: Adverse event; SVR: Sustained virological response.

Treatment

With the exception of 1 patient, all responders were treated for 48 weeks (genotype 3 patients with detectable HCV RNA at week 4 or >F3 cirrhosis at baseline).

A total of 57 patients reached SVR24 (56%), of whom 18 (47%) had genotype 1, 29 (63%) genotype 3, 8 (57%) genotype 6, and 2 (67%) had a mixed genotype. Liver enzymes recovered for the patients with SVR, median (IQR) AST and ALT of these patients at 24 weeks of follow up were 29 (23-36) and 31 (23-42) U/L, respectively.

Peg-interferon alfa 2a was used by 6 patients (6%) and 2b was used by 95 patients (94%). All patients received, according to protocol, weight-based ribavirin (Table 2).

Table 2: Overview HCV treatment.

HCV drug	Number	Dosage-median (IQR)
Start dose: Peg-interferon alfa 2a	6	180 mcg/week
# patients discontinued	1	-
# patients needed dose reduction	3	-
Week 48 dose	5	180 (135-180) mcg/week
Peg-interferon alfa 2b	95	1.43 (1.33-1.50) mcg/kg/week
# patients discontinued	26[b]	-
# patients needed dose reduction	52(1-4[a])	-
Week 48 dose	69[b]	1.37 (1.06-1.53) mcg/kg/week
Ribavirin	101	14.28 (13.33-15.22) mg/kg/day
# patients discontinued	27	-
# patients needed dose adjustment	38[b, d] (1-4[a])	-
Week 48 dose	74[b]	13.84 (12.39-15.05) mg/kg/day

[a]Number of dose reductions varied from 1 to 4 dose reductions per patient.
[b]1 patient was treated for 24 weeks.
[c]1 patient needed a dose increase.
HCV: Hepatitis C virus; IQR: Interquartile range.

The median (IQR) start dosage of ribavirin was 14.28 (13.33-15.22) mg/kg/day. During treatment, 38 patients needed a dose reduction and 1 patient received an increased dose of ribavirin due to weight gain. Overall, this resulted in a lower week 48 median (IQR) ribavirin dosage: 13.84 (12.39-15.05) mg/kg/day (Table 2, Figure 3). During treatment, anemia was reported by 28 patients (28%).

For peg-interferon 2b the median start dose (IQR) was 1.43 (1.33-1.50) mcg/kg/week and 52 patients needed a dose reduction, resulting in a median (IQR) week 48 dose of 1.37 (1.06-1.53) mg/kg/day. For peg-interferon 2a, all patients started with a dose of 180 mcg/week and the dose was reduced for two patients to 90 mcg/week and for one patient to 135 mcg/week.

In Figure 3, the course of ribavirin dosage, plasma hemoglobin, and serum creatinine concentrations during treatment are presented. The median (IQR) hemoglobin concentration dropped from 14.70 (13.40-16.00) g/dL at baseline to 12.20 (11.13-12.90) g/dL at week 48 of treatment. Median (IQR) serum creatinine remained stable: 0.86 (0.78-1.00) mg/dL at baseline and 0.79 (0.74-0.89) mg/dL at week 48 of treatment.

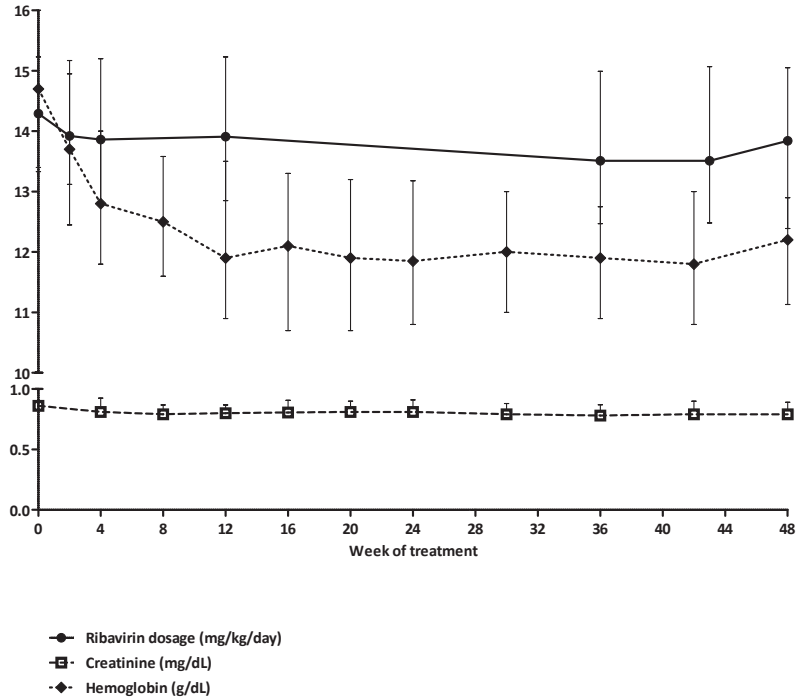

Figure 3: Course of hemoglobin levels, ribavirin dosages, and creatinine levels during HCV treatment.

Median (interquartile range) values are shown and data from all available patients were used (Figure 1). Error bars represent interquartile ranges.

A total of 752 adverse events (AEs) were reported by 101 patients of which 20 serious adverse events (SAEs). The number of AEs varied from 1 to 22 AEs per patient. A total of 20 patients discontinued treatment, of which 7 patients due to (serious) adverse events ([S]AEs). During the study, three patients deceased. One due to a heroin overdose (unrelated to study medication), one to pulmonary tuberculosis (unrelated to study medication), and one to thrombocytopenia which was probably related to the study medication.

The majority of AEs were graded to be mild or moderate severity: 383 (51%) and 306 (41%), respectively. AEs were judged severe and potentially life threatening in 48 (6%) and 10 (1%) of the cases, respectively (Table 3).

Table 3: Safety analysis: overview of the reported (serious) adverse events during the study.

	Times reported count	Grade severe count	Grade moderate count	Grade mild count
Serious adverse event n = 11	**20**			
Anemia	5	2	3	0
Thrombocytopenia	1	1	0	0
Hemoptysis	1	1	0	0
Neutropenia	1	1	0	0
Hyperglycemia	1	1	0	0
Uncontrolled diabetes mellitus	1	1	0	0
Schizoaffective disorder	1	0	1	0
Hypokalemia	1	0	1	0
Pulmonary Tuberculosis	1	0	1	0
Vomiting	1	0	1	0
Headache	1	0	1	0
Fever	1	0	1	0
Cataract surgery	1	0	1	0
Death due to overdose	1	-	-	-
Death due to thrombocytopenia	1	-	-	-
Death due to pulmonary tuberculosis	1	-	-	-
Adverse event (≥10 times reported) n = 101	**732**			
Fever	107	0	15	92
Neutropenia	62	27	29	6
Alopecia	41	1	10	30
Fatigue	34	2	12	20
Weight loss	27	3	18	6
Myalgia	25	0	11	14
Pain/rash at injection site	24	1	7	16
Anemia	23	0	2	21
Elevated AST	21	5	16	0
Decreased lymphocytes	20	2	18	0
Anorexia	18	0	8	10
Dizziness	18	0	3	15
Nausea	17	0	6	11
Thrombocytopenia	14	0	14	0
Insomnia	14	0	6	8
Flu-like symptoms	13	1	4	8
Loss of appetite	12	0	4	8
Depression	12	0	8	4

AST: Aspartate aminotransferase.

Ribavirin pharmacokinetics

In Figure 4, we present the results of the pharmacokinetics of ribavirin. Ribavirin plasma concentrations were available for 39 up to 64 patients, from week 2 to week 48 respectively. Visual inspection of the data reveals that ribavirin steady-state plasma concentrations were reached at week 8 of treatment and the geometric mean (IQR) ribavirin plasma concentration at that time point was 1.81 (1.42-2.32) mg/L. The inter-subject CV% was 34, 57, and 29% at week 8, 12, and 24 respectively. The intra-subject CV% was much smaller: 18% (week 8, 12, 24).

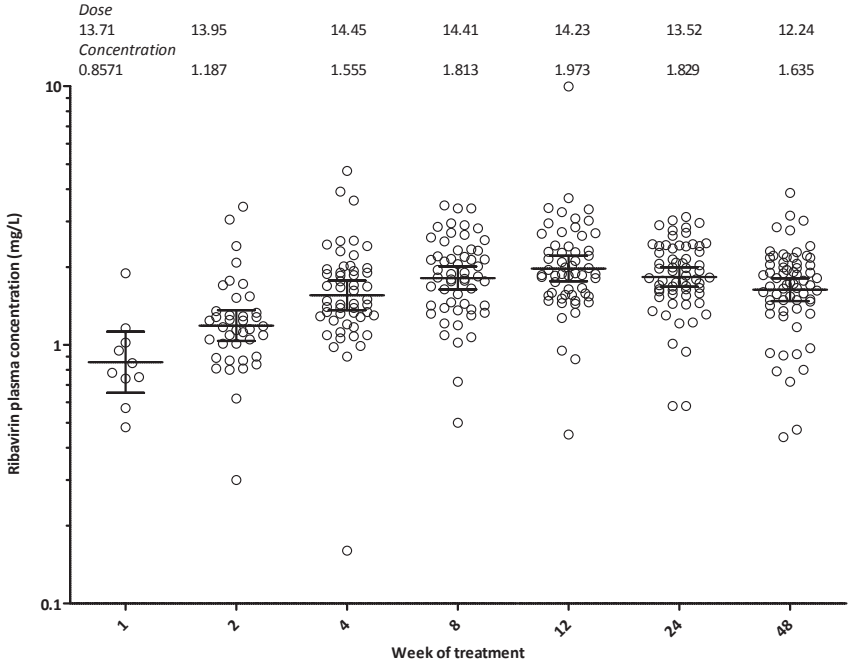

Figure 4: Ribavirin plasma concentrations per patient in each visit.
Dose: Geometric mean ribavirin dose in mg/kg/day.
Concentration: Geometric mean ribavirin plasma concentration in mg/L.
The bars are show the 95% confidence intervals.

Pharmacodynamics

Sustained virological response

Table 4 shows the geometric mean ribavirin plasma concentration per week of treatment for patients with and without SVR24. It was observed that patients with SVR24 had slightly higher ribavirin plasma concentrations throughout treatment. This difference only reaches statistical significance at week 24 of treatment.

Table 4: Ribavirin plasma concentrations per week presented for patients with and without sustained virological response and anemia.

Treatment week	Number	Ribavirin plasma concentration, mg/L (SD)	Number	Ribavirin plasma concentration, mg/L (SD)	p-value
	SVR		**No SVR**		
2	22	1.25 (1.58)	13	1.07 (1.51)	0.326
4	28	1.57 (1.73)	19	1.53 (1.48)	0.883
8	32	1.91 (1.45)	19	1.74 (1.43)	0.378
12	35	2.12 (1.58)	17	1.76 (1.41)	0.145
24	39	1.97 (1.38)	21	1.59 (1.41)	0.020
48	45	1.69 (1.46)	19	1.51 (1.58)	0.293
	Anemia		**No anemia**		
2	15	1.45 (1.43)	24	1.05 (1.52)	0.017
4	19	2.01 (1.45)	33	1.34 (1.59)	0.002
8	17	2.29 (1.37)	38	1.63 (1.43)	0.002
12	17	2.56 (1.56)	39	1.76 (1.44)	0.002
24	16	2.00 (1.49)	46	1.77 (1.37)	0.216
48	17	1.36 (1.67)	47	1.75 (1.40)	0.028

Results of the independent t-tests are given. Values are geometric means of the ribavirin plasma concentrations. SVR: Sustained virological response; SD: Standard deviation.

Anemia

Table 4 demonstrates that patients suffering from anemia had higher ribavirin plasma concentrations than patients without anemia. Until week 24, all patients with anemia had statistically significant higher ribavirin plasma concentrations than patients without anemia. Strikingly, at week 48 patients with anemia had lower ribavirin plasma concentrations.

DISCUSSION

This is the first Thai prospective cohort study describing efficacy and safety of PR treatment in combination with pharmacokinetics of ribavirin in HIV/HCV co-infected patients. In this study, an overall SVR24 rate of 56% was achieved after 48 weeks of PR treatment. The overall SVR-rate was relatively low, but comparable with the SVR24 rates described in genotype 2 and 3 Western co-infected patients treated with PR[4]. This could be explained by the fact that in our cohort the majority of patients was infected with genotype 3, of whom slightly more patients achieved SVR24 compared to genotype 1 (63% versus 47%). The SVR24 rate of 56% is lower than in mono-infected patients[4]. It remains unclear why co-infected patients have lower response rates with PR treatment than mono-infected patients. Possibly HIV increases HCV replication,

induces hepatic inflammation and the release of pro-inflammatory cytokines, increases hepatic apoptosis, and leads to an impaired HCV specific immune response[12]. Remarkably, these differences in SVR-rates disappeared with the introduction of interferon-free therapy[7]. As mentioned before, lower ribavirin plasma concentrations in co-infected versus mono-infected patients may play a role too.

As expected with PR treatment, many AEs were reported. Most commonly reported AEs were fever, neutropenia, alopecia, fatigue, weight loss, and myalgia. These are in line with previously reported studies and resemble the known toxicity profile of PR[13, 14]. Depression and other mental disorders were only reported 21 times during this study, possibly because patients with psychiatric illnesses were excluded from this study[13, 14]. A total of three patients died during treatment, two of these deaths were unrelated to PR treatment; one patient had severe thrombocytopenia (without bleeding), which was probably related to PR treatment (peg-interferon alfa dose was previously reduced). Lastly, anemia was the most reported SAE ($n = 5$). All but one of these patients experiencing anemia as a SAEs needed dose reductions of peg-interferon alfa and/or ribavirin.

We showed that at week 8 of treatment, ribavirin steady-state plasma concentrations were reached, which fits the long elimination half-life of 300 hours[15]. The steady-state ribavirin plasma concentrations found in this study were in the same range as previously published in Western HIV/HCV co-infected patients[8, 16, 17]. However, these ribavirin plasma concentrations remained lower than reported ribavirin plasma concentrations in mono-infected patients[8, 18]. We showed that hemoglobin concentrations dropped inversely with the raise of ribavirin plasma concentrations. In addition, when steady-state was achieved hemoglobin concentrations remained stable.

These findings fit the observation that patients with anemia had significantly higher ribavirin plasma concentrations than patients without anemia (until week 12). At week 24, ribavirin plasma concentrations dropped in patients with anemia, which could be explained by the high number of dose reductions that were necessary because of low hemoglobin concentrations, resulting in reduced week 48 daily dosages and ribavirin plasma concentrations. In addition, creatinine concentrations, and thus renal function, remained stable during treatment. So, ribavirin renal clearance was not altered, giving an extra argument that the altered ribavirin plasma concentration is caused by the dose alterations.

Only at week 24, ribavirin plasma concentrations were higher for responders than for non-responders. This could be driven by the increasing number of patients of which

ribavirin plasma concentrations were available towards week 48. The relation between ribavirin plasma concentrations and SVR remains controversial, as both negative and positive results have been published on this topic[16, 17, 19, 20, 21]. These inconsistent results might be caused by, for example, differences in treatment, heterogeneity of populations, variation in analytical assays, and small sample sizes.

A limitation of this study is that the number of ribavirin samples were varying and the number was increasing during the study. This could potentially influence the (statistical) analysis. In addition, this was not a randomized trial comparing efficacy and safety in mono and co-infected patients. Lastly, the drugs used in this trial are considered outdated in high income countries, because of the availability of DAAs which have better safety and efficacy profiles. However, to date, access to these expensive DAAs is still limited, but could greatly improve SVR-rates among HIV/HCV co-infected individuals[7]. Especially, velpatasvir combined with sofosbuvir (Epclusa®, Gilead Sciences, Foster City, CA) as this combination is highly active against genotype 3[22]. The introduction of (generic) velpatasvir in Thailand would not only greatly improve SVR-rates, it will shorten treatment duration and there will be less toxicity, compared with the current treatment of PR[22].

CONCLUSION

PR treatment in Thai HIV/HCV co-infected patients resulted in an overall SVR-rate of 56%, including hard-to-treat genotype 1 patients. As known from PR therapy, toxicity was severe, but only a limited numer of patients dropped out because of toxicity issues. Ribavirin plasma concentrations were comparable with previously published studies in HIV/HCV co-infected patients, but were lower than in mono-infected patients.

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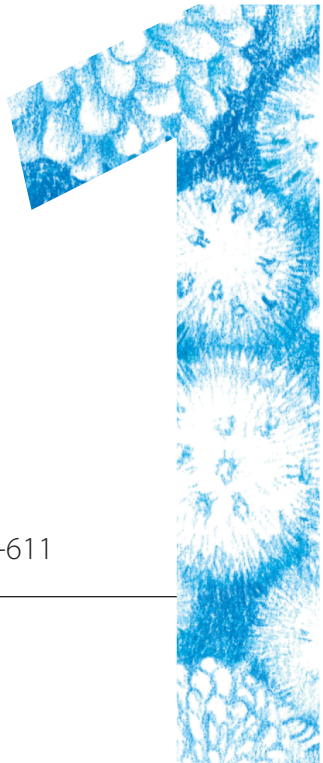
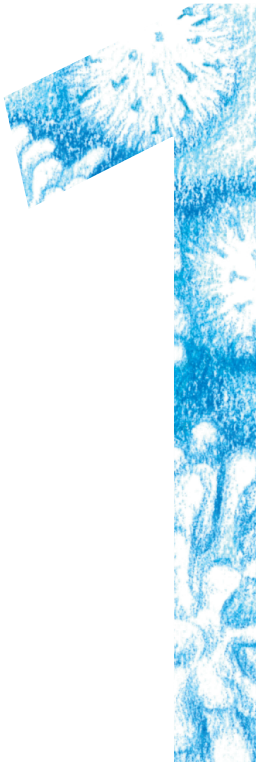
Part 3

Treatment of hepatitis C virus in special patient populations

Pharmacokinetics, efficacy, and safety of HCV drugs in patients with liver and/or renal impairment

EJ Smolders
CTMM de Kanter
B van Hoek
JE Arends
JPH Drenth
DM Burger





Drug Saf. 2016 Jul; 39 (7): 589-611

ABSTRACT

Hepatitis C virus (HCV) infected patients often suffer from liver cirrhosis, which can be complicated by renal impairment. Therefore, in this review we describe the treatment possibilities in HCV patients with hepatic and renal impairment.

Cirrhosis alters the structure of the liver, which affects drug-metabolizing enzymes and drug-transporters. These modifications influence the plasma concentration of substrates of drugs metabolized/transported by these enzymes. The direct-acting antivirals (DAAs) are substrates of, for example, cytochrome P450 enzymes in the liver. Most DAAs are not studied in HCV-infected individuals with decompensated cirrhosis, and therefore awareness is needed when these patients are treated. Most DAAs are contra-indicated in cirrhotic patients; however, patients with a Child-Pugh class of B or C can be treated safely with a normal dose sofosbuvir plus ledipasvir or daclatasvir, in combination with ribavirin.

Patients with renal impairment (Glomerular Filtration Rate [GFR] <90 mL/min) or who are dependent on dialysis often tolerate ribavirin treatment poorly, even after dose adjustments. However, most DAAs can be used at the normal dose because DAAs are not renally excreted. To date, grazoprevir plus elbasvir is the preferred DAA regimen in patients with renal impairment, as data are pending for sofosbuvir patients with GFR <30 mL/min (as for ledipasvir and velpatasvir). However, sofosbuvir has been used in a small number of patients with severe renal impairment and, based on these trials we recommend sofosbuvir 400 mg every day, when no other DAA regimen is available. Ledipasvir and velpatasvir are not recommend in patients with severe renal impairment.

KEY POINTS

- All drugs used in hepatitis C virus (HCV) treatment can be used in patient with compensated liver cirrhosis (Child-Pugh class A).
- All drugs used in HCV treatment can be used in patients with moderate renal insufficiency (Glomerular Filtration Rate [GFR] ≥30 mL/min).
- In patients with GFR ≤29 mL/min or advanced liver disease, HCV drugs might be contra-indicated or dosage adjustments may be necessary.

INTRODUCTION

Chronic hepatitis C virus (HCV) related liver cirrhosis is the leading cause of liver transplantation in many countries[1-3]. Eventually, 15-30% of chronically infected HCV patients develops liver cirrhosis[4, 5]. Symptoms of decompensated cirrhosis are portal hypertension (with increased risk for variceal bleedings), ascites, hepatic encephalopathy, and hepato-renal syndrome. In addition, cirrhotic patients have an enhanced risk of hepatocellular carcinoma, which is an important cause of mortality[6, 7].

HCV is associated with both renal and hepatic impairment, and care must be taken when prescribing direct-acting antivirals (DAAs) in these patients. The drugs described in this review are ribavirin and the novel DAAs.

Impaired kidney or liver function may result in altered drug concentrations, causing either toxicity or subtherapeutic levels, because these organs are mainly responsible for metabolizing and excreting drugs. For instance, patients with reduced renal function have a decreased ability to eliminate water soluble agents[8] and patients with impaired liver function have reduced expression of drug-metabolizing enzymes and thus reduced metabolizing capacity[6].

There is only limited information on the pharmacokinetics, safety, efficacy, and dosage in these special populations. Moreover, this information is often difficult to find and not presented in a comprehensive manner. Therefore, the aim of this review is to give an overview of the pharmacokinetics, efficacy, and safety of drugs used for HCV treatment in patients with renal or hepatic impairment and to provide dose recommendations for prescribing these drugs in these special populations.

METHODS

An extensive search was performed using PubMed (1946-October 2015) and Embase (1947-October 2015) to identify peer-reviewed studies containing information on pharmacokinetics, efficacy, and safety in patients with impaired renal or hepatic function and HCV medication. Search terms contained generic and brand names. Various general search terms were used describing impaired renal and hepatic function, e.g., 'end stage renal disease' (ESRD), 'dialysis', 'cirrhosis', and 'hepatic impairment'.

Google, Google Scholar, and ClinicalTrials.gov were used to identify conference papers and abstracts. All searches were performed in the English language. Additional articles and primary sources were identified with citation snowballing. Lastly, the Summary of

Product Characteristics (SmPC) approved by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) Prescribing Information were main sources of information for this review.

This review focuses on the novel DAAs, e.g., simeprevir, paritaprevir, asunaprevir, grazoprevir, daclatasvir, ombitasvir, ledipasvir, elbasvir, velpatasvir, sofosbuvir, and dasabuvir. To date, velpatasvir is not yet licensed. The included DAAs are used in international guidelines[9, 10] or were submitted for registration up to November 2015 by the EMA and/or FDA. Ribavirin is also discussed, because it is still a component of the therapy in cirrhotic patients. We omitted telaprevir and boceprevir from the review, as their current use is limited. Additionally, we do not describe peg-interferon alfa as we believe it should not be used in patients with cirrhosis or renal impairment.

PHARMACOKINETICS

This section summarizes the pharmacokinetics of drugs used in HCV treatment, in both healthy subjects and in patients with impaired renal or hepatic function: DAAs (protease inhibitors [PIs], NS5A inhibitors, NS5B polymerase inhibitors, and fixed-dose regimens) and other antivirals (ribavirin). The clinical consequences and dosage recommendations based on these observations are summarized in Tables 1 and 2. Figure 1 gives an overview of the hepatic and renal metabolism of these drugs.

Protease inhibitors

Simeprevir

Simeprevir is a second wave, first generation PI and is prescribed at a dose of 150 mg once daily (QD). Simeprevir is highly bound to plasma proteins (>99.9%) and is a substrate of various drug-transporters such as P-glycoprotein (P-gp), organic anion-transporting polypeptide (OATP) 1B1, OATP1B2, OATP2B1, and multidrug resistance protein (MRP) 2, and different cytochrome P450 (CYP) enzymes (intestinal CYP3A4, CYP2C19, and CYP2C8). The plasma concentration of simeprevir was two- to three-fold higher in HCV-infected patients than in healthy subjects[11].

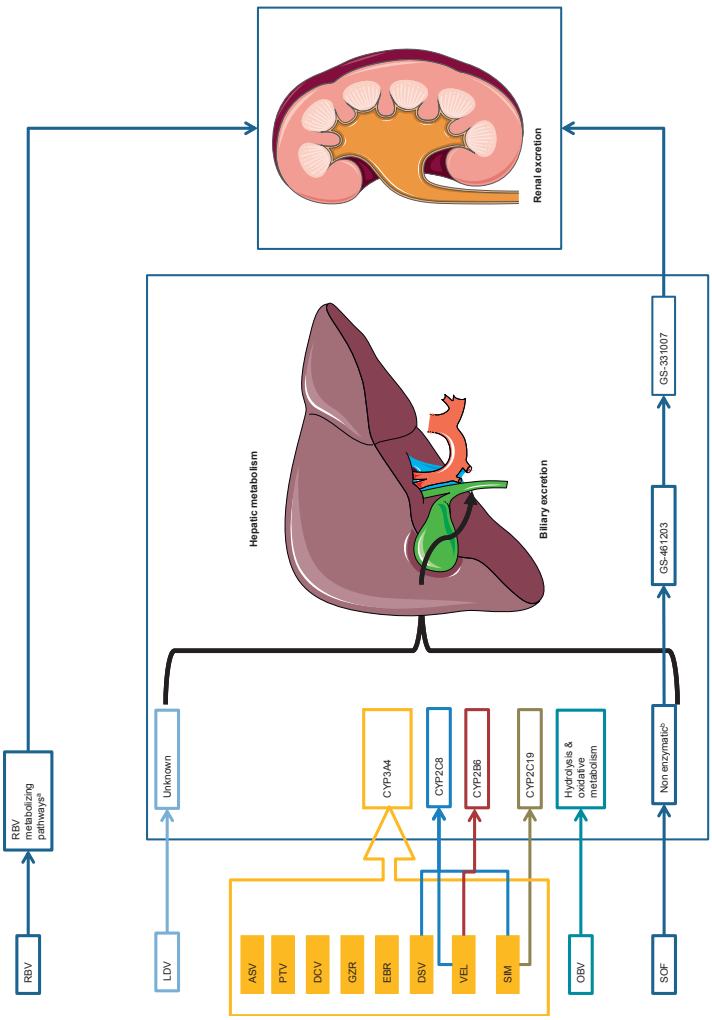


Figure 1: Overview of the hepatic or non-enzymatic metabolism of drugs used for the treatment of hepatitis C: cytochrome P450 enzymes involved and biliary and/or renal excretion of drug (metabolites).

[a]The site of metabolism is unknown but two metabolizing pathways are involved: (1) a reversible phosphorylation pathway and (2) a degradative pathway involving deribosylation and amide hydrolysis.

[b]Sofosbuvir is extensively metabolized in the liver in the active metabolite GS-461203, followed by dephosphorylation which results in the inactive compound GS-331007.

ASV: Asunaprevir; CYP: Cytochrome P450; DCV: Daclatasvir; DSV: Dasabuvir; EBR: Elbasvir; GZR: Grazoprevir; LDV: Ledipasvir; OBV: Ombitasvir; PTV: Paritaprevir; RBV: Ribavirin; SIM: Simeprevir; SOF: Sofosbuvir; VEL: Velpatasvir.

Table 1: Dosage recommendations for patients with Child-Pugh class A, B, or C.

HCV drug	Normal hepatic function	Degree of cirrhosis		
		Child-Pugh class A	Child-Pugh class B	Child-Pugh class C
Simeprevir	150 mg QD [11]	150 mg QD [11]	Contra-indicated [11, 18]a	Contra-indicated [11, 18]a
Asunaprevir[b]	100 mg BID [15, 14]	100 mg BID [15, 14]	Contra-indicated [15, 14]	Contra-indicated [15, 14]
Daclatasvir	60 mg QD [19]	60 mg QD [19]	60 mg QD [19]	60 mg QD [19]
Sofosbuvir	400 mg QD [20]	400 mg QD [20]	400 mg QD [20]	400 mg QD [20]
Ledipasvir/sofosbuvir	90 mg QD/ 400 mg QD [21]	90 mg QD/ 400 mg QD [21]	90 mg QD/ 400 mg QD [21]	90 mg QD/ 400 mg QD [21]
Velpatasvir/sofosbuvir[b]	100 mg QD/ 400 mg QD [22]	100 mg QD/ 400 mg QD [23, 22]	100 mg QD/ 400 mg QD [23, 22]	Unknown
Grazoprevir/elbasvir	100 mg QD/ 50 mg QD [24, 25]	100 mg QD/ 50 mg QD [24, 25]	Contra-indicated [26]	Contra-indicated [26]
Paritaprevir/ Ritonavir/ Ombitasvir	150 mg QD/ 100 mg QD/ 25 mg QD [27, 28]	150 mg QD/ 100 mg QD/ 25 mg QD [27, 28]	Contra-indicated [29, 30]c	Contra-indicated [27]
Dasabuvir	250 mg BID [31, 28]	250 mg BID [31, 28]	Contra-indicated [29, 30]c	Contra-indicated [31]
Ribavirin	<75 kg = 500 mg BID ≥75 kg = 600 mg BID [32]	<75 kg = 500 mg BID ≥75 kg = 600 mg BID [32]	<75 kg = 500 mg BID ≥75 kg = 600 mg BID [32]	<75 kg = 500 mg BID ≥75 kg = 600 mg BID [32]

[a]The US FDA Prescribing Information for Olysio™ comments that: the potential risks and benefits of OLYSIO™ (Janssen Therapeutics, Titusville, NJ, USA) should be carefully considered prior to use in patients with moderate or severe hepatic impairment. The EMA SmPC comments that: the safety and efficacy of OLYSIO™ have not been studied in HCV-infected patients with moderate or severe hepatic impairment (Child-Pugh class B or C); therefore, particular caution is recommended when prescribing OLYSIO™ to HCV-infected patients with moderate or severe hepatic impairment.

[b]No SmPC or Prescribing Information was available at time of publication.

[c]The SmPC Viekierax® (AbbVie, North Chicago, IL, USA) and Exviera® (AbbVie, North Chicago, IL, USA) states that efficacy and safety are not studied in Child-Pugh class B patients. The US Prescribing Information has been updated and both Child-Pugh class B and C are contra-indicated.

BID: Twice daily; EMA: European Medicines Agency; FDA: Food and Drug Administration; HCV: Hepatitis C virus; QD: Once daily; SmPC: Summary of Product Characteristics.

Compared with healthy individuals, simeprevir steady-state area under the concentration-time curve (AUC) was 2.4- and 5.2-fold higher in Child-Pugh class B (CP-B) and C (CP-C) patients, respectively. Therefore, the manufacturer recommends that simeprevir should not be used CP-C patients and that caution should be taken in CP-B patients[11]. Another trial reported similar results: non-HCV CP-B patients had twofold increased exposure compared with healthy individuals and CP-C patients had twofold higher exposure to simeprevir than CP-B patients[12]. After a dose of 150 mg, Sekar and co-authors[13] observed equal exposure and protein binding between non-HCV Child-Pugh class A (CP-A) and CP-B subjects.

The steady-state AUC of simeprevir increased (62%) in patients with severe renal impairment (Glomerular Filtration Rate [GFR]: 15-29 mL/min). This may indicate that exposure may increase in patients with severe renal impairment and ESRD (GFR ≤15 mL/min). Thus, caution is needed in these patients. However, the label states that simeprevir can be used by patients with all grades of renal impairment. At last, simeprevir is not removed by dialysis[11].

Asunaprevir

Asunaprevir is a PI that has activity against multiple genotypes. It is used at a dose of 100 mg twice daily (BID), is metabolized by the liver (CYP3A4), and mainly excreted through the biliary system. Asunaprevir is 98.8% bound to serum proteins[14, 15].

The pharmacokinetics of asunaprevir were studied in non-HCV infected subjects with CP-A/B/C and compared with healthy volunteers; they were comparable in CP-A subjects and controls. Maximum plasma concentration (C_{max}) and AUC increased 10- and 5-fold in CP-B subjects and 23- and 32-fold in CP-C subjects, respectively. Therefore, it is not recommended that CP-B/C patients be treated with asunaprevir. Protein binding in all groups was >99.5% and the unbound fraction was ± 0.004[14].

Asunaprevir was studied in non-HCV subjects dependent on dialysis compared with healthy controls. Protein binding, C_{max}, AUC, and trough concentration (C_{trough}) were not affected by dialysis[16]. Comparable results were presented in an open-label study in HCV-uninfected subjects with normal (GFR >90 mL/min), mild (GFR 50-89 mL/min), moderate (GFR 30-49 mL/min), or severe (GFR <30 mL/min) renal disease or patients dependent on dialysis. Subjects received asunaprevir, daclatasvir, and beclabuvir (NS5B inhibitor). ESRD subjects had slightly decreased asunaprevir concentrations. Subjects with moderate and severe renal impairment had increased C_{max} (65 and 100%) and AUC (50 and 76%) values, respectively, compared with controls[17].

NS5A inhibitors

Daclatasvir

Daclatasvir is an NS5A inhibitor that is administered at a dosage of 60 mg QD. Daclatasvir is highly bound to plasma proteins (99%). It is hepatically metabolized (CYP3A4) and is a substrate of P-gp. Biliary excretion is the major route of elimination.

Compared with healthy volunteers, C_{max}, and AUC (total daclatasvir = unbound and bound drug) values were lower in non-HCV patients with CP-A/B/C after a single dose of daclatasvir 30 mg. However, there was no influence on the unbound fraction of daclatasvir when CP-B/C patients were compared with HCV-infected controls[19, 33].

Patients with mild, moderate, severe, or end-stage renal disease had increased unbound daclatasvir AUCs of 18, 39, 51, and 20%, respectively, compared with normal renal function. A similar trend was seen in total daclatasvir exposure. Although the exposure was affected, the authors concluded that no dose adjustments are necessary in patients with renal impairment and that these differences are within the high inter- individual variability of daclatasvir pharmacokinetics[19, 34].

The steady-state pharmacokinetics of daclatasvir 60 mg have been studied in combination with asunaprevir and beclabuvir in patients with moderate and severe renal impairment, showing increased exposure of daclatasvir (C_{max} 35 and 45%, and AUC 50 and 65%, respectively). Patients undergoing dialysis had comparable pharmacokinetic parameters as healthy subjects[17].

NS5B polymerase inhibitors

Sofosbuvir

Sofosbuvir is an NS5B polymerase inhibitor that is administered at 400 mg QD. Sofosbuvir is intracellular metabolized into the active metabolite GS-461203, followed by dephosphorylation resulting in the inactive compound GS-331007. GS-331007 is primarily renally excreted (78% of the administered dose). Sofosbuvir is a substrate of P-gp and Breast Cancer Resistance Protein (BCRP) and is 61-65% bound to plasma proteins. GS-331007 is minimally bound to plasma proteins[21, 35].

In a study of the pharmacokinetic properties of sofosbuvir, the steady-state AUC of 400 mg sofosbuvir following a 7-day dosing in CP-B and CP-C patients increased 126 and 143%, respectively, relative to control subjects. The GS-331007 AUC was slightly increased: 18 and 9%[20]. Lawitz and co-authors[36] reported increased C_{max} and AUC values

of sofosbuvir 80 and 130%, respectively, in patients with hepatic impairment (CP-B and CP-C) compared with non-cirrhotic controls. The pharmacokinetics of GS-331007 were similar in these three groups.

In patients with mild, moderate, and severe renal insufficiency sofosbuvir, AUC values were elevated by 61, 107, and 171% compared with controls. GS-331007 AUC values were 55, 88, and 451% higher in these patients. Administration before and after dialysis influenced the exposure to GS-331007 as it is removed during dialysis. After 4 hour of dialysis, 18% of the administered dose had been removed[20, 37]. A study comparing sofosbuvir 400 mg every day or only on the day of dialysis showed that there was no accumulation of sofosbuvir or GS-331007 in both treatment groups[38]. Gane and co-authors treated patients with severe renal impairment with daily sofosbuvir 200 mg and low-dose ribavirin. Compared with historical controls the patients had comparable sofosbuvir exposure and fourfold higher GS-331007 concentrations[39]. A recently presented study of ten patients describing the steady-state pharmacokinetics of sofosbuvir in patients with a GFR <30 mL/min (mean creatinine clearance 26.2 mL/min) showed comparable results. Exposure to GS-331007 and sofosbuvir increased 6- and 1.4-fold, respectively, compared with patients with normal renal function[40].

The manufacturer does not recommend using sofosbuvir in patients with severe renal impairment or ESRD, since studies are still ongoing (NCT01958281[41]). The main issue might be the increased exposure to GS-331007 (AUC 451%). This is caused by decreased clearance of GS-331007. However, increased exposure of GS-331007 is not associated with increased toxicity[42].

Several small studies and case reports have shown that both low-dose (200 mg) and normal-dose (400 mg) sofosbuvir were overall well-tolerated[38, 43]. Pending more definite results of ongoing studies, we recommend patients be treated with sofosbuvir 400 QD (GFR <30 mL/min or ESRD) in case there is no safer DAA option available. We base this advice on a number of arguments. First, accumulation of sofosbuvir does not take place in patients dependent on dialysis, suggesting that a standard dosage of 400 QD will produce similar concentrations of active intracellular metabolites independent of renal function[38]. Secondly, (interim) analyses of small studies show that sofosbuvir at standard doses is well-tolerated in these patient groups. Lastly, data are available for the sustained virological response at week-12 (SVR12) of patients treated with half-dose sofosbuvir, which varied from 40 to 90%[40, 44]. Patients treated with sofosbuvir 400 QD reached SVR12 in 60-100% of cases[40, 45]. These results suggest that a reduced dose of the prodrug sofosbuvir may result in lower concentrations of active intracellular metabolites.

Fixed-Dose regimens

Ledipasvir/sofosbuvir

Ledipasvir is an NS5A inhibitor available in a fixed-dose tablet with sofosbuvir containing sofosbuvir 400 mg and ledipasvir 90 mg. The metabolism of ledipasvir is unknown but unchanged ledipasvir is mainly found in feces, indicating biliary excretion. It is a substrate of P-gp and BCRP and it is >99.8% bound to plasma proteins[21].

No relevant differences were seen in between pharmacokinetics of control patients with normal hepatic function and CP-C patients after a dose of ledipasvir 60 mg[21]. Single and multiple doses of ledipasvir 30 mg (in combination with 200 mg of the investigational PI vedroprevir) resulted in a reduction of the C_{max} (36%) and an extended elimination half-life ($T_{1/2}$) in CP-C patients (84.4 versus 45.7 hours in healthy subjects). The free fraction of ledipasvir increased in patients with severe hepatic impairment (0.21 versus 0.11% in healthy subjects). No significant changes were seen between CP-B patients and control subjects[46].

No pharmacokinetic differences were observed between healthy subjects and patients with severe renal impairment, although no safety data are available for patients with GFR <30 mL/min or ESRD[21, 47].

The pharmacokinetics of sofosbuvir in patients with impaired renal and hepatic function are discussed previously.

Velpatasvir/sofosbuvir

Velpatasvir is a novel NS5A inhibitor that will probably be licensed in a fixed-dose tablet with sofosbuvir (100 mg/400 mg)[22, 48]. Velpatasvir is primarily metabolized by the liver and excreted through the biliary system. Velpatasvir is substrate of P-gp and OATPs, and strong inducers or inhibitors of CYP influence the plasma concentration of velpatasvir, suggesting it is a substrate of CYP enzymes[49, 50].

Non-HCV subjects with CP-B and CP-C received a single dose of velpatasvir 100 mg and the AUC from time zero to infinity (AUC_{∞}) was comparable with subjects with normal hepatic function: AUC_{∞} decreased 17% and increased 14%, respectively. However, C_{max} in both groups decreased ~50% and the unbound fraction increased with decreasing hepatic function[51].

Table 2: Dosage recommendations for patients with mild, moderate, severe renal insufficiency or end-stage renal disease.

HCV drug	Normal renal function	Degree of renal impairment				Removed by dialysis?
	GFR >90 mL/min	Mild (GFR 50-89 mL/min)	Moderate (GFR 30-49 mL/min)	Severe (GFR 15-29 mL/min)	ESRD (GFR <15 mL/min)	
Simeprevir	150 mg QD [11]	150 mg QD [11]	150 mg QD [11]	150 mg QD [11]a	150 mg QD [11]a	No
Asunaprevir[b]	100 mg BID [17]	100 mg BID [17]	100 mg BID [17]	100 mg QD [17]	100 mg BID [67, 17]	Unknown
Daclatasvir	60 mg QD [19]	60 mg QD [19]	60 mg QD [19]	60 mg QD [19] or 30 mg QD in combination with Asunaprevir [17]	60 mg QD [19]	No
Sofosbuvir	400 mg QD [20]	400 mg QD [20]	400 mg QD [20]	400 mg QD [44, 43]c	400 mg QD [44, 38, 43]c	Yes, administer after dialysis
Velpatasvir/ sofosbuvir[b]	100 mg QD/ 400 mg QD [22]	Unknown	Unknown	Unknown	Unknown	Unknown
Ledipasvir/ sofosbuvir	90 mg QD/ 400 mg QD [21]	90 mg QD/ 400 mg QD [21]	90 mg QD/ 400 mg QD [21]	Unknown	Unknown	Ledipasvir = no[21] GS-331007 = yes[20]
Grazoprevir/ elbasvir	100 mg QD/ 50 mg QD [26]	100 mg QD/ 50 mg QD [26]	100 mg QD/ 50 mg QD [26]	100 mg QD/ 50 mg QD [26]	100 mg QD/ 50 mg QD [26]	Elbasvir = no Grazoprevir = negligible[44, 26]
Paritaprevir/ Ritonavir/ Ombitasvir	150 mg QD/ 100 mg QD/ 25 mg QD [27]	150 mg QD/ 100 mg QD/ 25 mg QD [27]	150 mg QD/ 100 mg QD/ 25 mg QD [27]	150 mg QD/ 100 mg QD/ 25 mg QD [27]	150 mg QD/ 100 mg QD/ 25 mg QD [68]	Unknown

Table 2: Dosage recommendations for patients with mild, moderate, severe renal insufficiency or end-stage renal disease. (*continued*)

HCV drug	Normal renal function	Degree of renal impairment				Removed by dialysis?
	GFR >90 mL/min	Mild (GFR 50-89 mL/min)	Moderate GFR 30-49 mL/min)	Severe (GFR 15-29 mL/min)	ESRD (GFR <15 mL/min)	
Dasabuvir	250 mg BID [31]	250 mg BID [31]	250 mg BID [31]	250 mg BID [31]	250 mg BID [68]	Unknown
Ribavirin	<75 kg = 500 mg BID ≥75 kg = 600 mg BID [32]	<75 kg = 500 mg BID ≥75 kg = 600 mg BID [32]	Loading dose: <75 kg = 500 mg BID for 1 day ≥75 kg = 600 mg BID for 1 day. Followed by alternating 200 and 400 mg QD[69] TDM ribavirin[d]	Loading dose: <75 kg = 500 mg BID for 1 day ≥75 kg = 600 mg BID for 1 day. Followed by 200 mg QD[69] TDM ribavirin[d]	Loading dose: <75 kg = 500 mg BID, for 1 day ≥75 kg = 600 mg BID for 1 day. Followed by 200 mg QD[69] TDM ribavirin[d]	No[32]

[a]The SmPC for OLYSIO™ states that exposure may be increased in HCV-infected patients with severe renal impairment, caution is recommended when prescribing OLYSIO™ to these patients.

[b]No SmPC or FDA Prescribing Information was available at the time of publication.

[c]The SmPC for Sovaldi® (Gilead Sciences, Inc, Foster City, CA, USA) states that the safety and appropriate dose of Sovaldi® have not been established in patients with severe renal impairment (eGFR <30 mL/min/1.73 m^2) or ESRD requiring hemodialysis. These recommendations are made when no other DAA regimen is available.

[d]The SmPC for Rebetol® (Merck & Co., Inc., Whitehouse Station, NJ, USA), states that patients with Cl_{cr} <50 mL/min must not be treated with Rebetol®. The Prescribing Information for Copegus® (Genentech USA, Inc., South San Francisco, CA, USA) states that the dose should be reduced in patients with Cl_{cr} <50 mL/min as described in the table. No loading dose is advised (expert opinion).

BID: Twice daily; Cl_{cr}:Creatinine clearance; DAA: Direct-acting antiviral; eGFR: estimated glomerular filtration rate; ESRD: End-stage renal disease; FDA: Food and Drug Administration; GFR: Glomerular filtration rate; HCV: Hepatitis C virus; SmPC: Summary of Product Characteristics; QD: Once daily.

A study in HCV-uninfected subjects with GFR <30 mL/min showed that renal insufficiency had a modest influence on the pharmacokinetics of velpatasvir (single dose 100 mg). C_{max} was increased 11% and AUC_{∞} increased 50%[52]. Further studies are ongoing and the results are still pending (NCT02185794[53]).

Grazoprevir/elbasvir

Grazoprevir (PI) and elbasvir (NS5A inhibitor) are newly licensed in the USA and data from phase-III studies were recently published. Grazoprevir is a substrate of CYP3A4, P-gp, and OATPs and prescribed in a dosage of 100 QD[54]. Exposure to grazoprevir was approximately one- to two-fold higher in HCV patients than in healthy controls[55-57].

Elbasvir is prescribed in a dose of 50 QD. It is a substrate of CYP3A4, P-gp, and OATP[58]. Both elbasvir and grazoprevir are highly hepatically metabolized and less than 1% is renally extracted[54, 59].

Jacobson and co-authors[60] presented pharmacokinetic data of grazoprevir plus elbasvir in HCV patients with CP-B. CP-B patients received grazoprevir 50 mg and elbasvir 50 mg and healthy controls received normal-dose grazoprevir and elbasvir. Despite the reduced dose, grazoprevir AUC and C_{trough} values were increased 30 and 73%, respectively, compared with controls. Elbasvir exposure was comparable between these two groups[60]. However, the fixed-dose combination is only available in a dose of grazoprevir 100 mg and elbasvir 50 mg; therefore, and due to a lack of safety and efficacy data, the combination is contra-indicated for CP-B and CP-C patients[26].

Pharmacokinetic data are available in non-HCV patients with GFR <30 mL/min and in patients dependent on dialysis. Dialysis did not influence the steady-state pharmacokinetics of both grazoprevir and elbasvir. Grazoprevir was slightly removed by dialysis (<0.5%) and elbasvir was not removed. Subjects with GFR <30 mL/min (not on dialysis) had increased grazoprevir and elbasvir exposure. AUC and C_{trough} values of grazoprevir were elevated 65 and 60%, compared with controls (GFR >80 mL/min). Elbasvir pharmacokinetics showed similar results: AUC was 86% higher and C_{trough} was 107% higher. The unbound fraction of grazoprevir was comparable between the three treatment groups. The unbound fraction of elbasvir was below the limit of detection[26, 44].

Paritaprevir/ritonavir, ombitasvir, and dasabuvir

The fixed-dose combination of paritaprevir (75 mg), ritonavir (50 mg), and ombitasvir (12.5 mg) is administered as two tablets QD with or without dasabuvir 250 mg BID.

Paritaprevir is a second-generation PI, which is a substrate of CYP3A4/5, P-gp, OATP1B1, and OATP1B3. Ritonavir is added to improve the pharmacokinetics of paritaprevir by inhibiting CYP3A4 ('boosting'). Paritaprevir itself also inhibits various drug-transporters and is 97-98.6% bound to plasma proteins. After hepatic metabolism paritaprevir is excreted through the biliary system. CP-C patients had 3.2- and 9.5-fold higher C_{max} and AUC values than control subjects[27, 28]. Paritaprevir is contra-indicated in CP-B/C patients. The unbound fraction was 1.1% in subjects with normal hepatic function and 0.78, 0.75, and 1.2% by patients with CP-A, CP-B, and CP-C, respectively.

In patients with mild, moderate, and severe renal insufficiency, the AUC of paritaprevir increased by 19, 33, and 45%. C_{max} was comparable with control subjects[27].

Ombitasvir is an NS5A inhibitor and highly metabolized: only 8.9% of the unchanged drug is excreted and a total of 13 metabolites were identified. Amide hydrolysis and oxidative metabolism are responsible for its biotransformation. Ombitasvir is >99.9% bound to plasma proteins and biliary excretion is the major elimination pathway. In CP-C patients, ombitasvir reduced the AUC and C_{max} values by 68 and 54%, respectively. The unbound fraction of ombitasvir increased from ~0.020% in control subjects and CP-A/B patients to 0.047% in CP-C patients[27, 28]. Ombitasvir exposure was not affected by any degree of renal insufficiency[27].

Dasabuvir is an NS5B polymerase inhibitor and a substrate of CYP2C8, CYP3A4, P-gp, BCRP, and organic cation transporter (OCT) 1. Dasabuvir is hepatically metabolized into seven metabolites, of which M1 accounts for 21% of the administered dose. However, unchanged dasabuvir accounts for 60% of the exposure. Dasabuvir is >99.5% and M1 94.5% bound to plasma proteins. The AUC values of dasabuvir and M1 were equal in healthy controls and CP-A patients. CP-B patients had reduced dasabuvir and M1 AUC values (16 and 57% respectively). CP-C patients had elevated AUCs for dasabuvir and M1: 325 and 77%, respectively[28, 31]. Dasabuvir unbound fractions were lower in patients with CP-A, CP-B, and CP-C: 0.29, 0.28, and 0.42% respectively (control subjects: 0.61%). The unbound fraction of M1 in control subjects was 5.8% and was 5.1, 5.4 and 6.8% in CP-A, CP-B and, CP-C patients[28]. Due to the elevated AUC of dasabuvir (and M1) in CP-C patients, dasabuvir is contra-indicated in these patients.

The AUC of dasabuvir decreased in patients with mild (21%), moderate (37%), and severe (50%) renal insufficiency. As exposure slightly declines in patients with renal impairment, no dose adjustments are required in these patients[31].

To conclude, paritaprevir/ritonavir plus ombitasvir with or without dasabuvir can be used safely in patients with any stage of renal impairment. Due to a recent FDA announcement, the label for this combination regimen has been updated, stating that paritaprevir/ritonavir, and ombitasvir with or without dasabuvir is contra-indicated for both CP-B and CP-C patients. These changes have been made based on results from post-marketing surveillance showing liver decompensation and liver failure in patients with advanced cirrhosis (CP-B/C) (n = 26) after 1-4 weeks of treatment[29, 30].

Other antivirals

Ribavirin

Ribavirin is a guanine analog with activity against a range of RNA and DNA viruses. Ribavirin is always prescribed as part of a combination therapy. In general practice, ribavirin is administered in a weight-based dose (<75 kg = 1,000 mg/day; ≥75 kg = 1,200 mg/day), although this may differ by genotype and commercial product[32, 61]. The $T_{1/2}$ of ribavirin is ~300 hours and approximately 61% of the administered dose is renally excreted, of which 17% was unchanged ribavirin. The site of metabolism is unknown but two metabolizing pathways are involved: (1) a reversible phosphorylation pathway: and (2) a degradative pathway involving deribosylation and amide hydrolysis[61]. It is notable that the ratio of whole blood:plasma is 60:1 and the volume of distribution (V_d) 5,000 L, which is caused by the extensive accumulation of ribavirin in the erythrocytes[32, 61].

The SmPC states that the pharmacokinetics of ribavirin are similar in control subjects and patients with CP-A/B/C and therefore no dose adjustments were deemed necessary in patients with cirrhosis[32]. By contrast, a single-dose study described an increased C_{max} with increasing severity of cirrhosis (the AUC was not significantly different between those patient groups)[62].

Patients with moderate or severe renal impairment had 20 to 30% higher ribavirin exposure despite adjusted daily doses of 600 and 400 mg, respectively. ESRD patients had 20% lower ribavirin plasma exposure when given 200 mg daily, than did subjects with GFR >80 mL/min receiving the standard dose[32].

Brennan and co-authors[63] studied steady-state plasma concentrations in patients with renal impairment. Data were hard to interpret, because many dose adjustments were necessary due to toxicity in patients with moderate and severe renal insufficiency. At week 12 of treatment, these patients had 36 and 25% higher AUCs, with adjusted daily doses of 600 and 400 mg, respectively, compared with control subjects. The apparent total clearance of ribavirin from plasma (CL/F) was 20.0 L/h in patients with normal

renal function but decreased in patients with renal insufficiency, ranging from 5 to 6 L/h[63]. In a single-dose study, increased AUC and decreased clearance were linearly correlated with the severity of renal dysfunction (single dose of 400 mg)[64].

Taking into account the information from the literature and our clinical experience with ribavirin[65, 66], we recommend a weight-based loading dose of ribavirin, followed by 200 mg QD in patients with severe renal dysfunction or ESRD. We also recommend alternating 200 and 400 mg QD in patients with moderate renal function. Steady-state plasma concentrations of ribavirin are directly achieved using a loading dose, which is necessary due to the long $T_{1/2}$. Ribavirin is not removed by dialysis and these patients often have lower hemoglobin levels. Caution is needed due to accumulation of ribavirin in the erythrocytes causing hemolysis. If available, therapeutic drug monitoring can be helpful to individualize treatment with ribavirin in patients with impaired and/or variable renal function.

EFFICACY AND SAFETY

HCV therapy consists of combinations of drugs, and therefore efficacy and safety are mostly evaluated in patients using combination therapy, making data hard to interpret for only one drug. Efficacy and safety data are given in Table 3 for CP-A/B/C patients. Table 4 presents the data for patients with mild, moderate, severe, and end-stage renal disease; only multiple-dose studies performed in HCV patients are included.

Protease inhibitors

Simeprevir

Adverse events (AEs) were retrospectively reported in 22% of CP-A/B HCV genotype (GT) 1 patients (n = 119) treated with simeprevir and sofosbuvir ± ribavirin. SVR was reached in 78% of the CP-A/B patients (n = 84), of whom 29% were CP-B patients[70]. Another retrospective study, in which CP-B/C patients were treated with sofosbuvir and simeprevir, reported that 9% of patients discontinue due to AEs (CP-A = 1% discontinued). These patients were hospitalized more often than CP-A patients. Furthermore, 91% of the CP-A patients reached SVR versus 73% of the CP-B/C patients[71]. Both the EMA and FDA have warned for possible safety issues with CP-B/C patients because simeprevir safety data are lacking[11, 18].

In an observational study including ESRD patients with or without dialysis (n = 17) treated with simeprevir and sofosbuvir, 23% of the patients reported AEs. No patient discontinued treatment due to an AE[45]. Trials describing treatment with a reduced dose of sofosbuvir and simeprevir are discussed below[72, 73].

Asunaprevir

An open-label, randomized, uncontrolled trial with daclatasvir 30 mg, asunaprevir 200 mg, and beclabuvir 75 mg, all BID, reported SVR-rates of ~94% in naive cirrhotic patients. Treatment-experienced patients had SVR-rates ≥87%. Ribavirin increased treatment response. Nine patients experienced a serious AE (SAE) and three patients discontinued the study due to AEs. The authors concluded that most AEs were caused by ribavirin and that there were no significant differences between cirrhotic and non-cirrhotic patients[74]. In a trial including genotype 1- and 4-infected cirrhotic patients (METAVIR score F3/4; n = 223), an SVR of 84% was reported after treatment with daclatasvir and asunaprevir. Pooled analyses of four phase-II/III studies showed that SVR was reached in 84% of genotype 1b cirrhotic patients (n = 229). No meaningful differences in safety

were described between cirrhotic and non-cirrhotic patients. Overall, most reported AEs were headache, fatigue, nausea, and diarrhea (≥10%)[75].

An SVR of 96% was reached in dialysis-dependent genotype 1 patients when treated with daclatasvir 60 mg and asunaprevir 100 mg (n = 21). Of these patients, 97% experienced any AE. Anemia (29%) and nasopharyngitis (29%) were most the commonly reported AEs[67].

NS5A inhibitors

Daclatasvir

The safety and efficacy of daclatasvir in cirrhotic patients was studied in combination with asunaprevir or sofosbuvir ± ribavirin. In combination with sofosbuvir 400 mg, an SVR of 83% was reached in CP-A/B/C patients (phase-III trial). SVR-rates in CP-B/C patients were lower: 56%. In this trial anemia (20%), fatigue (18%), and nausea (17%) were the most commonly reported AEs, of which 18% were grade 3-4[76]. Another open-label, phase-III study included patients with cirrhosis/advanced fibrosis (genotype 3) who were treated with sofosbuvir 400 mg and daclatasvir 60 mg (n = 50). These patients most commonly reported insomnia (30%), headache (24%), and fatigue (20%) as AEs[77]. No SVR was reported in this preliminary analysis.

Daclatasvir, in combination with asunaprevir and beclabuvir, was overall well-tolerated by patients with renal impairment. SAEs were reported in 67% of the patients and SVR was ≥96% (n = 21)[67].

NS5B polymerase inhibitors

Sofosbuvir

No SAEs were reported in a multiple-dose study where 400 mg of sofosbuvir was administered to HCV-infected CP-B/C patients (n = 17)[36]. Sofosbuvir-containing regimens were in general well-tolerated in patients with advanced cirrhosis. Fatigue, nausea, headache, and anemia were the most frequently reported AEs (≥10%)[78, 79]. SVR-rates varied from 29 to 91%, depending on genotype, Child-Pugh score, and other DAAs (Table 3)[70, 71, 78, 79].

Table 3: Overview of efficacy and safety of hepatitis C virus medication in hepatitis C virus infected patients with Child-Pugh class A, B, or C.

Population and treatment (dose)	Study design	Number	SVR-rates (%)	Patients with SAEs (%)	Patients with AEs (%)	Patients who: discontinued (%) / discontinued due to (S)AEs (%)	(S)AEs reported with rates ≥10%	Ref
CP-A/B, GT 1 Simeprevir 150 mg QD, sofosbuvir 400 mg QD ± weight-based ribavirin	Retrospective	119	CP-A: 78 CP-B: 29	1	22	NR / 3	Anemia (72% ribavirin group)	[70]
Cirrhosis, GT 1 and 4 Simeprevir 150 mg QD, sofosbuvir 400 mg QD ± ribavirin	Prospective, open-label	108	77	NR	NR	NR / 0	Fatigue (40%) Nausea (10%) Headache (10%)	[79]
CP-A, GT 1 **CP-B/C, GT 1** Simeprevir 150 mg QD, sofosbuvir 400 mg QD ± ribavirin	Retrospective	101 55	91 73	NR NR	NR NR	1 / 1 11 / 9	NR NR	[71]
Cirrhosis, GT 2 Sofosbuvir 400 mg QD ± weight-based ribavirin[b]	Phase-IV, open-label	66	79	12	88	10 / 5	Fatigue (33%) Anemia (24%) Nausea (18%) Headache (17%) Hemoglobin <10 g/dL (12%)	[78]
CP-A/B/C, GT 1-6 Daclatasvir 60 mg QD, sofosbuvir 400 mg QD + 600 mg ribavirin potential adjustments up to 1,000 mg	Phase-II, prospective, open-label	60	83	17	18 (grade 3/4)	NR / 2	Anemia (20%) Fatigue (18%) Nausea (17%) Total bilirubin >2.5 X ULN (15%) Headache (15%) Lymphocytes <0.5 x 10^9/l (10%)	[76]

Table 3: Overview of efficacy and safety of hepatitis C virus medication in hepatitis C virus infected patients with Child-Pugh class A, B, or C. (*continued*)

Population and treatment (dose)	Study design	Number	SVR-rates (%)	Patients with SAEs (%)	Patients with AEs (%)	Patients who: discontinued (%) / discontinued due to (S)AEs (%)	(S)AEs reported with rates ≥10%	Ref
Cirrhosis, advanced fibrosis, GT 3 Daclatasvir 60 mg QD, sofosbuvir 400 mg QD + weight-based ribavirin	Phase-III open-label, randomized	50	SVR4 ≥88	NR	NR	NR / 0	Insomnia (30%) Fatigue (26%) Headache (24%)	[77]
Cirrhosis -Fibroscan ≥14.6 kPa. GT 1b Daclatasvir 60 mg QD, asunaprevir 100 mg BID	Phase-III	223	84	6	NR	NR / 1	Headache (25%) Fatigue (20%) Diarrhea (16%) Nausea (11%)	[75]
Compensated cirrhosis (F4), GT 1 Daclatasvir 30 mg BID, asunaprevir 200 mg BID, beclabuvir 75 mg BID ± weight-based ribavirin[b]	Phase-III, open-label, randomized, uncontrolled	202	≥87	5	NR	NR / 4	Headache (20%) Fatigue (12%) Nausea (15%) Diarrhea (11%) Insomnia (10%) Pruritus (10%)	[74]
CP-B, GT 1, 3 **CP-C, GT 1, 3** Ledipasvir 90 mg QD, sofosbuvir 400 mg QD ± weight-based ribavirin[b]	Phase-II, open-label, randomized	59 49[a]	≥82 ≥91	22 35	98 100	NR / 5 NR / 10	NR NR	[80]

Table 3: Overview of efficacy and safety of hepatitis C virus medication in hepatitis C virus infected patients with Child-Pugh class A, B, or C. (*continued*)

Population and treatment (dose)	Study design	Number	SVR-rates (%)	Patients with SAEs (%)	Patients with AEs (%)	Patients who: discontinued (%) / discontinued due to (S)AEs (%)	(S)AEs reported with rates ≥10%	Ref
CP 5-7, GT 1 and 4 Ledipasvir 90 mg QD, sofosbuvir 400 mg QD, ± weight-based ribavirin[b]	Randomized, double-blind, placebo-controlled	155	≥96	NR	91	NR / 1	Asthenia (52%) Headache (34%) Pruritus (19%) Insomnia (19%) Nausea (14%) Fatigue (14%) Cough (14%) Diarrhea (12%) Myalgia (11%) Arthralgia (11%) Bronchitis (11%) Back pain (10%) Dry skin (10%) Irritability (10%)	[81]

Table 3: Overview of efficacy and safety of hepatitis C virus medication in hepatitis C virus infected patients with Child-Pugh class A, B, or C. (*continued*)

Population and treatment (dose)	Study design	Number	SVR-rates (%)	Patients with SAEs (%)	Patients with AEs (%)	Patients who: discontinued (%) / discontinued due to (S)AEs (%)	(S)AEs reported with rates ≥10%	Ref
CP-B, GT 1-4, and 6 Velpatasvir 100 mg QD, sofosbuvir 400 mg QD (12 weeks)	Phase-III, open-label, randomized.	90	83	19	81	NR / 1	Fatigue (26%) Nausea (24%) Headache (26%) Platelets 25,000 to <50,000 per mm^3 (17%) Lymphocytes 350 to <500 per mm^3 (11%) Pruritus (11%) Insomnia (10%)	[23]
Velpatasvir 100 mg QD, sofosbuvir 400 mg QD ± weight-based ribavirin[b]		87	94	16	91	NR / 5	Fatigue (39%) Nausea (25%) Anemia (31%) Lymphocytes <500 per mm^3 (28%) Hemoglobin <10 g/dl (23%) Headache (21%) Diarrhea (21%) Insomnia (14%) Muscle spasm (11%)	
Velpatasvir 100 mg QD, sofosbuvir 400 mg QD (24 weeks)		90	86	18	81	NR / 4	Platelets 25,000 to <50,000 per mm^3 (11%) Fatigue (23%) Nausea (20%) Platelets 25,000 to <50,000 per mm^3 (20%) Headache (19%) Insomnia (10%)	

Table 3: Overview of efficacy and safety of hepatitis C virus medication in hepatitis C virus infected patients with Child-Pugh class A, B, or C. (*continued*)

Population and treatment (dose)	Study design	Number	SVR-rates (%)	Patients with SAEs (%)	Patients with AEs (%)	Patients who: discontinued (%) / discontinued due to (S)AEs (%)	(S)AEs reported with rates ≥10%	Ref
CP-A, GT 1c Grazoprevir 100 mg QD, elbasvir 50 mg QD ± ribavirin: 51-65 kg 800 mg, 66-80 kg 1,000 mg 81-105 kg 1,200 mg 106-125 kg 1,400 mg	Phase-II, parallel group, open-label, randomized	253	≥90	3	79	NR / 1	Fatigue (26%) Headache (23%) Asthenia (14%)	[82]
CP-A, GT 1 Grazoprevir 100 mg QD, elbasvir 50 mg QD, sofosbuvir 400 mg QD	Phase-II, open-label, interim analysis	41	SVR8 = 95 (n = 19)	5	NR	NR / 3	NR	[25]
CP-B, GT 1 Grazoprevir 50 mg QD, elbasvir 50 mg QD	Phase-II, interim analysis	30	EOT = 100 (n = 25)	13	87	NR / 0	Fatigue (30%) Arthralgia (17%) Hyperbilirubinemia grade 3/4 (13%) Nausea (10%) Pyrexia (10%) Headache (10%)	[60]
CP-A, GT 1 Paritaprevir 150 mg QD, ritonavir 100 mg QD, ombitasvir 25 mg QD, dasabuvir 250 mg BID ± weight-based ribavirin[b]	Phase-III, randomized	380	~94	6	91	NR / 2	Fatigue (39%) Headache (29%) Nausea (19%) Pruritus (19%) Insomnia (17%) Diarrhea (16%) Asthenia (13%) Rash (13%) Irritability (10%)	[83]

Table 3: Overview of efficacy and safety of hepatitis C virus medication in hepatitis C virus infected patients with Child-Pugh class A, B, or C. (*continued*)

Population and treatment (dose)	Study design	Number	SVR-rates (%)	Patients with SAEs (%)	Patients with AEs (%)	Patients who: discontinued (%) / discontinued due to (S)AEs (%)	(S)AEs reported with rates ≥10%	Ref
CP ≥5 and ≤7, GT 4 Paritaprevir 150 mg QD, ritonavir 100 mg QD, ombitasvir 25 mg QD ± weight-based ribavirin	Phase-II, randomized, open-label. Ongoing study	111	SVR not reported in abstract	4	NR	2 / 0	Fatigue (~15%) Headache (~15%)	[84]

[a]Pre-transplant patients.

[b]Weight-based ribavirin = 500 mg BID for <75 kg or 600 mg BID ≥75 kg.

[c]Data reported of total cohort, including non-cirrhotic patients.

AE: Adverse event; BID: Twice daily; CP-A: Child-Pugh class A; CP-B: Child-Pugh class B; CP-C Child-Pugh class C; EOT: End-of-treatment; GT: Genotype; NR: Not reported; QD: Once daily; SAE: Serious adverse event; SVR: Sustained virological response; SVR4: Sustained virological response at week 4; SVR8: Sustained virological response at week 8; ULN: Upper limit of normal; Ref: Reference.

Sofosbuvir was well-tolerated in dialyses-dependent patients treated with sofosbuvir 200 mg daily or 400 mg every day or on the day of dialysis[38]. However, as discussed, the SVR12 of patients treated with half-dose sofosbuvir, varied from 40 to 90%[40, 44, 72, 73]. Two other recently presented studies (*n* = 17 and 10) concluded that full-dose sofosbuvir (400 QD) had good tolerability and was safe in patients with GFR <30 mL/min with and without dialysis. SVR12 was reached in 60-100% of the patients[40, 45]. There was no evidence of an elevated risk of sofosbuvir-related toxicity. No cardiac toxicity was reported[40]. A longitudinal, observational cohort reported SVR-rates of 85% (*n* = 18), 81% (*n* = 63), and 88% (*n* = 168) in patients with severe, moderate, and mild renal insufficiency, respectively, who were treated with sofosbuvir-containing regimens[43].

Fixed-Dose regimens

Ledipasvir/sofosbuvir

A phase-II study with ledipasvir and sofosbuvir ± ribavirin reported AEs in 98% of the CP-B (*n* = 59) and 100% of the CP-C (*n* = 49) patients. Of these patients, 22 and 35%, respectively, experienced an SAE. SVR was reached in ≥82% and ≥91% of the CP-B and CP-C patients, respectively[80]. A randomized, double-blind, placebo controlled trial reported >96% SVR. These patients had a CP-score of 5-7. Asthenia (52%) and headache (34%) were the most common AEs in this trial[81].

Studies in patients with renal impairment and ledipasvir are still ongoing (NCT01958281[41]).

Velpatasvir/sofosbuvir

A randomized, double-blind, placebo-controlled trial described the use of velpatasvir in HCV patients with CP-B in combination with sofosbuvir (genotype 1-6). Different treatment regimens were used (Table 3) and SVRs ≥83% were reported. Overall, this combination was well-tolerated. The most reported AEs were fatigue, nausea, and headache (≥20%). In total, nine patients discontinued treatment due to an AE[23].

No studies have been published yet describing the safety of velpatasvir in HCV patients with renal insufficiency.

Table 4: Overview of efficacy and safety of hepatitis C virus medication in hepatitis C virus infected patients with mild, moderate, severe renal insufficiency, and end-stage renal disease.

Population and treatment (dose)	Study design	Number	SVR-rates (%)	Patients with SAEs (%)	Patients with AEs (%)	Patients who: discontinued (%) / discontinued due to (S)AE s (%)	(S)AEs reported with rates ≥10%	Ref
GFR ≤15 mL/min or dialysis, GT 1 Sofosbuvir 400 mg every other day	Open-label, real-life experience	4	91	0	0	NR / 0	Fatigue (20%) Anemia (13%) Rash/itching (13%)	[72]
Sofosbuvir 200 mg QQ, simeprevir 150 mg QD		11	75					
GFR <30 mL/min or ESRD, GT 1-3 Sofosbuvir 200 mg QD Sofosbuvir 400 mg every other, simeprevir 150 mg QD	Observational, interim-analysis	28	90 (n = 21/23)	10	NR	NR / NR	Fatigue (20%) Anemia (15%) Rash/itching (10%)	[73]
GFR <15 mL/min or ESRD, GT 1a Sofosbuvir 400 mg QD, simeprevir 150 mg QD	Observational cohort	17	94	NR	23	0 / 0	Insomnia (12%)	[45]
GFR <30 mL/min, GT 1, 3 Sofosbuvir 200 mg QD, ribavirin 200 mg QD	Open-label	10	40	20	100	NR / 10	Anemia (50%) Headache (40%) Pruritus (30%) Rash (30%) Muscle spasms (20%) Hypoesthesia 20%) Insomnia (20%) Irritability (20%)	[39]

Table 4: Overview of efficacy and safety of hepatitis C virus medication in hepatitis C virus infected patients with mild, moderate, severe renal insufficiency, and end-stage renal disease. (*continued*)

Population and treatment (dose)	Study design	Number	SVR-rates (%)	Patients with SAEs (%)	Patients with AEs (%)	Patients who: discontinued (%) / discontinued due to (S)AE s (%)	(S)AEs reported with rates ≥10%	Ref
GFR <30 mL/min, GT 1 and 3 Sofosbuvir 400 mg QD, ribavirin 200 mg QD	Open-label	10	SVR not reported in abstract	20	NR	20 / 20	Anemia (40%) Dizziness (20%) Grade 3 laboratory AEs (60%) Grade 4 laboratory AEs (10%)	[40]
GFR ≤30 mL/min Sofosbuvir-containing regime for GT 1-6 + ribavirin	Longitudinal, observational cohort	18	85 (n = 11/13)	19	NR	9 / 6	Anemia (31%) Fatigue (19%) Nausea (19%)	[43]
GFR 31-45 mL/min Sofosbuvir-containing regime for GT 1-6 + ribavirin		63	81 (n = 30/37)	24	NR	8 / 5	Fatigue (37%) Anemia (28%) Headache (16%)	
GFR 46-60 mL/min Sofosbuvir-containing regime for GT 1-6 + ribavirin		168	88 (n = 108/123)	5	NR	4 / 2	Nausea (15%) Fatigue (33%) Anemia (23%) Nausea (19%) Headache (12%)	
Dialysis, GT 1 Daclatasvir 60 mg QD, asunaprevir 100 mg BID	Prospective, observational	21	96	5	67	NR / 5	Nasopharyngitis (29%) Anemia (29%) Increased ALT (14%) Pyrexia (10%)	[67]
GFR <29 mL/min, GT 1a Grazoprevir 100 mg QD, elbasvir 50 mg QD	Phase-III, randomized, placebo-controlled	111	~95	15	76	NR / 0	Headache (17%) Nausea (15%) Fatigue (10%)	[59]

Table 4: Overview of efficacy and safety of hepatitis C virus medication in hepatitis C virus infected patients with mild, moderate, severe renal insufficiency, and end-stage renal disease. (*continued*)

Population and treatment (dose)	Study design	Number	SVR-rates (%)	Patients with SAEs (%)	Patients with AEs (%)	Patients who: discontinued (%) / discontinued due to (S)AE s (%)	(S)AEs reported with rates ≥10%	Ref
GFR <30mL/min, GT 1 Paritaprevir 150 mg QD, ritonavir 100 mg QD, ombitasvir 25 mg QD, dasabuvir 250 mg BID ± ribavirin 200 mg QD	Phase-I, open-label, interim analysis	20	EOT = 100 (n = 14) SVR4 = 100 (n = 10)	10	NR	0 / 0	Anemia (40%) Fatigue (30%) Nausea (25%) Diarrhea (25%) Dizziness (15%) Headache (15%) Decreased appetite (10%) Irritability (10%) Edema peripheral (10%) Weight decrease (10%)	[68]

[a]Immediate treatment group.

AE: Adverse event; ALT: Alanine aminotransferase; BID: Twice daily; EOT: End-of-treatment; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; GT: Genotype; NR: Not reported; QD: Once daily; SAE: Serious adverse event; SVR: Sustained virological response; SVR4: Sustained virological response at week 4; Ref: Reference.

Grazoprevir/elbasvir

The safety and efficacy of grazoprevir plus elbasvir ± ribavirin in CP-A patients was studied in a phase-II trial. SVR was reached in more than 90% of the patients (*n* = 253). More discontinuations due to AEs (2 versus 0%) and drug-related AEs (71 versus 54%) were seen in the patients treated with grazoprevir, elbasvir, and ribavirin. The regimen was well-tolerated by the patients[82]. Another phase-II trial showed that a reduced dose of grazoprevir (50 mg) and normal-dose elbasvir was well tolerated in CP-B patients (*n* = 30) despite the increased exposure. Fatigue (30%) was the most reported AE and end-of-treatment (EOT) response was 100% (*n* = 25/30)[60].

As described earlier, increased exposure to grazoprevir and elbasvir was reported in patients with a GFR <30 mL/min without dialysis. A phase-III study presented safety data for grazoprevir 100 mg and elbasvir 50 mg in patients with GFR ≤29 mL/min (*n* = 111). High rates of AEs were reported (76%), but these were comparable with the placebo group (84%). SAEs and laboratory abnormalities were also comparable between groups. Taking these results in combination with high SVR-rates (~95%) it was concluded that this regimen is safe and effective for the use in patients with renal insufficiency[59].

Paritaprevir/ritonavir, ombitasvir, and dasabuvir

The fixed-dose combination of paritaprevir/ritonavir, ombitasvir, and dasabuvir plus ribavirin in CP-A patients was studied by Poorded et al[83]. Of these patients, 91% reported AEs, of whom 6% had an SAE. Only 2% of patients discontinued the study due to AEs. More AEs were seen during 24 weeks of treatment than during 12 weeks of treatment (phase-III trial) and SVR-rates were approximately 94% (*n* = 380). As discussed, the label of this combination regimen was adjusted due to information that became available during post-marketing surveillance.

During a phase-I trial, this combination was studied in patients with severe renal insufficiency (*n* = 20). An interim analysis shows, that EOT response was 100% (*n* = 14/20). All patients completed the trial but experienced AEs such as anemia (40%), fatigue (30%), nausea (25%), and diarrhea (25%)[68].

Other antivirals

Ribavirin

In the past, ribavirin was frequently used in combination with peg-interferon alfa, but it is used now as part of DAA regimens. Anemia was frequently reported in trials where cirrhotic patients were treated with ribavirin, varying from 2 to 40% dependent on the combination treatment[70, 72, 76, 85, 86].

Patients with severe and moderate renal impairment who were treated with peg-interferon alfa and a daily dose of ribavirin 400 or 600 mg, respectively, frequently needed dosage reductions (71 and 54%). Dosage reductions were required in 22 and 23% of ESRD patients treated with a daily dose of ribavirin 200 mg and subjects with normal renal function, respectively. ESRD patients had a safety profile comparable with that of subjects with normal renal function[32, 63].

DISCUSSION

Influence of hepatic impairment on drug pharmacokinetics

Liver cirrhosis is the main complication of chronic HCV infection. Cirrhosis changes the liver architecture, into one with nodules causing reduction in hepatic blood flow, portal systemic shunting, capiliarization of sinusoids, and loss in number and function of hepatocytes. The liver is the main metabolizing organ, and therefore these changes have a profound influence on metabolism and elimination of drugs[6].

Cirrhosis curtails the metabolizing capacity of the liver, due to decreased levels of CYP enzymes. Various CYP enzymes are affected (e.g., CYP3A4, CY2A6, CYP2C9), but the susceptibility depends of the type and severity of the liver disease. For example, CYP1A2 and CYP2C19 are sensitive to liver disease, whereas CYP2E1 is less susceptible. These changes in CYP enzymes may cause increased drug concentrations of enzyme substrates. This explains the increased exposure of grazoprevir and paritaprevir in cirrhotic patients, because these drugs are metabolized by CYP3A4. Likewise, the increased sofosbuvir concentration or increased $T_{1/2}$ of ledipasvir might be related to the reduced capacity of the liver to metabolize drugs by enzymes other than CYP enzymes, e.g., uridine 5'-diphospho-glucuronosyltransferase (UGT) conjugation is affected in cirrhotic livers. In addition, efflux transporters may be upregulated, while uptake transporters may be downregulated. These alterations are not yet fully understood[87]. However, they provide an explanation of increased simeprevir and grazoprevir concentrations, because OATP uptake transporters may be down-regulated, causing decreased uptake from the circulation into hepatocytes, resulting in increased plasma concentrations.

Reduced hepatic metabolism affects the first-pass effect. This pre-systemic metabolism is responsible for metabolizing orally administered drugs prior to entering the systemic circulation. Decreased pre-systemic metabolism results in elevated bioavailability, as seen with paritaprevir and grazoprevir. In addition, portal shunting affects hepatic blood flow. Blood bypasses the liver, leading to an increased systemic drug concentration, as a result of decreased hepatic metabolism.

In cirrhosis, the liver produces less drug-binding proteins (albumin, alpha$_1$-acid glycoprotein). Fewer proteins are available, and thus the unbound fraction of drugs may be elevated. Only this unbound fraction of drugs is available for uptake in the tissues and therefore is responsible for the pharmacological effect of a drug. The increased unbound fraction might even cause toxicity despite the total exposure being decreased. This was seen in ombitasvir: the unbound fraction increased twofold in patients with severe hepatic impairment but the total AUC was decreased[28]. On the other hand, the AUC of total daclatasvir was decreased, but the unbound fraction of daclatasvir remained unchanged in patients with cirrhosis due to increased clearance of free daclatasvir. This means that no differences were found in the active concentration of daclatasvir and therefore there was no need for dose modifications[33].

Finally, the ribavirin C_{max} was increased in patients with cirrhosis compared with controls. Ribavirin is renally cleared and extensively metabolized (site unknown). These changes in C_{max} may be caused by alterations in, for example, hepatic drug-transporter activity; however, these findings could also be caused by the higher inter-individual variability of ribavirin. Since other pharmacokinetic parameters were not affected, no dose adjustments are necessary when using ribavirin in cirrhotic patients.

Figure 2 summarizes the physiological alterations during cirrhosis that influence drug concentrations and Table 1 shows the recommended doses or contra-indications for CP-A/B/C patients.

Influence of renal impairment on drug pharmacokinetics

The prevalence of HCV in dialysis patients in Europe and the USA varies from 3 to 20%. In 2002, 8% of the dialyzed patients in the USA were infected with HCV[88]. HCV is both a cause and a consequence of renal impairment: first, patients on dialysis have an increased infection risk due to medical procedures and, secondly, HCV causes pathological changes to the kidneys[88, 89].

Renal dysfunction influences the renal clearance of drugs. Glomerular filtration, tubular secretion, and tubular reabsorption are responsible for renal clearance of drugs. The clearance may be altered due to damage to glomeruli or by altered activity of drug-transporters in tubular cells. In general, renal impairment results in increased drug concentrations of renally cleared drugs.

Patients with ESRD are often dependent on dialysis. An important factor that affects the clearance of drugs is the molecular weight of the drug in relation to the pore size of the membrame in the dialyzer. Other parameters influencing drug clearance during hemo-

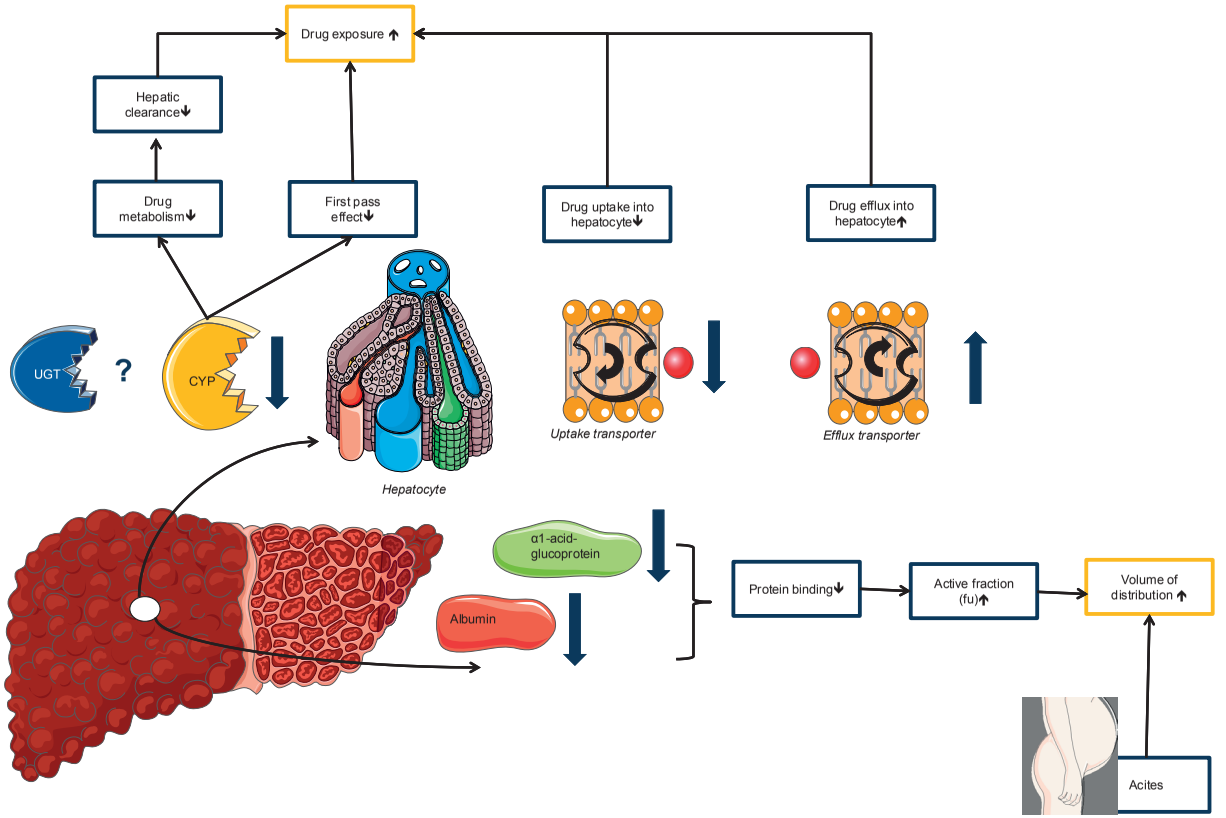


Figure 2: Overview of the pathophysiological changes in patients with liver cirrhosis that influence drug-metabolism and therefore the pharmacokinetics of drugs.

CYP: Cytochrome P450; UGT: Uridine 5′-diphospho-glucuronosyltransferase; ↓ indicates decrease; ↑ indicates increase.

dialysis are protein binding, V_d, water solubility, and plasma clearance. Characteristics of the dialyzer are also important for the pharmacokinetics of drugs, e.g., the flow of the blood and dialysate, and the concentration gradient. As described previously most HCV drugs are highly bound to plasma proteins, which explains why these drugs are not removed by dialysis (Table 4) as only unbound drugs can be removed by dialysis. Additionally, most drugs are metabolized by the liver, and therefore the contribution of hemodialysis to the clearance of drugs is relatively low[90].

In general, DAAs are hepatically cleared, meaning no dose modifications are necessary in patients with renal impairment. However, sofosbuvir and ribavirin, which are primarily renally eliminated, are exceptions.

Exposure to GS-331007 increased with a decreasing GFR, but -as explained earlier- dose modification may not always be advisable. Sofosbuvir is removed during dialysis and thus it is recommended that it is administered after dialysis[20].

Ribavirin is mainly situated in the erythrocyte and not effectively cleared from the body during hemodialysis[63]. This causes increased plasma concentrations, which are related to (severe) anemia. However, while higher ribavirin plasma concentrations are related to anemia, they are also associated with improved SVR. Plasma concentrations of ribavirin can explain toxicity or give information regarding whether the ribavirin exposure is sufficient. In other words: is the patient treated with the right dose[66, 91-93]? Due to toxicity, dose modifications are needed in ribavirin-treated patients who have a GFR ≤50 mL/min and in ESRD patients.

A second consideration is that non-renal clearance is affected in patients with renal disease. It should be noted that this phenomenon is mostly studied in patients with ESRD. CYP enzymes, UGT enzymes, and drug-transporters have altered activity in patients with renal insufficiency, but protein-binding may also decrease. One hypothesis is that the uremic toxins cause these alterations in drug-transporters and enzymes; dialysis then removes these toxins, which improves CYP3A- and transporter-related clearance[94]. These alterations in hepatic function may affect hepatically cleared drugs such as daclatasvir, grazoprevir, and elbasvir. Daclatasvir exposure (bound and unbound) was elevated in patients with decreasing renal function[34]. Similarly, grazoprevir and elbasvir exposure increased significantly in patients with a GFR <30 mL/min[26, 44].

In conclusion, it is hard to predict what the influence of renal function on the exposure of drugs is. This should be taken into account when interpreting the dose recommendations in Table 2.

Studies in patients with renal and hepatic impairment during clinical development

The effect of renal- or hepatic impairment on the pharmacokinetics of HCV drugs is often studied in single-dose studies in HCV-negative patients with renal insufficiency or liver cirrhosis. These studies give an idea of the pharmacokinetics in these patient groups, but the influence of HCV is missing. The virus itself may also influence drug-metabolism, as inflammation and infection are known to affect CYP activity in the liver[95, 96].

Therefore, studies in HCV-infected patients with renal or hepatic impairment are performed after licensing and post-marketing surveillance takes place (e.g., collecting AEs). These findings might then be used to change the Prescribing Information. For example, the labels of both simeprevir and paritaprevir/ritonavir, plus ombitasvir with or without dasabuvir were recently changed due to information that became available after licensing. In our opinion, these studies should be part of the pre-registration process, since HCV patients suffer from these conditions and therefore they will be treated with the novel DAAs. In comparison, sofosbuvir is contra-indicated for patients with a GFR <30 mL/min, because no safety studies have been performed and data are missing. This might exclude patients unnecessarily from treatment.

CONCLUSION

This review described the pharmacokinetics, efficacy, and safety of HCV drugs in patients with renal and hepatic dysfunction. All of the available drugs for the treatment of HCV can be used in patients with CP-A and in those with a GFR ≥30 mL/min. Some drugs are contra-indicated in patients with advanced liver disease (CP-B or CP-C), and sofosbuvir plus ledipasvir or daclatasvir are the best options for this group. Patients with GFR <30 mL/min can be treated with grazoprevir plus elbasvir or paritaprevir/ritonavir, and ombitasvir with or without dasabuvir. Sofosbuvir is an important part of HCV therapy, and therefore data on its use renally impaired patients is essential information; however, data on sofosbuvir are still pending. Lastly, it would be helpful if more pharmacokinetics, efficacy, and safety data became available for the treatment of patients with advance liver disease or severe renal impairment. These patients might benefit the most from therapy, possibly preventing the need for liver transplantation.

CONFLICT OF INTEREST

EJS and CTMMK declare that they have no conflicts of interest that are directly relevant to the content of this review. BH has been a member of advisory boards of AbbVie, BMS, Falk, Janssen, and MSD and has participated recently in clinical trials for AbbVie, BMS, Gilead, Janssen, and Merck. JEA has been a member of advisory boards of AbbVie, BMS, Gilead, Janssen, Merck, and ViiV and has received sponsorship/research grants from AbbVie and BMS. JPHD has been a member of advisory boards of AbbVie, BMS, Gilead, Janssen, and Merck and received sponsorship/research grants from AbbVie and Janssen. DMB has been a member of advisory boards of AbbVie, BMS, Gilead, Janssen, and Merck and has received sponsorship/research grants from BMS, Janssen, Merck, and ViiV.

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Pharmacokinetics of daclatasvir in cirrhotic patients: challenges in physiology-based pharmacokinetic modeling

EJ Smolders
EPH Colbers
DM Burger
R Greupink

In preparation

ABSTRACT

Daclatasvir is an NS5A inhibitor used for the treatment of chronic hepatitis C virus (HCV) infection. Being a substrate of cytochrome P450 (CYP) 3A4 and P-glycoprotein (P-gp), hepatic metabolism and biliary excretion are important for daclatasvir clearance. Since HCV eventually leads to liver cirrhosis, physiology-based pharmacokinetic (PBPK) modeling was used to describe daclatasvir pharmacokinetics (PK) in healthy volunteers and cirrhotic patients. The Simcyp population-based PBPK modeling platform was used to investigate performance of a daclatasvir PBPK model. PK was simulated following single dosages (SD; 10, 30, 100 mg) and multiple dosages (MD; 30, 60 mg) in healthy volunteers. MD simulations were also performed in presence of CYP3A4 inhibitors darunavir/ritonavir and atazanavir /ritonavir. Simulated data were compared to reported clinical values. AUC-ratios (simulated/observed) of SD daclatasvir in healthy subjects varied from 0.74-0.89 and C_{max}-ratios varied from 0.93-1.13. MD simulations and interaction studies resulted in AUC and C_{max}-ratios which were considered acceptable, ranging from 0.37-0.87 and 0.72-1.43, respectively. Subsequently, SD of 30 mg daclatasvir were simulated in the Simcyp virtual Child-Pugh class A, B, and C populations. However, we were not able to recover *in vivo* exposure in cirrhotic patients with this model. Given the involvement of intestinal and hepatic drug-transporters in daclatasvir clearance, and the fact that changes in transporter expression levels are not captured in the current (patho)physiological PK model, a more detailed charting of the impact of cirrhosis on these aspects of daclatasvir disposition appears required before its PK and drug-drug interactions may be simulated reliably within this patient population.

INTRODUCTION

Hepatitis C virus (HCV) infection is transmitted through blood-blood contact and is nowadays effectively treated with direct-acting antivirals (DAAs). Daclatasvir is a DAA and, in combination with sofosbuvir, high cure rates are achieved (>90%). Daclatasvir is, among others, a substrate of cytochrome P450 (CYP) 3A4, P-glycoprotein (P-gp), and organic cation transporter (OCT) 1. It is actively and passively transported into the hepatocyte where it is metabolized. Daclatasvir is mainly cleared from the body via biotransformation and biliary excretion, as a mass-balance study showed a recovery of 88% of a single oral dose in feces of which 53% was the unchanged drug[1, 2].

HCV replicates in hepatocytes, ultimately leading to hepatocyte cell death, causing chronic liver injury which may subsequently progress to liver cirrhosis. Patients with cirrhosis have abnormal liver structure caused by scarring of the liver tissue. Cirrhosis leads to reduced liver blood flow (e.g. portal-systemic shunting) and reduced liver function (e.g., decreased number of hepatocytes and production of albumin). These changes could eventually lead to clinical manifestations such as ascites and encephalopathy[3-5]. According to international guidelines, the treatment goal of HCV treatment is reduction of HCV transmission and decreasing the mortality caused by liver diseases (cirrhosis and hepatocellular carcinoma)[6, 7].

The liver is the main drug-metabolizing organ and loss of hepatic function influences the pharmacokinetics (PK) of a compound that is subject to biotransformation and/or biliary excretion; for example, in cirrhotic patients, metabolism by CYP enzymes, first pass effect, protein binding, and hepatic uptake by transporters may be altered[8-12]. As a result, drugs that are likely to be used in cirrhotic patients, or that are >20% metabolized in the liver must be studied in cirrhotic patients, during drug development or post-marketing studies. The importance of this is recognized by the regulatory authorities: both the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) recommend to study drugs in patients with Child-Pugh (CP) class A, B and C[13, 14].

There are some concerns when studying DAAs in cirrhotic patients. First of all, it is challenging to recruit HCV-infected patients with compensated or decompensated liver disease, i.e. covering the range of CP classifications. In addition, studying PK of these drugs in these patients, could also be considered unethical, as exposure to the compound could possibly be sub- or supratherapeutic, causing viral resistance or toxicity, respectively. Therefore, non-HCV-infected cirrhotic patients are usually included in these kind of PK studies, which are, of course, also vulnerable patients exposed to a compound from which they will not benefit.

A tool that can help to better understand the exposure of compounds in cirrhotic patients, without any *in vivo* study, is physiology-based pharmacokinetic (PBPK) modeling. This is a mechanistic approach in which absorption, distribution, metabolism, and excretion (ADME) as well as the resulting clinical pharmacokinetic profile of a compound can be predicted by combining physicochemical and *in vitro* pharmacokinetic data from a compound, with human anatomical and (patho)physiological information in a mathematical model[9, 11]. *In vitro* to *in vivo* extrapolation (IVIVE) of PK is also mentioned by the EMA for these patients, whereas the FDA has drafted a white paper, outlining the requirements of such models in order to use the approach in pharmaceutical drug development[14, 15].

We now explored a mechanistic model to simulate the PK of daclatasvir in healthy volunteers and cirrhotic patients. The objective was to simulate and predict the PK of total and unbound daclatasvir in these patient populations, including drug-drug interactions (DDI) taking place at the level of CYP3A4. Secondly, we aimed to identify knowledge gaps that hamper a more accurate PBPK modeling and simulation effort of daclatasvir PK in cirrhotic patients.

MATERIALS & METHODS

PBPK modeling platform

Simcyp Population-Based Pharmacokinetic Simulator version 15, release 1 (Simcyp Limited, a Certara company, Sheffield, UK) was used as a PBPK platform. Simulations were performed using Simcyp virtual populations of healthy volunteers, as well as CP-A, B, and C liver cirrhotic patients. For each simulation, the number of patients, gender, and age range of the virtual population were matched with clinical data sets used for validation.

PBPK workflow

Two available populations in the database of Simcyp were used: healthy volunteers and cirrhotic patients. Simulations in healthy volunteers were done to determine the performance of the model. We used single dosages of daclatasvir (10, 30, and 100 mg) and multiple dosage of daclatasvir (30 mg and 60 mg once daily [QD]). In addition, we used 30 mg daclatasvir in combination with two strong CYP3A4 inhibitors to confirm robustness of the model and to test the influence of alterations in CYP3A4/5 metabolism (1) darunavir/ritonavir (800/100 mg QD) and (2) atazanavir/ritonavir (300/100 mg QD). The effect of these interactions on daclatasvir systemic exposure were studied at steady-state concentrations.

After establishing adequately simulated daclatasvir plasma concentrations in single dose, multiple dose, and DDI studies in healthy volunteers, we moved to the simulation of 30 mg daclatasvir in CP-A, B, and C patients. Subsequently, we simulated the PK of CYP3A4 (probe) substrate midazolam in the same populations (15 mg oral QD). We included midazolam for comparison purposes as midazolam is cleared via biotransformation only, in contrast to daclatasvir which is also actively transported by hepatic drug-transporters. The midazolam model available in Simcyp was used for these simulations.

Physicochemical and in vitro pharmacokinetic parameters of daclatasvir

For this study, we used a modified model which was previously presented[16, 17], but which was modified in order to accommodate an active liver sinusoidal uptake transport step. The physicochemical and *in vitro* PK parameters of daclatasvir used to parameterize the model are presented in Table 1. Main assumptions were that next to CYP3A4, CYP2C8 contributed to metabolic clearance, as fraction of the dose metabolized could not be explained by bottom-up CYP3A4 clearance only. In order to estimate daclatasvir distribution, we assumed the drug followed general lipid:water partitioning, as well as protein, phospholipid binding rules[18], except for distribution to the liver where transporters were assumed to be involved in daclatasvir hepatic uptake. In addition, P-gp was included in the model, being involved in intestinal absorption and biliary efflux[16, 17].

P-gp canalicular efflux in the liver was described to be 88 pmol/min/million cells with a K_m of 8.16 µM and a relative activity factor/relative expression factor (RAF/REF) of 1.6.

The previously published model did not include active sinusoidal uptake into the liver, despite that both passive and active uptake is described in the product label[1]. Therefore, we added a sinusoidal hepatic uptake clearance of 177 µL/min/million cells, based on the mean of two reported *in vitro* uptake experiments with hepatocytes. The involved transporters remain unknown from the experiment[1]. We used a passive diffusion uptake clearance of 0.0412 mL/min/million cells based on observed uptake in an experiment in hepatocytes in which active uptake processes via OCT1 and organic anion transporter (OAT) 2 were assumed to be fully blocked with pyrimethamine and probenecid, respectively[1].

For simulations, we used a full PBPK distribution model with an Advanced Dissolution, Absorption, and Metabolism model (ADAM) absorption module and permeability-limited liver model. Volume of distribution at steady-state (V_{ss}) was calculated using the prediction method described by Rodgers and Rowland[18]. This overestimated V_{ss} was subsequently scaled to the clinically observed value of 0.5507 L/kg (Kp scalar 0.1565) [16, 17].

Table 1: Physicochemical and *in vitro* pharmacokinetic parameters of daclatasvir.

Parameters	Values	Ref[16, 17]
Physicochemical and blood binding		
Molecular Weight	738.96	
log $P_{o:w}$	4.05	
Compound Type	Diprotic base	
pKa 1	5.6	
pKa 2	4.9	
Blood:plasma ratio	0.8	
f_u	0.006	
Absorption: ADAM model		
Predicted $f_u$gut	0.0007854	
Predicated permeability	8.9783	
PAMPA (x10^{-6} cm/s)	49	
Distribution: Full PBPK Model		
Predicted V_{ss} (l/kg)	0.5507 (Method 2: Rodgers and Rowland)	
Kp Scalar	0.1565	
Elimination: enzyme kinetics, recombinant		
Unidentified CYP: CYP2C8		
CL_{int} (µL/min/pmol)	0.32	
$f_u$mic	0.35	
CYP3A4		
V_{max} (pmol/min/pmol)	0.575	
K_m (µM)	2.53	
CYP3A5		
V_{max} (pmol/min/pmol)	0.0957	
K_m (µM)	9.14	
Available for re-absorption (%)	80	
Transport – Liver		
CL_{pd} (ml/min/million cells)	0.0412	[1]
Canalicular efflux liver: P-gp		
J_{max} (pmol/min/million cells)	88	
K_m (µM)	8.16	
RAF/REF	1.6	
Sinusoidal uptake liver		[1]
CL_{int} (µL/min/1x x10^{-6} cells)	177	
$f_u$inc	1	
RAF/REF	1	

CYP: Cytochrome P450; CL_{int}: Intrinsic clearance; CL_{pd}: Passive diffusion; f_u: Fraction unbound; $f_u$inc: Fraction unbound in the incubation medium; $f_u$mic: Fraction unbound microsomes; J_{max}: Maximum observed flux; k_a: Absorption rate constant; K_i: Inhibitory constant; Log P: Partition coefficient; PAMPA: Parallel artificial membrane permeability assay; P-gp: P-glycoprotein; p*K*a: Acid dissociation constant; V_{ss}: Volume of distribution at steady-state; K_m: Michaelis-Menten constant; V_{max}: Maximum rate; RAF/REF: Relative activity factor / relative expression factor; Ref: Reference.

Simulation of drug-drug interactions

To study the interactions of daclatasvir with darunavir/ritonavir and atazanavir/ritonavir, four compound files were created. Two compound files were created for ritonavir, one in addition with darunavir, and another in addition with atazanavir, as ritonavir PK parameters are different when ritonavir is combined with either darunavir or atazanavir[19]. We used the 50% inhibitory concentrations (IC_{50}) of darunavir, atazanavir, and ritonavir against the different CYP enzymes and transporters in order to mechanistically simulate the influence of these compounds on daclatasvir metabolic clearance. Note that in this respect we adopted a semi-mechanistic approach as we did not build full PBPK models for the interacting drugs, and we only aimed to describe the influence of these inhibitors on daclatasvir PK.

For the compound file of darunavir and ritonavir we used information described by our group before[20]. We included *in vitro* data on drug-metabolizing enzymes and transporters, in order to simulate the interaction with daclatasvir PK (Table 2)[20]. Secondly, compound files of both atazanavir and ritonavir were created using data available in the public domain (Table 3).

Liver cirrhosis virtual populations

Disease severity of cirrhotic patients is clinically classified using the CP-score, which contains three classes of severity: CP-A, B, and C. This system was developed to determine short term prognosis in cirrhotic patients and one must consider that this classification is not based on etiology of the liver disease. The CP-score is calculated using five clinical parameters: albumin, prothrombin time, bilirubin, ascites, and encephalopathy. CP-A patients have a score of 7-9 (well compensated disease), CP-B a score of 10-15 (functional compromised disease), and CP-C a score >15 (decompensated disease)[35].

Table 2: Physicochemical and *in vitro* pharmacokinetic parameters of darunavir/ritonavir.

Parameters	Darunavir values	Ref	Ritonavir values	Ref
Physicochemical and blood binding				
Molecular Weight	548	[21]	720.95	[22]
log $P_{o:w}$	1.8	[23]	4.3	a
Compound Type	Monoprotic base		Monoprotic base	
pKa 1	2.39	[23]	2	a
Blood:plasma ratio	0.64	[24, 25]	0.587	a
f_u	0.06	[24, 25]	0.02	
Absorption	ADAM		First-Order Absorption Model	
f_a	–		1	a
k_a (1/h)	–		0.24 CV%:30	a
$f_u$gut	0.05	[20]	0.02	a
Q_{gut}	–		11.16	a
Predicted permeability	3.40		3.10	
Caco-2 (10^{-6} cm/s)	18.9	[26]	–	
Apical pH 6.5: basolateral pH 7.4 (Passive & Active)				
Distribution	Full PBPK model		Minimal PBPK model	
V_{ss} (l/kg); CV%	Predicted: 1.24 (method 2)		0.41; 30	a
Kp Scalar	6	[20]	–	
Elimination: In vivo clearance				
CL_{po} (L/h); CV (%)	–		16; 30	[27]
CL_r (L/h)	–		0.32	[20]
CL_{iv} (L/h); CV (%)	5.9; 30	[28]		
Available for re-absorption (%)	80	[20]		
Interaction				
Competitive inhibition				
CYP2B6 (K_i (μM); $f_u$mic)	24; 1	[25]	–	
CYP2C9 (K_i (μM); $f_u$mic)	24; 1	[25]	4; 0.29	[20]
CYP3A4 (K_i (μM); $f_u$mic)	0.4; 1	[25]	0.03; 0.976	[20]
CYP2D6 (K_i (μM); $f_u$mic)	24; 1	[25]	10; 0.29	[20]
CYP2C19 (K_i (μM); $f_u$mic)	24; 1	[25]		
Mechanism-based inhibition				
CYP3A4 (K_{app}; k_{inact} (1/h); $f_u$mic)	–		0.1; 0.32; 0.91	[29]
Induction				
CYP3A4 ($IndC_{50}$^ (μM; CV%; $f_u$inc)	–		0.5; 30; 1	a
Transporter				
OATP1B1 (K_i (μM); $f_u$inc)	–		1.4; 1	[30, 20]b
OATP1B3 (K_i (μM); $f_u$inc)	–		2.5; 1	[30, 20]b
P-gp (K_i (μM); $f_u$inc)	–		0.2; 0.233	[31]

[a]Simcyp compound library.

[b]Based on reported K_i for organic anion transporting polypeptide 1B3 (2.5 μM).

CL_{po}: Oral clearance; CL_r: Renal clearance; CL_{iv}: Intravenous clearance; CYP: Cytochrome P450; f_a: Fraction absorbed; f_u: Fraction unbound; $f_u$inc: Fraction unbound in the incubation medium; $f_u$mic: Fraction unbound microsomes; $IndC_{50}$: The concentration that yields half of the maximum fold induction; k_a: Absorption rate constant; K_{app}: Apparent absorption; K_i: Inhibitory constant; k_{inact}: Maximum inactivation rate; Log P: Partition coefficient; P-gp: P-glycoprotein; p*Ka*: Acid dissociation constant; V_{ss}: Volume of distribution at steady-state; Ref: Reference.

Table 3: Physicochemical and *in vitro* pharmacokinetic parameters of atazanavir/ritonavir.

Parameters	Atazanavir values	Ref	Ritonavir values	Ref
Physicochemical and blood binding				
Molecular Weight	704.856	[32]	720.95	[22]
log $P_{o:w}$	4.08	[33]	4.3	a
Compound Type	Monoprotic Base		Monoprotic base	
pKa 1	4.2	[33]	2	a
Blood:plasma ratio	Predicted: 0.55		0.587	a
f_u	0.14	[33]	0.02	
Absorption: First-Order Absorption Model				
f_a	Predicted: 0.998		1	a
k_a (1/h)	Predicted: 3.07		0.24 CV%:30	a
$f_u$gut	1	a	0.02	a
Q_{gut}	Predicted: 14.78		11.15705	a
Predicted permeability	7.46	a	3.096	
MechPeff model: P_{trans}, 0 (10^{-6} cm/s)	1188.166	a	–	
Distribution: Minimal PBPK model				
V_{ss} (l/kg); CV%	1.44 CV V_{ss}%: 35	[34]b	0.7; 29.3	[34]b
Elimination: In vivo clearance				
CL_{po} (L/h); CV (%)	5.41; 26	[34]b	8.83; 29.4	[34]b
CL_r (L/h)	–		0.32	[20]
Interaction				
Competitive inhibition				
CYP1A2 (K_i (μM); $f_u$mic)	12; 1	[32]	–	
CYP2C9 (K_i (μM); $f_u$mic)	12; 1	[32]	4; 0.29	[20]
CYP3A4 (K_i (μM); $f_u$mic)	2.35; 1	[32]	0.03; 0.976	[20]
UGT1A1(K_i (μM); $f_u$mic)	1.9; 1	[32]	–	
CYP2D6 (K_i (μM); $f_u$mic)	–		10; 0.29	[20]
P-gp	29; 1	[32]	–	–
Mechanism-based inhibition				
CYP3A4 (K_{app}; k_{inact} (1/h); $f_u$mic)	–		0.1; 0.32; 0.91	[29]
Induction				
CYP3A4 ($IndC_{50}$^ (μM; CV%; $f_u$inc)	–		0.5; 30; 1	a
Transporters				
OATP1B1 (K_i (μM); $f_u$inc)	–		1.4; 1	[30, 20]c
OATP1B3 (K_i (μM); $f_u$inc)	–		2.5; 1	[30, 20]c
P-gp (K_i (μM); $f_u$inc)	–		0.2; 0.233	[31]

[a]Simcyp compound library.

[b]Data on file.

[c]Based on reported K_i for organic anion transporting polypeptide 1B3 (2.5 μM).

CL_{po}: Oral clearance; CL_r: Renal clearance; CV: Coefficient of variation; CYP: Cytochrome P450; f_a: Fraction absorbed; f_u: Fraction unbound; $f_u$inc: Fraction unbound in the incubation medium; $f_u$mic: Fraction unbound microsomes; $IndC_{50}$: The concentration that yields half of the maximum fold induction; k_a: Absorption rate constant; K_{app}: Apparent absorption; K_i: Inhibitory constant; k_{inact}: Maximum inactivation rate; Log P: Partition coefficient; P-gp: P-glycoprotein; p*K*a: Acid dissociation constant; UGT: Uridine 5′-diphospho-glucuronosyltransferase; V_{ss}: Volume of distribution at steady-state; Ref: Reference.

Three virtual populations available in Simcyp correspond to these three CP-classes of cirrhotic patients. The virtual populations were based on the demographic, genetic, anatomic, and physiological parameters described in literature. In brief, the parameterization of the cirrhotic populations were used: demographics were adapted for cirrhotic patients. Because liver cirrhosis is more prevalent in males than in females, so the proportion of males was set on 66%. Secondly, decreased liver size is described for cirrhotic patients, and thus the availably of functional hepatocytes. Therefore, the liver volume of CP-A, B, and C patients was set on 81, 65, and 53% compared with the controls (100%). Important for daclatasvir metabolism is the expression of CYP enzymes which alters with increasing severity of cirrhosis. The CYP3A4 activity in the control population was set on 137 pmol/mg. For CP-A, B, and C patients this was reduced to 80.8, 53.2, and 34.2 pmol/mg. Comparable for CYP2C8, the activity in the control population was 24 pmol/mg which reduced to 7.9 pmol/mg in CP-C patients. The amount of CYP3A4 in nmol per total gut was lowered: from 70 to 59, 40 and 25 for respectively controls, CP-A, B, and C subjects. Albumin plasma concentrations were 40 g/L in the control group and declined to 26.3 g/L in the CP-C patients. Also, alpha$_1$-acid glycoprotein, hematocrit, cardiac output, portal blood flow, and glomerular filtration rate (GFR) decreased with severity increasing CP-class. Hepatic arterial blood flow and villous blood flow increased with severity of cirrhosis. When no data was available on possible changes in physiological and biochemical parameters, data from Caucasian healthy volunteers were used[11].

Evaluation of model performance

The performance of the model was checked by visual inspection of the PK curves and the simulated PK parameters area under the concentration-time curve (AUC) and maximum plasma concentration (C_{max}) were compared with *in vivo* data from literature. Acceptance criteria for the model were defined as follows: both the geometric mean of C_{max} and AUC should not deviate more than 2-fold from the observed PK parameters, as is commonly applied in assessing PBPK model performance. We calculated an AUC and C_{max}-ratio by dividing the simulated data with the observed data. The simulated data are presented as geometric mean (GM) with 95% confidence intervals (CI) of both unbound and total (bound + unbound) daclatasvir concentrations. The fraction unbound (f_u) is calculated dividing the unbound concentration with the total concentration.

All the simulated data were compared with *in vivo* (observed) data available in literature. Daclatasvir simulations of single dosages 10 and 100 mg were compared with Wang et al[16, 17] and 30 mg with Bifano et al[36]. Multiple dose simulations were compared with data from Wang et al[16, 17] (30 and 60 mg). Daclatasvir PK parameters with darunavir/ritonavir and atazanavir/ritonavir were compared with results from the study of Gandhi

et al[37] and Smolders et al[34], respectively. The simulations of daclatasvir in the cirrhotic population, were compared with the studies from Bifano et al[36] and Lawitz et al[38].

RESULTS

Simulation of daclatasvir pharmacokinetics single and multiple dose in healthy volunteers

Daclatasvir simulations of single dosages of 10, 30, and 100 mg were done and presented in Table 4 and Figure 1. The simulated PK parameters and the shape of the curves for all dosages were comparable with the observed *in vivo* PK results of total daclatasvir in healthy subjects[16, 17, 36]. The AUC-ratios varied from 0.74-0.89 and C_{max}-ratios varied from 0.93-1.13 for these three dosages of daclatasvir. These results were within our acceptance criteria.

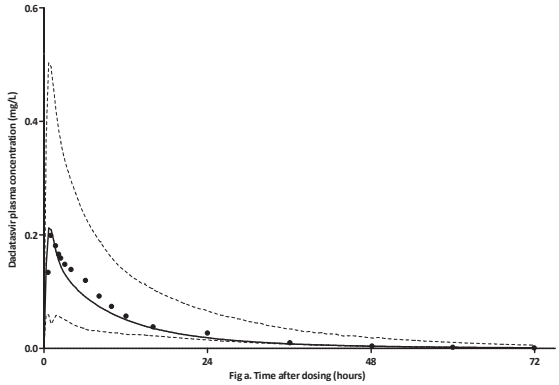
Table 4: Pharmacokinetic parameters calculated from single and multiple dose simulations of daclatasvir.

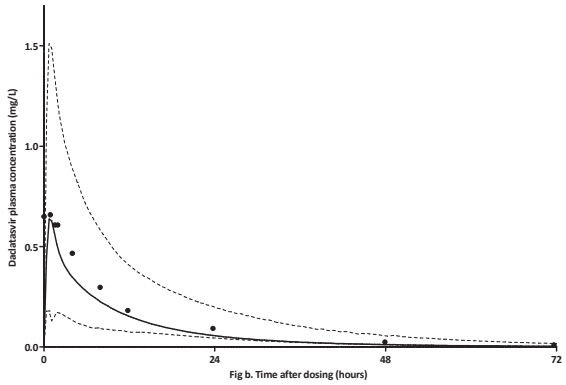
Dosage	Simulated AUC ($h^{-}mg/L$)	Ref AUC ($h^{-}mg/L$)	AUC-ratio[a]	Simulated C_{max} (mg/L)	Ref C_{max} (mg/L)	C_{max}-ratio[a]
Single dose						
10 mg						
Total	1.78 (1.67-1.91)	2.05[16, 17]	0.89	0.22 (0.21-0.22)	0.20[16, 17]	1.10
Unbound	0.011	–	–	0.0013	–	–
30 mg						
Total	5.36 (5.01-5.73)	7.29[36]	0.74	0.65 (0.62-0.67)	0.70[36]	0.93
Unbound	0.033	–	–	0.0038	–	–
100 mg						
Total	17.95 (16.77-19.22)	22.24[16, 17]	0.78	2.16 (2.08-2.25)	1.92[16, 17]	1.13
Unbound	0.11	–	–	0.013	–	–
Multiple dose						
30 mg						
Total	5.43 (5.06-5.82)	6.27[16, 17]	0.87	0.71 (0.68-0.75)	0.74[16, 17]	0.96
Unbound	0.037	–	–	0.0042	–	–
60 mg						
Total	10.89 (10.15-11.69)	15.66[16, 17]	0.70	1.43 (1.37-1.49)	1.58[16, 17]	0.91
Unbound	0.073	–	–	0.0084	–	–

[a]Ratio was calculated as: simulated/reference with an acceptance range of 50-200%.

Reported values are geometric mean with 95% confidence intervals. Healthy volunteer populations, Caucasian, age 20-49, 50% females, 6 trials of 14 subjects. Single dose represents 72 hours sampling and multiple dose 380 hours sampling.

AUC: Area under the concentration-time curve; C_{max}: Maximum plasma concentration; Ref: Reference.





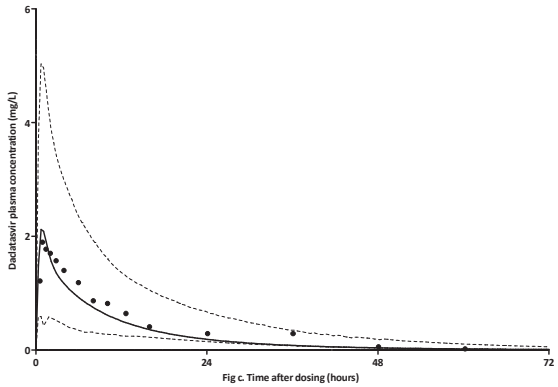


Figure 1: Simulations of single dosages 10 mg (A), 30 mg (B), and 100 mg (C) daclatasvir in healthy subjects.

Lines: simulations showing total daclatasvir concentrations.

Dashed lines: 95% confidence interval

Data points reflect the observed data by Wang et al[17, 16] and Bifano et al[36].

Healthy volunteer populations, Caucasian, age 20-49, 50% females, 6 trials of 14 subjects. Single dose represents 72 hours sampling.

Multiple dose simulations (Table 4, Figure 2) were done and the shape of the PK curve and the visual time to reach steady-state concentrations were comparable with the *in vivo* results[16, 17]. The AUC and C_{max}-ratio for 30 mg QD daclatasvir were 0.87 and 0.96, respectively. For 60 mg QD the AUC-ratio was 0.70 and C_{max} 0.94. These results were within our acceptance criteria. Bioavailability (F) was simulated at approximately 97% for both the single and multiple dose simulations, which was an overestimation as *in vivo* was determined at 67%[2].

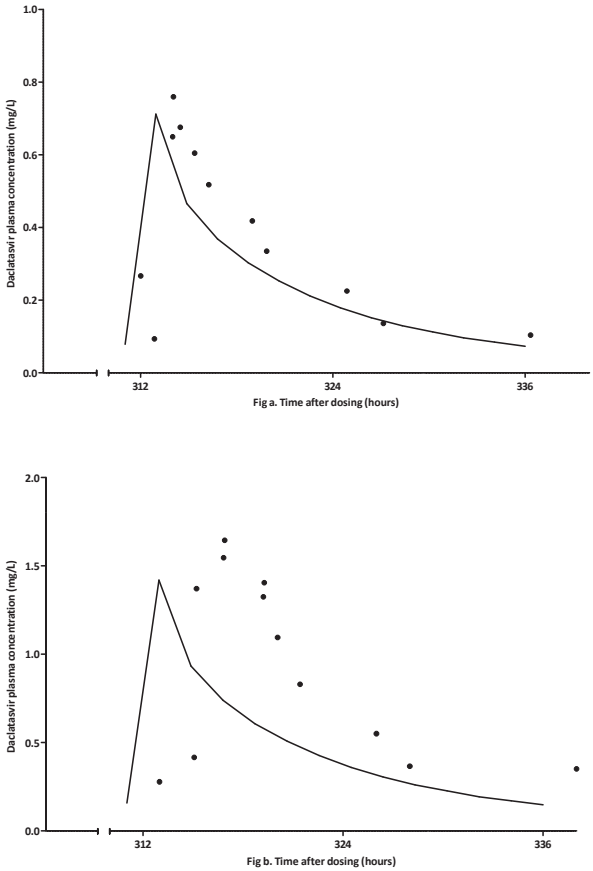


Figure 2: Last dose intervals of the multiple dosage simulations of 30 mg (A) and 60 mg (B) daclatasvir once daily (QD) in healthy subjects.

Lines: simulations showing total daclatasvir concentrations.
Data points reflect the observed data by Wang et al[17, 16].
Healthy volunteer populations, Caucasian, age 20-49, 50% females, 6 trials of 14 subjects.
Multiple dose represents 380 hours sampling.

Simulation of daclatasvir pharmacokinetics in combination with CYP3A4 inhibitors in healthy volunteers

To validate the performance of the model, we simulated the interaction with daclatasvir at steady-state in combination with darunavir/ritonavir (Table 5, Figure 3a) and atazanavir/ritonavir (Table 5, Figure 3b).

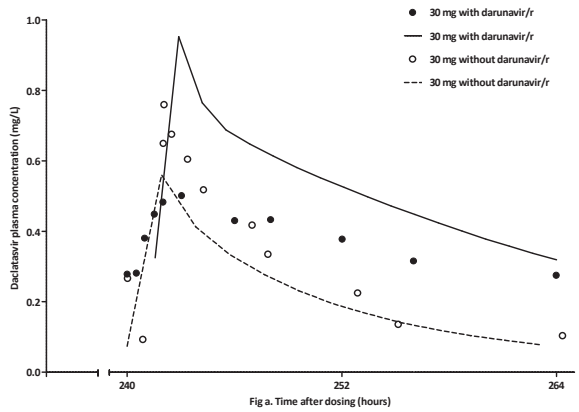
The darunavir AUC and C_{max}-ratios of 800 mg darunavir in combination with 100 mg ritonavir were 1.77 and 1.43, respectively. For ritonavir, these values were 1.47 and 1.12. Together with darunavir/ritonavir, the shape of the simulated daclatasvir PK curve was comparable with *in vivo* data[37]. The ratio for AUC and C_{max} were 0.63 and 1.43, respectively, for 30 mg daclatasvir QD.

The atazanavir AUC and C_{max}-ratio were 1.05 and 0.73 respectively. For ritonavir, these values were 1.11 and 0.43, respectively. Daclatasvir PK parameters with atazanavir/ritonavir were compared with values obtained from the clinical study by Smolders et al[34]. The shape of the curve and value of C_{max} were comparable, however the AUC of 30 mg daclatasvir was more than 2-fold lower (AUC-ratio: 0.37).

Table 5: Pharmacokinetic parameters calculated for 30 mg once daily (QD) daclatasvir in combination with 800/100 mg QD darunavir/ritonavir (11 days) and 300/100 mg QD atazanavir/ritonavir (8 days).

Drug and dosage	Simulated AUC (h·mg/L)	Ref AUC (h·mg/L)	AUC-ratio[a]	Simulated C_{max} (mg/L)	Ref C_{max} (mg/L)	C_{max}-ratio[a]
800 mg darunavir/ 100 mg ritonavir						
Darunavir	141.52	80.0[39]	1.77	10.68	7.45[39]	1.43
Ritonavir	8.36	5.69[40]	1.47	0.84	0.75[40]	1.12
Daclatasvir 30 mg						
Total	5.22 (4.56-5.97)	8.29[37]	0.63	0.70 (0.63-0.77)	0.49[37]	1.43
Unbound	0.082	–	–	0.0058	–	–
300 mg atazanavir/ 100 mg ritonavir						
Atazanavir	58.38	55.56[34]	1.05	3.98	5.46[34]	0.73
Ritonavir	12.65	11.37[34]	1.11	0.82	1.87[34]	0.43
Daclatasvir 30 mg						
Total	5.27 (4.63-5.99)	14.18[34]	0.37	0.70 (0.64-0.77)	0.97[34]	0.72
Unbound	0.071	–	–	0.0056	–	–

[a]Ratio was calculated as: simulated/reference with an acceptance range of 50-200%.
Reported values are geometric mean with 95% confidence intervals.
Healthy volunteer population, Caucasian, age 20-49, 50% females, 1 trial of 14 subjects.
AUC: Area under the concentration-time curve; C_{max}: Maximum plasma concentration; Ref: Reference.



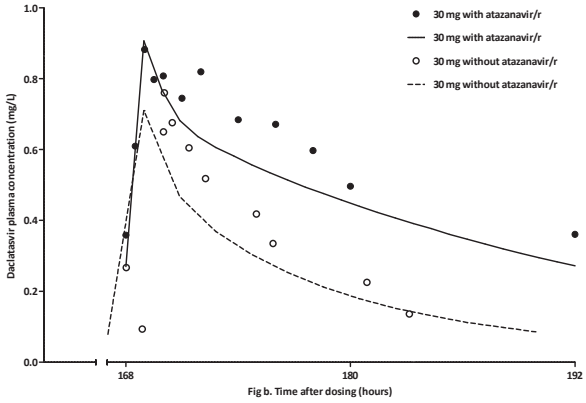


Figure 3: Last dose intervals of the multiple dosage simulations of 30 mg once daily (QD) daclatasvir in combination with 800/100 mg QD darunavir/ritonavir (11 days, A) and 300/100 mg QD atazanavir/ritonavir (8 days, B).

Lines: simulations showing total daclatasvir concentrations.

• reflect the observed daclatasvir concentrations in the presence of the interacting drugs by Gandhi et al[37] and Smolders et al[34]. ° reflect the 30 mg daclatasvir concentrations adapted from Wang et al[16, 17].

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Simulation of daclatasvir and midazolam pharmacokinetics in cirrhotic patients

Bifano et al[36] published data on 30 mg single dose daclatasvir exposure in non-HCV-infected CP-A, B, and C patients, which were used as a comparison for the performance of our model. The simulated exposure of daclatasvir (for 30 mg single dose) was higher than observed exposure (Table 6, Figure 4). With increasing severity of cirrhosis, the simulated/observed AUC-ratio of total daclatasvir increased, starting at a ratio of 1.85 in

CP-A patients and ending with a ratio of 4.66 in CP-C patients. This was in line with the decreasing apparent clearance (CL/F) from the simulated data starting at 24 mL/min to 9 mL/min in CP-A and CP-C patients, respectively. Along the same line, the *in vivo* data showed CL/F values of 120 mL/min and 108 mL/min in these patients. Comparable results are shown for the unbound AUC values of daclatasvir. C_{max}-ratios were 1.74 and 1.81 for CP-A patients for total and unbound daclatasvir, 1.88 and 1.60 for CP-B patients, and 1.99 and 2.45 for CP-C patients, respectively.

Midazolam simulations were included for comparison purposes as midazolam is only cleared via biotransformation, in contrast to daclatasvir which is also being actively transported by hepatic drug-transporters. Midazolam exposure was simulated and compared with a study of Andersen et al[41]. This study included CP-A, B, and C patients who received 15 mg midazolam QD (Table 6). The AUC and C_{max} in the cirrhotic patients increased with increasing severity of disease and these patients had higher exposure than healthy volunteers. The simulated AUC and C_{max} for healthy volunteers were 0.22 h·mg/L and 0.07 mg/L. For CP-A patients the simulated AUC is 0.31 h·mg/L and C_{max} 0.08 is mg/L resulted in a AUC-ratio of 2.03 and a C_{max}-ratio of 1.81. CP-B patients had a simulated AUC and C_{max} of 0.69 h·mg/L and 0.12 mg/L resulting in a AUC-ratio of 1.68 and a C_{max}-ratio of 1.33, respectively. CP-C patients had a simulated AUC of 1.09 h·mg/L and a C_{max} of 0.14 mg/L resulting in a AUC-ratio of 2.66 and a C_{max}-ratio of 1.55.

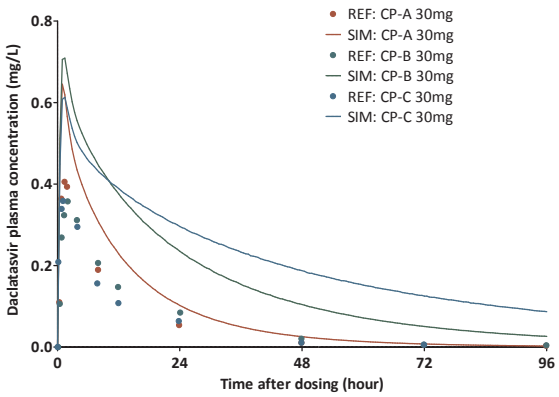

Figure 4: Simulations of single dosages 30 mg daclatasvir in Child-Pugh class A, B, and C patients.
Lines: simulations showing total daclatasvir concentrations.
Data points reflect the observed data of Bifano et al[36].
Cirrhotic populations, Caucasian, age 20-49, 50% females, 1 trial of 14 subject

Table 6: Pharmacokinetic parameters for single dosages 30 mg daclatasvir and 15 mg midazolam in cirrhotic patients.

Dosage	Simulated AUC (h·mg/L)	Ref AUC (h·mg/L)	AUC-ratio[a]	Simulated C_{max} (mg/L)	Ref C_{max} (mg/L)	C_{max}-ratio[a]	Simulated CL/F[b] (mL/min)	Ref CL/F[b] (mL/min)
CP-A								
Daclatasvir								
Total	7.74 (7.72-8.31)	4.17	1.85	0.66 (0.63-0.69)	0.38	1.74	24	120
Unbound	0.052	0.0256	2.03	0.0042	0.00233	1.81	–	–
Midazolam[c]								
Total	0.31	0.41[d][41]	0.76	0.08	0.09[d][41]	0.88	–	–
CP-B								
Daclatasvir								
Total	14.69 (13.81-16.20)	4.55	3.23	0.72 (0.70-0.76)	0.382	1.88	14	110
Unbound	0.127	0.04157	3.05	0.0056	0.00349	1.60	–	–
Midazolam								
Total	0.69	0.41[d][41]	1.68	0.12	0.09[d][41]	1.33	–	–
CP-C								
Daclatasvir								
Total	19.85 (18.23-21.62)	4.649	4.66	0.63 (0.60-0.66)	0.317	1.99	9	108
Unbound	0.229	0.0401	6.08	0.0066	0.00273	2.45	–	–
Midazolam								
Total	1.09	0.41[d][41]	2.66	0.14	0.09[d][41]	1.55	–	–

[a]Ratio was calculated as: simulated/reference with an acceptance range of 50-200%.

[b]CL/F = Clearance of the drug from plasma after oral administration; CL = $CL_{total/F}$ and $CL_{unbound/F}$.

[c]Reference values healthy volunteers: AUC: 0.143 h·mg/L and C_{max}: ~0.045 mg/L[42]; simulated values healthy volunteers: AUC: 0.22 h·mg/L C_{max}: 0.07 mg/L; AUC-ratio: 1.54 C_{max}-ratio: 1.55.

[d]Mixed group of patients; CP-A, B, and C.

Cirrhotic populations, Caucasian, age 20-49, 50% females, 1 trial of 14 subjects.

Reported values are geometric mean with 95% confidence intervals.

CP: Child-Pugh class; AUC: Area under the concentration-time curve; C_{max}: Maximum plasma concentration; Ref: Reference.

DISCUSSION

We evaluated a mechanistic model to predict daclatasvir exposure in healthy subjects and patients with CP-A, B, or C cirrhosis.

We were able to adequately recover the exposure in healthy volunteers compared with the data from literature. This was the case for both the single and multiple dose simulations and the simulation in combination with the CYP3A4 inhibitors. We must notice that both the metabolism by CYP2C8 and canalicular efflux by P-gp in our model, were based on sensitivity analysis/fitting as presented in the previously published papers[16, 17]. These aspects improved the performance of the model. CYP2C8 contributes in the model to the metabolism of daclatasvir (CL_{int} = 0.32 µL/min/pmol CYP). It has been described that *in vitro* CYP2C8 has only minor contribution in the formation of daclatasvir metabolite M1 and that CYP3A4 is the main metabolizing enzyme (*in vitro* and *in vivo*)[1].

The interactions between daclatasvir and darunavir/ritonavir and atazanavir/ritonavir were studied to test the susceptibility of the daclatasvir model for impaired CYP3A4 activity. *In vivo* studies demonstrated the need for combining darunavir/ritonavir with 60 mg daclatasvir, whereas atazanavir/ritonavir is to be combined with 30 mg daclatasvir. This was remarkable as it was expected that both boosted protease inhibitors would increase daclatasvir plasma concentrations, resulting in a need for a dose reduction. The different influence of darunavir/ritonavir and atazanavir/ritonavir on daclatasvir exposures, is probably caused by different *in vivo* ritonavir concentrations (Table 5). When ritonavir is combined with darunavir, the C_{max} and AUC of ritonavir were ~2-fold lower, compared when combined with atazanavir[34, 37]. Another explanation can be that next to being a CYP3A inhibitor, darunavir may also induce CYP3A (Table 2).

We were able to simulate ritonavir AUCs adequately with both dosing regimens, although the increased C_{max} of ritonavir when combined with atazanavir was not captured (C_{max}-ratio was 0.43). This is of importance as exposure to the interacting drug clearly determines the magnitude of the effect on daclatasvir exposure and we were therefore probably not able to capture the different effect on daclatasvir PK by darunavir/ritonavir and atazanavir/ritonavir. The interaction studies resulted in a daclatasvir AUC-ratio of 0.63 and 0.37 and C_{max}-ratio of 1.43 and 0.72 when combined with respectively darunavir/ritonavir and atazanavir/ritonavir. Despite the deviations of the simulated daclatasvir concentrations with observed values, we considered these simulations to be appropriate for current purposes.

The simulations of daclatasvir in the cirrhotic population showed increased daclatasvir exposure with increasing severity of cirrhosis, which is in line with the simulated findings of midazolam. For midazolam, these findings were comparable with observed clinical findings[8, 11, 41, 42]. For daclatasvir the model overestimated the daclatasvir exposure compared with observed findings by Bifano et al[36], as exposure decreased in cirrhotic patients compared to healthy individuals. When the results of the model are compared with another study, also presenting *in vivo* PK data in CP-A and CP-B patients[38], the model also overpredicts exposure. However, these authors showed that for CP-B patients with HCV infection at steady-state daclatasvir AUC and C_{max} were approximately 1.2-fold higher than these parameters in CP-A patients with HCV infection, an increase in plasma concentration which is in the same direction shown by the simulations of the model[38]. The evaluation of PK in the study of Lawitz et al, was part of studying efficacy and safety of triple therapy with simeprevir, sofosbuvir and daclatasvir[38]. These results must be interpreted with care in respect to our simulations, as simeprevir is an inhibitor of intestinal CYP3A4 which increases AUC, C_{max}, and C_{min} of daclatasvir by 96, 50, and 168% *in vivo*, respectively[2]. The discrepancies in outcome between the two clinical studies hampered the evaluation of our model in cirrhotic patients. Despite this, it appeared that predictions for daclatasvir were less accurate than simulation of midazolam PK in cirrhotics.

In the study of Bifano et al, 30 mg QD daclatasvir was studied in non-HCV CP-A, B, and C patients[36] and the study of Lawitz et al studied 60 mg multiple dose in HCV CP-A and CP-B patients. The discrepancy in outcome points out the variation of *in vivo* studies, and shows that non-HCV and HCV cirrhotic patients are a different population. Especially, when PK parameters are evaluated we must be careful by extrapolating these results to other populations. The differences were previously described by Morcos et al showing that the HCV infection itself also has influence on the metabolism of midazolam[43], however, *in vivo* daclatasvir PK was not different in healthy volunteers compared with non-cirrhotic HCV-infected patients[2]. So, when we extrapolate non-HCV cirrhotic simulations to cirrhotic HCV populations we should take these differences between patients into account.

As with drug-metabolizing enzymes, the cause of cirrhosis (e.g., alcohol induced or HCV) also has influence on the expression drug-transporters[44, 45]. Daclatasvir is a substrate of OCT1, P-gp and other unidentified transporters. It is described that both P-gp and OCT are down-regulated (assessed via quantitative proteomics) during HCV induced cirrhosis[44]. P-gp is, among others, important for the canalicular efflux of daclatasvir, and thus the biliary clearance of the drug and OCT is responsible for the uptake into the hepatocyte.

However, hepatic transporter expression is not well captured in the cirrhotic pathophysiological PK model. This may be a contributing factor in the disposition of daclatasvir, but not midazolam, and in line with the fact that the changes in midazolam PK in cirrhotic patients were better predicted, whereas the changes in daclatasvir exposure were not. Secondly, transporters are of course also located on membranes of cells in other organs[46, 47]. As it has been previously described that fibrotic rats have upregulated P-gp and CYP expression (mRNA) in the intestine compared with non-fibrotic rats[48]. Still, there is only limited and sometimes conflicting evidence for this process which makes it difficult to include these aspects in the PBPK model[48, 49]. At the same time this stresses the fact that more detailed experimental data on transporter expression across various CP classes would be valuable in order to improve predictions.

Note that it is common to use the CP classification system to identify severity of cirrhosis. However, this system is based on clinical parameters instead of etiology of liver cirrhosis. As said, etiology of the disease (e.g., non-alcoholic steatohepatitis [NASH], alcoholic steatohepatitis [ASH], HCV) might differently affect the relevant determinants of drug disposition (e.g., transporters, CYPs). Hence, this may result in additional variation in PK which may add to differences found between the *in vivo* and *in silico* findings, when the latter are based the characteristics obtained from overall CP-classes, while the former are obtained in a patient group with specific disease etiology.

The EMA describes that PBPK modeling can be a tool for the evaluation of the PK of a drug in patients with hepatic impairment. We believe, that PBPK is a valuable tool in this population, as these are vulnerable and compromised (especially CP-C) patients which you preferably do not expose to a full drug-interaction study. We used Simcyp to gather more information about the influence of cirrhosis on the PK of daclatasvir. However, with the available cirrhotic model, we were not able to recover *in vivo* findings due some discussed shortcomings. Clearly, the use of the hepatically impaired population in e.g. Simcyp for registration purposes could be useful for hypothesis finding and understanding PK of a compound. For other purposes, such as DDI studies in healthy volunteers for which the substrates/inhibitors are verified and robust, PBPK can be a useful additional tool during drug development. In this respect, predictions for drugs that are only subject to biotransformation are generally in better agreement with observed PK profiles than we found for daclatasvir, a drug that is also subject to active transport.

In conclusion, we were able to adequately model daclatasvir PK in healthy volunteers using a PBPK model that included both hepatic metabolism as well as hepatic drug-transporters. However, there remained a discrepancy between the observed and simu-

lated daclatasvir exposure in cirrhotic patients. We propose that future studies address hepatic and intestinal transporter mediated daclatasvir disposition in more detail, with a particular focus on the influence of cirrhosis on transporter expression and function.

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Part 4

A pharmacist's contribution to eradicate hepatitis C

Clinical pharmacology in individual patients

This chapter is based on the following papers

Sixty milligram daclatasvir is the right dose for hepatitis C virus treatment in combination with etravirine and darunavir/ritonavir

EJ Smolders
CTMM de Kanter
K Grintjes
A D'Avolio
G Di Perri
R van Crevel
JPH Drenth
DM Burger

AIDS. 2016 Jun; 30 (9): 1491-1493

Effective treatment of hepatitis C virus infection with sofosbuvir and daclatasvir 90 mg in a patient with severe epilepsy on oxcarbazepine

EJ Smolders
CTMM de Kanter
N van 't Veer
A D'avolio
G Di Perri
DM Burger
P van Wijngaarden

Int J Antimicrob Agents. 2016 Sep; 48 (3): 347-348

Decreased tacrolimus plasma concentrations during HCV therapy: a drug-drug interaction or is there an alternative explanation?

EJ Smolders
S Pape
CTMM de Kanter
AP van den Berg
JPH Drenth
DM Burger

Int J Antimicrob Agents. 2016 Sep; 48 (3): 347-348

The observed effect of gastric bypass surgery on direct-acting antiviral treatment: a case report

EJ Smolders
SB Willemse
O El-Sherif
S Khoo
DM Burger

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INTRODUCTION

Patients chronically infected with hepatitis C virus (HCV) in the Netherlands are treated according to a national guideline[1]. This guideline is periodically updated using several international guidelines such as EASL[2] and AASLD[3]. Information is given about which direct-acting antivirals (DAAs) can be used for which genotype, but also information about dose adaptations in renal- and hepatic impairment and drug-drug interactions (DDIs) are given. However, it is not possible to treat all patients according to these guidelines, for example due to complex DDIs or other special conditions.

Here, we describe five patients who could not be treated according to the guideline, but were all in need of HCV treatment. Knowledge of pharmacology and DDIs were necessary to optimally treat these patients.

SIXTY MILLIGRAM DACLATASVIR IS THE RIGHT DOSE FOR HEPATITIS C VIRUS TREATMENT IN COMBINATION WITH ETRAVIRINE AND DARUNAVIR/RITONAVIR

Patient and treatment

In May 2015, a 54-year-old Ethiopian man with a 20-year history of HIV infection presented with progressive pulmonary hypertension and ascites. These symptoms were attributed to his liver cirrhosis (Child-Pugh class B [CP-B]; alanine transaminase [ALT] 37 U/l; aspartate transaminase [AST] 27 U/l; γ-glutamyl transpeptidase [γGT] 54 U/l). Cirrhosis was caused by an HCV genotype 4 infection, for which he had been treated unsuccessfully with peg-interferon (Peg-IFN) and ribavirin in 2006. Treatment with diuretics led to progressive kidney function loss, after which was decided to initiate HCV therapy.

The proposed 12-week HCV treatment was a daily regimen containing 400 mg sofosbuvir, 800 mg ribavirin, and daclatasvir, in line with international[4] and Dutch guidelines[1]. For his HIV infection, he received 400 mg etravirine and 800/100 mg darunavir/ritonavir once daily (QD), after experiencing mitochondrial and other toxicity on nucleoside reverse transcriptase inhibitor-based combinations. Thus, the HIV regimen could not be changed. His HIV viral load was undetectable.

We examined potential DDIs that could arise from both regimens. Etravirine and darunavir/ritonavir induce and inhibit cytochrome P450 (CYP) 3A4, respectively, and daclatasvir is a CYP3A4 substrate. Experimental studies have shown that daclatasvir pharmacokinetics is minimally affected by darunavir/ritonavir, obviating the need for

dose adjustment. Co-administration of daclatasvir with etravirine may lead to decreased daclatasvir concentrations[5, 6]. Although this has not been shown in humans, the US Food and Drug Administration (FDA) advises increasing daclatasvir dose to 90 QD when co-administered with etravirine[6]. The normal daclatasvir dosage is 60 QD.

In view of the uncertain interaction between daclatasvir, etravirine, and darunavir/ritonavir, we started treatment with 60 mg QD daclatasvir reasoning that CYP3A4 induction by etravirine would be compensated by inhibition of this enzyme by darunavir/ritonavir. However, no dose adjustment of daclatasvir is necessary; CYP3A4 is inhibited by darunavir/ritonavir. The area under the concentration-time curve (AUC) and maximum plasma concentration (C_{max}) of daclatasvir decreased with 41 and 23% when co-administered, respectively[5].

The pharmacokinetic curve of daclatasvir was recorded on steady-state. Blood samples were taken at $t = 0$ (pre-dose), 2, 6, 8, and $t = 24$ hours (C_{trough}) after daclatasvir intake. Plasma concentrations were determined with a validated liquid chromatography-mass spectrometry (LC-MS) method and pharmacokinetic parameters were calculated using WinNonlin[7].

Figure 1 shows the pharmacokinetic curve and parameters of daclatasvir from our patient. C_{max} and $AUC_{0\text{-}tau}$ were 59 and 20% decreased and C_{trough} was 11% increased, compared with reference values obtained from literature[8]. The elimination half-life ($T_{1/2}$) was 12.6 hours, which is similar to literature[8].

The patient completed a 12-week course of treatment and after follow-up sustained virological response (SVR12) was achieved in January 2016.

AUC and C_{trough} are the most important pharmacokinetic parameters to attain antiviral efficacy and both were similar with reference values[9-11]. C_{trough} should be high enough to maintain viral inhibition throughout the complete dose interval, whereas a decreased C_{trough} potentially leads to viral failure or induction of resistant strains.

C_{max} was reduced in our patient, which is comparable with a trial describing decreased C_{max} and AUC in CP-B patients. Additionally, the unbound daclatasvir fraction was equivalent between non-cirrhotic HCV and CP-B patients, meaning exposure to pharmacological active daclatasvir was unaffected[12]. Extrapolating these results to our patient; we believe the patient is treated with the right dose daclatasvir.

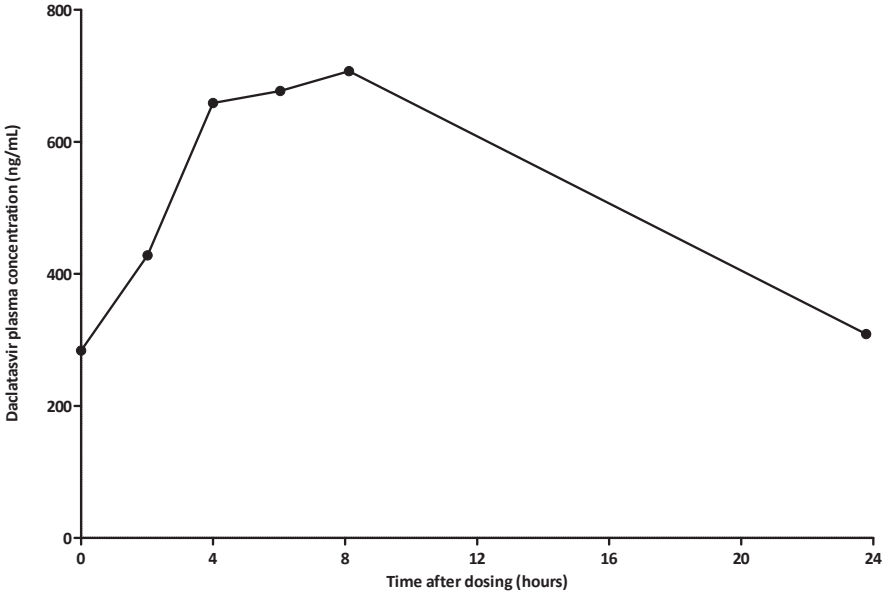

Figure 1: Pharmacokinetic curve 60 mg daclatasvir QD in combination with 400 mg etravirine QD and 800/100 mg darunavir/ritonavir QD.

C_{max}: 707 ng/mL; $AUC_{0\text{-}tau}$: 12,037 h·ng/mL; C_{trough}: 284 ng/mL; T_{max}: 8.0 hours; $T_{1/2}$: 12.6 hours.
Reference values in HCV patients without cirrhosis derived from Nettles et al: C_{max}: 1,726 ng/mL; $AUC_{0\text{-}tau}$: 15,121 h·ng/mL; C_{trough}: 254 ng/mL: T_{max}: 1-2 hours; $T_{1/2}$: 12-15 hours[8].

Our pharmacokinetic data suggest that the inducing effect of etravirine can be mitigated by darunavir/ritonavir. This is analogous to the interaction between maraviroc (CYP3A4 substrate), efavirenz (CYP3A4 inducer), and protease inhibitors (CYP3A4 inhibitors). Boosted protease inhibitors mitigated the inducing effect of efavirenz on maraviroc, and hence the dose of maraviroc should be based on the presence of the boosted protease inhibitor[13].

Based on the pharmacokinetic parameters and the achievement of SVR12, we believe that exposure to daclatasvir was adequate. This fuels our hypothesis that CYP3A4 induction by etravirine can be compensated through CYP3A4 inhibition by darunavir/ritonavir. Daclatasvir should be administered in a dose of 60 mg QD when combined with etravirine and darunavir/ritonavir.

EFFECTIVE TREATMENT OF HEPATITIS C VIRUS INFECTION WITH SOFOSBUVIR AND DACLATASVIR 90 MG IN A PATIENT WITH SEVERE EPILEPSY ON OXCARBAZEPINE

Patient and treatment

A 63-year-old, treatment-naïve male patient was seen because of a chronic genotype 1b HCV-infection. He had treatment-resistant epilepsy that ultimately stabilized with lacosamide 100 mg two times daily (BID), oxcarbazepine 600 mg three times daily plus 300 mg before sleeping, and clobazam 5 mg BID.

Liver function test were slightly abnormal with an ALT of 100 U/L and a γGT of 258 U/L. Baseline HCV RNA was $1.5x10^6$ IU/mL (Real*Time* HCV Genotype II, m2000sp instrument, Abbot Molecular Inc, Des Plaines, USA). Biopsies showed extensive fibrosis and some focal slight cirrhosis. The patient's METAVIR score was F3-F4.

Potential drug-interactions were checked and all available regimes were contra-indicated with oxcarbazepine. The patient could not switch to another anticonvulsant in view of the severity of his epilepsy. After careful consideration, the proposed treatment consisted of sofosbuvir, daclatasvir, and ribavirin.

Oxcarbazepine is a P-glycoprotein (P-gp) and CYP3A4 inducer and is contra-indicated with sofosbuvir and daclatasvir because these DAAs are substrates of P-gp and CYP3A4, respectively[14, 15]. Therefore, oxcarbazepine potentially decreases daclatasvir and sofosbuvir plasma concentrations. Reduced levels of DAAs may lead to virological failure and/or resistance because they might affect the ability of the drugs to maintain viral inhibition throughout the complete dose interval.

Therefore, we increased the daclatasvir dosage to 90 mg (normal dose, 60 mg)[15] based on a study with efavirenz, a moderate CYP3A inducer[15]. We did not alter the sofosbuvir dosage because only 400 mg tablets were available and we did not want to crush or split tablets. Furthermore, the patient had good prognostic factors (e.g., genotype 1b, treatment-naïve) and we added ribavirin 500 mg BID (patient weight, 58 kg) to enhance the potency of the regimen.

The patient started his 12-week treatment and at steady-state (week 7) an intensive pharmacokinetic curve was recorded. Blood samples were taken at $t = 0$ (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, and 24 hours after dosing of daclatasvir and sofosbuvir. Plasma concentrations of daclatasvir, sofosbuvir, and the main metabolite of sofosbuvir (GS-331007) were determined using a validated LC-MS method[16]. Pharmacokinetic

parameters for both DAAs were calculated using Phoenix WinNonlin (Certara USA, Inc., Princeton, NJ).

The AUC, as well as the C_{max} and $C_{trough,}$ of daclatasvir, were 54, 45, and 37% decreased compared with reference values despite the increased dose (Figure 2a). Sofosbuvir exposure was comparable with literature; however, C_{max} was increased approximately three-fold. The AUC and C_{max} of GS-331007 were both increased ca. 53% (Figure 2b). Lastly, the oxcarbazepine concentration was within references values during treatment (19 mg/L; reference: 7-35 mg/L).

During treatment, ALT and γGT decreased to 22 and 85 U/L (week 1) and 14 and 44 U/L (week 4), respectively. Twelve weeks after finishing DAA treatment, the patient had undetectable HCV RNA, meaning that he reached SVR12 (ALT: 16 U/L; γGT: 84 U/L).

The interaction study between daclatasvir and efavirenz showed that daclatasvir C_{max}, C_{trough}, and AUC were 17, 59, and 32% decreased in combination with efavirenz. To compensate for this reduced exposure, daclatasvir 90 mg is recommended[15]. However, our patient had still decreased daclatasvir exposure compared with reference values. This suggests that CYP3A4 induction of oxcarbazepine was not completely abolished by increasing the daclatasvir dose to 90 mg[8]. On the other hand, a previous study showed that in patients with cirrhosis, daclatasvir exposure was reduced compared with non-cirrhotic patients. Also, the unbound (active) fraction of daclatasvir was unaffected[12]. This could mean that in our patient the reduced exposure of total daclatasvir fits his cirrhosis, and that the unbound fraction of daclatasvir could be adequate. One could also argue that an even higher dose should be administered when daclatasvir is combined with moderate enzyme inducers (e.g., 120 mg QD or 60 mg BID), but this warrants further studies.

Sofosbuvir is a substrate of P-gp, but GS-331007 is not a substrate of P-gp and therefore is not affected by oxcarbazepine[14]. Apparently, in this patient sofosbuvir levels were unaffected by oxcarbazepine. This could be explained by the fact that oxcarbazepine is a mild/moderate inducer of P-gp.

This case report suggests that daclatasvir 90 mg and sofosbuvir 400 mg were the correct dosages for this patient in combination with oxcarbazepine. Sufficient sofosbuvir concentrations were reached, but daclatasvir exposure was reduced in this patient. Alternatively, daclatasvir dose could be increased in patients with moderate enzyme inducers. However, we advise prescribers to be cautions until this is confirmed with a drug-interaction study. Nevertheless, our patient achieved SVR12, so it can be defended to use daclatasvir 90 mg in combination with sofosbuvir 400 mg and ribavirin in similar patients.

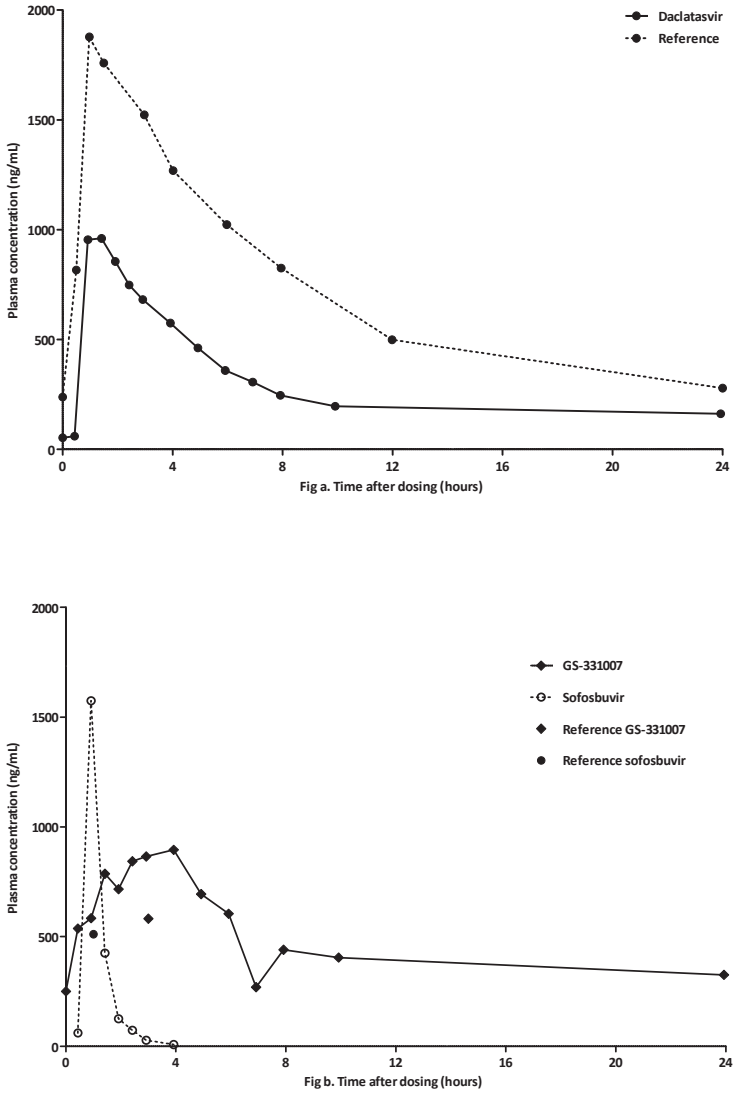

Figure 2: Pharmacokinetic curve of daclatasvir 90 mg (A) and sofosbuvir and GS-331007 (B) in combination with oxcarbazepine. Reference curve shows daclatasvir 60 mg and the reference values show C_{max} of both sofosbuvir and GS-331007.

A: T_{max}: 1.4 hours; C_{max}: 960: ng/mL; C_{trough}: 162 ng/mL; $T_{1/2}$: 32 hours; $AUC_{0\text{-}tau}$: 7,069 h·ng/mL.

Reference values and curve adapted from Nettles et al. Daclatasvir 60 mg QD (steady-state concentrations) hepatitis C virus (HCV) genotype 1-infected patients without cirrhosis: T_{max}: 1-2 hours; C_{max}: 1,726 ng/mL; C_{trough}: 254 ng/mL; $T_{1/2}$: 12 hours; $AUC_{0\text{-}tau}$: 15,120 h·ng/mL[8].

B: T_{max}: 0.92 hours; C_{max}: 1,574 ng/mL; C_{trough}: 8 ng/mL; $T_{1/2}$: 0.48 hours; $AUC_{0\text{-}tau}$: 1,063 h·ng/mL. GS-331007: T_{max}: 3.9 hours; C_{max}: 895 ng/mL; C_{trough}: 251 ng/mL; $T_{1/2}$: 39 hours; $AUC_{0\text{-}tau}$: 11,033 h·ng/mL.

Reference values adapted from Kirby et al. Sofosbuvir 400 mg QD treatment-naïve HCV genotype 1-infected subjects without cirrhosis. Sofosbuvir: T_{max}: 0.5-1.5 hours; C_{max}: 511 ng/mL; $AUC_{0\text{-}tau}$: 1,030 h·ng/mL. GS-331007: T_{max}: 2-4 hours; C_{max}: 582 ng/mL; $AUC_{0\text{-}tau}$: 7,120 h·ng/mL[14].

DECREASED TACROLIMUS PLASMA CONCENTRATIONS DURING HCV THERAPY: A DRUG-DRUG INTERACTION OR IS THERE AN ALTERNATIVE EXPLANATION?

Patient 1 and treatment

The first patient was a 56-year-old male with a chronic HCV-infection genotype 3. He was diagnosed in 2009 and received prior treatment with Peg-IFN and ribavirin but failed to respond (2010 and 2011). He rapidly progressed to cirrhosis and end-stage liver disease, resulting in a liver transplantation (LT) in 2012. He received 2 mg tacrolimus QD (target value: 2-3 µg/L) and 500 mg mycophenolic acid BID. These doses had been stable since August 2013. In 2015, he was re-treated for his HCV-infection with 400 QD sofosbuvir and 600 mg ribavirin BID for 24 weeks. At start of therapy, tacrolimus C_{trough} was 5.5 µg/L (estimated Glomerular Filtration Rate [eGFR] 61 mL/min), followed by a decreased C_{trough} of 2.4 µg/L at week 4. This required an increase of tacrolimus dosage to 2.5 mg QD. At week 8, the C_{trough} was 2.5 µg/L and tacrolimus dosage was further elevated to 3 mg QD, resulting in an increased C_{trough} of 3.8 µg/L. The dosage was subsequently lowered to 2.5 mg QD.

The patient remained on that dosage during the rest of HCV treatment. The patient reached SVR after 12 weeks after completing treatment. His tacrolimus plasma concentration has been stable since, and the patient is still treated with a dosage of 2.5 mg QD (Figure 3).

Patient 2 and treatment

Patient 2 was a 74-year-old male with a chronic HCV genotype 1b infection. He was treated twice with Peg-IFN and ribavirin (2002 and 2004) and relapsed both times. Cirrhosis was diagnosed in 2008 and he developed hepatocellular carcinoma for which he received chemoembolization and radio frequent ablation. In June 2010, he underwent his first liver transplantation, which was complicated by a grade 2 rejection. A second liver transplantation was performed in August 2010. Since his transplantation, he has been on mycophenolic acid 1,000 mg BID and tacrolimus 4 mg QD (target value: 5-10 µg/L). In May 2015, he developed mild ascites and HCV therapy was initiated (eGFR: 60 mL/min). He received sofosbuvir 400 mg QD, daclatasvir 60 mg QD, and ribavirin 500 mg BID for 12 weeks. His tacrolimus C_{trough} was 17.9 µg/L at start of therapy and had decreased to 5.3 µg/L at week two of therapy. His tacrolimus dosage was increased to 5 mg QD but at week 4 his C_{trough} had dropped to 3.1 µg/L and dosage was increased again to 6 mg QD. From this moment on, his tacrolimus C_{trough} rose gradually to 7.2 µg/L at the end of therapy (Figure 3). His treatment was complicated due to anemia (hemoglobin 6.1 g/dL) necessitating blood transfusions and temporary withdrawal of ribavirin. In November 2015, he achieved SVR12.

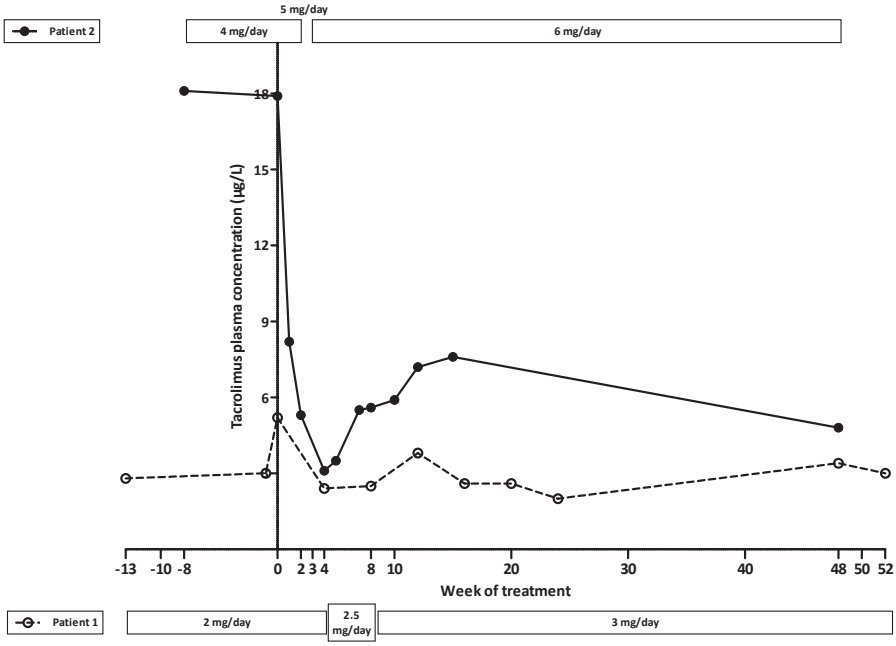

Figure 3: Tacrolimus plasma concentrations and tacrolimus dosages during hepatitis C virus (HCV) treatment for patients 1 and 2.

The figure shows the tacrolimus plasma concentration (µg/L) (left y-axis) and the tacrolimus dosage in mg/day. The weeks of treatment are shown on the x-axis. Patient 1 was treated for 24 weeks (follow-up data are missing). Patient 2 was treated for 12 weeks; follow-up data are shown at week 24. The estimated glomerular filtration rate (eGFR) remained stable during treatment in both patients.

DISCUSSION

This part describes two liver transplant recipients receiving tacrolimus who have been treated for HCV successfully. Tacrolimus is a substrate for CYP3A4 and P-gp. It has a narrow therapeutic range and pharmacokinetics show a high inter-patient variability requiring close TDM with dose adjustment.

At start of treatment, no DDIs between tacrolimus, sofosbuvir, daclatasvir, and ribavirin had been described. However, to maintain target tacrolimus C_{trough} plasma concentrations, we found that the tacrolimus dosage had to be increased during HCV treatment. This was an unexpected observation, in view of the fact that none of the DAAs influenced CYP3A4, the main metabolizing enzyme of tacrolimus.

We hypothesize that decreased tacrolimus plasma concentrations resulted from repression of drug-metabolizing enzymes, such as CYP3A4, by an inflammatory/infectious

stimulus. Pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), inhibit CYP3A4 enzymes[17, 18]. This has been studied both *in vitro*[17, 18] and *in vivo* and is described in infectious (HIV[19]) and inflammatory (rheumatoid arthritis[20]) conditions. Morcos et al[21] found decreased midazolam (CYP3A4 substrate) metabolism in HCV-infected patients compared with healthy volunteers. The midazolam metabolic ratio for HCV treatment-naïve patients was 37% lower and was even 54% lower in null-responders compared with healthy subjects. This is in line with increased midazolam plasma levels seen in chronically HCV-infected patients[21].

Additional evidence for this concept comes from trials, which show that the DAAs simeprevir and grazoprevir reach higher blood concentrations in HCV patients compared with healthy volunteers[22, 23]. This is also described for the HIV protease inhibitors, which are all CYP3A4 substrates[24].

Table 1 shows the results of previously published studies showing a similar decrease in tacrolimus plasma concentrations during HCV therapy[25-30]. For example, the study of Saab et al[25] described that there was a statistically significant decrease in tacrolimus daily dose adjusted per weight and tacrolimus serum levels during HCV therapy with novel DAAs. In addition, van den Berg et al showed in liver transplant patients with and without HCV that patients with recurrent HCV infection after transplantation needed a lower dose of tacrolimus as trough levels remained high (20% compared with liver transplant patients without HCV)[31].

Lastly, tacrolimus plasma concentrations were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with a quantitative range of 1.0-25 µg/L. Repeatability varied from 5.7 to 8.2% and reproducibility varied from 4.5 to 7.3%. As this is a validated assay with good precision and little variation, we believe that the decrease in C_{trough} was not caused by any variability of the analytical assay. Second, as the plasma concentrations dropped ca. 50%, this was not thought to be caused by intra-subject variability of tacrolimus trough levels (coefficient of variation: 13.4% in healthy subjects[32]).

The current cases are in line with previous reported studies and we argue that before DAA treatment was initiated in these two patients, CYP3A4 activity was reduced under the influence of the infection. The tacrolimus dosage was titrated under these infectious conditions using TDM. During HCV treatment with DAAs, HCV RNA becomes rapidly undetectable (<4 weeks), which is shown in different studies[33]. Similarly, ALT decreased quickly in our patients. The ALT level in patient 1 was 40 U/L at the start of therapy and was decreased to 31 U/L at week 2 and to 25 U/L at week 12. For patient

2, baseline ALT was 41 U/L and decreased to 16 U/L at week 2 (upper limit normal: <45 U/L). Normalization of the liver enzymes in our patients is undeniably due to clearance of the virus and a consequent decrease in liver cell damage. This potentially leads to normalized CYP3A4 activity because the infectious stimulus that downregulates CYP enzymes becomes absent, resulting in increased metabolism and thus decreased tacrolimus plasma concentrations.

In the Netherlands, it is clinical practice to adjust the tacrolimus dosage based on C_{trough} concentrations. In general, the AUC is a more reliable value for the determination of drug exposure. However, the relationship between tacrolimus AUC and C_{trough} is still under debate and therefore C_{trough} is still used in TDM[34].

Novel HCV therapies have become easier and more available. Therefore, we believe that awareness among clinicians for the dynamics of tacrolimus levels during DAA therapy is necessary. Not only could tacrolimus levels be influenced, but mycophenolic acid levels might also be altered, as this is a uridine 5'-diphospho-glucuronosyltransferase (UGT) substrate. Other substrates of liver enzymes with narrow therapeutic ranges might also require dose adjustments.

In conclusion, the plasma concentration of drugs that are extensively metabolized by the liver might be influenced by DAA therapy even when there is no DDI. We suggest that physicians treating patients with DAAs are aware of the dynamics of CYP substrates such as tacrolimus. Patients receiving tacrolimus starting with DAA treatment should be closely monitored. TDM of tacrolimus is an effective option to monitor the plasma concentration so that dosage adjustments can be made.

Table 1: Overview from literature. Previously published studies showing a decrease in tacrolimus plasma concentration during hepatitis C virus (HCV) therapy.

Author	Study design	Number of LT patients	HCV therapy	Tacrolimus dose and plasma levels
Saab et al, 2016[25].	Retrospective cohort. Dosages were collected 3 months before DAA therapy and 3 months after HCV therapy.	52	All-oral HCV therapy	Total daily dose was decreased in 41%, equivalent in 36% and increased in 23% when compared before and after start of DAA treatment. Comparable tacrolimus serum levels were decreased in 80%, equivalent in 0%, and increased in 21% of the patients.
Ueda and Uemoto, 2016[26].	Case series. Patients treated with asunaprevir and daclatasvir.	10	Asunaprevir, daclatasvir	Before DAA treatment: 3.95 ng/mL per mg Week 1: 5.2 ng/mL per mg Week 2: 2.975 ng/mL per mg Five patients needed a dose increase
Raschzok et al, 2016[27],	Cohort	21	Sofosbuvir, simeprevir/ daclatasvir ±ribavirin	Dose ratios are given. Start of DAA treatment: 4.68 Week 12: 2.72. Tacrolimus dose start: 1.8 mg/day Tacrolimus dose week 12: 1.7 mg/day
Kawaoka et al, 2016[28].	Case report	3	Asunaprevir, daclatasvir	Patient 1: Start dose: 1 mg/day and plasma level: 2.4 ng/mL Weeks 4, 8, 12, 16, 20, and 24 Plasma levels were 3.0, 3.2, 3.4, 3.0, 2.9, 3.3 ng/mL. Patient 2: Start dose: 1.5 mg/day and plasma level: 10.9 ng/mL. Weeks 4 and 8 plasma levels were 5.7 and 3.4 ng/mL. Dose increased to 2.0 mg/day. Weeks 12, 16, 20, 24 Plasma levels were 6.6, 6.4, 6.9, and 5.2 ng/mL.
Lee et al, 2010[29].	Case control	35	Peg-IFN alpha 2a ribavirin (n = 25)	Start of DAA treatment: tacrolimus median (IQR) 6.9 (6.0-8.9) ng/mL End-of-treatment: median (IQR) 3.8 (3.6-5.0) ng/mL Dosages adjusted according to AST/ALT.
Fontana et al, 2013[30].	Case report	1	Daclatasvir, sofosbuvir	Tacrolimus dose was not adjusted. Plasma concentrations were stable during DAA treatment: 4-5 ng/mL.

LT: Liver transplant; DAA: Direct-acting antiviral; Peg-IFN: Peg-interferon; IQR: Interquartile range; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; No: Number.

THE OBSERVED EFFECT OF GASTRIC BYPASS SURGERY ON DIRECT-ACTING ANTIVIRAL TREATMENT: A CASE REPORT

Patient and treatment

We describe a 61-year old Brazilian female patient who presented to our outpatient clinic in 2011 with chronic HCV genotype 1b infection. She was diagnosed with HCV-infection in 2008, but the transmission route was unknown. Possible sources of infection included dental treatment or a caesarean section in Brazil.

The patient was severely obese with a body mass index (BMI) of 35.4 kg/m^2 (weight 84 kg, height 1.54 meters). Ultrasound demonstrated hepatic steatosis without any ultrasonographic signs of cirrhosis. Evaluation of liver stiffness using Fibroscan®[35, 36] showed a value of 13.6 kPa, consistent with METAVIR fibrosis score F3 (severe fibrosis) [37, 38]. A liver biopsy showed moderately active periportal inflammation and moderate periportal fibrosis with formation of septae in less than 50% of portal fields (METAVIR score A2/F2-3) and macrovascular steatosis in 40-50% of hepatocytes with minimal pericellular fibrosis (Brunt score steatosis grade 2, fibrosis stage F1)[39]. Laboratory testing showed mildly elevated liver enzymes with an ALT of 77 U/L, AST of 51 U/L, and γGT of 57 U/L. Serum bilirubin, prothrombin time, albumin, creatinine, thrombocytes, and fasting blood glucose values were all normal. HCV RNA was 9.56x10^5 IU/mL and HBsAg and anti-HIV 1 & 2 antibodies were negative.

The patient was a non-responder to treatment with Peg-IFN alfa and ribavirin in 2009. In 2013, she was included in a clinical trial and was treated with DAAs daclatasvir and asunaprevir for 24 weeks. A relapse occurred after this treatment.

Whilst waiting for registration and reimbursement of the first DAAs in the Netherlands, the patient decided to undergo gastric bypass surgery in 2014 (Roux-and-Y gastric bypass). She came back to our outpatient clinic in 2015 for (re-)treatment of the chronic HCV-infection. Her weight had reduced to 59 kg (BMI 24.9 kg/m^2), and transaminases had improved (ALT 48 U/L; AST 39 U/L). Other liver enzymes and liver function tests were not altered. HCV RNA load was 5.64 x10^6 IU/mL and Fibroscan® showed a value of 7.8 kPa. Sequencing of the viral genome was performed on the regions NS5A and NS3 (as she had received a NS5A inhibitor and a protease inhibitor), which showed a high level of resistance-associated substitutions (RAS) to NS5A inhibitors on the loci L31M/I and Y93H. There were no RAS present in the NS3 gene of the viral genome. For these reasons, we decided to treat the patient with 400 mg sofosbuvir QD (Sovaldi®, Gilead Sciences, Cambridge, United Kingdom), 150 mg simeprevir QD (Olysio®, Janssen-Cilag International, Beerse, Belgium), and 1,000 ribavirin per day, for a total of 24 weeks[1, 40, 41].

Simeprevir and ribavirin in particular must be taken with food for adequate plasma concentrations[42]. However, due to the bariatric surgery, the patient was not able to eat large meals. To study the exposure of the DAAs and ribavirin in this patient, a pharmacokinetic curve was obtained at week 3 of DAA treatment. Blood was sampled at t = 0 (pre-dose), 2, 3, 5, 6, 8, and 24 hours after intake of the DAAs. DAA plasma concentrations were determined using an in-house made, validated high performance liquid chromatography (HPLC)-MS/MS assay. Pharmacokinetic parameters were calculated using WinNonlin/Phoenix version 6.3, Pharsight Corporation, St. Louis, MO, USA. The assay lower limits of quantification for sofosbuvir, GS-331007, and simeprevir were 2.5 ng/mL, 10 ng/mL, and 10 ng/L respectively. The precision for low, medium, and high quality control (QCs) samples was <10% for all analytes. Ribavirin plasma concentrations were determined using validated HPLC assay with ultraviolet detection[43, 44].

At week 3, the AUC_{last} for sofosbuvir was 0.63 h·mg/L, the C_{max} was 0.35 mg/L, and the C_{trough} was 0.0013 mg/L. For the main inactive metabolite of sofosbuvir, GS-331007, the $AUC_{0\text{-}24}$ was 21.02 h·mg/L, C_{max} was 1.55 mg/L, and the C_{trough} was 0.35 mg/L (Figure 4a).

For simeprevir, at week 3 of treatment, the $AUC_{0\text{-}24}$ was 9.42 h·mg/L, the C_{max} was 1.21 mg/L, and the C_{trough} was 0.046 mg/L (Figure 4b). Ribavirin concentration was 2.5 mg/L. Sofosbuvir and ribavirin concentrations were considered adequate but simeprevir concentrations were subtherapeutic compared with those described in literature[45]. As a result, at week 10 of treatment, the simeprevir dose was doubled to 150 mg BID (taken together with food). At week 14, trough concentrations of ribavirin and simeprevir were determined again and were 0.532 mg/L and 3.5 mg/L, respectively. The hemoglobin concentration had dropped from 12.3 g/dL to 9.8 g/dL.

HCV RNA was undetectable during treatment at week 3, 4, 12, 24 (end-of-treatment) and 12 weeks after end-of-treatment (SVR12).

During treatment, the main side effect was extreme fatigue. Liver enzymes, liver function tests, and renal function were all normal during treatment. Twelve weeks after completion of treatment, Fibroscan® showed a value of 4.6 kPa.

For this case report, no formal ethical approval was obtained as all procedures were performed for regular health care purposes. The patient did not have to comply with certain extra examinations of life style rules. However, the patient gave consent for performing the pharmacokinetic curve and publication of this paper. This was recorded in the patient chart.

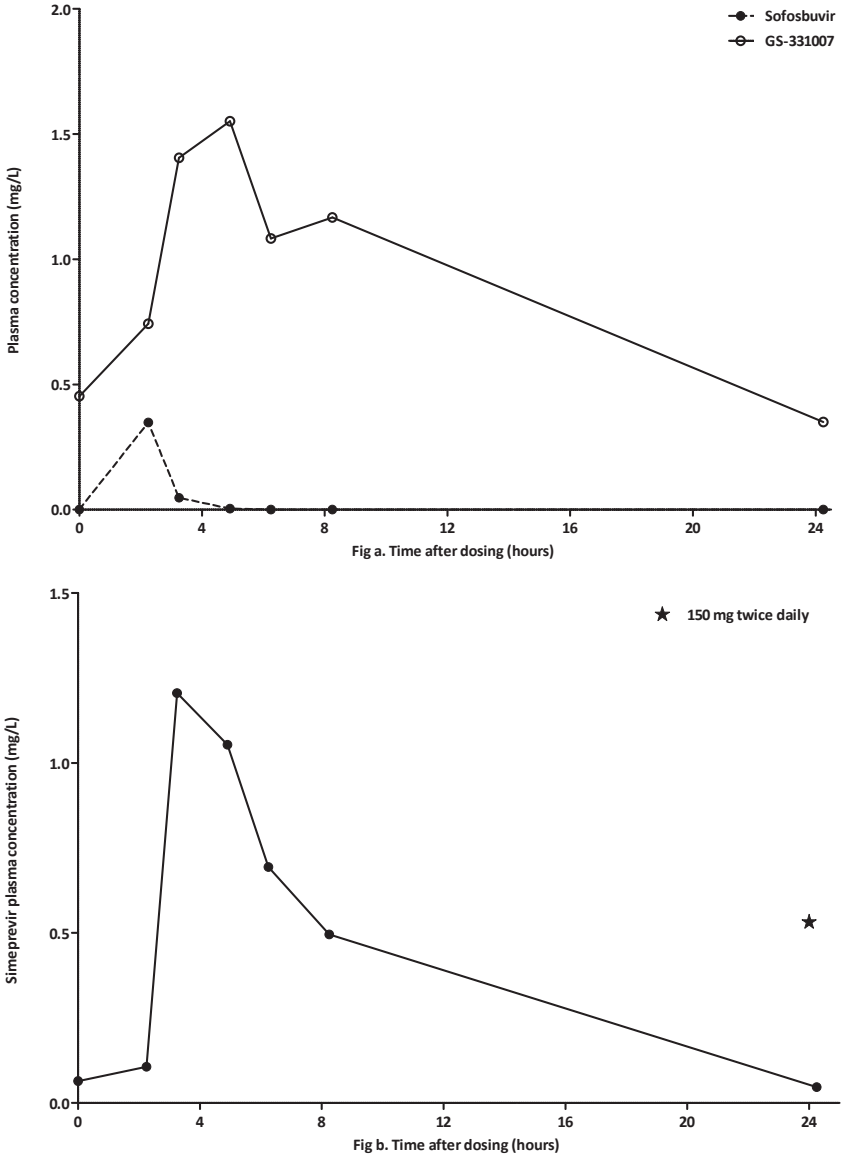

Figure 4: Plasma concentrations of 400 mg sofosbuvir QD and GS-331007 (A) and 150 mg and 300 mg simeprevir QD (B).

A: Sofosbuvir: C_{trough}: 0.001 mg/L; C_{max}: 0.35 mg/L; T_{max}: 2.25 hours; AUC_{last}: 0.63 h·mg/L; $T_{1/2}$: 0.5 hours.
GS-331007: C_{trough}: 0.35 mg/L; C_{max}: 1.55 mg/L; T_{max}: 4.91 hours; AUC_{0-24}: 21.02 h·mg/L; $T_{1/2}$: 10.3 hours.
Sofosbuvir reference values (400 mg QD treatment-naïve HCV genotype 1-infected subjects without cirrhosis).
Sofosbuvir: T_{max}: 0.5-1.5 hours; C_{max}: 0.55 mg/L; AUC_{0-tau}: 1.03 h·mg/L. GS-331007: T_{max}: 2-4 hours; C_{max}: 582 mg/L; AUC_{0-tau}: 7.12 h·mg/L[14].
B: Simeprevir: C_{trough}: 0.046 mg/L; C_{max}: 1.20 mg/L; T_{max}: 3.25 hours; AUC_{0-24}: 9.41 h·mg/L; $T_{1/2}$: 4.6 hours.
Week 14: C_{trough}: 0.532 mg/L.
Simeprevir reference values (treatment-experienced patients 150 mg QD): C_{trough}: 1.41 mg/L; C_{max}: 4.38 mg/L; T_{max}: 2.03-9.87 hours; AUC_{0-24}: 57.4 h·mg/L[45].

DISCUSSION

We are the first to describe a patient who was successfully treated with DAAs including an adjusted dose of simeprevir after undergoing gastric bypass surgery. Although simeprevir was not deemed ideal in this patient, given the food-dependent uptake, there was no alternative due to existing resistance to NS5A inhibitors.

We treated the patient for 24 weeks, according to national and international guidelines, as she relapsed to earlier dual NS3/NS5A DAA therapy[1, 40, 41]. We also tried to enhance the potency of the treatment by adding ribavirin (at a weight-based dose).

According to the simeprevir label, the AUC increases by 60% when administered with a fatty meal or normal breakfast[42]. We measured simeprevir C_{trough} levels that were 97% lower than comparable reference values, and the $AUC_{0\text{-}24}$ was 84% lower. Our patient was not able to have large or 'normal sized' meals (i.e. a high intake of calories) anymore and we postulate that this resulted in the extremely low exposure to simeprevir. Despite the fact that HCV RNA was undetectable at that time, we doubled the dose of simeprevir to increase the plasma exposure and possibly efficacy. This dose was well tolerated and the C_{trough} plasma concentration at week 14, 4 weeks after doubling the dose, was approximately 11-fold higher than the week 3 C_{trough} level (62% lower than the reference value). This extreme increase is the result of the non-linear pharmacokinetics of simeprevir.

For ribavirin, we strived to attain a plasma concentration of 2.0-3.0 mg/L at steady-state[46]. At week 3 of treatment the plasma concentration was already 2.5 mg/L, which is remarkable as the patient had a low intake of food[47]. These high ribavirin levels caused anemia and the patient suffered from extreme fatigue. It was considered to lower the dose of ribavirin, but because the hemoglobin levels remained stable throughout the whole course of treatment and the patient did not want a dose reduction, so the starting dose of 1,000 mg per day was continued. The high plasma concentrations of ribavirin (compared to the low plasma concentrations of simeprevir) could also be related to the low body weight of <60 kg of the patient after gastric bypass surgery. The fact that a large or 'normal' meal could not be consumed seems less important for an adequate ribavirin level as the initial dose was already relatively high.

Sofosbuvir pharmacokinetics were not affected by the gastric bypass or the low intake of food as the exposure to both sofosbuvir and GS-331007 (the main inactive metabolite of sofosbuvir) were sufficient. This was as expected because it was earlier described

that a high-fat meal does not influence the plasma concentration of sofosbuvir or GS-331007[48].

This case report describes a patient with chronic HCV-infection genotype 1b without liver cirrhosis, but with a relapse after earlier dual DAA-treatment, who was successfully treated with simeprevir, sofosbuvir, and ribavirin for 24 weeks after undergoing gastric bypass surgery. Adequate sofosbuvir and ribavirin plasma concentrations were achieved, however, simeprevir plasma concentrations were low when simeprevir was dosed according to the drug label (150 mg QD)[42]. Both bariatric surgery and low intake of food can influence drug absorption and drug exposure. Awareness is needed when patients who underwent bariatric surgery are treated with certain drugs without any experience in this specific condition. This is especially the case for simeprevir, as absorption is dependent of food intake; it has non-linear pharmacokinetics and possibly more severe side effects when given in high dosages. Patients with a history of bariatric surgery who are treated with simeprevir should be closely monitored using, for example, therapeutic drug monitoring.

CONCLUSION

A lot of information about the pharmacology of drugs is available when new compounds access the market. However, not every single unique situation has been studied and therefore some patients cannot be treated according to (inter)national guidelines. An option is to abstain patients in these situations from treatment. However, with good cooperation between physicians and pharmacists and knowledge of pharmacology, we were able to treat these patients effectively as they all achieved SVR12. Of course, this chapter contains experimental data, and therefore, the patients were well informed about the risk of their individualized treatment.

These four case reports were all published in the public domain, so that other healthcare providers can benefit from our experience when they are dealing with a patient in a comparable situation.

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General discussion

GENERAL DISCUSSION

The overall objective of this thesis was to address relevant pharmacological issues concerning current hepatitis C virus (HCV) therapy including novel direct-acting antivirals (DAAs) and ribavirin. **Part 1** focuses on drug-drug interactions (DDIs) with novel DAAs. **Part 2** discusses the pharmacokinetics (PK) and use of therapeutic drug monitoring (TDM) of ribavirin and in **Part 3** the use of DAAs in special patient populations is debated. Lastly, **Part 4** illustrates the contribution of the expertise of pharmacists in the proper use of DAAs by discussing four real-world examples.

This general discussion discusses these four parts combined with future perspectives concerning clinical pharmacology for DAAs and HCV therapy.

Table 1 gives an overview of the aims, main findings, and conclusions of this thesis.

Table 1: Overview of the aims, main findings, and conclusions of the 13 chapters in this thesis.

Part	Chapter	Aim(s)	Main findings and conclusion	Comments
Drug-drug interactions involving DAAs	1	• Overview of the interaction mechanisms of DAAs and psychoactive agents. • Overview of DDIs between DAAs and psychoactive agents. • Identify safe options for simultaneous treatment of mental illnesses and HCV infection.	• Only a limited number of DDI studies between psychoactive agents and DAAs are performed. • No studies are done with ledipasvir, sofosbuvir, and elbasvir. • Safe combinations of DAAs and psychoactive agents are those without any theoretical DDIs or DDIs studied in humans.	• There is a paucity of experimental data on DDIs between psychoactive agents and DAAs. • More in vivo or in vitro interaction studies are necessary.
	2	• To comment on the process of drug licensing using the product label of velpatasvir/sofosbuvir and grazoprevir/elbasvir as examples.	• There is no clear list of strong enzyme inducers or inhibitors available and there is discrepancy between drug labels which of these compounds are used. • 'contra-indicated', 'avoid use' or 'use with caution' are used in the product labels, however are these recommendations the same? • Only two drugs that are listed by the FDA as a drug with a narrow therapeutic range are discussed in the label of grazoprevir/elbasvir. • Of the top 10 most frequently used drugs by the HCV-infected population, only the proton-pump inhibitors were mentioned in the labels.	• Is there a need for a Consortium for Optimal Management of drug-drug Interactions in patient Care (COMIC)?
	3	• To evaluate the effect of the proposed OCT inhibitor daclatasvir on the PK and PD of the OCT substrate metformin.	• Daclatasvir does not influence the PK of metformin in healthy subjects. • PD parameters were comparable between treatments.	• The use of PD outcomes together with PK outcomes remains challenging. • The understanding of the role of drug-transporters increases however, it must become common knowledge for pharmacists and physicians and implemented in daily clinical care.

Table 1: Overview of the aims, main findings, and conclusions of the 13 chapters in this thesis. (*continued*)

Part	Chapter	Aim(s)	Main findings and conclusion	Comments
Drug-drug interactions involving DAAs	4	• Determining if atazanavir/cobicistat has a comparable influence on daclatasvir PK as atazanavir/ritonavir.	• Atazanavir/cobicistat and atazanavir/ritonavir had a similar influence on daclatasvir PK in healthy volunteers.	• Is it possible to predict DDIs between comparable compounds without any in vivo information?
	5	• Predicting DDIs between DAAs and co-medication used by HCV-infected patients.	• Co-medication use is rich in frequency and diversity in HCV patients. • 60% of patients are at risk for DDIs which may affect efficacy or toxicity of DAAs or co-medication.	• Many DDIs are predicted by the interaction checkers and not studied in vivo. • Some DDIs cannot be predicted on theoretical grounds but do occur in clinical practice.
	6	• Predicting DDIs between DAAs and non-antiretroviral co-medication/cART used by HIV/HCV-infected patients.	• 63% of the population used non-antiretroviral co-medication. • 38% of the patients were at risk for a DDI between DAAs and non-antiretroviral co-medication and 75% of the patients must alter their cART. • From the perspective of potential DDIs with non-antiretroviral co-medication and/or cART, the most favorable regimen seems to be sofosbuvir/daclatasvir.	• What is the influence of all these DDIs on efficacy (SVR), HIV viral load, and tolerability of co-medication?
	7	• How do physicians manage DDIs between DAAs and non-antiretroviral co-medication/cART?	• Dutch physicians seem well aware of possible DDIs and prevent the large majority of these. • All patients switch cART to non-interacting regimens prior to DAA treatment.	• Improvement can be made for the limited number of patients (4/6) who continued contra-indicated medications.

Table 1: Overview of the aims, main findings, and conclusions of the 13 chapters in this thesis. (*continued*)

Part	Chapter	Aim(s)	Main findings and conclusion	Comments
Ribavirin pharmacokinetics and therapeutic drug monitoring	8	• Describing the results of the first year of the ribavirin PT program.	• 22/28 of the ribavirin samples were measured accurately.	• A part of the ribavirin samples measured were inaccurate which could have clinical consequences. For instance, subtherapy or unnecessary dose increase.
	9	• To assess the influence of ribavirin steady-state plasma concentrations on SVR in HCV patients treated with DAAs.	• Overall, an SVR-rate of 89% was achieved. • Multivariable analysis showed that ribavirin levels are independent predictors of SVR. • A steady-state target range of 2.3-3.6 mg/L can be defined with the novel DAAs.	• Do we need to use TDM to improve the response rates in hard-to-cure patients?
	10	• Evaluating the efficacy and safety of HCV treatment with peg-interferon and ribavirin and the PK of ribavirin in HIV/HCV co-infected Thai patients.	• Overall, an SVR-rate of 56% was achieved. • HIV/HCV patients had lower ribavirin exposure when compared with mono-infected HCV patients from literature.	• To improve response rates, do we need to dose higher in HIV/HCV patients treated with ribavirin? • It is time that oral DAAs become available in low and middle-income countries.

Table 1: Overview of the aims, main findings, and conclusions of the 13 chapters in this thesis. (*continued*)

Part	Chapter	Aim(s)	Main findings and conclusion	Comments
Treatment of HCV with DAAs in special patient populations	11	• Describing treatment possibilities in HCV patients with hepatic and renal impairment.	• All drugs used in HCV treatment can be used in patients with compensated cirrhosis. • All drugs used in HCV treatment can be used in patients with moderate renal insufficiency. • In patients with GFR ≤29 mL/min and/or advanced liver disease, HCV drugs might be contra-indicated or dosage adjustments may be necessary.	• Studies in these special populations are challenging as these patients are vulnerable, however, there is a need for information to optimally treat these patients. • There is still no answer about the use of sofosbuvir in patients with decreased renal function.
	12	• Exploring a mechanistic model to simulate the PK of daclatasvir in healthy volunteers and cirrhotic patients and describing the PK of bound and unbound daclatasvir in these patient populations.	• Daclatasvir PK was accurately modeled in healthy volunteers. • A discrepancy remained between the observed and simulated daclatasvir exposure in cirrhotic patients.	• PBPK modeling is a useful tool for understanding the PK of drugs; however, the use in special patient populations needs more work. For cirrhotic patients we need more information about the expression of drug-transporters. • It is challenging to incorporate the influence of drug-transporters in a PBPK model.

Table 1: Overview of the aims, main findings, and conclusions of the 13 chapters in this thesis. (*continued*)

Part	Chapter	Aim(s)	Main findings and conclusion	Comments
A pharmacist's contribution to eradicate hepatitis C	13	Patient 1: Treated with sofosbuvir, daclatasvir, plus ribavirin while on etravirine and darunavir/ritonavir for his HIV infection.	• 60 mg daclatasvir is the right dose in combination with etravirine, and darunavir/ritonavir as the patient reached SVR and adequate exposure of daclatasvir.	• Despite all the available information about DDIs for some unique patients, special dosages are needed.
		Patient 2: Treated with sofosbuvir and daclatasvir in combination with oxcarbazepine for his epilepsy.	• Daclatasvir 90 mg and sofosbuvir 400 mg were for this specific patient the right dosages in combination with oxcarbazepine as the patient reached SVR, however exposure of daclatasvir was lower than expected.	• More information is needed about using strong CYP3A4 and P-gp inducers in combination with DAAs, especially for patients with epilepsy. • Rifampicin is not the only strong inducer that must be studied in phase-I trials.
		Patients 3 and 4: Patients undergoing liver transplantations that used tacrolimus simultaneously with daclatasvir, sofosbuvir, and ribavirin. There were no DDIs; however, decreased tacrolimus plasma concentrations were reported.	• Plasma concentrations of drugs that are extensively metabolized by the liver (tacrolimus), might be influenced by DAA therapy even when there is no DDI.	• Physicians treating patients with DAAs must be aware of the dynamics of CYP-substrates, such as tacrolimus.
		Patient 5: A treatment-experienced patient with chronic HCV infection genotype 1b, treated with simeprevir, sofosbuvir, and ribavirin after a Roux-and-Y gastric bypass.	• The patient was successfully treated with DAAs after undergoing gastric bypass surgery. • Adequate sofosbuvir and ribavirin levels were achieved. Simeprevir exposure was low with a standard dose, for which the dose was doubled.	• In general, little is known about the effects of a Roux-and-Y gastric bypass on drug exposure. • What do we do with drug dosing after bariatric surgery?

DDIs: Drug-drug interactions; OCT: Organic cation transporter; HCV: Hepatitis C virus; DAAs: Direct-acting antivirals; PT: Proficiency testing; cART: combination antiretroviral therapy; GFR: Glomerular filtration rate; SVR: Sustained virological response; TDM: Therapeutic drug monitoring; PK: Pharmacokinetics; PD: Pharmacodynamics; CYP: Cytochrome P450; P-gp: P-glycoprotein; FDA: US Food and Drug Administration.

DRUG-DRUG INTERACTIONS

In this thesis DDIs are a focus point of discussion. It is important to study DDIs of DAAs, as most DAAs are substrates of a large number of drug-transporters and drug-metabolizing enzymes. Substrates of enzymes and transporters are prone to be a victim of DDIs which might affect the plasma concentration of the substrate. Especially reduced plasma concentrations might be a problem in case of the DAAs, since it could possibly be that subtherapeutic levels cause therapeutic failure. Secondly, DAAs themselves also can influence some of these same drug-transporters and drug-metabolizing enzymes which makes them potential perpetrators of DDIs, causing increased or decreased plasma concentrations of co-medication **(Chapters 5, 6, and 7)**.

Before drugs are licensed, DDIs are extensively studied, both *in vitro* and *in vivo*. Drug-metabolizing enzymes and drug-transporters involved in the metabolism and distribution of the novel compound are identified *in vitro*. This *in vitro* information is, among others, used to design DDI studies in humans (*in vivo*). In addition, DDI studies are executed with drugs that are frequently used by the target population. Especially the impact and the character of the DDIs are of interest in these *in vivo* studies.

In vitro interaction studies

An important aspect of preclinical studies is mapping which drug-metabolizing enzymes are involved in the metabolism of a compound, using for example human hepatocytes or liver microsomes. These experiments quantify the contribution of a certain cytochrome P450 (CYP) or uridine 5'-diphospho-glucuronosyltransferase (UGT) enzyme to the metabolism of the compound. Comparably, over expression systems can identify possible drug-transporters involved in the disposition of the compound[1].

Another important preclinical experiment aims to identify whether a compound is an inducer or inhibitor of a certain CYP enzyme. These experiments can be done in human hepatocytes measuring the metabolism of a model substrate with and without the study compound. Similar studies can be done for drug-transporters. A caveat is that experiments studying induction of drug-transporters and enzymes challenging in view of the necessity of genetranscription[1].

In vivo interaction studies

In vivo DDI studies (humans) are mostly performed in healthy volunteers giving a good impression of the clinical relevance and character of the DDI. These results are used to make dose recommendations (increase/decrease) or can even lead to a contra-indication (do not use simultaneously) for combinations of drugs. Studying DDIs in

healthy volunteers **(Chapter 3 and 4)** gives the researcher the opportunity to control parameters such as the use of other co-medication and the existence of co-morbidities. However, healthy volunteers are different from the target population, in case of this thesis, HCV patients with diabetes **(Chapter 3)** or HIV/HCV co-infected patients **(Chapter 4)**. For example, drug-metabolism can be altered in patients, resulting in a different exposure of a compound when compared with healthy volunteers **(Chapter 13)**. This has been described for simeprevir: the exposure of simeprevir was approximately 3-fold higher in HCV patients compared with healthy volunteers[2], and this is probably caused by altered expression of CYP enzymes and/or drug-transporters. This altered metabolism might also affect the magnitude of a DDI[3]. In addition, healthy volunteers and patients have different characteristics such as age, weight, gender, renal, and hepatic function, which are known covariates responsible for variation in PK.

In my opinion, the preferable option is to study DDIs in the target population, although, it could be unethical to include patients in a DDI study as they can possibly be exposed to unnecessary subtherapy or even toxicity. The study with metformin and daclatasvir **(Chapter 3)** could have been conducted in patients with diabetes. However, these patients often suffer from many other diseases, making it hard to interpret results as there could be many other confounding factors. For example, co-medication used by patients would complicate the interpretation of the results of such a DDI study.

The reasoning that supported the design of the interaction study between daclatasvir and metformin was that daclatasvir, as an OCT1 and 2 inhibitor might influence the excretion of metformin. However, we did not detect a clinically relevant DDI, which was bolstered by subsequent published studies showing that the unbound half maximal inhibitory concentration (IC_{50}) of daclatasvir for OCT1 and 2, was higher than the *in vitro* maximum plasma concentration (C_{max}). One could argue that this DDI study in healthy volunteers would have been unnecessary as the IC_{50} value gave no indication for a clinical relevant interaction. However, at time of the study, this information was not available in the public domain[4-6]. Now we can state that the *in vitro* data are consistent with the clinical data, which is not always the case.

There are different options to evaluate the outcome parameters (PK values) from an *in vivo* DDI study. **Chapter 3** and **4** are randomized, cross-over studies in healthy volunteers. Outcome parameters were evaluated with the bioequivalence approach. This approach is most commonly used to determine if the PK profile of a generic drug is comparable with the PK profile of a branded drug[7]. Bioequivalence can also be used to evaluate DDI studies, resulting in geometric mean ratios (GMR) of PK parameters (test versus reference). The GMR must meet acceptance criteria that are defined *a priori*.

In the study protocols of **Chapter 3** and **4** it was defined that bioequivalence was established if the GMR, with its 90% confidence interval, was within 80-125%. This range of 80-125% takes, among others, the inter-subject variability of the compound into account. Of course, for some drugs, for example, with a narrow therapeutic range or severe toxicity, other acceptance criteria must be defined.

Drug labels often use a different method when showing interaction data. Frequently a percentage increase or decrease in concentration of the substrate in combination with the inhibitor or inducer is presented. No acceptance criteria are given. How the results from DDI studies are interpreted and presented in the label, is decided by the manufactures. This could result in deviating descriptions in the drug labels, which is addressed in **Chapter 2** of this thesis. For instance, rifampicin causes similar reduced exposures of grazoprevir/elbasvir (82%) and velpatasvir/sofosbuvir (90%)[8-11], however, the interpretations in the labels are different. The label of grazoprevir/elbasvir lists a contra-indication with rifampicin, while velpatasvir/sofosbuvir labels the combination with rifampicin as 'not recommended'. In my opinion, the recommendations should be similar and regulatory agencies must be challenged to provide more guidance in these situations.

Which drug-drug interactions must be studied?

Another important question regarding DDI studies is which co-medications must be studied in combination with the new drug. A mechanism-based approached is discussed earlier. Another approach is to study possible DDIs with frequently used co-medication of the target population. Both interaction studies with daclatasvir (atazanavir/ritonavir or atazanavir/cobicistat and metformin) are examples of combinations of drugs that are frequently used by HCV- or HIV/HCV-infected patients. In **Chapters 5, 6, and 7** it is presented which drugs are most frequently used by Dutch HCV- and HIV/HCV-infected patients. Checking these drugs with the DAA drug labels (January 2017) shows that the majority of the top 10 co-medications of both studies are not mentioned in the drug labels (Table 2). The label of paritaprevir/ritonavir, ombitasvir with or without dasabuvir contains the most information about the listed co-medication.

Table 2: Overview of top 10 drugs used by Dutch HCV mono-infected and HIV/HCV co-infected patients and their presence in the label of the DAA.

	Dacla-tasvir [4, 5]	Simepre-vir [12, 13]	Ledipasvir/ Sofosbuvir [14, 15]	Paritaprevir/ ritonavir, ombitasvir [16]	Velpatasvir/ sofosbuvir [10, 11]	Grazoprevir/ elbasvir [8, 9]
Methadon[a]	F/E	F/E	F/E	F/E	F/E	F/E
Omeprazole[a]	F/E	F/E	F/E	F/E	F/E	
Pantoprazole[a]		E	E		E	F/E
Oxazepam[b]						
Hydrochlorothiazide[b]						
Metformin[b]				E		
Temazepam[b]						
Paracetamol[b]				E		
Salbutamol[b]						
(Es)citalopram[b]	F/E	F/E		F/E		
Calcium supplements[c]						
Salbutamol[c]						
Sulfamethoxazole/ Trimethoprim[c]				E		
Valaciclovir[c]						
Metoprolol[c]				E		
Diazepam[c]				E		
Lisinopril[c]						

[a]In top 10 of both mono and co-infection patients.
[b]In top 10 of mono-infected patients.
[c]In top 10 of co-infected patients.
F: US Food and Drug Administration; E: European Medicine Agency.

It is of particular interest that the drugs listed in the labels are not always comparable for US Food and Drug Administration (FDA) and European Medicine Agency (EMA). Additionally, *in vivo* DDI studies have not been performed for all listed co-medications. DDIs can be predicted based on the known characteristics of a compound, but can also be extrapolated from a DDI study with a drug in the same class. This was for example done for the benzodiazepines as **Chapter 1** of this thesis describes. However, to which extent the results of drugs within a class can be extrapolated is uncertain, especially if you take DAAs as an example. For instance, the DDI profile of the NS5A inhibitors daclatasvir and ledipasvir are different.

Almost all labels have inserted information of a DDI study with omeprazole (proton pomp inhibitor [PPI]). For example, ledipasvir and velpatasvir are dependent on a low intra gastric pH for its solubility and therefore acid-reducing agents, such as the PPIs

and H_2-antagonists, could influence absorption. The European label of ledipasvir states: 'PPI doses comparable to omeprazole 20 mg can be administered simultaneously with Harvoni® (ledipasvir/sofosbuvir). PPIs should not be taken before Harvoni' and 'H_2-receptor antagonists may be administered simultaneously with or staggered from Harvoni at a dose that does not exceed doses comparable to famotidine 40 mg twice daily'[14].

These are also fine examples where the information of a specific drug is extrapolated to the total drug class (PPIs and H_2-antagonists). In this case, the extrapolation is right as all drugs that decrease gastric pH, decrease the solubility of ledipasvir.

The statement about the acid-reducing agents in the label of ledipasvir/sofosbuvir raises a number of questions: 1) What is an equivalent dose of 20 mg omeprazole or 40 mg famotidine? 2) What if patients use a PPI twice daily? 3) What if the patient uses a dosage of 40 mg omeprazole?

As these questions emerged in clinical practice, we tried to enhance the information from label. Here, we show our contribution as pharmacists to optimize DAA therapy, by translating the information in the drug label to practical recommendations concerning ledipasvir and PPIs[17].

1. If possible, discontinue the PPI a few weeks prior to ledipasvir treatment.
2. If discontinuing the PPI is not possible: use only 20 mg once daily omeprazole together with ledipasvir.
3. If PPI dosages cannot be adapted, switch to another DAA regimen.

The most important question is still unanswered: does a possible reduced ledipasvir plasma concentration affect efficacy (sustained virological response [SVR])? This is a discussion in the scientific community, and the debate is ongoing[18-20].

A last topic I would like to discuss in this section is the interaction between the DAAs and strong CYP3A4 and P-gp inducers oxcarbazepine **(Chapter 13)** and carbamazepine. Oxcarbazepine and carbamazepine are used for the treatment of epilepsy and it the estimated that in 2010 there were 84,000 epilepsy patients in the Netherlands. About 50% of these patients were treated with carbamazepine in 2009 (44,000)[21-23]. Patients treated with oxcarbazepine and carbamazepine are excluded from HCV treatment as these drugs are (strong) CYP3A4/P-gp inducers, and therefore the plasma concentration of all DAAs decreases when combined (CYP3A4/P-gp substrates).

This was among others shown by a DDI study between carbamazepine and paritaprevir/ritonavir, ombitasvir, and dasabuvir showing a decrease in AUC of 31% for paritaprevir and 70% for both ombitasvir and dasabuvir. The other DAAs are not studied in combination with carbamazepine, and therefore the contra-indication is based on studies with rifampicin. Rifampicin is also a strong inducer of CYP3A4 and P-gp, and it significantly reduces the plasma concentration of all DAAs[4, 8, 10, 12, 16, 24, 25].

This boils down to a clinical issue: how do we treat epilepsy patients that are not able to switch their carbamazepine or oxcarbazepine therapy? As there is a paucity of data, physicians are struggling how to treat these patients. Therefore, the HepNed (hepatitis C Nederland; http://www.hepned.nl) initiative started to collect data on efficacy, safety, and PK of patients that are experimentally treated with strong inducers (carbamazepine) and DAAs. The first patient treated with oxcarbazepine and daclatasvir/sofosbuvir is discussed in **Chapter 13**. Four other patients were recently treated with carbamazepine in combination with an increased dose of daclatasvir (60 mg two or three times daily [BID or TID]) and 400 mg sofosbuvir. These patients were treated with 1) 400 mg/day carbamazepine; 60 mg daclatasvir BID and 2) 1,000 mg/day carbamazepine and 60 mg daclatasvir TID; 3 and 4) 1,200 mg/day carbamazepine and 60 mg daclatasvir TID. So far, patient 1 and 2 reached SVR and had daclatasvir trough plasma concentrations of approximately 0.1 mg/L, which is 50% lower than previously described[4, 5]. One must consider that these are preliminary results and more patients need to be included in this study. For now, no recommendations can be formulated.

HepNed is a research cooperation between all academic centers in the Netherlands and all disciplines involved in HCV treatment joined: hepatologists, infectious diseases specialists, microbiologists, and pharmacists. In my opinion, collaboration between these specialists is key to exchange knowledge and to coordinate nationwide research. This will probably improve research and answer questions from which patients eventually will benefit.

Management of drug-drug interactions

The interaction checkers from the University of Liverpool (http://www.hep-druginteractons.org and http://www.hiv-druginteractions.org) are useful tools for the management of DDIs and frequently used by physicians, nurse specialists, and pharmacists. These websites are part of three chapters in this thesis and contains information about possible DDIs between DAAs, cART, and other co-medications. Over 600 drugs are available and every combination of drugs is accompanied by an advice:

- Green: No interaction expected.
- Yellow: Potential interaction, likely to be of weak intensity where additional action/ monitoring or dosage adjustment is unlikely to be required (added to the website February 2017).
- Amber: Potential interaction, likely to require additional monitoring, alteration of drug dosage or timing of administration.
- Red: Contra-indication; do not co-administer.

The management of DDIs becomes much easier with the use of this tool. Also other sources for DDI checking are available online, for example the Epocrates application or Fungal Pharmacology. These websites and applications have in common that they are developed by pharmacologists to improve the knowledge of DDIs among healthcare professionals. Also, pharmacists can use it for medication monitoring. However, part of the displayed interactions are predictions, based on what is known of the metabolic profile of the drugs, and thus not based on *in vivo* DDI data. It is simply not feasible to study all possible DDIs (costs, time). Consequently, these tools fill in a gap of missing information. However, the indicated DDIs are not checked by any regulatory agency and one could argue how reliable these tools are? I think that these tools are very reliable and the recommendations are as evidenced-based as possible, using all information available in the public domain: product labels, publications, and poster presentations. Still, anybody using these websites must always interpret the information by themselves, as the results of the interaction checkers are usually 'general' recommendations, which must be adapted for an individual patient.

Especially, in case of the 'amber' interactions on the websites of the University of Liverpool. These DDIs can often be avoided and this is where the pharmacist comes to the aid of physicians. Pharmacists can interpret the data presented, give alternatives for interacting drugs, or can explain the interaction mechanism. This is where pharmacists do best and where they really can contribute to optimize pharmaceutical care of HCV-infected patients treated with DAA therapy. Two examples where we contribute to the treatment of HCV patients with complex DDIs are described in **Chapter 13.** With knowledge of pharmacology and good collaboration with the physicians, we were able to treat the patients safely and successfully resulting in clearance of the virus.

As indicated before, many DDIs can be predicted on the basis of already available information. There are a number of notable exceptions. There appears to be a DDI of daclatasvir/ledipasvir with sofosbuvir and amiodarone[26-30]. The DDI led to mortality and this outcome was not predicted and incompletely understood. These examples show that despite the fact that the scientific community, manufacturers, and regulatory

agencies do their best, DDIs are sometimes unpredictable. Studying novel compounds focuses mainly on absorption, metabolism, distribution, and elimination of drugs. However, Back and Burger suggested that the combination of highly protein-bound drugs (DAAs and amiodarone), could have caused this interaction by protein displacement, resulting in temporarily increased unbound amiodarone plasma concentrations[28]. It may be necessary to study highly protein-bound drugs in more detail. Or must we deal with the fact that with the introduction of novel drugs there will always be safety concerns, which we must reduce if possible? In my opinion, we must accept that we simply do not know everything at the time of drug licensing.

RIBAVIRIN PHARMACOKINETICS AND THERAPEUTIC DRUG MONITORING

The exact mechanism of action of ribavirin against the HCV virus remains unclear, but as it is a guanine analogue it is expected to interfere with the RNA synthesis of the virus. Ribavirin is metabolized through two pathways: 1) a reversible phosphorylation pathway; 2) a degradative pathway involving deribosylation and amide hydrolysis to yield a triazole carboxyacid metabolite. Because no CYP enzymes are involved, ribavirin is not prone to be involved in 'metabolic' DDIs. Ribavirin has an absolute bioavailability of 45-65%, and this reduced availability is probably caused by first pass metabolism and/or saturation of the uptake transporters in the intestine (N1 sodium-dependent nucleoside transporters)[31, 32]. Ribavirin accumulates in erythrocytes leading to a volume of distribution of 5,000 L, but it does not bind to any plasma proteins. Both ribavirin and its metabolite are renally excreted[33].

Combining treatment of peg-interferon (Peg-IFN) and ribavirin (duo-therapy) increased response rates compared with Peg-IFN alone (~48 versus 38%, respectively[34-36]). Adding ribavirin reduces among others the risk on viral relapse after treatment[37]. **Chapter 10** describes an HIV/HCV co-infected cohort of Thai patients who were treated with Peg-IFN and ribavirin. SVR-rate was 56%, which is comparable with previously published studies of genotype 1 and 3 HIV/HCV co-infected patients treated with duo therapy[38].

Ribavirin remains part of current DAA therapy. It is recommended to add ribavirin to DAAs to increase SVR-rates or to shorten treatment duration[39, 40]. This is particularly true for patients with genotype 1a and 3, in patients with cirrhosis, or patients who are treatment-experienced. However, other promising DAAs show in phase-II/III studies high cure rates in difficult-to-cure patient populations, possibly reducing the need for ribavirin in HCV therapy.

Chapter 9 shows the importance of ribavirin concentrations in a Dutch cohort treated with DAAs and ribavirin. Ribavirin steady-state concentration was a predictor for SVR and a therapeutic range of 2.3-3.6 mg/L was defined. The relationship between SVR and ribavirin concentration remain controversial, both in combination with Peg-IFN and DAA treatment, as conflicting results are published[41-48]. These inconsistent results might be caused by differences in treatments, heterogeneity of populations, variation in analytical assays, and small sample sizes.

Ribavirin meets a number of criteria for TDM. For example, it has a high inter-subject variation and a low intra-subject variation, which is confirmed in **Chapter 9 and 10**[33, 46]. This means that it is hard to predict what the plasma concentration for an individual patient will be, but once you know the ribavirin plasma concentration at steady-state, the variation within that patient is small (without any dose adjustments and stable renal function).

Secondly, analytical assays are available for determination of ribavirin plasma concentrations. **Chapter 8** describes the results of a first-year proficiency testing (PT) program. These PT programs are important for the quality control of methods developed by laboratories. However, not all participating laboratories were able to accurately determine ribavirin plasma concentrations. Samples were accurately measured when they were within 80-120% of the spiked (weighed-in) 'expert' concentration. Previously published studies showed that methodological problems, technical problems, or clerical errors could explain the deviating results[49]. It is of importance that the reported value is accurate because pharmacists and clinicians rely on these values for their clinical decision making. In my opinion, pharmacists (laboratories) must strive to measure ribavirin samples and interpret results as soon as possible, because of the relatively short HCV treatment time (12-16 weeks) and possible lack of effect/adverse events can be prevented.

The added value of TDM in ribavirin treatment has never been established through a randomized controlled trial. The preferred trial design would be one with two randomized groups in a multicenter setting. One patient group will be treated with ribavirin and dosages will be adjusted based on TDM results; the other group will be treated with ribavirin and no TDM is performed. The efficacy and safety results of both groups can be compared to show if TDM is beneficial, in terms of higher efficacy (higher SVR-rates) or less toxicity (less anemia). Probably, this kind of trial will not be conducted as efficacy rates of current DAA treatment with or without ribavirin is over 90%.

Because we do not have these kinds of studies, we must decide whether we would use TDM in patient care. In my opinion, TDM of ribavirin could be useful, especially since **Chapter 9** shows that in a large prospective cohort adequate ribavirin levels were an independent predictor of SVR. So, I believe that physicians must aim to achieve a steady-state ribavirin plasma concentration of at least 2.3 mg/L (when tolerated). Patients who are nowadays treated with ribavirin are the patients that are hardest to cure: patients with liver cirrhosis, resistance-associated substitutions (RAS), genotype 1a or 3 infection, or those who have failed prior antiviral therapy[37]. Therefore, we must balance efficacy with safety. Subtherapy is a risk for patients that are treated with a normal dose of ribavirin with the absence of toxicity. It could be that these patients have adequate exposure, but it could also be that the ribavirin plasma concentrations are not high enough. Using TDM helps tracking down patients with subtherapy followed by a ribavirin dosage increase. Another option is to dose based on toxicity. In other words, anemia is the dose-limiting factor. With this strategy, also extreme plasma concentrations could occur, causing severe anemia with even an indication for blood transfusion. Due to the long elimination half-life of ribavirin, plasma concentrations will slowly drop in these cases which cannot be accelerated by dialysis. Additionally, dosing on toxicity is not an option for vulnerable patients, such as patients with renal impairment and the transplant patients. These patients should be closely monitored and they must start with a reduced ribavirin dose according to AASLD guidelines[40].

SPECIAL PATIENT POPULATIONS

After discovery of a potential effective compound (a drug), this compound is extensively studied by the manufacturer. The first part of development is the pre-clinical phase, using *in vitro* experiments unraveling the characteristics of a drug (e.g., antiviral potency, mechanism of action). Next, the studies continue in humans and we can distinguish four phases.

1) First in human trials. Depending on the type of drug, but mostly done in little groups of healthy volunteers. During this phase dose finding and safety of the compound are main subjects of interest.
2) First in patient trials. Efficacy and safety of a compound are studied in a selected target population. In addition, DDIs and dose-response is studied. This is usually a small number of patients (<100).
3) Large patient trials (>100 patients) which are used to monitor efficacy and safety and compare the treatment with the current standard of care. Again, selected patients are included.

These three phases are needed for market authorization; however, we can add a last phase of drug development:

4) Post approval trials, which are conducted to further evaluate efficacy and safety of a compound.

Special patients discussed in this thesis are mainly hepatic and renal impaired patients **(Chapter 11** and **12)**. There is no consistency of the data that becomes available about the use of these drugs when the DAAs were approved. For instance, in the drug label of simeprevir, PK data was available for non-HCV cirrhotic subjects (Child-Pugh (CP) class A, B, and C). However, efficacy and safety data are not available for cirrhotic HCV patients[12]. Another example, patients with an estimated glomerular filtration rate (eGFR) below 30 mL/min, patients on hemodialysis, or with end-stage renal disease were not discussed in the labels of sofosbuvir, velpatasvir/sofosbuvir, and ledipasvir/sofosbuvir, but data is available for daclatasvir, simeprevir, and grazoprevir/elbasvir[4, 8, 10, 12, 14, 25].

The efficacy and safety data for these patient groups becomes mostly available during post approval studies. Studying these patient groups is important as patients with hepatic or renal impairment could have altered PK as these two organs are important for the metabolism and excretion of drugs **(Chapter 11** and **12)**. Subsequently, patients with severe disease might have higher plasma concentrations of some drugs, as there could be decreased excretion/metabolism, causing more toxicity. This is relevant for HCV-infected patients, as they are at risk for developing cirrhosis and/or declined renal function.

As efficacy, safety, and PK data in special patient populations is sometimes missing or insufficient, physicians thus treat these patients off-label and based on little information. Because they are in need of treatment the risk on a possibly unknown event is taken. This may have severe consequences as we have seen with the combination of paritaprevir/ritonavir, ombitasvir with or without dasabuvir. After registration, the FDA warned for serious liver injury in patients with advanced disease with this combination[50]. This safety issue was probably caused by the protease inhibitor (paritaprevir). The safety and efficacy of this combination was only studied in CP-A patients (PK data was available for CP-A/B/C patients[51, 52]), but in real-life the combination of drugs was used in patients with more advanced liver disease.

Often, PK data obtained in pre-approval trials comes from non-HCV cirrhotic patients. As discussed above, PK of DAAs can be different between HCV and non-HCV cirrhotic. For drugs that are likely to be used in cirrhotic patients (DAAs), a possibility to gather more PK information, is to combine PK with safety and efficacy studies. For example,

by including cirrhotic patients in phase-III and/or phase-IV trials. Full PK curves could be obtained (non-compartmental analysis) or limited sample strategies can be used. These samples can be evaluated using population pharmacokinetic modeling. Another tool to understand the PK of a compound better in cirrhotic patients is compartmental analysis (for example physiology-based pharmacokinetic [PBPK] modeling). This is described in **Chapter 12**, where a cirrhotic model available in Simcyp was used to simulate the exposure of daclatasvir in CP-A, B, and C patients[53]. The simulated daclatasvir plasma concentrations were not in line with *in vivo* results. In my opinion, the cirrhotic Simcyp model can be useful for all kinds of drugs in explaining and understanding the PK of a compound in the cirrhotic population, however improvements of the model are needed. These improvements include transporter expression in cirrhotic patients and a more physiological classification instead of the use of the CP classification system.

Patients with decompensated cirrhosis remain hard to treat, but there are several safe DAA options. However, it is unknown what the right timing for treatment is, before or after transplantation? When treated, these patients can be cured, without improvement in hepatic function. A total of 17% of the decompensated patients had an unchanged model for end-stage liver disease (MELD) score and 23% of the patients had a worsened MELD score after DAA therapy. The MELD score is used as an indicator for liver transplantation (MELD >15). So, we can cure decompensated patients from HCV, but treatment may worsen the hepatic disease with risk on further complications. Most patients (60%), however, had improved MELD scores. The median improvement in MELD is +2 points, which means that patients could lose their indication for a transplantation (MELD <15) but are still at risk for progression of their liver disease and hepatocellular carcinoma[54]. When to treat these patients is under debate. In the Netherlands, we aim to treat patients who are on the waiting list before transplantation[17].

Patients with renal insufficiency can be treated safely and effectively with paritaprevir/ritonavir, ombitasvir, with or without dasabuvir or with grazoprevir/elbasvir[16, 24,55]. For all the sofosbuvir combinations, efficacy, safety, and PK data in patients with eGFR <30 mL/min are still pending[10, 14, 25]. However, there are some studies indicating that sofosbuvir can be safely and effectively used in patients with all degrees of renal insufficiency[56-61].

Ribavirin remains a drug that is difficult to dose in these patients. Ribavirin and its main metabolite are primarily eliminated through the kidneys and with decreasing renal function the clearance of ribavirin decreases[33]. The Dutch guidelines recommends to start with a weight-based loading dose and to adapt the daily dose to 200-400 mg dependent on the renal function (eGFR <50 mL)[17]. Despite this advice, **Chapter 9** shows that this is not always done in these patients resulting in anemia and further

dose reductions as a consequence. TDM can definitely help optimize ribavirin dosing for these patients. In addition, applications such as MwPharm, Dose Me, and InsightRX could be helpful, because these programs use population PK models of a compound in combination with TDM results and patient characteristics to predict PK in an individual patient. These predictions help finding the right dose.

Of course, there are also other special populations who might be treated with DAAs and for whom no efficacy, safety, or PK data is available. Two examples are given in **Chapter 13**. The patient with gastric bypass surgery had decreased simeprevir exposure when treated with 150 once daily (QD). Therefore, the dose was increased to 300 QD. Optimal DAA exposure is important for two reasons 1) subtherapy could cause treatment failure and possibly RAS and 2) supratherapy could cause toxicity. The minimum target concentration (C_{min}), or another PK parameter we must achieve to accomplish SVR and to avoid RAS is unknown. Therefore, for all the case reports in this thesis, the area under the concentration-time curve (AUC) and C_{max} of comparable patient populations were used to determine whether the exposure was adequate. Other special populations that might be treated for HCV in the future, but without data available are for example: children (except ledipasvir/sofosbuvir[62, 63]), pregnant women, and obese patients. Previous studies from our department show that dose adaptations in these populations might be necessary to accomplish the correct exposure of drugs[64-78].

A PHARMACIST'S CONTRIBUTION TO ERADICATE HEPATITIS C

The four case reports in **Chapter 13** present the actual contribution of our work as pharmacists to the eradication of HCV. As discussed previously in this general discussion, these patients all needed extra attention prior to DAA treatment. Due to the extensive knowledge of (HCV) pharmacology and good collaboration with physicians we were able to treat and cure these patients.

Of course, most of these treatments were off-label and based on predictions rather than on evidence, so, together with the treating physicians, we had to make risk assessments prior to treatment initiation. In my opinion, this kind of cooperation between physicians, nurses, and pharmacists really improves therapy as DDIs are managed or other (experimental) treatment options are evaluated.

For example, the patient with a gastric bypass was an interesting but complex patient to treat. Especially in view of the altered absorption together with RAS. I believe that these kinds of case reports add significant value to the scientific community and must

be published to give insight to these treatments for physicians and pharmacists, because these cases, represent the gaps in our knowledge.

ERADICATION OF HEPATITIS C VIRUS

At the end of 2016, the Dutch government released a national plan to reduce transmission, morbidity, and mortality caused by the hepatitis viruses, using five themes: awareness, identification, diagnostics and adequate treatment, improved organization of the chain of care and improved surveillance, and monitoring of HCV in order to gain insight in the cascade of care. This strategy focuses on six risk groups: immigrants, children of HCV-infected parents, men who have sex with men, (ex)drug users, sex workers, and patients who received blood products[69].

This strategy is the first step towards eradication of HCV in the Netherlands. Important for eradication of HCV is that we must identify all infected patients, as individuals might be unaware of their infection. Therefore, screening is advised for the six risk groups. Secondly, everybody diagnosed must have access to care, treatment must be affordable and easy[70]. Probably, in high-income countries we can achieve most of these aspects. In the Netherlands identification of the patients is the limiting step in this process. The costs of DAAs are also high (see introduction) and under debate. The Dutch Minister of Health has negotiated special price arrangements with the pharmaceutical companies, which resulted in non-transparent prices, but making all DAAs available for all HCV patients.

For governments in low and middle-income countries eradication of HCV will probably be much harder to achieve, which is contradictory because most HCV-infected patients live in these countries[70]. The costs of the DAAs influence the accessibility to treatment around the world. Thai patients **(Chapter 10)** are still treated with inferior therapy resulting in lower response rates than HIV/HCV co-infected patients treated with DAAs in high-income countries. There are several initiatives advocating for reducing the costs. For example, in Egypt a combination of ravidasvir and sofosbuvir is developed and will be marketed for ~$300[71]. Another example is the use of parallel import of (generic) DAAs which first started in Australia, because DAAs were unavailable (http://www.fixhepc.com).

We must mention that the costs of DAAs varies extremely among low, middle, and high-income countries, which was shown by Andrieux-Meyer et al[72]. Generic sofosbuvir is available for $343 in Egypt and in India the price of sofosbuvir varies from ~$160 to $312 (patent sold by Gilead). However, branded sofosbuvir in Malaysia costs

$18,000 which is even higher than in some high-income countries. Sofosbuvir was the best available DAA throughout the low and middle-income countries questioned. The availability of generic sofosbuvir in some of these countries is a result of licensing agreements between Gilead and generic companies[72].

Except pricing, other aspects mentioned in the Dutch national plan are also of importance. One thing that is already achieved is that treatment of HCV is very effective and easy. With the introduction of the fixed-dose combination velpatasvir/sofosbuvir, all patients can be treated without the need for genotyping when this product is reasonably priced and available. NS5A RAS could also be important, especially for genotype 3, when treatment-experienced patients are re-treated. These patients can for example be re-treated with ledipasvir/sofosbuvir (genotype 1, 4, 5, and 6) or velpatasvir/sofosbuvir with ribavirin for 24 weeks[40]. Other promising studies are done with sofosbuvir, velpatasvir, and voxilaprevir, which is a pan-genotypic regimen showing SVR-rates of 96% in NS5A experienced patients (41% cirrhosis[73]). Also, other manufactures have presented data on novel compounds showing high SVR-rates in difficult-to-cure patients. For example, glecaprevir/pibrentasvir for compensated genotype 1 and 3 cirrhotic patients (SVR 96%)[74]. All these developments and other novel compounds will probably result in easier, cheaper, and even more effective therapy, possibly without the need for ribavirin. This all will hopefully contribute to the target of the World Health Organization to eliminate HCV as a global treat 2030[75].

With the introduction of all these highly effective drugs, choices must be made. For example, which DAAs are going to be used for which patients? In my opinion, pharmacists can help in selecting the most suitable regimen for each individual patient by interpreting the information from the drug labels. Not only based on efficacy and safety but also considering drug-interactions, contra-indications, co-morbidities, and recommendations considering special populations.

CONCLUDING REMARKS

The aim of this thesis was to address pharmacological issues concerning current HCV therapy including novel DAAs and ribavirin, which can be used for the optimization of HCV treatment. The first part of this thesis focuses on DDIs and showed that patients undergoing DAA treatment are at risk for DDIs. In addition, this part shows that the Dutch infectious disease specialists are keen on preventing DDIs. This second part discusses the pharmacokinetics and the role of ribavirin in combination with DAA and Peg-IFN therapy. The third part of thesis focused on the use of DAAs in patients with renal and hepatic disease. With these chapters and the general discussion, the contribution of a

pharmacist towards more understanding of the pharmacology of DAAs and ribavirin is shown. This will hopefully improve DAA therapy in the future.

This thesis is finalized with five examples of individual patients that were treated with DAAs. These patients all received individualized treatments with DAAs. Adaptations were made to therapy, trying to increase the chance of reaching an SVR. These cases are the icing on the cake of this thesis and examples of: *a pharmacist's contribution to eradicate hepatitis C.*

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Summary & Samenvatting

SUMMARY

INTRODUCTION

The hepatitis C virus (HCV) is an RNA virus which replicates in the hepatocyte. Therefore, HCV causes liver inflammation and damage which can eventually lead to cirrhosis. HCV is transmitted through blood-blood contact and the main risk populations are men who have sex with men, patients that received blood products in the past, or intravenous drug users. Nowadays, HCV can be treated with direct-acting antivirals (DAAs). These oral drugs are highly effective and >95% of the patients treated with these DAAs clear the virus. At least two DAAs are combined for the treatment with a duration varying from 6 to 24 weeks. Most commonly used regimens are: daclatasvir + sofosbuvir, and the fixed-dose products ledipasvir + sofosbuvir, velpatasvir + sofosbuvir, grazoprevir + elbasvir, and paritaprevir/ritonavir, ombitasvir, and dasabuvir.

The first selection criterion for a DAA regimen is the HCV genotype (1-6). Other factors influencing the regimen of choice are: whether the patient is treatment-experienced (e.g. resistance); the patient has cirrhosis; or if there are any drug-drug interactions (DDIs). Because DAAs are substrates/influencers of several drug-transporters and drug-metabolizing enzymes they are known to cause drug-interactions. **Part 1** of this thesis focuses on these DDIs with the DAAs.

Ribavirin is an antiviral drug (not an DAA) that is added to DAA therapy in patient populations that are hard-to-cure such as patients with cirrhosis, treatment-experienced patients, or patients with genotype 1a or 3. Ribavirin is added to improve treatment response and to shorten treatment duration. **Part 2** discusses the pharmacokinetics of ribavirin and the use of therapeutic drug monitoring (TDM) for ribavirin.

Part 3 focuses on special patient populations, such as patients with renal and hepatic impairment. These conditions can influence the pharmacokinetics of a drug and therefore may lead to dose alterations or increased awareness when treating these patients.

Part 4 describes the contribution of a pharmacist to the eradication of HCV by presenting four case reports about patients with special DDIs or adjusted DAA treatment.

The overall aim of this thesis was to *answer pharmacological issues concerning current HCV therapy including novel DAAs and ribavirin, which can be used for the optimization of HCV treatment*.

PART 1:
Drug-drug interactions involving direct-acting antivirals

Chapter 1 is a review describing DDIs between DAAs and psychoactive medications. Mental disorders are highly prevalent in chronic HCV patients. Therefore, these two classes of drugs are likely to be combined. **Chapter 1** starts with an overview of the metabolism of both DAAs and psychoactive agents. Subsequently, the DDI studies performed between DAAs and psychoactive agents are presented. This information was combined and used to predict safe options for the simultaneous treatment with DAAs and psychoactive agents. We discussed that escitalopram and citalopram have been studied with most DAAs and either drugs can be safely combined with HCV treatment. Sofosbuvir and ledipasvir are not studied with any psychoactive agents, but based on their metabolism we expect no DDIs with most psychoactive agents. Simeprevir and the combination of paritaprevir/ritonavir plus ombitasvir with dasabuvir are most likely to cause drug-interactions via the inhibition of cytochrome P450 (CYP) 3A4. Therefore, caution is needed when CYP3A4 substrates such as midazolam and/or quetiapine are co-administered with these DAAs. The conclusion is that the number of *in vivo* DDI studies between DAAs and psychoactive medicines is small and that theoretical predictions of DDIs must be backed with actual *in vivo* studies.

Chapter 2 is a commentary on how informative drug labels are concerning DDIs. These labels are the first source for DDI information when new drugs are marketed. Often information is missing (as not everything can be studied before market access) or statements in the labels are unclear. **Chapter 2** discusses four aspects where the labels could be more informative using the labels of velpatasvir + sofosbuvir and grazoprevir + elbasvir as an example.

1. In case a new drug is a CYP3A substrate, with which CYP3A inducers is an interaction mentioned in the label?
2. In case of interaction with CYP3A inducers, when will this lead to a contra-indication or labeled as 'not recommended'; is this the same or not?
3. In case a new drug is a (moderate) CYP3A inhibitor, which CYP3A substrates with a narrow therapeutic range are listed in the drug label?
4. New drugs are usually tested with relevant co-medication in the target patient population, but how is this set of 'relevant' medication selected?

The potential DDI between metformin (diabetes mellitus type 2) and daclatasvir (DAAs) is described in **Chapter 3** and studied in healthy volunteers. The aim of this study was to evaluate the effect of the proposed organic cation transporter (OCT) inhibitor daclatasvir on the pharmacokinetics and pharmacodynamics of the OCT substrate metformin. A total of 20 subjects received 1,000 mg metformin (treatment A) followed by 1,000 mg

metformin and 60 mg daclatasvir (treatment B). Pharmacokinetic curves were recorded at steady-state. Geometric mean ratios (GMR) with 90% confidence intervals (CI) of metformin area under the concentration-time (AUC) $_{0\text{-}12}$, maximum plasma concentration (C_{max}), and last plasma concentration (C_{last}) (B versus A) were 109% (102-116%), 108% (101-116%), and 111% (103-120%). This showed that daclatasvir does not influence the pharmacokinetics of metformin in healthy subjects. Pharmacodynamic parameters were also comparable between treatments.

Chapter 4 studies whether the fixed-dose combination of atazanavir/cobicistat has a comparable influence on daclatasvir pharmacokinetics as the separate agents atazanavir and ritonavir. Atazanavir is an HIV protease inhibitor that is boosted with CYP3A4 inhibitor ritonavir. When combined with the CYP3A4 substrate daclatasvir, the daclatasvir dosage should be reduced from 60 mg to 30 mg once daily. Recently, cobicistat was licensed as a CYP3A booster and used in combination with atazanavir. It was unknown whether atazanavir/cobicistat had comparable influence on daclatasvir exposure as atazanavir/ritonavir. This interaction study was performed in 16 healthy volunteers receiving 300/100 mg atazanavir/ritonavir in combination with 30 mg daclatasvir once daily (treatment A), followed by, 300/150 mg of atazanavir/cobicistat plus 30 mg daclatasvir once daily (treatment B). The GMRs (90% CI) of daclatasvir AUC_{tau} and C_{max} (B versus A) were 101% (92-111%) and 97% (89-106%), respectively. We showed that atazanavir/cobicistat and atazanavir/ritonavir had a similar influence on daclatasvir pharmacokinetics in healthy volunteers. Daclatasvir at 30 mg once daily is the correct dose when combined with atazanavir/cobicistat.

Chapter 5 studies the risk on DDIs in HCV patients. We predicted the number of DDIs with co-medication used by HCV mono-infected patients. We assembled a nationwide cohort of HCV patients and collected cross-sectional data on co-medication use. This data was used to cross-check for potential DDIs between DAAs and used co-medication using the database of the University of Liverpool (http://www.hep-druginteractions.org). Four DDI categories were defined: (1) no clinically relevant DDI; (2) possible DDI; (3) contra-indication; or (4) no information available. We defined category 2 and 3 as clinically relevant DDIs. We found that 77% of the 461 patients used co-medication. Antidepressants (7.4%), proton pump inhibitors (7.1%), and benzodiazepines (7.1%) were most frequently used. We predicted that 60% of the patients were at risk for a clinically relevant DDI with at least one of the available DAA regimens. Interactions were most common with paritaprevir/ritonavir, ombitasvir ± dasabuvir and least interactions were predicted with grazoprevir/elbasvir.

Chapter 6 described a comparable experiment with HIV/HCV co-infected patients from the Netherlands. Information from the ATHENA observational HIV database was used. This is a database including all Dutch HIV-infected patients (except if they opt out). Patients with an HIV/HCV co-infection at 1 January 2015 were selected and a list of non-antiretroviral co-medication and combination antiretroviral therapy (cART) registered in the ATHENA database was compiled. The potential for DDIs between DAAs and co-medication/cART was predicted, using the database of the University of Liverpool. A total of 777 HIV/HCV co-infected patients were included of whom 63% used non-antiretroviral co-medication and 98% were treated with cART. At risk for a category 2/3 DDI with non-antiretroviral co-medications were 38% of the patients. Concerning cART, daclatasvir + sofosbuvir is the most favorable combination as no cART is contra-indicated with this combination. In genotype 1/4 patients grazoprevir/elbasvir is least favorable as 75% of the patients must alter their cART.

In **Chapter 5 and 6,** the number of DDIs is predicted and it was shown that ~50% of the patients were at risk for a DDI when treated with DAAs. In **Chapter 7** we mapped how physicians manage these DDIs between DAAs and co-medication including cART. Again, the ATHENA cohort was used to select HIV/HCV co-infected patients treated with DAAs between January 2015 and May 2016. Interactions were checked using the database of the University of Liverpool. Subsequently, analysis was performed to determine whether the cART regimen was changed and co-medication discontinued. A total of 423 patients were included, of whom 418 (99%) were treated with cART. Of these patients 251 (59%) used non-antiretroviral co-medication. Before commencing DAA treatment, in 17/84 (20%) patients the non-antiretroviral co-medications, which would result in a category 2 or 3 DDI, were discontinued before DAA initiation, including 2/6 (33%) prescriptions of category 3 drugs.
196/418 (47%) patients had a category 2/3 DDI between their DAA regimen and cART. Category 2 and 3 DDIs were prevented by switching cART in 78/147 (53%) and 47/49 (96%) patients, respectively. 367/423 (87%) patients have reached SVR (33 in follow-up). We found that the DDIs did not influence the HIV viral load and all patients with a clinically relevant DDI achieved SVR. In addition, combined treatment with ledipasvir + sofosbuvir and a proton pump inhibitor, did not influence SVR. We concluded that Dutch physicians are well aware of potential DDIs with DAAs, in particular when it concerns cART. Improved awareness is needed for non-antiretroviral co-medication and DAA category 3 interactions.

PART 2:
Ribavirin pharmacokinetics and therapeutic drug monitoring

Ribavirin is added to DAA therapy to increase efficacy rates in hard-to-cure patients. These patients are among others cirrhotic patients, treatment-experienced patients, or patients with genotype 1a or 3.

Ribavirin has a strong concentration-effect relationship, and TDM of ribavirin can be used to individualize the dose of ribavirin. Therefore, several laboratories developed ribavirin assays. To ensure the accuracy of these bioanalytical methods and to alert laboratories to previously undetected problems an international external quality control (QC) or proficiency testing (PT) program for measurement of ribavirin is developed. In **Chapter 8** we described the results of the first year of this ribavirin PT program (2015).

Per round two samples were dispatched (2 rounds in total) to the participating laboratories. For these samples, bovine serum was spiked with low and high concentrations of ribavirin. These samples were freeze-dried and measured by the laboratories. Accuracy was considered to be acceptable if measurements were within the 80-120% limits of the spiked (weighed-in) 'expert' concentrations. Eight laboratories participated in the program of which only two participants completed one round. In round 1, 81% of the samples (i.e. 13 out of 16 samples) were determined accurately and the variation in accuracy of samples with low concentrations was 86-336%. The samples spiked with high ribavirin concentrations varied from 55-160% in accuracy. In round 2, a total of 75% samples (i.e. 9 out of 12) were determined accurately within 80-120% of the weighed-in concentrations. Accuracy for samples with low and high concentrations varied from 97-303% and 97-148%, respectively.

The aim of a PT program is to provide external validation of bioanalytical assays to assure and improve quality. The laboratories with a poor performance should improve their analyses.

Chapter 9 describes the association between ribavirin steady-state plasma concentrations and SVR in the DAA era. Therefore, HCV patients were included who were treated with DAAs and ribavirin in four academic centers in the Netherlands. At steady-state (at least week 8 of treatment) ribavirin plasma concentrations were determined. A total of 183 patients were included of which the majority was treated with daclatasvir and sofosbuvir. The mean ribavirin start dosage was 13.6 mg/kg/day, which was decreased to 13.1 mg/kg/day at week 8. The median week 8 ribavirin plasma concentration was 2.66 mg/L. Multivariable analysis showed that a higher ribavirin plasma concentration

was a predictor of SVR. We defined a therapeutic range of 2.28 to 3.61 mg/L. A total of 89% of the patients reached SVR.

Chapter 10 describes an HIV/HCV co-infected population from Thailand that was treated with peg-interferon alfa and ribavirin. With this therapy, HIV/HCV co-infected patients have lower response rates than with DAAs. A total of 101 patients were included of whom 56% reached SVR. Ribavirin steady-state plasma concentrations (week 8 of treatment) were 1.81 mg/L and we calculated an inter-subject variability of 29% and intra-subject variability of 18%. Patients with anemia (hemoglobin <10 g/dL) had higher ribavirin plasma concentrations throughout treatment than patients without anemia. However, at week 24, due to dose adaptations ribavirin plasma concentrations were comparable in these two groups. No difference in ribavirin plasma concentrations was found for patients with and without SVR.

PART 3:
Treatment of HCV in special patient populations

HCV replicates in the liver and can therefore cause liver inflammation and cirrhosis, which is often complicated by renal impairment. **Chapter 11** is a review where we describe the treatment possibilities in HCV patients with hepatic and/or renal impairment. Dose adaptations could be necessary as cirrhosis alters the structure of the liver, which affects drug-metabolizing enzymes and drug-transporters. These modifications influence the plasma concentration of substrates of drugs metabolized/transported by these enzymes. Comparable, drugs that are eliminated by the kidneys might need dose adjustments in patients that have decreased renal function. We found that cirrhotic patients with Child-Pugh class (CP) A can be treated with all DAAs, but that patients with decompensated cirrhosis (CP-C) are harder to treat. All drugs used in HCV treatment can be used in patients with moderate renal insufficiency (Glomerular Filtration Rate [GFR] ≥30 mL/min). Grazoprevir + elbasvir can be used in patients with severe renal impairment; ledipasvir + sofosbuvir and velpatasvir + sofosbuvir are both not recommended in patients with severe renal impairment as data is still lacking. However, some evidence is available that patients with renal insufficiency tolerate a normal dose of sofosbuvir.

Chapter 12 describes a physiology-based pharmacokinetic (PBPK) model of daclatasvir pharmacokinetics in healthy volunteers and cirrhotic patients. Unfortunately, we were not able to mimic *in vivo* exposure with our model in cirrhotic patients. With increasing severity, the AUC-ratio of daclatasvir increased, varying from ~2-fold to ~5-fold increase in CP-A and CP-C patients. We argue that this issue might be caused by altered intestinal and hepatic transporter expression in cirrhotic patients which was not captured in the model. We proposed future studies that must address these issues in more detail.

PART 4:
A pharmacist's contribution to eradicate hepatitis C

Chapter 13 describes four case reports of individual HCV patients treated with DAAs. All had adjusted DAA treatment or something unexpected occurred during DAA treatment. With this chapter, we showed the added value of a pharmacist in the treatment of HCV patients.

The first patient was effectively treated with 60 mg daclatasvir in combination with etravirine and darunavir/ritonavir. Etravirine is an inducer of CYP3A4 and darunavir/ritonavir an inhibitor to the same enzyme. In combination with etravirine the daclatasvir dose must be increased to 90 mg, but with darunavir/ritonavir the normal dose of 60 mg must be given. Therefore, we treated this patient with 60 mg, as we believed that induction of CYP3A4 was mitigated by the inhibition of CYP3A4. Fortunately, the patient achieved SVR.

The second patient was treated with oxcarbazepine for epilepsy, which is a strong inducer of CYP3A4. As no DDI studies are available, it is unknown what the right dose of daclatasvir and sofosbuvir should be. We treated the patient with 90 mg daclatasvir in combination with normal dose sofosbuvir. This was effective as the patient reached SVR; however, the daclatasvir exposure was somewhat low so caution is needed when treating patients with strong inducers.

Thirdly, two patients were described that both had a liver transplantation in the past for which they were treated with tacrolimus. During DAA therapy there was a need to increase the dose of tacrolimus. There is no DDI between the DAAs and tacrolimus, but we hypothesized that the plasma concentration of tacrolimus decreased due to normalization of CYP enzyme activity. Tacrolimus is metabolized by CYP3A4 and we argued that patients with HCV have decreased CYP expression. By clearing the HCV virus, there will be a normalization of CYP activity, which results in lower plasma concentrations of CYP substrates.

The last patient was a female that underwent a gastric bypass before DAA therapy. The patient was treatment-experienced and had resistance-associated substitutions. Therefore, the only treatment available was simeprevir plus sofosbuvir with ribavirin. We obtained a pharmacokinetic curve of simeprevir, showing decreased plasma concentrations. This resulted in an increased simeprevir dose of 300 mg a day. Exposure increased and the patient reached SVR.

GENERAL DISCUSSION

In the general discussion four parts of the thesis are discussed. The discussion starts with DDIs: how are DDI studies designed for new compounds (*in vitro* to *in vivo*) and which drugs must be studied in combination with the new compound. Secondly, the use of (online) interaction checkers was discussed in respect to management of drug-interactions in daily practice. In addition, it is discussed how pharmacist could help in managing these DDIs.

Ribavirin pharmacokinetics and the use of TDM for dose adjustment are discussed. Especially, TDM of ribavirin remains under debate. We argue that ribavirin is added to therapy in the hardest to cure patients and TDM can help to treat these patients optimally, as the dosages can be individualized based on the ribavirin plasma concentration (decreased or increased). Especially for patients that do not experience any toxicity TDM can be useful.

Lastly, we discuss the use of novel compounds in special populations, focusing on patients with renal and hepatic impairment. We discuss that HCV genotype 1 and 4 patients with renal impairment can be treated with grazoprevir + elbasvir but ribavirin treatment remains difficult. Also for these patients, ribavirin TDM could be useful. Secondly, data are lacking for the sofosbuvir-containing regimens, making patients with renal failure and other HCV genotypes harder to treat.

Compensated cirrhotic patients can be safely treated with most DAAs, however for decompensated patients there is a treatment paradigm. When do we need to treat these patients? Before or after liver transplantation? The timing could be essential for a patient; however, this issue is still under debate.

The general discussion is finished with a section on what is needed for the eradication of the HCV virus. First step is to identify the patients, followed by access to care and treatment. As treatment remains to be expensive this could be a limiting factor. However, some pricing agreements are made which improved the availability of the DAAs in low and middle-income countries.

The discussion of this thesis is ended with a statement that all the chapters in this thesis, but especially the chapters concerning the individual patients are examples of *a pharmacist's contribution to eradicate hepatitis C.*

SAMENVATTING

INTRODUCTIE

Het hepatitis C-virus (HCV) is een ribonucleïnezuur (RNA) virus dat zich vermenigvuldigt in de lever (levercel [hepatocyt]). Doordat het virus zich vermenigvuldigt in de lever veroorzaakt het een ontsteking en schade aan de lever. Dit kan uiteindelijk leiden tot levercirrose, wat uiteindelijk een indicatie kan zijn voor levertransplantatie.

HCV is een virus dat wordt overgedragen via bloed-bloed contact, vergelijkbaar met het hiv of het hepatitis B-virus. Risicogroepen zijn dan ook mensen die in het verleden een bloedtransfusie hebben ondergaan, mensen die intraveneus drugs spuiten, of mannen (voornamelijk hiv positieve) die onveilige seks hebben met mannen.

Door de recente ontwikkeling van uiterst effectieve orale geneesmiddelen, is HCV te genezen. Deze geneesmiddelen worden de direct-acting antivirals (DAAs) genoemd. Gemiddeld genomen wordt meer dan 95% van de behandelde patiënten genezen. De behandeling bestaat altijd uit een combinatie van minimaal twee DAAs. De meest voorkomende behandelcombinaties zijn: daclatasvir + sofosbuvir, ledipasvir + sofosbuvir, velpatasvir + sofosbuvir, grazoprevir + elbasvir, en paritaprevir/ritonavir, ombitasvir, en dasabuvir.

De behandeling is onder andere afhankelijk van het HCV-genotype (genotype 1 tot en met 6 en verschillende subtypen). Dit komt doordat niet alle DAAs voldoende werkzaam zijn tegen alle genotypen. Andere factoren waar men rekening mee dient te houden bij het kiezen van een DAA-regime zijn onder andere: falen van eerdere behandeling, de mate van levercirrose en het gebruik van andere medicatie in verband met geneesmiddel-geneesmiddel interacties. De DAAs worden gemetaboliseerd door verschillende enzymen, maar de DAAs beïnvloeden zelf ook deze enzymen. Door deze beide mechanismen zijn de DAAs betrokken bij geneesmiddel-geneesmiddel interacties. Deze wisselwerking tussen geneesmiddelen kan leiden tot een verminderde werkzaamheid of tot extra bijwerkingen. Het eerste deel van het proefschrift gaat over geneesmiddel-geneesmiddel interacties met HCV-medicatie.

Het tweede deel van het proefschrift wordt de behandeling met ribavirine besproken in combinatie met DAAs, evenals of de hoeveelheid ribavirine in het bloed invloed heeft op de genezing van het virus. Ribavirine is een oud geneesmiddel, maar wordt vandaag de dag nog steeds toegevoegd aan de behandeling met de DAAs. Dit wordt

voornamelijk gedaan om de kans op genezing te vergroten bij patiënten met levercirrose, patiënten bij wie eerdere behandeling heeft gefaald, of patiënten met genotype 1a of 3. Dit zijn patiëntengroepen bij wie het virus moeilijk is uit te roeien. Tevens kan door het toevoegen van ribavirine de behandeling worden verkort: 12 weken ten opzichte van 24 weken behandeling.

Het derde deel van dit proefschrift beschrijft het gebruik van de DAAs en ribavirine in speciale populaties. De twee populaties die uitgebreid worden besproken, zijn patiënten met verminderde leverfunctie en patiënten met verminderde nierfunctie. Beide organen zijn betrokken bij de eliminatie (verwijdering) van geneesmiddelen uit het lichaam. Wanneer de lever en nieren minder goed werken, kunnen geneesmiddelen zich anders gedragen in het lichaam. De concentratie van een geneesmiddel in het bloed kan bijvoorbeeld toe- of afnemen wat weer invloed heeft op bijvoorbeeld de werkzaamheid of de toxiciteit (bijwerkingen) van geneesmiddelen.

In het laatste deel van het proefschrift wordt ingegaan op vijf speciale patiënten. Twee patiënten hadden ingewikkelde interacties, twee patiënten hadden een levertransplantatie ondergaan en één patiënt had bariatrische chirurgie (maagverkleining/ gastric bypass) ondergaan. Bij al deze patiënten waren apothekers betrokken om de behandeling te optimaliseren.

Het doel van dit proefschrift is o*m farmacologische vragen met betrekking tot de huidige HCV-behandeling te beantwoorden, zodat de behandeling kan worden geoptimaliseerd.*

DEEL 1:
Geneesmiddel-geneesmiddel interacties

Geneesmiddel-geneesmiddel interacties ontstaan wanneer twee of meer geneesmiddelen tegelijkertijd worden gebruikt door een patiënt. Er is sprake van een interactie indien er een wisselwerking tussen twee geneesmiddelen is. Door deze wisselwerking kan de geneesmiddelconcentratie in het bloed stijgen, waardoor de kans op bijwerkingen toeneemt. De geneesmiddelconcentratie in het bloed kan ook lager worden. Dit kan mogelijk ook leiden tot onder behandeling (sub therapie). **Hoofdstuk 1** is een review waarin geneesmiddelinteractie onderzoeken tussen de DAAs en psychoactieve medicatie wordt beschreven. Psychoactieve medicatie wordt veel gebruikt door HCV-patiënten aangezien mentale stoornissen veelvuldig voorkomen in deze populatie. Bij psychoactieve medicatie kan gedacht worden aan slaapmiddelen of geneesmiddelen die worden gebruikt bij depressie of psychoses. In **hoofdstuk 1** geven we een overzicht van het metabolisme van zowel de DAAs als de psychoactieve medicatie. Het metabolisme van geneesmiddelen is belangrijk voor het voorspellen van geneesmid-

delinteracties, omdat het metabolisme essentieel is voor de afbraak van geneesmiddelen. Daarnaast beïnvloeden de DAAs de enzymen die verantwoordelijk zijn voor het metabolisme van andere geneesmiddelen, waardoor interacties ontstaan.

Tevens is er een overzicht gemaakt van de onderzoeken die de wisselwerking tussen de DAAs en psychoactieve medicatie hebben bestudeerd. Als er geen onderzoeken naar bepaalde combinaties zijn gedaan, werden deze overzichten over het metabolisme van de DAAs en psychoactieve medicatie, gebruikt om voorspellingen te doen welke geneesmiddelen veilig samen gebruikt kunnen worden. Hieruit blijkt dat escitalopram en citalopram (beide geneesmiddelen gebruikt voor onder andere depressie) veilig gebruikt kunnen worden in combinatie met de DAAs. Opvallend was dat met ledipasvir en sofosbuvir geen interactie onderzoeken zijn uitgevoerd. Echter, we voorspellen weinig tot geen interacties tussen psychoactieve medicatie en ledipasvir en sofosbuvir. Dit komt doordat deze middelen een gunstig interactieprofiel hebben. Simeprevir en paritaprevir/ritonavir, ombitasvir en dasabuvir zijn de geneesmiddelen waarbij de meeste interacties zijn voorspeld. Dit komt doordat ze cytochroom P450 enzymen (CYP3A4) in de lever beïnvloeden. Dit zijn de meest belangrijke enzymen betrokken bij geneesmiddelmetabolisme. De conclusie van **hoofdstuk 1** is dat er weinig onderzoeken in mensen zijn gedaan waarin de interacties tussen DAAs en psychoactieve medicatie worden onderzocht. Deze onderzoeken zijn nodig om de voorspellingen te verifiëren.

Wanneer geneesmiddelen op de markt komen wordt er een professionele bijsluiter gepubliceerd. Dit wordt het geneesmiddel label genoemd. In dit label staan alle onderzoeken beschreven, inclusief de informatie over geneesmiddelinteracties. In **hoofdstuk 2** bediscussiëren we hoe informatief dit label eigenlijk is voor artsen en apothekers. Dit doen we aan de hand van twee recent geregistreerde DAA combinaties: velpatasvir + sofosbuvir en grazoprevir + elbasvir. In dit hoofdstuk stellen we ons vier vragen:

1) In het geval dat een nieuw geneesmiddel wordt gemetaboliseerd door CYP3A4 (substraat), welke CYP-inductoren (versnellen geneesmiddel afbraak) en remmers worden genoemd in het label?
2) In het geval een geneesmiddel een CYP inductor is, wanneer leidt dit tot een 'contra-indicatie' met een ander geneesmiddel en wanneer is een combinatie 'niet aanbevolen'. Zijn beide aanbevelingen hetzelfde of is dit niet het geval?
3) Wanneer een geneesmiddel een CYP3A4 remmer is, welke CYP3A4 substraten met een nauwe therapeutische breedte worden genoemd in het geneesmiddel label?
4) Geneesmiddelinteracties worden doorgaans bestudeerd met comedicatie die wordt gebruikt door de doelgroep populatie; op basis waarvan is deze lijst samengesteld?

In **hoofdstuk 3** beschrijven we vervolgens een geneesmiddelonderzoek met gezonde vrijwilligers, waarbij de interactie tussen metformine (bij diabetes mellitus type 2) en daclatasvir (DAA) is bestudeerd.

Naast interacties waarbij enzymen betrokken zijn, vinden interacties plaats op het niveau van geneesmiddeltransporters. Deze transporters zijn betrokken bij de verdeling en de eliminatie van geneesmiddelen in het lichaam.

Metformine is onder andere substraat van organic cation transporters (OCTs). Daclatasvir is een mogelijke remmer van deze transporters. Hierdoor kan de metformine concentratie in het bloed stijgen, omdat er minder metformine wordt uitgescheiden wanneer OCT wordt geremd. Om dit te onderzoeken zijn 20 vrijwilligers behandeld met 1000 mg metformine voor 7 dagen gevolgd door 7 dagen 1000 mg metformine en 60 mg daclatasvir (of in omgekeerde volgorde). Op dag 7 van elke behandeling werd gedurende 24 uur bloed afgenomen waarin de metformine concentraties zijn gemeten. Deze uitslagen zijn gebruikt om farmacokinetische parameters te berekenen. De uitslagen zijn gebruikt om een beeld te krijgen van de blootstelling van metformine in het lichaam. Uit ons onderzoek bleek dat zowel de totale blootstelling, de maximale en minimale plasmaconcentratie van metformine niet waren beïnvloed door daclatasvir. Daarom concluderen we in **hoofdstuk 3** dat metformine en daclatasvir veilig kunnen worden gecombineerd.

In **hoofdstuk 4** wordt een tweede interactie onderzoek met daclatasvir beschreven. In dit geval met atazanavir/ritonavir en atazanavir/cobicistat. Beide combinaties worden gebruikt voor de behandeling van hiv. Een lage dosering ritonavir en cobicistat zijn zelf niet werkzaam tegen hiv, maar zijn toegevoegd om het farmacokinetisch profiel van atazanavir te verbeteren. Hierdoor kan atazanavir eenmaal daags worden gebruikt en is er minder kans op resistentievorming. Zowel ritonavir als cobicistat zijn sterke remmers van CYP3A4. Hierdoor wordt ook het metabolisme van daclatasvir geremd en neemt de concentratie van daclatasvir toe in het bloed. In combinatie met atazanavir/ritonavir moet de dosering van daclatasvir daarom worden verlaagd van 60 mg per dag naar 30 mg per dag. De vraag was of dezelfde dosisverlaging ook nodig zou zijn wanneer daclatasvir wordt gecombineerd met atazanavir/cobicistat. Daarom kregen 16 gezonde vrijwilligers 30 mg daclatasvir en 300/100 mg atazanavir/ritonavir voor 10 dagen gevolgd door 30 mg daclatasvir en 300/150 mg atazanavir/cobicistat voor 10 dagen (of andersom). Wederom werd op dag 10 bloed afgenomen zodat de farmacokinetische parameters berekend konden worden. De totale blootstelling, de maximale en minimale plasmaconcentratie van daclatasvir was vergelijkbaar tussen beide groepen.

Daarom kan worden geconcludeerd dat 30 mg daclatasvir de juiste dosering is met gelijktijdig gebruik van atazanavir/cobicistat.

In **hoofdstuk 5** is onderzocht hoe groot het risico voor HCV-patiënten op een geneesmiddelinteractie is. Hiervoor hebben we de medicatiegegevens verzameld die Nederlandse HCV-patiënten gebruikten, en dus geen HCV-medicatie. We hebben de interacties tussen deze comedicatie en de beschikbare DAA-regimes voorspeld met behulp van de database van de Universiteit van Liverpool (http://www.hep-druginteractions.org). Wanneer een combinatie van geneesmiddelen wordt gecontroleerd op interacties kunnen er vier adviezen worden gegeven: categorie 1) er is geen interactie, geen actie nodig; categorie 2) er is een mogelijke interactie, verlaag/verhoog de dosering of monitor de patiënt; categorie 3) er is een contra-indicatie, gebruik de geneesmiddelen niet gelijktijdig; categorie 4) er is geen informatie over de combinatie aanwezig. Categorie 2 en 3 werden klinisch relevant beschouwd. In ons onderzoek zijn 461 patiënten geïncludeerd waarvan 77% geneesmiddelen gebruikten. Antidepressiva (7,1%), protonpomp remmers (7,1%) en benzodiazepinen (7,1%) waren de meest gebruikte geneesmiddelgroepen. We voorspelden dat 60% van de patiënten een klinisch relevante interactie zou hebben met minimaal een van de DAA-regimes. De meeste interacties werden voorspeld met paritaprevir/ritonavir, ombitasvir en dasabuvir; de minste interacties met grazoprevir en elbasvir.

In **hoofdstuk 6** hebben we een vergelijkbare analyse uitgevoerd met patiënten met zowel een hiv als een HCV-infectie. We voorspelden hoeveel patiënten een interactie zouden hebben met combinatie antiretrovirale therapie (cART) en andere comedicatie. cART wordt gebruikt voor de behandeling van een hiv-infectie. Hiervoor is het ATHENA-cohort gebruikt, beheerd door de Stichting hiv-monitoring (http://www.hivmonitoring.nl). Dit is een database waarin alle Nederlandse hiv-patiënten zijn geregistreerd, met uitzondering van de patiënten die hebben aangegeven niet te willen deelnemen. Alle patiënten met een co-infectie op 1 januari 2015 werden geïncludeerd en informatie over zowel de cART en de andere comedicatie werd verzameld. Interacties met de verschillende DAA-regimes zijn wederom gecontroleerd met behulp van de database van de Universiteit van Liverpool. In totaal konden we de gegevens van 777 patiënten evalueren waarvan 98% met cART werd behandeld. 63% van de patiënten gebruikte comedicatie. Met deze comedicatie had 38% van de patiënten een klinisch relevante interactie met één van de DAA-regimes (categorie 2 of 3). Daclatasvir + sofosbuvir is het regime dat het makkelijkst is te combineren met cART. Wanneer daclatasvir wordt gebruikt voor de HCV-behandeling behoeft de cART geen aanpassing. Grazoprevir + elbasvir is het DAA-regime dat het lastigste is te combineren met cART; 75% van de patiënten zou dan hun cART moeten aanpassen.

In **hoofdstuk 5** en **6** hebben we het risico van geneesmiddel-geneesmiddel interacties tussen DAAs en comedicatie/cART voorspeld. In **hoofdstuk 7** hebben we bestudeerd wat er daadwerkelijk voor interacties waren tijdens de behandeling met de DAAs. Dit is wederom bestudeerd in hiv/HCV-patiënten waarvan data beschikbaar waren in het ATHENA-cohort. Dit waren patiënten die zijn behandeld met DAAs tussen januari 2015 en mei 2016. Van al deze patiënten verzamelden we informatie over het DAA-regime, cART en andere comedicatie. Met behulp van de Liverpool database is gekeken naar de interacties tussen het gekozen DAA-regime, cART en andere comedicatie. In totaal zijn er 423 patiënten behandeld met DAAs, waarvan 418 (99%) cART gebruikten en 251 (59%) comedicatie. Voor de gekozen behandeling met DAAs hadden 84 patiënten een categorie 2 of 3 interactie met comedicatie en de DAA-behandeling. Bij 17 (20%) patiënten is de interactie voorkomen door de comedicatie te stoppen. Wat betreft de cART, 196 patiënten gebruikten een cART regime dat een interactie had met het DAA-regime. Categorie 2 interacties werden voorkomen bij 78/147 (53%) en categorie 3 bij 47/49 (96%) van de patiënten, doordat bij deze patiënten de cART werd aangepast naar een regime zonder een interactie. We hebben in dit hoofdstuk laten zien dat de Nederlandse hiv-behandelaren goed op de hoogte zijn van de mogelijke interacties tussen DAAs, comedicatie en cART en deze ook goed kunnen voorkómen.

DEEL 2:
Ribavirine farmacokinetiek en 'therapeutic drug monitoring'

Ribavirine wordt toegevoegd aan de behandeling met DAAs om de effectiviteit te bevorderen bij patiënten die moeilijker te genezen zijn. Het gaat dan om patiënten met cirrose, bij wie een eerdere behandeling gefaald heeft, of die geïnfecteerd zijn met HCV-genotype 1a of 3.

Ribavirine heeft een sterke concentratie-effect relatie. Dit betekent in het geval van ribavirine dat zowel de effectiviteit als de toxiciteit (bijwerkingen; anemie [bloedarmoede]) gerelateerd zijn aan de plasmaconcentratie van ribavirine. Hoe hoger de plasmaconcentratie hoe beter de effectiviteit is, maar ook een hogere kans op bijwerkingen.

Ribavirine is een kandidaat voor zogenaamde therapeutic drug monitoring (TDM). Bij TDM worden geneesmiddelconcentraties in het bloed bepaald, welke worden gebruikt voor het aanpassen van de dosering. Ribavirine is een kandidaat voor TDM omdat er sprake is van een concentratie-effect relatie en er grote inter-patiënt variatie is. Dit betekent dat eenzelfde dosis bij twee patiënten voor verschillende blootstelling kan zorgen. Om ribavirine concentraties te meten in het bloed is door verschillende laboratoria een methode ontwikkeld om ribavirine te meten. Om de kwaliteit van deze bepalingen extern te controleren is er een internationaal kwaliteitsprogramma voor ribavirine

ontwikkeld. De resultaten van het eerste jaar van het programma zijn beschreven in **hoofdstuk 8**. Dit kwaliteitsprogramma, uitgevoerd door de sectie Kwaliteitsbewaking Klinische Geneesmiddelanalyse en Toxicologie (http://www.kkgt.nl), is onderdeel van de Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek (SKML, http://www.skml.nl).

Via de post is er kalfsplasma verstuurd waaraan een bekende hoeveelheid ribavirine was toegevoegd. Er zijn monsters gemaakt met zowel een lage als een hoge concentratie ribavirine. In 2015 hebben er twee rondes plaatsgevonden. De monsters zijn op de gebruikelijke manier geanalyseerd door de deelnemende laboratoria. De ribavirine concentraties zijn vervolgens gerapporteerd aan de KKGT. Een monster was juist geanalyseerd wanneer de gerapporteerde concentratie viel binnen 80-120% van de ingemeten concentratie. In totaal hebben 8 laboratoria meegedaan, waarvan twee deelnemers maar aan 1 ronde hebben meegedaan. In ronde 1, was 81% (13/16) van de monsters juist gemeten. De variatie in de metingen van de lage monsters was 86-336% en bij de monsters met hoge concentratie 55-160%. In ronde twee was 75% (9/12) van de monsters juist gemeten. De monsters met lage concentratie hadden een variatie van 97-303% en monsters 97-148% met de hoge concentratie. Het doel van een kwaliteitsprogramma is om analysemethodes extern te valideren zodat de kwaliteit van de methodes verbeterd wordt. Laboratoria die ribavirine niet juist hebben bepaald, wordt geadviseerd de analysemethode aan te passen.

In **hoofdstuk 9** is de relatie tussen ribavirine concentratie en effectiviteit van de HCV-behandeling bestudeerd. Patiënten die zijn behandeld met de nieuwe DAAs en ribavirine werden geïncludeerd in deze studie. Tijdens de behandeling is bloed afgenomen (week 8, steady-state) om de ribavirine concentratie te bepalen. De gemiddelde ribavirine startdosering was 13,6 mg/kg/dag. Op week 8 was de gemiddelde ribavirine plasmaconcentratie 2,66 mg/L. In multivariabele analyse hebben we laten zien dat een hogere plasmaconcentratie van ribavirine sustained virological response (SVR [genezing van het virus]) voorspelt. Hieruit kon een therapeutisch venster van 2.28-3.61 mg/L worden afgeleid. In totaal hebben 89% van de patiënten een SVR behaald, wat betekent dat 89% van de behandelde patiënten was genezen van HCV.

In **hoofdstuk 10** is een vergelijkbare analyse gedaan, echter zijn nu hiv/HCV-patiënten uit Thailand geëvalueerd. Deze patiënten zijn behandeld met oude therapie bestaande uit peginterferon en ribavirine. Het is bekend dat hiv/HCV-patiënten die met deze therapie worden behandeld een lagere kans hebben op respons en dat de hiv/HCV-patiënten ook lagere ribavirine plasmaconcentraties hebben. In totaal hebben we 101 patiënten geëvalueerd waarvan 56% een SVR heeft bereikt. De steady-state concentra-

tie was 1.81 mg/L met een inter-patiënt variabiliteit van ongeveer 29% en intra patiënt variabiliteit van 18%.

DEEL 3:
Behandeling van HCV in speciale patiënten populaties

Het HCV-virus repliceert zich in de lever waardoor het leverontsteking en levercirrose kan veroorzaken. HCV patiënten hebben ook regelmatig nierfunctiestoornissen. **Hoofdstuk 11** is een review waarin de behandelingsmogelijkheden voor patiënten met lever- of nierfunctiestoornissen wordt besproken. Dosisaanpassingen kunnen nodig zijn aangezien de lever en nieren belangrijk zijn voor de eliminatie van geneesmiddelen.

Patiënten met gecompenseerde (Child-Pugh klasse A) levercirrose kunnen worden behandeld met alle DAAs, echter patiënten met gedecompenseerde cirrose (Child-Pugh klasse C) zijn moeilijker te genezen. Voor patiënten met nierfunctiestoornissen (nierfunctie <30 mL/min) geldt dat grazoprevir + elbasvir gebruikt kan worden bij patiënten met een genotype 1 of 4 HCV-infectie. Ledipasvir + sofosbuvir en velpatasvir + sofosbuvir wordt afgeraden bij deze patiënten.

In **hoofdstuk 12** hebben we een computermodel (physiology-based pharmacokinetic [PBPK] model) gebruikt om de farmacokinetiek van daclatasvir te voorspellen bij mensen met levercirrose. Helaas waren we niet in staat om het computermodel te valideren met humane data. De gesimuleerde en gemeten daclatasvir plasmaconcentraties kwamen niet overeen. Deze verschillen worden mogelijk veroorzaakt door veranderingen in de expressie van transporters die aanwezig zijn in de darm en in de lever bij patiënten met levercirrose.

DEEL 4:
De bijdrage van een apotheker aan de behandeling van hepatitis C

In het laatste deel van het proefschrift worden vier case reports besproken. Dit zijn vijf individuele patiënten die behandelend zijn met DAAs. Bij al deze patiënten was iets bijzonders aan de hand. Uit deze casussen blijkt des te meer hoe een apotheker kan bijdragen aan de optimale en veilige behandeling van HCV.

De eerste patiënt werd behandeld met 60 mg daclatasvir in combinatie met etravirine en darunavir/ritonavir voor zijn hiv-infectie. Etravirine versnelt (induceert) het metabolisme van daclatasvir via CYP3A4 waardoor de geadviseerde dosering voor daclatasvir 90 mg is in plaats van 60 mg. Darunavir/ritonavir remt dit metabolisme enigszins maar de geadviseerde dosering daclatasvir is de normale dosering van 60 mg. De combinatie

van daclatasvir, etravirine en darunavir/ritonavir is niet onderzocht. We hebben deze patiënt behandeld met 60 mg daclatasvir aangezien we vermoedden dat de remming de inductie van CYP3A4 zou opheffen. We hebben de plasmaconcentratie gemeten en de blootstelling van daclatasvir was vergelijkbaar met waarden bekend in de literatuur. In combinatie met etravirine en darunavir/ritonavir lijkt 60 mg daclatasvir de juiste dosering vanwege de blootstelling en omdat de patient het virus heeft geklaard.

De tweede patiënt had epilepsie waarvoor de patiënt werd behandeld met oxcarbazepine. Ook oxcarbazepine is een sterke inductor van CYP3A4 en P-gp waardoor de plasmaconcentraties van daclatasvir en sofosbuvir mogelijk zouden dalen. Deze patiënt is effectief behandeld met 90 mg daclatasvir en een normale dosering sofosbuvir. De blootstelling van daclatasvir was lager dan verwacht. Daarom blijft voorzichtigheid geboden bij patiënten die worden behandeld met sterke inductoren.

Het derde case report beschrijft twee patiënten die beide in het verleden een levertransplantatie hebben ondergaan. Na een orgaantransplantatie gebruiken deze patiënten immunosuppressiva die de weerstand onderdrukken zodat de nieuwe lever niet wordt afgestoten door het lichaam. Beide patiënten gebruikten hiervoor tacrolimus (immunosuppressief geneesmiddel). Tacrolimus wordt gemetaboliseerd door CYP3A4. Beide patiënten waren ook geïnfecteerd met HCV, waarvoor ze werden behandeld met DAAs. Er is geen interactie tussen de door deze patiënt DAAs en tacrolimus. Echter tijdens de behandeling hadden beide patiënten een hogere dosering tacrolimus nodig, omdat de plasmaconcentratie van tacrolimus lager was dan verwacht. We bediscussiëren dat deze verhoogde behoefte aan tacrolimus wordt veroorzaakt door de normalisatie van de activiteit van CYP-enzymen in de lever gedurende de behandeling met DAAs. HCV zelf heeft invloed op de CYP-enzymen waardoor bij genezing het metabolisme van geneesmiddelen kan veranderen.

De laatste patiënt die wordt besproken is eerder behandeld met DAAs. Tijdens deze eerdere behandelingen heeft de patiënt resistentie ontwikkeld. Daarnaast heeft de patiënt recent bariatrische chirurgie ondergaan. Dit betekent dat er een bypass van de maag naar de darm is gecreëerd. Hierdoor worden voedingsstoffen minder goed opgenomen wat resulteert in gewichtsverlies. Dit heeft echter ook invloed op de blootstelling van geneesmiddelen, deze worden mogelijk ook minder goed opgenomen. Deze patiënt is behandeld met simeprevir + sofosbuvir + ribavirine voor de HCV-infectie. Dit regime was de enige optie vanwege de resistentie die eerder was ontwikkeld. Tijdens de behandeling is bloed afgenomen om de blootstelling van simeprevir te volgen. Het bleek dat de blootstelling lager was ten opzichte van de referentiewaarden. Hierom is

besloten de dosering van simeprevir te verdubbelen, waardoor de blootstelling toe nam. De patiënt heeft SVR behaald en is dus genezen.

DISCUSSIE

In het laatste hoofdstuk van dit proefschrift worden bovenstaande delen bediscussieerd. De discussie wordt gestart met de geneesmiddelinteracties. We bespreken hoe geneesmiddelinteractie onderzoeken worden opgezet (zowel in het laboratorium als in de mens) en welke geneesmiddelen er eigenlijk moeten worden bestudeerd in combinatie met de nieuwe DAAs. Daarnaast wordt besproken hoe apothekers bijdragen aan het management van geneesmiddelinteracties.

Ten tweede wordt de rol van ribavirine TDM besproken. Wij denken dat moeilijk te genezen patiënten (zoals patiënten met levercirrose of nierfunctie problemen) mogelijk voordeel hebben van TDM. Vooral patiënten die geen bijwerkingen ervaren van ribavirine. Deze patiënten hebben mogelijk een te lage plasmaconcentratie en profiteren van een dosisverhoging om zo de kans op SVR te verhogen.

Als laatste worden de patiënten met lever- en nierfunctiestoornissen besproken. Er is nog geen geschikte behandeling voor patiënten met nierfunctiestoornissen die niet in aanmerking komen voor behandeling met grazoprevir + elbasvir. Tevens is er voor de patiënten met gedecompenseerde cirrose een behandelingsdilemma. Wanneer moeten deze patiënten worden behandeld? Voor of na de levertransplantatie?

De discussie wordt afgesloten met een sectie waarin de eradicatie van HCV centraal staat. HCV is vandaag de dag goed te behandelen, maar het meest belangrijk is dat alle patiënten worden opgespoord en dat vervolgens al deze patiënten toegang krijgen tot zorg en de geneesmiddelen. Omdat de DAAs op dit moment erg duur zijn, blijft de prijs een beperkende factor in veel landen.

De discussie wordt afgesloten met een opmerking dat het hele proefschrift, maar vooral het laatste deel waarin de individuele patiënten worden besproken goede voorbeelden zijn van a *pharmacist's contribution to eradicate hepatitis C*.

Epilogue

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gezegd over hepatitis C. We hebben elk hoogtepunt gevierd met jouw champagne. Jullie zijn er allebei altijd voor ons drieën. Dank jullie wel.

Arjan, *dear Arie*, hoe kan ik jou nu bedanken. Je bent degene die me altijd steunt in mijn ambities, de volste vertrouwen in me heeft en zo trots is als een pauw. Je hebt presentaties aangehoord, plaatjes gemaakt, overuren getolereerd. Bedankt dat je bent wie je bent en je leven met mij wilt delen. Jij maakt mij gelukkig en laat me lachen. Samen met jou is het leven geweldig! Het afgelopen jaar was moeilijk. Veel is er veranderd. Omringd door onze lieve vrienden en familie, kunnen we samen de wereld aan. 2018 wordt ons jaar! To the moon and back, in de groene kia <3.

CURRICULUM VITAE

Elise Smolders werd op 28 maart 1988 geboren in Nijmegen. Op 1-jarige leeftijd verhuisde ze naar Oss waar ze opgroeide en in 2001 het vwo ging doen op het Mondriaan Collega. In 2006 behaalde ze haar vwo-diploma. Aansluitend begon Elise met haar studie Farmacie aan de Rijksuniversiteit in Groningen waar ze in 2009 haar bachelor behaalde en in 2013 haar masterdiploma ontving. Elise schreef haar bacheloronderzoek over de lokale toediening van amiodaron op de boezem van het hart. Dit onderzoek voerde Elise uit de afdeling Farmaceutische Technologie en Biofarmacie onder leiding van prof. dr. Erik Frijlink. Het masteronderzoek van Elise richtte zich op het chronischevermoeidheidssyndroom welke ze uitvoerde op de afdeling laboratorium geneeskunde onder leiding van prof. dr. Frits Muskiet. Na haar afstuderen verhuisde Elise naar Genève, Zwitserland, om daar te stage te lopen bij de World Health Organization op de afdeling Essential Medicines. Gevolgd door een stage bij Health Action International in Amsterdam. Hierna is Elise gaan werken in het Amphia ziekenhuis te Breda.

In 2015 begon Elise met haar promotie traject in de apotheek van het Radboud universitair medisch centrum te Nijmegen, onder leiding van prof. dr. David Burger, prof. dr. Joost Drenth en dr. Klaartje de Kanter. Het onderzoek richtte zich op de farmacologie van de direct-acting antivirals welke worden gebruikt voor de behandeling van hepatitis C. Dit onderzoek is beschreven in dit proefschrift.

Sinds 1 maart 2017 is Elise in opleiding tot ziekenhuisapotheker in het Isala ziekenhuis in Zwolle onder de leiding van dr. Peter ter Horst.

LIST OF PUBLICATIONS

Prevalence and predictors of over-the-counter medication use among pregnant women: a cross-sectional study in the Netherlands.
Verstappen GMPJ, Smolders EJ, Munster JM, Aarnoudse JG, Hak E. *BMC Public Health. 2013 Mar 2;13:185.*

Pharmacokinetics, Efficacy, and Safety of Hepatitis C Virus Drugs in Patients with Liver and/or Renal Impairment.
Smolders EJ, de Kanter CTTM, van Hoek B, Arends JE, Drenth JPH, Burger DM. *Drug Saf. 2016 Jul;39(7):589-611.*

Sixty milligram daclatasvir is the right dose for hepatitis C virus treatment in combination with etravirine and darunavir/ritonavir.
Smolders EJ, de Kanter CTTM, Grintjes KJT, D'Avolio A, Di Perri G, van Crevel R, Drenth JPH, Burger DM. *AIDS. 2016 Jun 1;30(9):1491-3.*

Drug-Drug Interactions Between Direct-Acting Antivirals and Psychoactive Medications.
Smolders EJ, de Kanter CTTM, de Knegt RJ, van der Valk M, Drenth JPH, Burger DM. *Clin Pharmacokinet. 2016 Dec;55(12):1471-1494.*

Effective treatment of hepatitis C virus infection with sofosbuvir and daclatasvir 90 mg in a patient with severe epilepsy on oxcarbazepine.
Smolders EJ, de Kanter CTTM, van 't Veer N, D'avolio A, Di Perri G, Burger DM, van Wijngaarden P. *Int J Antimicrob Agents. 2016 Sep;48(3):347-8.*

Geneesmiddelinteracties met direct-werkzame antivirale middelen tegen hepatitis C virus infectie.
Burger DM, Smolders EJ, Grintjes KJT, Dofferhoff ASM, de Kanter CTMM, Drenth JPH. *Tijdschr Infect 2016;11(2):45-51*

Measuring Plasma Concentrations of Ribavirin: First Report From a Quality Control Program.
Smolders EJ, Kan R, de Kanter CTTM van Luin M, Aarnoutse RE, Touw DJ, Burger DM. *Ther Drug Monit. 2016 Oct;38(5):646-7*

Daclatasvir 30 mg/day is the correct dose for patients taking atazanavir/cobicistat.
Smolders EJ, Colbers EPH, de Kanter CTTM, Velthoven-Graafland K, Drenth JPH, Burger DM. *J Antimicrob Chemother. 2017 Feb;72(2):486-489.*

A call for a consortium for optimal management of drug-drug interactions in patient care.
Burger DM, Smolders EJ, Schapiro J, Drenth JPH, Back DJ. *Clin Pharmacol Ther. 2017 Feb 4.*

Decreased tacrolimus plasma concentrations during HCV therapy: a drug-drug interaction or is there an alternative explanation?
Smolders EJ, Pape S, de Kanter CTTM, van den Berg AP, Drenth JPH, Burger DM. *Int J Antimicrob Agents. 2017 Mar;49(3):379-382.*

Metformin and daclatasvir: absence of a pharmacokinetic-pharmacodynamic drug-interaction in healthy volunteers.
Smolders EJ, Colbers EPH, de Kanter CTMM, Velthoven-Graafland K, Wolberink LT, van Ewijk-Beneken Kolmer N, Drenth JPH, Aarnoutse RE, Tack CJ, Burger DM. *Br J Clin Pharmacol. 2017 May 5.*

High need to switch cART or co-medication with the initiation of DAAs in elderly HIV/HCV co-infected patients.
Smolders EJ, Smit C, de Kanter CTMM, Dofferhoff ASM, Arends JE, Brinkman K, Rijnders B, Van Der Valk M, Reiss P, Burger DM. *J Acquir Immune Defic Syndr. 2017 Jun 22.*

The majority of hepatitis C patients treated with direct acting antivirals are at risk for relevant drug-drug interactions.
Smolders EJ, Berden FAC, de Kanter CTMM, Kievit W, Drenth JPH, Burger DM. *United European Gastroenterol J. 2017 Aug;5(5):648-657.*

PHD PORTFOLIO

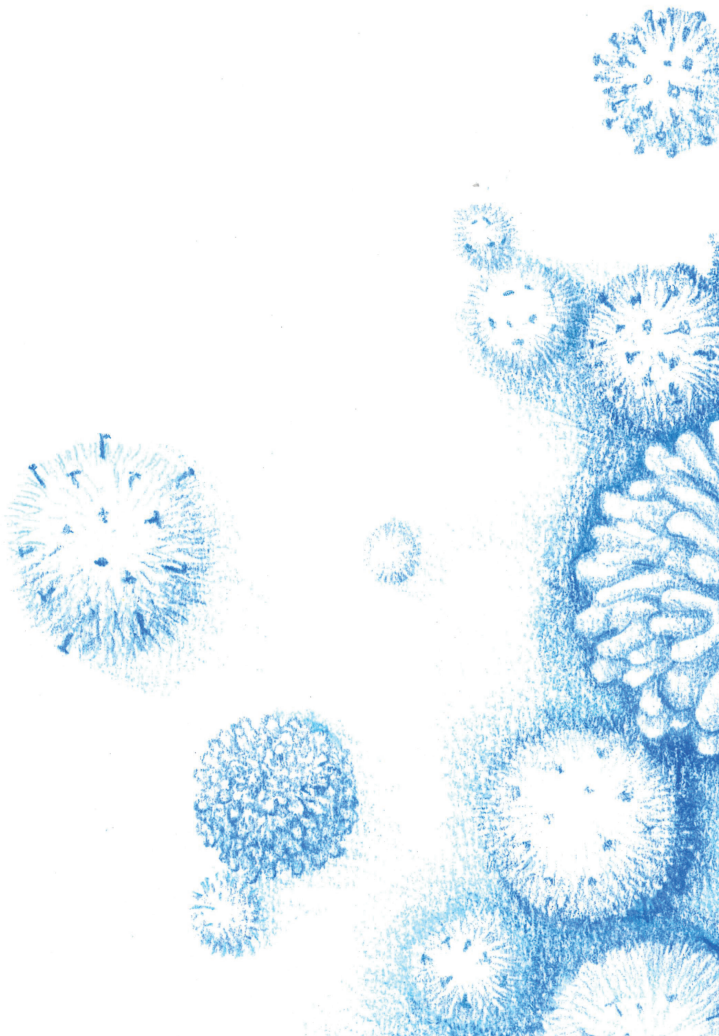
Institute for Health Sciences
Radboudumc

Name PhD candidate: E.J. Smolders **Department: Pharmacy** **Graduate School: Radboud Institute for Health Sciences**	**PhD period: 01-01-2015 – 01-03-2017** **Promotor(s): Prof. D.M. Burger, prof. J.P.H. Drenth** **Co-promotor(s): Dr. C.T.M.M. de Kanter**

	Year(s)	ECTS
TRAINING ACTIVITIES		
Courses & Workshops		
Introduction day Radboudumc.	2015	0.8
RIHS graduate school specific introductory course.	2015	0.5
Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers (BROK), PAO Heyendaal, Nijmegen.	2015	2.5
Interpretation of population pharmacokinetics, NVZA, Leiden.	2015	0.2
PhD retreat + poster presentation.	2015	0.75
Management for PhD students, PAO Heyendaal, Nijmegen.	2015	2.0
SPSS introduction course, PAO Heyendaal, Nijmegen.	2015	0.6
Academic writing for PhD students, PAO Heyendaal, Nijmegen.	2015-2016	3.0
Hepatitis C masterclass, Virology Education, Utrecht.	2015-2016	1.4
Scientific integrity course, POA Heyendaal, Nijmegen.	2016	1.5
Proficiency English, Radboud in'to Languages, Nijmegen.	2016-2017	2.8
Seminars & lectures		
Lecture: Aangrijpingspunten van antiretrovirale middelen en het ontstaan van resistentie - focus op HIV en HCV. Anselmus Colloquium, Utrecht.	2015	0.5
Lecture: Hepatitis C casuïstiek. HCV speakers tour, Nijmegen.	2016	0.45
Lecture: Clinical case presentation: drug-drug interactions in hepatitis C patients. 17th International Workshop on Clinical Pharmacology of Antiviral Therapy of HIV & Hepatitis, Washington DC, USA.	2016	-
Lecture: Drug-drug interactions, het richtsnoer hepatitis C in de praktijk. Zwolle.	2017	0.45
Lecture: Drug-drug interactions in ageing hepatitis C patients. 18th International Workshop on Clinical Pharmacology of Antiviral Therapy, Chicago, USA.	2017	-
Symposia & congresses		
Accepted abstracts		
Oral presentation: Ribavirin plasma level is an independent predictor for efficacy in hepatitis C patients treated with DAAs and ribavirin (HepNed study 002). Nederlandse Ziekenhuisfarmaciedagen, Amersfoort.	2015	-
Oral presentation: Ribavirin plasma level is an independent predictor for sustained virologic response in difficult-to-treat hepatitis C-infected patients treated with direct-acting antivirals + ribavirin combination (HepNed study 002). Najaarsvergadering Nederlandse Vereniging voor Gastroenterologie, Veldhoven.	2016	-

Oral presentation: Steady state ribavirin pharmacokinetics in chronic hepatitis C infected patients with moderate renal impairment taking modern DAA combinations: are we dosing too high?! 17th International Workshop on Clinical Pharmacology of Antiviral Therapy of HIV & Hepatitis, Washington DC, USA.	2016	-
Poster presentation: Ribavirin plasma level is an independent predictor for sustained virologic response in difficult-to-treat hepatitis C-infected patients treated with direct-acting antivirals + ribavirin combination (HepNed-002 study). The Liver Meeting, American Association for the Study of Liver Diseases, Boston, USA.	2016	-
Poster presentation: High risk of drug-interactions in hepatitis C treatment: a nationwide cohort. Nederlandse Ziekenhuisfarmaciedagen, Den Bosch.	2016	-
Poster presentation: Measuring plasma concentrations of ribavirin: first report from a quality control. 17th International Workshop on Clinical Pharmacology of Antiviral Therapy of HIV & Hepatitis, Washington DC, USA.	2016	-
Poster presentation: Evaluation of ribavirin plasma concentrations in HIV/HCV co-infected patients from. 17th International Workshop on Clinical Pharmacology of Antiviral Therapy of HIV & Hepatitis, Washington DC, USA.	2016	-
Poster presentation: Daclatasvir 30 mg/day is the correct dose for patients taking atazanavir/cobicistat. 17th International Workshop on Clinical Pharmacology of Antiviral Therapy of HIV & Hepatitis, USA.	2016	-
Poster presentation: High risk on drug-drug interactions during hepatitis C treatment: a nationwide cohort. 17th International Workshop on Clinical Pharmacology of Antiviral Therapy of HIV & Hepatitis, Washington DC, USA.	2016	-
Poster presentation: Pharmacokinetics of daclatasvir in cirrhotic patients: challenges in PBPK modeling. 18th International Workshop on Clinical Pharmacology of Antiviral Therapy, Chicago, USA.	2017	-
Poster presentation: Treating the "untreatable": Adjusted doses of daclatasvir with the anti-epileptic drug carbamazepine (HepNED study 003). 18th International Workshop on Clinical Pharmacology of Antiviral Therapy, Chicago, USA.	2017	-
Poster presentation: Management of drug-interactions with DAAs in Dutch HIV/HCV co-infected patients: adequate but not perfect. International Liver Congress, European Association for the Study of the Liver, Amsterdam.	2017	-
Symposia & Congresses		
Nationale hepatitis dag, Virology Education, Amsterdam.	2015-2017	0.75
Post CROI, Virology Education, Utrecht.	2015-2016	0.6
Post EASL, Virology Education, Utrecht.	2015-2016	0.4
Peer-to-peer review HCV, Virology Education, Utrecht.	2015	0.2
International Liver Congress, European Association for the Study of the Liver, Vienna, Austria.	2015	1.5
16th and 17th International Workshop on Clinical Pharmacology of Antiviral Therapy of HIV & Hepatitis, Washington DC, USA.	2015-2016	4.5

HCV Advances Workshop, Virology Education, Amsterdam.	2016	0.5
De behandeling van chronische hepatitis C in Nederland, Arnhem.	2016	0.2
Pediatric drug development: a field in maturation, Dutch Society of Toxicology, Nijmegen.	2016	0.2
International Liver Congress, European Association for the Study of the Liver, Barcelona, Spain.	2016	1.5
The Liver Meeting, American Association for the Study of Liver Diseases, Boston, USA.	2016	1.75
Najaarsvergadering Nederlandse Vereniging voor Gastroenterologie, Veldhoven.	2016	0.45
CROI conference, Seattle, USA.	2017	1
International Liver Congress, European Association for the Study of the Liver, Amsterdam.	2017	1.75
18th International Workshop on Clinical Pharmacology of Antiviral Therapy, Chicago, USA.	2017	2.25
Other		
Journal club, Department of Pharmacy, Radboudumc.	2015-2017	3
Grant: studying the interaction between daclatasvir and metformin, Bristol-Myers Squibb.	2015	-
Grant: studying the interaction between daclatasvir and atazanavir/ritonavir and atazanavir/cobicistat, Bristol-Myers Squibb.	2015	-
Clinical trial monitoring during the CRUSTRI trial.	2015	-
TEACHING ACTIVITIES		
Lecturing		
Clinical pharmacology lectures (lecturing on research or a clinical topic), department of Pharmacology and Toxicology and Department of Pharmacy, Radboudumc.	2015-2017	0.75
'Snoepje van de week' (lecturing on a clinical topic), Department of Pharmacy.	2016, 2017	0.75
Supervision of internships / other		
8-week internship, master student Pharmacy, University of Groningen.	2015	1
6-month internship, bachelor student Biomedical Sciences, Radboud university.	2016	1
TOTAL		40.5



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