Exposure to antiretroviral agents in HIV-infected pregnant women: not too much – not too little

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Introduction

HIV epidemic

Worldwide around 35 million people are living with HIV of which approximately 16 million (almost 50%) are female. Of these women 1.5 million gave birth to a child in 2013.[1] HIV can be transmitted from mother to child. The highest chance of this mother-to-child transmission (MTCT) of HIV-1 occurs during delivery (10-20%), but there is also a chance of transmission during pregnancy (5-10%). Furthermore, during the breast feeding period an additional risk of 10-20% has been observed.[2]
Without intervention the chance of MTCT of the virus is 25-40% during pregnancy and delivery. This risk can be reduced to <2% if the mother and infant are treated with antiretroviral therapy.[2] This great reduction of MTCT is driven by two mechanisms:

1) minimizing the chance of infection during a possible blood-blood contact during delivery because of suppression of the virus in the mother by treating the mother (and achieving an undetectable viral load in blood).
2) some antiretrovirals cross the placenta barrier and reach the foetus, protecting the foetus against the virus at the moment of possible blood-blood contact.[3]

Treatment of HIV

At this moment 24 different antiretrovirals are available, disrupting different phases of the reproduction cycle of the virus. This leads to an allocation into six different classes of antiretroviral medication: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PI), integrase inhibitors (II), entry inhibitors (EI) and fusion inhibitors (FI). Antiretroviral drugs are generally used in combinations of three or more drugs from more than one class to optimally suppress the virus and prevent drug resistance. This is called "combination AntiRetroviral Therapy": cART. Table 1 lists all available antiretrovirals per class and also includes two "boosting" agents (ritonavir and cobicistat). These compounds inhibit the CYP3A4 enzyme, and through this mechanism increase the exposure of antiretrovirals which are substrates for the CYP3A4 enzyme. This approach reduced the number of tablets or capsules to be used of the CYP3A4 substrates.
cART is also recommended for the treatment of pregnant women with HIV. When a woman is already using cART for her own health when she becomes pregnant this treatment regimen is mostly continued during pregnancy. However, treatment guidelines for middle- and high income countries recommend to prevent the use of efavirenz during conception and early pregnancy due to potential teratogenicity.[3,6] Treatment-naive HIV infected pregnant women should start cART early in the second trimester with cART in the same regimen as non-pregnant patients. The preferred regimens consist of two NRTIs

Table 1. Drugs for HIV treatment, available in 2015[4,5]

Class	Mechanism of action	Drugs
Nucleoside/nucleotide reverse transcriptase inhibitors NRTI	NRTIs interrupt the HIV replication cycle via competitive inhibition of HIV reverse transcriptase and termination of the DNA chain. NRTIs are administered as prodrugs, requiring host cell entry and phosphorylation.	Abacavir Didanosine Emtricitabine Lamivudine Stavudine Tenofovir Zidovudine
Non-nucleoside reverse transcriptase inhibitors NNRTI	NNRTIs inhibit HIV-1 reverse transcriptase by binding and inducing the formation of a hydrophobic pocket proximal to, but not overlapping the active site.	Efavirenz Etravirine Nevirapine Rilpivirine
Protease inhibitors PI	HIV protease inhibitors function as competitive inhibitors that directly bind to HIV protease and prevent subsequent cleavage of polypeptides.	Atazanavir Darunavir Fosamprenavir Indinavir Lopinavir Ritonavir Saquinavir Tipranavir
Boosters, CYP3A4 inhibitors	Inhibitors of the cytochrome P450 3A4 liver enzyme, reducing the metabolism of CYP3A4 substrates.	Ritonavir Cobicistat
Integrase inhibitors II	HIV integrase is responsible for the transport and integrated attachment of proviral DNA to host-cell chromosomes, allowing subsequent transcription by host enzymes of messenger and viral RNA and translation of viral proteins essential for the assembly of virus particles. IIs competitively inhibit the strand transfer reaction by binding metallic ions in the active site.	Dolutegravir Elvitegravir Raltegravir
Entry inhibitors (CCR5 binding), EI	Binding the CCR5 co-receptor selectively and reversibly, blocking the V3 loop interaction and inhibiting fusion of the cellular membranes.	Maraviroc
Fusion inhibitors FI	Fusion inhibitors are peptides that bind to gp41 extracellularly to prevent the fusion of HIV envelope to the CD4 or other target cell wall.	Enfuvirtide

with either a boosted PI or NNRTI. The European guideline includes the option to add raltegravir to the combination therapy if the viral load is not undetectable in the third trimester.[6]

In the choice of a regimen for a pregnant woman many factors should be included: co-morbidities, convenience, adverse effects, drug interactions, resistance testing results, pharmacokinetics, information on safety for mother and foetus and experience with use in pregnancy. Physiological changes in pregnancy may lead to pharmacokinetic alter-

ations, i.e. lower plasma levels of drugs and may necessitate increased dosages, more frequent dosing, or boosting, especially of protease inhibitors.[3] The next paragraph summarizes the mechanisms behind these pharmacokinetic changes.

Physiological changes in pregnancy affecting pharmacokinetics

Several physiological changes during pregnancy may influence absorption, distribution, metabolism and/or excretion of drugs. Increased gastric pH can decrease gastric absorption of weak acids and increase absorption of weak bases. Slower intestinal motility might influence absorption, in most cases increasing absorption. An increased volume of distribution during pregnancy can lead to a lower peak concentration (C_{max}) at steady state. Changes in protein concentrations in the blood (albumin and alpha 1 glycoprotein acid) may lead to higher unbound concentrations of highly protein bound drugs. Hepatic clearance is dependent on protein binding, activity of metabolic enzymes and liver blood flow, all of which change during pregnancy. Protein binding decreases, metabolic enzyme activity is induced for most enzymes and liver blood flow increases. All factors can lead to increased clearance of drugs during pregnancy. Glomerular filtration rate and renal blood flow are increased during pregnancy, possibly decreasing in the third trimester, an effect which also increases clearance of renally excreted drugs. The influence of pregnancy on tubular secretion and reabsorption is not known, but changes are suggested as pregnancy influences the renal handling of endogenous substances such as uric acid and glucose. The influence of pregnancy on transporters is getting more attention lately, because this might be an additional factor influencing excretion of drugs.[7-10]
Most of the alterations mentioned above lead to lower plasma concentrations of drugs during pregnancy, and possibly to below the effectiveness threshold level. A decrease in exposure during pregnancy has been described to be the case for several antiretroviral drugs, especially protease inhibitors,[11,12] leading to recommendations to increase the dose during the third trimester of pregnancy. Sub-therapeutic concentration of antiretroviral agents is of particular concern as this not only may lead to development of resistance in the woman but also to HIV MTCT. It is therefore of great importance that our knowledge on the pharmacokinetics of antiretroviral medications in pregnancy is extended. This thesis contributes to this increased knowledge.

Pharmacokinetics of antiretroviral medication in pregnancy

This thesis starts with Chapter 2: "Pharmacological considerations on the use of antiretrovirals in pregnancy" which summarizes and discusses publications concerning pharmacokinetic changes of antiretroviral drugs during pregnancy which were recently

published. In this review the articles published in 2012 were described.
At that time, for the majority of antiretrovirals information on pharmacokinetics during pregnancy was either not available, very limited, or contradictory. As changes in pharmacokinetics should be included in the choice of the antiretroviral regimen during pregnancy, we felt that there was a need to explore this further.
Pharmacokinetic data in pregnancy (or safety data) are not collected by the pharmaceutical industry during the development of a new drug. Pregnant women are simply excluded from registrational trials. Post-marketing studies investigating pharmacokinetic changes of drugs during pregnancy are set up by independent, academic, groups. In the US a study has been set up by the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) to study pharmacokinetics of antiretroviral drugs during pregnancy (P1026 protocol, clintrials.gov reference NCT00042289). The population studied within this network is mainly black or Hispanic, with only a limited number of white patients included. Because the IMPAACT group includes a limited number of patients per compound in the study, and the population studied probably differs from the European population, we felt there was a need for an additional study. After successfully performing a proof of concept study, investigating the pharmacokinetics of saquinavir/ritonavir in pregnant women, presented in chapter 3 of this thesis, an initiative was taken in 2008 to set up a network of hospitals investigating pharmacokinetics in pregnancy in Europe. A protocol entitled "Study on Pharmacokinetics of newly developed ANtiretroviral agents in HIV-infected pregNAnt women (PANNA)" was developed by the Department of Pharmacy of the Radboud university medical center. Investigators treating HIV infected pregnant women, interested in pharmacokinetics and having the facilities to perform pharmacokinetic studies, located at different hospitals in Europe were invited to participate. A study group, consisting of infectologists, internists, gynaecologists and pharmacologists gave input in the set-up of the study. The first patients were recruited in 2009 and in 2015 the PANNA network comprises of 21 hospitals in 7 European countries (The Netherlands, Belgium, Germany, UK, Spain, Italy, Ireland), with central management at the Department of Pharmacy of the Radboud university medical center Nijmegen, the Netherlands.
The study was conducted in compliance with the principles of the "Declaration of Helsinki". Informed consent was obtained from each participant before entering the study. Approval of the Medical Ethics Committee from each individual centre involved and the national authorities if applicable was obtained. The study is registered at ClinicalTrials.gov under number NCT00825929.
A selection of antiretrovirals to be studied was made based on the limited availability or absence of pharmacokinetic information in pregnancy. Compounds initially under investigation were: etravirine, efavirenz (UK/Ireland only), emtricitabine, tenofovir, atazanavir, darunavir, fosamprenavir, tipranavir, indinavir, raltegravir, maraviroc, enfuvirtide and maraviroc. In a later stage abacavir, rilpivirine, elvitegravir, cobicistat and dolutegravir were added.

Pregnant women using (at least) one of the antiretrovirals indicated in the protocol (prescribed by their treating physician) can be included. If they use more than one compound from the list, all compounds will be used for analysis. Blood samples are taken for a full pharmacokinetic curve in the third trimester of pregnancy (preferably around week 33), during delivery a cord blood and matching maternal sample are collected and after delivery (preferably 4-6 weeks) a full pharmacokinetic curve is taken (postpartum curve). The postpartum curve serves as the control curve representing the non-pregnant situation. The women are their own control, which decreases the variation due to which the number of patients to be included in the study can be limited. For each compound we aim to collect data from 16 women. Pharmacokinetic parameters are calculated using the non-compartmental analysis. The comparison between the third trimester pharmacokinetic parameters and postpartum pharmacokinetic parameters is done using analysis of variance for paired samples, or using a t-test for paired samples.
Next to the pharmacokinetic information, safety and efficacy data are collected. At each visit blood samples are taken for haematology and biochemistry analysis and for viral load and CD4 T cell counts which are performed at the local hospital. The major part of this thesis comprises of the first results of the PANNA study. The study is still ongoing, but some medication arms have been closed and analysed, amendments have been drafted to include new antiretrovirals to the list of medication to be investigated.
In this thesis we present the results of this study for two protease inhibitors: atazanavir/r (ritonavir) in chapter 4 and darunavir/r in chapter 5; two NRTIs: tenofovir and emtricitabine in chapter 6 and the integrase inhibitor raltegravir (chapter 7). For rilpivirine no information was reported in the public domain, therefore we published the pharmacokinetics and placental passage of the first two patients included using this compound in the PANNA study as a case report, see chapter 8 of this thesis.
Some antiretrovirals under study are rarely used in pregnancy, for example maraviroc. The PANNA network included seven patients over a period of 5 years. The P1026 study, initiated by the IMPAACT group, encountered the same problem. This led to a co-operation between the two networks, starting with a joint presentation of the preliminary data at CROI 2013, resulting in a joint paper describing the pharmacokinetics and placenta passage of maraviroc, presented in chapter 9.

Physiologically based pharmacokinetic modelling

Executing these pharmacokinetic studies involves much time, effort and commitment of the investigators as well as the patients participating in such a study, and these studies are expensive to perform.
Physiologically based pharmacokinetic (PBPK) modelling is a mathematical (computer) technique to predict absorption, distribution, metabolism and excretion (ADME) of for example medication, based on physicochemical properties and *in vitro* biotransforma-

tion. The model consists of different compartments corresponding to the different organs and tissues, connected by blood or lymph flows. Recently, a PBPK model including physiological changes in relation to duration of pregnancy (pregnancy PBPK model) has been developed by Simcyp. The pregnancy PBPK model can be used to predict exposure to drugs during pregnancy at any gestational age, and eventually predict exposure of increased doses of medication, and indicate the gestational age at which a dose increase should be suggested. The foeto-placental unit (combination of foetus, placenta, amniotic fluid, membranes and umbilical cord) is included as a perfusion-limited compartment running in parallel with the other maternal compartments.[13]
The data derived from the PANNA study were used to verify whether darunavir pharmacokinetic parameters in pregnancy can reliably be predicted by the SimCYP pregnancy PBPK model, see chapter 10.

In conclusion, the aim of this thesis was:

to describe pharmacokinetic alterations of specified antiretroviral agents during pregnancy, and to indicate efficacy, safety and cord blood/maternal ratios.

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Pharmacological Considerations on the Use of Antiretrovirals in Pregnancy

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Abstract

Purpose of review: Treatment with combination antiretroviral therapy during pregnancy reduces the chance of mother to child transmission of HIV. Physiological changes during pregnancy can lead to lower exposure to antiretrovirals, possibly resulting in virological failure. For most antiretrovirals, data on exposure during pregnancy and transplacental passage is limited. This review summarizes the most recent information on pharmacokinetics (including transplacental passage), efficacy, as well as the safety of antiretrovirals during pregnancy.

Recent findings: Intensive-sampling pharmacokinetic studies as well as observational studies using sparse sampling were performed to explore the exposure to antiretrovirals during pregnancy. Transplacental passage, efficacy (viral load at delivery and infection status of the newborn) and safety information were evaluated for several antiretrovirals.

Summary: For most nucleoside/nucleotide reverse transcriptase inhibitors and protease inhibitors, recent research shows a decreased exposure during pregnancy. However, the advantage of a general dose increase during pregnancy still remains unclear. For newer compounds and efavirenz, limited or no data on pharmacokinetics during pregnancy or transplacental passage are available, while the mechanisms of transplacental passage also remain unknown. For safety reasons, it will be important to monitor pregnancy outcomes in resource-limited settings during the implementation of the WHO guidelines (including the use of efavirenz during pregnancy).

Introduction

Treatment with antiretrovirals, especially when used as combination antiretroviral therapy (cART), dramatically reduces the chance of mother to child transmission (MTCT) of HIV from 20% to less than 1%. Current perinatal guidelines recommend to start cART at 12-14 weeks of pregnancy, or earlier in case of a CD4 count below 350-500 cells/μL. Preferred agents to include in cART are: lamivudine, zidovudine, nevirapine, ritonavir-boosted lopinavir or atazanavir/r.[1,2] According to the US Department of Health & Human Services (DHHS) and British HIV Association guidelines, or triple therapy including nevirapine or efavirenz[3] may be used according to the WHO guidelines.

Physiological changes occurring during pregnancy can alter exposure to drugs. Examples include increased gastric pH, volume of distribution, glomerular filtration and cardiac output, decreased protein binding and alteration of cytochrome P450 activity. Recently, a meta-analysis of these changes during pregnancy was published.[4] The changes lead to lower exposure to antiretrovirals during (late) pregnancy in most cases. In turn, subtherapeutic drug levels could lead to virological failure and development of resistant virus and eventually to MTCT of HIV. In some studies, antiretroviral dose was increased in the third trimester of pregnancy to compensate for lower maternal exposure. Also, therapeutic drug monitoring (TDM) is recommended to check antiretroviral levels and perform dose increases on an individual level. Besides considerations with regard to maternal exposure, antiretrovirals can pass the blood-placenta barrier and might cause teratogenicity, induce premature birth or cause low birth weight. Yet, placenta passage of antiretrovirals can also ensure infant pre-exposure prophylaxis.

During clinical development exposure of pregnant women to new drugs is avoided, whereas after reaching the market they will be used by pregnant women. Hence, post-marketing studies are being performed focusing on pharmacokinetics during pregnancy, including transplacental passage. Also, the effects of antiretroviral use on MTCT, preterm delivery and teratogenicity are important issues here.

We now give an update of the most recent (since 2012) publications on these topics.

Pharmacokinetics and efficacy of antiretrovirals during pregnancy

An extensive review has recently been published,[5] covering pharmacokinetic studies on antiretrovirals in pregnancy until 2012. This earlier review concluded that, to optimize antiretroviral therapy during pregnancy, pharmacological changes during pregnancy and transplacental transfer should be taken into account. TDM of antiretroviral exposure in pregnant women was mentioned as an intervention to optimize therapy.

Two other reviews were published in 2012 on this subject.[6,7] Furthermore, Eley *et al.* published a meta-analysis of pharmacokinetic data of atazanavir during pregnancy.[8]

In the following section, an overview of recent studies published on pharmacokinetics and efficacy of antiretrovirals during pregnancy is given for each antiretroviral class; a summary of the results and conclusions can be found in Table 1.[9,10-12,13,14-17,18-20,21,22,23]

Nucleoside/Nucleotide reverse transcriptase inhibitors

Two intensive-sampling pharmacokinetic studies, collecting pharmacokinetic curves during pregnancy (second and/or third trimester) and postpartum (in the same women), were published: emtricitabine[9] was described by the IMPAACT group and tenofovir and emtricitabine pharmacokinetics by the PANNA network.[10] Both studies observed decreased exposure (by approximately 25%) to these NRTIs during pregnancy, but conclude that dose adaptation seems not to be necessary during pregnancy. This conclusion was based on absence of an association with virological failure or MTCT,[10] or C_{24h} exceeding the IC_{50} in all individuals.[9] The clinical relevance of IC_{50} is uncertain, as it only reflects 50% inhibitory concentrations, whereas 100% inhibition of the virus is the aim *in vivo*.

Benaboud *et al.* reported two population-pharmacokinetic studies, describing lamivudine[11] and tenofovir[12] in pregnancy. The developed population-pharmacokinetic models were based on blood samples obtained just before dosing (C_{trough}) of pregnant women and non-pregnant women (controls).

The lamivudine exposure observed was close to the exposure of non-pregnant women and no dosage adjustment was advised.[11] For tenofovir, a 39% increase of clearance was observed during pregnancy. To guarantee similar C_{trough} as nonpregnant adults, an increase in tenofovir dose should be considered for women from the second trimester to delivery.[12] Strength of these studies is that they use TDM data of non-pregnant HIV-infected women as control, and not only reference values in literature that are mostly based on pharmacokinetic studies in male patients or healthy volunteers. No information was given on MTCT and dosing advice was based on (not yet clinically validated) population-pharmacokinetic models.

Non-Nucleoside Reverse Transcriptase Inhibitors

An intensive-sampling pharmacokinetic study was published on the pharmacokinetics of efavirenz 600mg once daily (q.d.) during pregnancy. Cressey *et al.*[13] compared second and third trimester efavirenz pharmacokinetic curves to postpartum curves of the same patients (n=25). This is of great interest because WHO treatment guidelines include efavirenz, tenofovir and emtricitabine as triple therapy to be used during pregnancy.

They found a slight increase of oral clearance and decreased predose and C_{trough} concentrations in the third trimester. Efavirenz exposure during pregnancy after standard dosing remained in the therapeutic range. A limitation of this study is that the majority of patients were Thai (83%), and one MTCT took place, without known reason. These pharmacokinetic data support the use of standard efavirenz dosing during pregnancy.

Table 1. Pharmacokinetic parameters of antiretrovirals during pregnancy

Parameter	Ref		Third trimester	Postpartum	GMR (90%CI)	Study conclusion
Nucleoside/Nucleotide reverse transcriptase inhibitors						
Emtricitabine 200mg q.d.	[9]	IS; GM (90% CI)	n=26	n=22	n=22	Because the FTC AUC decrease during pregnancy was less than 20% and C_{24} exceeded the IC50 in all subjects, dosing adjustment during pregnancy does not appear to be necessary.
AUC (mg·h/L)			8 (7.1-8.9)	9.7 (8.6-10.9)	0.82 (0.71-0.96)	
C_{trough} mg/L			0.058 (0.037-0.063)	0.085 (0.07-0.1)	0.68 (0.54-0.85)	
Emtricitabine 200mg q.d.	[10]	IS; GM (95% CI)	n=27	n=24	n=24	Although PK exposure of FTC during pregnancy is approximately 25% lower, this was not associated with virological failure in this study and did not result in MTCT of HIV.
AUC (mg·h/L)			9.56 (8.99-10.48)	13.0 (11.8-14.3)	0.75 (0.68-0.82)	
C_{trough} (mg/L)			0.052 (0.043-0.073)	0.073 (0.054-0.098)	0.77 (0.52-1.12)	
Tenofovir 245mg q.d.	[12]	CC; mean (95% CI)	n=46	n=158		As TFV clearance was increased by 39%, TFV dose escalation should be considered for women from the 2nd trimester to delivery in order to obtain exposure similar to exposure in non-pregnant adults.
pregnant vs. controls						
AUC (mg·h/L)			1.6 (0.9-3.3)	2.4 (1.1-5.2)		
C_{trough} (mg/L)			0.039 (0.022-0.092)	0.061 (0.031-0.164)		

Table 1. - continued Pharmacokinetic parameters of antiretrovirals during pregnancy

Parameter	Ref	Third trimester	Postpartum	GMR (90%CI)	Study conclusion
Nucleoside/Nucleotide reverse transcriptase inhibitors					
Tenofovir 245mg q.d.	[10] IS; GM (95% CI)	n=34	n=27	n=27	Although PK exposure of TDF during pregnancy is approximately 25% lower, this was not associated with virological failure in this study and did not result in MTCT of HIV.
AUC (mg·h/L)		2.46 (2.23-2.66)	3.17 (2.86-3.52)	0.77 (0.71-0.83)	
C_{trough} (mg/L)		0.052 (0.047-0.059)	0.066 (0.058-0.076)	0.79 (0.70-0.90)	
Lamivudine 150mg b.i.d.	[11] CC; median	n=114	n=46		As rates of exposure in pregnant women are close to values reported previously for nonpregnant adults, no dose adjustment should be needed.
AUC (mg·h/L) vs controls		10.3	12.7		
CL/F (L/h)		29.12	23.67		
Non-nucleoside reverse transcriptase inhibitors					
Efavirenz 600mg q.d.	[13] IS; median (range)	n=25	n=25	n=25	Although EFV oral clearance is increased and predose and trough concentrations are decreased during pregnancy, EFV exposure during pregnancy seems adequate.
AUC (mg·h/L)		55.4 (13.5-220.3)	58.3 (22.7-214.4)	0.97 (0.83-1.13)	
C_{trough} (mg/L)		1.60 (0.23-8.13)	2.05 (0.31-8.43)	0.88 (0.73-1.06)	
Met EFV C_{trough} target 1.0 mg/L		22/25 (88%)	23/25 (92%)		

Table 1. - continued Pharmacokinetic parameters of antiretrovirals during pregnancy

Parameter	Ref	Third trimester	Postpartum	GMR (90%CI)	Study conclusion
Protease Inhibitors					
Lopinavir/r 400/100mg b.i.d.	[14] CC; GM (CV%)	n=21	n=20		The standard dose of LPV/r tablets appears appropriate to provide adequate plasma levels during the 3rd trimester of pregnancy. However, large PK studies are required.
C_{trough} mg/L pregnant vs non-pregnant		4.205 (123%)	5.098 (147%)		
Lopinavir/r 400/100mg b.i.d. soft gel capsule	[15] GM (95% CI)	n=6			Despite a significant reduction in LPV exposure in pregnancy, standard dose of the tablet formulation showed adequate LPV conc. for for treatment naive patients. TDM is warranted for treatment-experienced patients, as levels can be below their target of 4.0 mg/L.
AUC (mg·h/L)		50.4 (36.5-75.3)			
C_{trough} (mg/L)		2.36 (1.57-4.09)			
Did not meet target 1.0 mg/L		1/6 (17%)			
Lopinavir/r 400/100mg b.i.d. melt-extruded tablet	[15] IS; GM (95% CI)	n=11	n=5		
AUC (mg·h/L)		58.0 (50.4-70.72)	89.4 (74.0-107.9)		
C_{trough} (mg/L)		2.50 (2.01-3.49)	4.65 (3.05-6.79)		
Did not meet target 1.0 mg/L		0	0		

Table 1. - continued Pharmacokinetic parameters of antiretrovirals during pregnancy

Parameter	Ref		Third trimester	Postpartum	GMR (90%CI)	Study conclusion
Protease Inhibitors						
Lopinavir/r 400/100mg b.i.d.	[17]	IS & CC; mean (CV%)	n=36	n=30	controls, n=34	Despite a reduction in LPV exposure in late pregnancy no dose adjustment is required for treatment navie patients. However, in treatment experienced patients, patients with high viral load a dose increase may be warranted bassed on TDM.
AUC (mg·h/L)			71.7 (22%)	97.3 (29%)	102.4 (32%)	
C_{trough} (mg/L)			4.51 (26%)	6.52 (37%)	6.66 (36%)	
CL/F (L/h)			5.8 (22%)	4.4 (25%)	4.4 (23%)	
Lopinavir/r 400/100mg b.i.d.	[16]	IS; median (IQR)	n=12	n=12	n=12	Total LPV exposure was significantly decreased throughout pregnancy despite the increased dose. Predose concentrations of unbound LPV were not affected by the additional dose and were 70-fold > minimum efficacy concentration. These findings suggest dose adjustments may not be necessary in all HIVinfected pregnant women.
			30 weeks 400/100 mg	32 weeks 500/125 mg	postpartum	
AUC (mg·h/L)			64.1 (51.3-69.7)	69.1 (55.2-78.2)	98 (67.1-115.1)	
C_{trough} (mg/L)			4 (3.4-5.4)	4.9 (4.4-6.0)	7.2 (6.1-9.3)	

Table 1. - continued Pharmacokinetic parameters of antiretrovirals during pregnancy

Parameter	Ref		Third trimester	Postpartum	GMR (90%CI)	Study conclusion
Protease Inhibitors						
Atazanavir 300/100mg q.d. or 400/100mg q.d.	[19]	IS; median (IQR)	n=37	n=34		These data suggest that a higher atazanavir/ritonavir dose should be used in third trimester pregnant women and also to be considered during the second trimester, and that postpartum atazanavir/ritonavir dosing can be reduced to standard dosing before 2 weeks after delivery.
			400/100mg without TDF	300/100mg without TDF		
AUC (mg·h/L)			45.7 (30.8-57.3)	48.8 (34.9-67.5)		
C_{trough} (mg/L)			0.71 (0.47-1.0)	0.90 (0.39-1.33)		
			n=32	n=34		
			400/100mg with TDF	300/100mg with TDF		
AUC (mg·h/L)			37.7 (25.7-44.4)	58.7 (41.4-80.5)		
C_{trough} (mg/L)			0.57 (0.39-0.81)	1.26 (0.69-1.95)		
Fosamprenavir/r 700/100mg b.i.d.	[20]	IS; GM (95% CI)	n=9	n=9	n=9	FPV/RTV 700/100-mg b.i.d. regimen provided adequate APV exposure during pregnancy. The observed reduction in APV concentrations seemed not to be clinically significant because all women remained virologically suppressed during the study, and no cases of vertical HIV transmission occurred. Close virologic monitoring is suggested with the use of FPV, as with the use of any PI, for p-MTCT in women with significant PI mutations.
AUC (mg·h/L)			30.1 (21.6-41.9)	39.9 (31.9-50.1)	0.75 (0.57-1.00)	
C_{trough} (mg/L)			1.34 (0.95 -1.89)	2.03 (1.46-2.83)	0.81 (0.62-1.06)	
					0.62 (0.48-0.92)	

Table 1. - continued Pharmacokinetic parameters of antiretrovirals during pregnancy

Parameter	Ref		Third trimester	Postpartum	GMR (90%CI)	Study conclusion
Protease Inhibitors						
Indinavir/r 400/100mg b.i.d.	[21]	median (range)	n=25	n=26	n=25	IDV exposure during pregnancy was significantly reduced compared to postpartum and ~30% of women fail to achieve a target trough concentration. Increasing the dose to 600/100 mg twice daily may be preferable to ensure adequate drug concentrations.
		historical nonpregn				
AUC (mg·h/L)		18.3 (11.1-33.0)	16.10 (7.50-39.9)	27.1 (18.6-44.7)	0.6 (0.53-0.68)	
C_{trough} (mg/L)		0.17 (0.10-0.39)	0.13 (0.07-0.60)	0.28 (0.14-0.71)		
Met IDV C_{trough} target		11/11 (100%)	19/25 (76%)	26/26 (100%)		
Nelfinavir 1250mg b.i.d. also free concentrations	[22]	IS; GM	n=14	n=10		ARV activity was maintained in the majority of subjects, with no evidence of vertical transmission or of any birth defects. On this basis, the nelfinavir 625 mg formulation is an acceptable component of cART when given during pregnancy, despite lower exposure during pregnancy.
AUC (mg·h/L) total nelfinavir			20.7	38.5	53.68 (37.21, 77.43)	
C_{trough} (mg/L)			0.38	1.59	24.14 (12.15, 47.96)	
AUC (mg·h/L) total M8			1.57	9.28	16.93 (8.38, 34.20)	

Table 1. - continued Pharmacokinetic parameters of antiretrovirals during pregnancy

Parameter	Ref	Third trimester	Postpartum	GMR (90%CI)	Study conclusion
Protease Inhibitors					
Darunavir/r 600/100mg b.i.d.	[23] IS; mean ±SD	n=11	n=11	n=11	Total darunavir exposure decreased during pregnancy. No clinically relevant change in unbound (active) darunavir occurred during pregnancy, suggesting that no dose adjustment is necessary for darunavir/ritonavir 600/100 mg b.i.d. in pregnant women.
Total DRV					
C_{max} (mg/L)		5.1±1.5	6.5±2.4	0.81 (0.69-0.96)	
C_{trough} (mg/L)		2.8±1.4	3.2±1.8		
AUC (mg·h/L)		43.7±16.4	55.3±27.0	0.83 (0.72-0.97)	
Free DRV		n=7	n=11		
C_{max} (mg/L)		0.92±0.29	1.17±0.48	0.82 (0.57-1.16)	
C_{trough} (mg/L)		0.52±0.34	0.46±0.24		
AUC (mg·h/L)		7.29±2.4	9.18±3.96	0.93 (0.69-1.24)	
Entry inhibitors					
Maraviroc	[26] GM (95% CI)	n=9	n=7	n=7	Exposure to MVC was 21% lower during pregnancy (3rd trimester) than postpartum. This is in line with previously reported data from other CYP3A substrates such as PIs.
AUC (mg·h/L)		2.15 (0.91-5.43)	2.40 (0.99-4.62)	0.79 (0.63-0.98)	
C_{trough} (mg/L)		0.074 (0.030-0.24)	0.094 (0.033-0.20)	0.85 (0.72-1.01)	

Concentrations reported were converted to mg/L for the purpose of consistency. /r, ritonavir boosted; APV, amprenavir; ARV, antiretroviral; AUC, area under the curve; b.i.d., twice daily; CC, case control; CI, confidence interval; CV, coefficient of variation; EFV, efavirenz; FPV, fosmaprenavir; FTC, emtricitabine; GM, geometric mean; GMR, geometric mean ratio; IDV, indivinavir; IQR, inter-quartile range; IS, intraindividual comparison; MVC, maraviroc; n, number; PI, protease inhibitor; PK, pharmacokinetics; q.d., once daily; SD, standard deviation; TDF, tenofovir diosproxil; TFV, tenofovir

Protease Inhibitors

Calza *et al.*[14] compared trough lopinavir concentrations of the 400/100mg lopinavir/r tablet in pregnant women (n=21) vs. non-pregnant women (n=20). In this study, a slight but non-significant decrease in lopinavir C_{trough} was found during the third trimester of pregnancy. Only virologically suppressed women could take part, possibly excluding women with subtherapeutic lopinavir levels. Furthermore, the number of patients included was very low for interindividual comparisons. Else *et al.*[15] compared the exposure of lopinavir soft gel capsules (SGCs) with the melt-extruded tablet during pregnancy. Despite a significant reduction in exposure during late pregnancy, the tablet formulation showed adequate concentrations (and higher than the SGC).

Patterson *et al.*[16] reported changes of unbound lopinavir plasma concentrations during different stages of pregnancy. The dose of lopinavir/r was empirically increased to 500/125mg twice daily (b.i.d.) after week 30 of pregnancy. Pharmacokinetic curves were collected (n=12), at second and third trimester (400/100mg and 500/125mg b.i.d.) and postpartum. A less-than-proportional increase in exposure was seen after dose increase; the reason is not clear. Lopinavir free fraction did not significantly change during the second and third trimesters or postpartum, regardless of dose. Fayet-Mello *et al.*[17] also described unbound lopinavir plasma concentrations during pregnancy. They performed sparse sampling during pregnancy and postpartum in 42 women using 400/100mg lopinavir/r b.i.d. Total lopinavir concentrations were moderately decreased during pregnancy (31-39%), whereas unbound concentrations were not significantly altered (lopinavir free fraction was higher during pregnancy). Unbound lopinavir concentrations (but not the total concentrations) reported in the studies differ to some extent. This difference might be due to different analysis methods. Patterson *et al.* used a rapid equilibrium dialysis, whereas Fayet-Mello *et al.* used ultrafiltration. Furthermore, the unbound concentrations were close to the lower limit of quantification and could possibly be less accurate.

All studies mentioned above conclude that for treatment-naive patients with wild-type virus, a dose increase of lopinavir is not necessary during pregnancy (using the tablet formulation). However, for treatment-experienced patients, generally needing higher antiretroviral concentrations, TDM during pregnancy is advised.[15]

Atazanavir has been upgraded to the preferred agent for use during pregnancy in the July 2012 revised DHHS perinatal guidelines.[1] Similar to other protease inhibitors, lower exposure during pregnancy has been reported for atazanavir. This is also described in the current product characteristics of atazanavir.[18]

A systematic review reports results of 13 studies performed on atazanavir during pregnancy up to April 2012.[8] Pharmacokinetic studies (nine) as well as studies on safety and efficacy were reported, including one study with an increased dose during the third trimester (400/100mg q.d. atazanavir/r). The increased dose resulted in therapeutic concentrations, but also a doubling of maternal grade 3-4 hyperbilirubinaemia.

As current cART includes compounds possibly reducing atazanavir C_{24h} (i.e. tenofovir), a dose increase during the third trimester may be required. If available, TDM is recommended to guide dose adaptations.

Recently, an intensive-sampling PK study applying higher atazanavir/r doses during pregnancy was published.[19] Pharmacokinetic curves were recorded in the second (300/100mg q.d.), third trimester after dose increase to 400/100mg q.d. and postpartum at the original dose. Postpartum atazanavir levels were higher than in nonpregnant adults. After dose increase, median atazanavir area under the curve (AUC) was similar to that seen in non-pregnant historical controls taking the standard dose. Concomitant tenofovir use seemed to reduce atazanavir exposure during the second and third trimester. These data suggest that a higher atazanavir/r dose should be used in the third trimester of pregnancy and is also to be considered during the second trimester, especially when tenofovir is coadministered and no TDM is available.

A study with fosamprenavir/r 700/100 mg b.i.d. in pregnant women, performing intensive-sampling pharmacokinetics in the second (n=6) and third (n=9) trimester and 4 weeks postpartum (n=9) was reported.[20] Amprenavir exposure was significantly lower in the second (35% lower) and third trimester (25% lower). For all patients viral load at delivery was <200 copies/mL and no MTCT was observed. Therefore, dose adjustment does not seem to be required for fosamprenavir/r b.i.d. administration during pregnancy. However, in pregnant women with significant protease inhibitor mutations, close virologic monitoring is suggested with the use of fosamprenavir.

A prospective, intensive-sampling pharmacokinetic study of indinavir/r 400/100mg b.i.d. in Thai pregnant women was performed by Cressey *et al.*[21] PK curves were collected in the second (n=13), third trimester (n=26) and postpartum (n=26). During pregnancy indinavir exposure was significantly reduced and approximately 30% of women did not achieve a target C_{trough} (0.1 mg/L), and none did postpartum. Nineteen percent of the women had a viral load of more than 40 copies/mL at delivery; no vertical transmission occurred. Increasing the dose of indinavir/r during pregnancy to 600/100 mg b.i.d. may be preferable to ensure adequate drug concentrations. No analysis was done linking high viral loads and plasma concentrations.

A study with limited pharmacokinetic sampling during the second and third trimesters, and 6 weeks postpartum was done in 16 pregnant women receiving 1250mg nelfinavir b.i.d.[22] Pharmacokinetic analysis of total and unbound nelfinavir and the M8 metabolite was performed. Compared with postpartum, AUC of total nelfinavir was reduced by 46% in the third trimester, total M8 by 83%, unbound nelfinavir by 39%, and unbound M8 by 79%. Despite this major reduction in exposure, no MTCT occurred. No dose recommendation was given on the basis of this finding, as the number of patients in the study was low and only limited sampling was performed.

Zorrilla *et al.*[23] published a study with intensive-sampling (second and third trimester and postpartum) in 11 women using darunavir/r 600/100mg b.i.d. The $AUC_{0\text{-}12h}$ for total darunavir was 17-24% lower during pregnancy compared to postpartum, for unbound

darunavir the $AUC_{0\text{-}12h}$ was only 7-8% lower during pregnancy (n=6). All 12 infants were HIV-negative. The authors suggest that, because of the nonclinically relevant change in unbound (active) darunavir, dose adjustment is not required for pregnant women receiving darunavir/r 600/100mg b.i.d.
In addition, a case has been reported of a pregnant woman failing on 600/100mg darunavir/r b.i.d. in late pregnancy. Darunavir C_{trough} values were lower than expected and etravirine (200mg b.i.d.) and maraviroc (150mg b.i.d.) were added to darunavir/r to ensure adequate treatment.[24]

Integrase Inhibitors

Croci *et al.*[25] described a case of a woman using lopinavir/r b.i.d. and raltegravir 400mg b.i.d. during pregnancy. In the third trimester a raltegravir C_{trough} of 0.21 mg/L was reported. The baby was born not HIV-infected at 39 weeks gestational age. Exposure, based on a single trough sample, to raltegravir in the third trimester was similar to nonpregnant historical controls in this case. More pharmacokinetic studies are needed to confirm this finding.

Entry Inhibitors

In 2012 a poster was presented by the IMPAACT group and PANNA network at CROI 2013 describing the first intensive-sampling pharmacokinetic data during pregnancy, including placenta passage information. Maraviroc exposure during pregnancy (third trimester) was 21% lower than postpartum (n=9). To make valid dosing recommendations, more data are needed.[26]

Transplacental passage

An extensive review was published in 2011, covering information available on transplacental passage of antiretrovirals as well as concentrations in amniotic fluid.[27] Most of the studies described in the pharmacokinetics section of this review also assessed transplacental passage. The results are summarized in Table 2.
Two recent papers described transplacental passage. Van Hoog *et al.*[28] summarized information on transplacental passage collected between 2003 and 2010 for nevirapine, nelfinavir and lopinavir.
Transplacental passage is not only influenced by physical-chemical properties of drugs, but drug transporters located in the placenta can also play a role in the passage of drugs across the placenta. The current knowledge on expression and function of ABC and SLC transporters in the trophoblast has been summarized.[29] Olagunju *et al.*[30] reviewed potential effects of pharmacogenetics on maternal, fetal and infant antiretroviral drug exposure during pregnancy and breastfeeding. The potential of SNPs in transplacental passage was described in detail for nevirapine, efavirenz, lopinavir and

Table 2. Transplacental passage of antiretrovirals

ARV	Cord blood : maternal blood ratio		n	ref
NRTIs				
tenofovir	0.82 (0.64-1.10)	median (range)	14	[10]
tenofovir	1.13		1	[24]
emtricitabine	1.63 (0.46-1.82)	median (range)	10	[10]
emtricitabine	1.66		1	[24]
NNRTIs				
efavirenz	0.49 (0.37-0.74)	median (range)	25	[13]
nevirapine	0.67 (±0.15)	median ± IQR	17	[28]
etravirine	0.51		1	[24]
PIs				
lopinavir	0.24 (±0.21)	median ± IQR	42	[28]
lopinavir total	0.17 ± 0.09	mean±SD	6	[15]
lopinavir unbound	0.31±0.09	mean±SD	6	[15]
total ritonavir	0.13 ±0.08	mean±SD	6	[15]
lopinavir total	0.16 (85%)	mean CV%	16	[17]
lopinavir unbound	0.43 (83%)	mean CV%	16	[17]
atazanavir - TDF	0.14 (0.05-0.84)	median (range)	>30	[19]
atazanavir + TDF	0.16 (0.03-4.08)	median (range)	>30	[19]
indinavir	0.12 (0.05-0.23).	median (range)	19	[21]
nelfinavir	0.14(±0.36)	median ± IQR	20	[28]
amprenavir	0.267 (0.241, 0.297)	GLS mean (95% CI)	7	[20]
darunavir	0.15 (range 0.014-0.36)	median (range)	9	[23]
darunavir	0.15		1	[24]
ritonavir	0.32		1	[24]
EI				
maraviroc	0.33 (0.03-0.56)	median (range)	6	[26]
maraviroc	0.37		1	[24]

ARV, antiretroviral; CI, confidence interval; CV, coefficient of variation; EI, entry inhibitors; GM, geometric mean; IQR, inter-quartile range; n, number; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI protease inhibitor; SD, standard deviation

atazanavir, as well as raltegravir. There is still insufficient knowledge about the pharmacogenetics possibly influencing antireroviral exposure in pregnant women.

Safety of antiretroviral use during pregnancy

In general, a doubling of the percentage of preterm births (<37 weeks gestational age) is seen in HIV-infected women compared with non-HIV-infected women. Whether this is due to HIV infection, the use of cART in general, or more specifically the use of protease inhibitors, is not clear. An analysis over time (1990–2009) indicates that the use of cART seems to contribute to this increase, as the percentage of prematurity in HIV-infected women was higher in 2005–2009 (routine, mainly protease inhibitor based cART) than in 1990–1993 (no therapy).[31]

As treatment duration during pregnancy increases (also in resource-limited settings), the issue of risks of adverse effects of cART during pregnancy (such as prematurity and congenital abnormalities) is becoming more important. Western countries do have facilities to handle prematurity, but in resource-limited settings this might be an important safety issue. Some articles warn about these safety concerns when implementing the new WHO guidelines to treat all HIV-infected patients (also during pregnancy) with efavirenz.[32,33]

A prospective study (in the US) in 183 HIV-infected pregnant women, all using cART, report that the increase of small-for-gestational-age (SGA) births (compared with the non-HIV-infected population) in pregnant women with HIV is related to the severity of HIV disease and not to antiretroviral therapy.[34] As there was no control group (without cART), it is unclear how this conclusion could be drawn.

In French cohorts (n=13 271), a remarkable increase was reported in premature deliveries when regimens recommended in pregnancy changed: 9.2% during 1990–1993 (no therapy), 9.6% during 1994–1996 (mostly zidovudine monotherapy) to 12.4% during 1997–1999 (dual-nucleoside analog therapy) and 14.3% during 2005–2009 (routine cART therapy).[31] Prematurity was associated with cART, compared to zidovudine monotherapy, when accounting for other factors. In 2005–2009, the prematurity rate was higher with boosted than with non-boosted protease inhibitor therapy (14.4% versus 9.1%). It should be noted that the non-boosted protease inhibitor used was nelfinavir and the majority of the patients using a boosted protease inhibitor used lopinavir/r.

Birth defects in pregnancies with exposure to antiretroviral drugs in Italy (1257 pregnancies) were reported over 2001–2011.[35] A birth defect prevalence of 3.2% for exposure during the first trimester was found (compared to 3.4% for no antiretroviral exposure during the first trimester). No associations were found between birth defects and antiretroviral therapy, main drug classes or individual drugs. Preterm delivery occurred in 20.9% of pregnancies.

Nucleoside/nucleotide reverse transcriptase inhibitors

Possible renal and bone/growth problems in newborns, exposed intrauterinally have been investigated in several articles, recently. With the use of tenofovir as preexposure prophylaxis, conceptions during tenofovir use might increase.

Two articles report tenofovir use to be well tolerated during pregnancy. Pregnancies and infant outcomes of the DART trial (Uganda/Zimbabwe, period 2003–2009) have been described.[36] There was no evidence that tenofovir exposure during the intrauterine period (n=111) had any adverse effects on pregnancy outcomes or on congenital, renal, bone, or growth abnormalities up to age 4 years of age.

In a prospective study, bone status of infants exposed to antiretrovirals (n=38) was compared with bone status of unexposed children from HIV-negative mothers (n=94).[37] Antiretroviral exposure *in utero* seems not to negatively affect bone metabolism and bone development, and changes in bone quantitative ultrasonography measurements during the first year of life in antiretroviral-exposed individuals are similar to those occurring in healthy controls.

No difference was seen between bone development of infants intrauterinally exposed to tenofovir (n=15) and not-exposed to tenofovir (n=23). Only a small group of children was exposed to tenofovir, and follow-up was only 1 year; however, a sensitive method quantifying bone status was used.

In contrast, Siberry *et al.*[38] found a lower mean height (0.41 cm shorter) and head circumference (0.32 cm smaller) at 1 year of age for infants exposed to tenofovir *in utero* (n=449) versus infants exposed to non-tenofovir-containing regimen (n=1580), independent of early and late exposure in pregnancy. The significance of this finding is uncertain, but this underscores the need for studies with a sufficiently large number of patients.

Non-nucleoside reverse transcriptase inhibitors

Because of the increased risk of potentially life-threatening hepatotoxicity in women with high CD4 T cell counts, nevirapine should be started in pregnant women with CD4 T counts >250 cells/μL only if benefit clearly outweighs risk.[1]

A systematic review and meta-analysis of the safety of nevirapine use during pregnancy was performed.[39] The analysis included 20 studies representing 3582 pregnant women. Adverse events reported were severe hepatotoxicity (3.2%), severe rash in 3.3% of patients. Around 6% of the patients discontinued nevirapine due to an adverse event. Pregnant women with a high CD4 T cell count may be at increased risk of adverse events, but evidence supporting this association is weak.

Efavirenz is suspected to cause teratogenicity based on animal studies and retrospective case reports showing that efavirenz may be associated with neural tube and/or central nervous system abnormalities. As WHO treatment guidelines include efavirenz,

tenofovir and emtricitabine as triple therapy during pregnancy, safety information on the use during pregnancy is important. The US Antiretroviral Pregnancy Registry (APR) did not find an increased prevalence of overall birth defects with first-trimester efavirenz exposure compared to the overall US population.
A case report of bilateral oblique clefts and extremity anomaly in an infant after intrauterine efavirenz exposure was recently published.[40] The mother used efavirenz (along with atorvastatin) at the time of conception until 5 weeks postconception, when she switched to nelfinavir and zidovudine/lamivudine. The relation between efavirenz exposure and this congenital anomaly cannot be confirmed, nor rejected.

Protease inhibitors

Results of the APR were published in 2012,[41] with more than 200 first trimester exposures to atazanavir reported (the threshold for performance of comparative analyses). A total of 698 pregnant women were exposed, 425 in the first trimester. Rates of birth defects after atazanavir exposure (2.3%) are not different from those noted for other antiretrovirals, nor from the reference population of the APR. As this is a voluntary registry, the number of anomalies found might be underreported.

Integrase inhibitors

Raltegravir is used in special cases during pregnancy only, that is in women presenting with HIV in late pregnancy with a high viral load or patients with no other options left. Recently, safety data on single and multiple cases were reported on regimens containing raltegravir as well as at least two other antiretrovirals.[25,42-46] They all report a viral load decline of approximately 1 log/week, being very effective in reaching an undetectable viral load around delivery. In total, 22 cases were described, with one case of likely in utero MTCT (maternal viral load at delivery was 64 copies/mL, delivery by caesarean section). Most case reports did report raltegravir use to be well tolerated during pregnancy, except for one case of increased serum aminotransferases during pregnancy.[45] In this patient, after 11 days of treatment with raltegravir, a substantial reduction in viral load was achieved, but she had a 23-fold increase in serum ALAT and a 10-fold increase in serum ASAT. Both returned to normal upon raltegravir discontinuation. No congenital abnormalities were reported.
These articles suggest that raltegravir could be an option for women presenting with HIV late in pregnancy or having no treatment options left, being very effective in fast decreasing viral load. However, further studies are required to establish safety and pharmacokinetics of raltegravir during pregnancy.

Conclusion

Physiological changes during pregnancy show a general trend to lower exposure to antiretrovirals, with the largest decrease for boosted protease inhibitors. Increased fraction of unbound concentrations does not seem to compensate completely for this decrease. However, virological failure or MTCT has not yet been associated with lower concentrations in pregnancy. This apparent discrepancy can be explained by the fact that the large majority of pregnant women harbour wild-type virus and antiretroviral C_{trough}, even when reduced by 23-35%, remains in the therapeutic range for this specific group of patients. Increasing the dose of lopinavir and atazanavir has been shown to effectively compensate for decreased exposure during pregnancy. Increasing the dose can be done guided by TDM in the second and third trimester, in the presence of tenofovir (boosted atazanavir) and in particular when patients have a history of virological failure on a previous cART regimen. For newer compounds and efavirenz, limited or no data on pharmacokinetics during pregnancy or transplacental passage are available.
The mechanisms of transplacental passage are not elucidated to date; further research on the role of transporters and placental metabolism is important to predict transplacental passage of new compounds. With regards to well tolerated use of antiretrovirals during pregnancy, monitoring of pregnancy outcomes in resource-limited setting during the implementation of the WHO-guidelines, allowing efavirenz during pregnancy, with emphasis on prematurity and congenital abnormalities, is important.
Raltegravir induces rapid viral decline during pregnancy and seems a fair option for women presenting with HIV in late pregnancy.

Key points

Physiological changes during pregnancy lead to lower exposure to antiretrovirals in most cases, especially for protease inhibitors.
The mechanism of transplacental passage should be subject of future research.
Adding raltegravir to cART seems an option for women presenting with HIV in late pregnancy.
Exposure to new antiretrovirals (and efavirenz) during pregnancy and transplacental passage should be subject of future research, also in resource-limited settings.

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3

The Pharmacokinetics, Safety and Efficacy of Boosted Saquinavir Tablets in HIV Type-1-infected Pregnant Women

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Abstract

Background: Pregnancy affects the pharmacokinetics of most protease inhibitors. Saquinavir, when administered in a tablet formulation, is not studied extensively in this setting.

Methods: A pharmacokinetic, prospective multicentre trial of HIV type-1-infected pregnant women treated with saquinavir (500mg tablets) boosted with ritonavir at a dose of 1,000/100mg twice daily plus a nucleoside backbone, was conducted. Pharmacokinetic curves were recorded for 12 h in the second trimester (week 20 GA ± 2), the third trimester (week 33 ± 2) and postpartum (weeks 4-6). Blood was sampled pre-dosing and at 1, 2, 3, 4, 6, 8, 10, and 12 h post-dosing. Pharmacokinetic parameters were calculated using WinNonlin software version 4.1.

Results: A total of 37 women were included in the analysis. Mean (±SD) values for saquinavir area under the curve ($AUC_{0\text{-}12h}$) were 23.47 h·mg/L (11.92) at week 20 GA (n=16), 23.65 h·mg/L (9.07) at week 33 (n=31), and 25.00 h·mg/L (11.81) postpartum (n=9). There was no significant difference in the saquinavir $AUC_{0\text{-}12h}$ when comparing the data during pregnancy and postpartum Subtherapeutic plasma concentrations of saquinavir (defined as < 0.10 mg/L) were not observed throughout the study. No major safety concerns were noted.

Conclusions: Saquinavir exposure in the new tablet formulation generates adequate saquinavir concentrations throughout the course of pregnancy and is safe to use; therefore, no dose adjustment during pregnancy is needed.

Introduction

At present, approximately 15.4 million women are infected with HIV, most of them of child-bearing age.[1] It is estimated that 39% of the European women with an HIV infection have a desire to bear children in the future, which is comparable to women without HIV infection.[2]

In order to prevent mother-to-child transmission of the virus, highly active antiretroviral therapy has shown to be the most effective strategy[3], reducing the chance of mother-to-child transmission from 15-40% to <2%.[4] Owing to the teratogenicity of efavirenz and the toxicity of nevirapine in women with CD4+ T-cell counts >250 cells/mm^3, the class of non-nucleoside reverse transcriptase inhibitors (NNRTIs) is contraindicated in most situations. Therefore, during pregnancy, a protease inhibitor (PI)-based regimen seems to be the most rational choice at present and is commonly used in the developed world (42% in the US and 65% in Europe).[5,6] At present, it is not clear what PI and at which dose results in the best outcome in terms of safety and efficacy during pregnancy.

According to the US Food and Drug Administration classification for medication use during pregnancy,[7] no category A drugs exist among the currently available PIs: (fos) amprenavir, indinavir, and lopinavir are classified as category C, whereas atazanavir, darunavir, nelfinavir (NFV) and saquinavir are all categorized as B.[8] Despite this classification, indinavir and lopinavir in particular, are commonly used in clinical practice.

During pregnancy the human physiology alters, possibly affecting the absorption, distribution, metabolism and excretion of several drugs.[9,10] The most profound changes that might interfere with the pharmacokinetics (PKs) are reduced intestinal motility, increased gastric pH, a larger plasma volume, decreased protein binding and induced hepatic enzymes. These changes all result in potentially lower exposure of certain medication during pregnancy. The use of drugs metabolized by the CYP3A4 iso-enzyme pathway is of special concern during pregnancy because its activity is substantially increased during gestation, resulting in lower drug concentrations and therefore possible decreased efficacy.[9,11] All PIs use this pathway to some extent and should be used with caution during pregnancy. Several PK studies have shown that, indeed, these changes have a significant impact on the PKs of different PIs when used during pregnancy [12]. However, it is questionable, whether all PIs are affected to the same extent.
Saquinavir has not yet been extensively studied in pregnancy, and published data for the new tablet formulation are lacking. Earlier data with the hard gel capsule (HCG) suggested that the concentrations of ritonavir-boosted saquinavir generate adequate concentrations during pregnancy.[13] Therefore, we studied the effect of pregnancy on the saquinavir concentrations of the new tablet formulation in an saquinavir/r 1,000/100mg twice-daily regimen.

Methods

This was a multiple-dose, open-label, non-comparative, multicentre phase II trial designed to determine the pharmacokinetic profile of saquinavir 500 mg tablet formulation combined with ritonavir (saquinavir/r 1,000/100 mg twice daily) in pregnant HIV type-1(HIV-1)-infected women.
The trial was performed in eight hospitals (seven in Europe and one in Thailand) between May 2005 and January 2008. Informed consent was obtained from each participant before entering the study. The trial was approved by the medical ethical committee from each individual centre involved.
Patients could be either treatment naive or treatment experienced, but without saquinavir failure or documented resistance in their history. For eligibility, HIV-1-infected women had to be between 18 and 40 years of age with a maximal gestational age (GA) of 31 weeks. Patients were treated with saquinavir 500 mg tablet formulation plus ritonavir 1,000/100 mg twice daily (with food) plus two nucleoside reverse transcriptase inhibitors (NRTIs). The choice of the two NRTIs was at the discretion of the investigator, with a preference for zidovudine plus lamivudine (300/150 mg Combivir®).

Safety and viral load assessments

Inclusion screening consisted of: medical history, physical examination, serum biochemistry, haematology and qualitative urinalysis, hepatitis B/C serology, HIV-1 RNA and CD4+ T-cell determination. Analyses were performed by local laboratories. Blood samples for laboratory safety were further taken at baseline and at 4-8 week intervals. During the study, HIV-1 RNA and CD4+ T-cell determination were performed at baseline, at weeks 20, 28, 33 at delivery and at 6 weeks postpartum. Patients were asked for adverse events at each visit. Retrospectively, the HIV status of the infants was collected from the treating physicians. The DAIDS toxicity table (2004) was used to grade the reported adverse events. Grade of at least 3 laboratory abnormalities were described.

Pharmacokinetic blood sampling

A 12 h pharmacokinetic curve was recorded after minimally 2 weeks of saquinavir treatment. For patients included before GA week 20, a 12 h pharmacokinetic curve was recorded during the second trimester at week 20 GA (± 2 weeks) and optionally during the third trimester at week 33 (± 2 weeks). For patients included after GA week 20, the first curve was recorded at week 33 (± 2 weeks). All patients who continued with the saquinavir/r regimen after delivery could consent to have a 12 h pharmacokinetic curve recorded at 6 weeks (± 2 weeks) postpartum.
A standard breakfast was served prior to dosing on the pharmacokinetic days, and 4 mL of blood was collected just before drug intake (pre-dose) and at 1, 2, 3, 4, 6, 8, 10, and 12 h post-ingestion (9 samples) at all pharmacokinetic study days. Plasma was

separated and stored at <-18°C until shipment on dry ice.

Analytical and pharmacokinetic methods

Plasma concentrations of saquinavir and ritonavir were assayed at the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre, using a validated high-performance liquid chromatography assay with ultraviolet detection.[14] The lower limit of quantification was 0.045 mg/L for both ritonavir and saquinavir.
Pharmacokinetic parameters were determined using WinNonlin version 4.1 (Pharsight Corporation, Mountain View, CA, USA). Area under the curve ($AUC_{0\text{-}12h}$), minimum concentration (C_{min}) defined as the sample taken at 12 h, maximum concentration (C_{max}), elimination half-life ($T_{1/2}$), time of maximum concentration (T_{max}) and clearance (CL/F) were determined per individual curve.

Statistical analysis data handling

Drop-outs were defined as patients who dropped out before at least one curve had been taken had to stop study medication because of toxicity. Patients not starting saquinavir treatment were not included in analyses.
As some visits were optional, percentages were calculated relative to the available data. The efficacy data are presented for week 20, 33 GA and 6 weeks postpartum for the patients with a recorded pharmacokinetic curve and expressed as proportion undetectable HIV-1 RNA plasma (< 50 copies/mL). Virological failure was defined as two consecutive viral load measurements >400 copies/mL for patients >6 months on treatment.

Pharmacokinetic parameters are reported as arithmetic means with standard deviation. As the sample size was rather small, both non-parametric (either Friedman or Wilcoxon signed-rank tests) and parametric (paired sample *t*-test and repeated-measure one-way ANOVA) tests were performed for comparioson within the group. As there were no large differences between these methods, the results of the *t*-test are presented in this paper for all analyses except for the comparison of the eight patients of whom three curves were available. Furthermore, the number of patients with saquinavir C_{min} values below the target threshold value of 0.1 mg/L[15] was reported.

Results

A total of 40 patients were enrolled in the study from eight different sites. Three patients withdrew their informed consent prior to starting saquinavir treatment; they were excluded from all analyses. The baseline characteristics of the remaining 37 subjects are depicted in Table 1. Among the patients on antiretrovirals (ARVs; n=20), 75% had an undetectable viral load (43% of the total group) and the mean ARV treatment duration of 153 weeks.

From these 20 patients 13 used a PI-based regimen when entering the study, 7 of which were already on a saquinavir-based regimen. The mean duration of saquinavir treatment prior to delivery was 19.1 weeks (range 6-40 weeks).
According to the protocol definition six patients dropped out, five before a pharmacokinetic curve was recorded and one patient after the week 20 GA curve. This latter patient stopped saquinavir/r due to hepatotoxicity. In the group of five patients who discontinued study medication before a pharmacokinetic curve was recorded, two patients discontinued the study due to grade 2 nausea and vomiting 2 weeks after study initiation, two patients withdrew consent before a pharmacokinetic curve was obtained and one had a miscarriage. As a result, at least one 12 h pharmacokinetic-curve was recorded for a total of 32 patients.
A total of 16 curves were collected at week 20 GA, 31 at week 33 and 9 at 6 weeks postpartum. All 3 curves were obtained for 8 patients, the week 20 GA and week 33 curves were obtained for 15 patients, and the week 33 and 6 weeks postpartum curves were available for 9 patients.

Pharmacokinetics

The mean plasma concentration-time profiles of saquinavir for the three different time points are presented in Figure 1A, and the summary statistics of the saquinavir pharmacokinetic parameters are listed in Table 2. With a mean $AUC_{0\text{-}12h}$ of 23.47 h·mg/L,

Table 1. Baseline Characteristics

Characteristics	Value
Patients, n	37
Age, years	29.6 (6.1)
Weight, kg	69.7 (17.1)
Height, cm	163.4 (7.2)
Race	
Caucasian, n	12
Black, n	14
Asian, n	9
Other, n	2
Gestational age, weeks	20 2/7 (8 3/7)
CD4+ T-cell count, cells/mm^3	440.5 (215.2)
HIV-1 RNA <50 copies/mL, %	43
Plasma HIV-1 RNA log_{10}, copies/mL	3.62 (0.87)
ARV treatment naive, %	46
Mean ARV treatment duration before study, weeks (range)	153 (2-260)

Unless otherwise indicated, all parameters are expressed as means (±SD). ARV, antiretroviral; HIV-1, HIV type-1

23.65 h·mg/L and 25.00 h·mg/L at week 20 GA, week 33 and 6 weeks postpartum, respectively, the exposure to saquinavir appears consistent over time. None of the patients had a saquinavir concentration below the therapeutic minimum concentration of 0.1 mg/L. The mean plasma concentration-time curves of ritonavir are presented in Figure 1B and the summary statistics for ritonavir pharmacokinetic parameters are depicted in table 2.

Saquinavir and ritonavir pharmacokinetic parameters for patients who had at least two pharmacokinetic curves taken were compared for the different time points (Table 3). No statistically significant differences were found for saquinavir between week 20 GA and week 33 GA, or between week 33 GA and 6 weeks postpartum. The C_{min}, usually related to antiviral efficacy, was found to be similar in both groups. In agreement with this, a Friedman analysis of the eight patients who had all three curves taken did not show a significant difference (p=0.135).

No differences between week 20 GA and week 33 GA were found for ritonavir. By contrast, the ritonavir AUC and $C_{max,}$ but not the $C_{min,}$ were significantly higher postpartum compared to week 33 (9 patients).

The findings above are consistent if compared with the group as a whole. A wide range of variation between and within the patients can be observed. This is also reflected in the coefficient of variation, which ranged from 39 to 51% for the saquinavir AUC.

Safety and Efficacy

Two patients developed a serious adverse event (SAE). One had a miscarriage at 11 5/7 weeks pregnancy, two weeks after initiation of the study medication. The study physician assessed it was not related to the study drugs because an ultrasound before the start of the study medication already showed a growth retardation of the embryo. The other SAE was a one night hospital admission due to diarrhoea. The patient was receiving treatment for 3 months without diarrhoea, so it was determined that the diarrhoea was not related to the study drugs.

Clinical adverse events higher than grade 2 were not observed. One patient developed

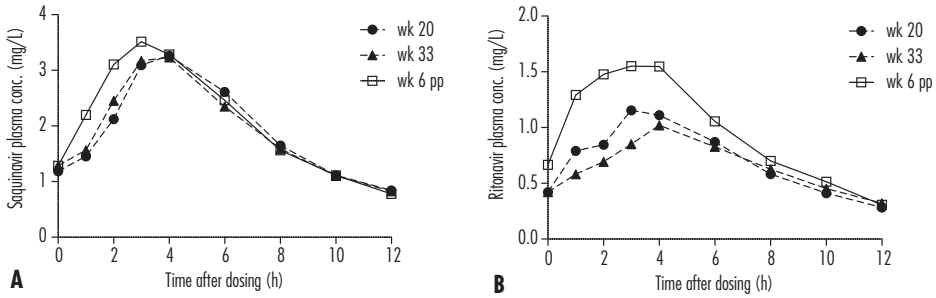

Figure 1. Mean steady-state concentration-time profile of saquinavir and ritonavir
A saquinavir B ritonavir

diabetes gravidarum.
One grade 3 cholesterol increase was documented. Three patients developed at least a grade 3 ASAT/ALAT elevation (2 naive to ARV at baseline, one ARV-experienced). The first patient had a grade 3 increase in ASAT/ALAT at delivery which was possibly related to the study drugs; therefore, the medication was permanently discontinued. The second patient already had grade 2 increases at baseline, but she was co-infected with hepatitis C. The liver enzyme elevations were assessed as possibly being related to the study drugs; however, the medication was continued. The third patient had an increase at 33 weeks GA and at delivery. This increase was possibly related to the study drugs, but the medication was continued until delivery.
The average GA at delivery was 38 3/7 weeks. Six infants were born before GA of 37 weeks, but none were earlier than 35 weeks. In total, 29 children tested HIV negative; the HIV status of the remaining three children is unknown, because they were lost during follow-up.
At, or close prior to delivery, the viral loads of 30 women were available. The viral load was detectable in only one of these women (189 copies/mL). At the delivery visit this patient received 72 days of treatment, the saquinavir C_{min} was 0.781 mg/L at 33 weeks GA. Among the patients who recorded the pharmacokinetic curves at GA of 20 weeks (n=15), week 33 (n=31) and 6 weeks postpartum (n=9, 67%, 93% and 100%

Table 2. Pharmacokinetic parameters of saquinavir/r 1,000/100 mg twice daily

	Saquinavir			Ritonavir		
	GA week 20	GA week 33	Week 6 pp	GA week 20	GA week 33	Week 6 pp
Patients, n	**16**	**31**	**9**	**16**	**31**	**9**
$AUC_{0\text{-}12h}$ (h·mg/L)	23.47 (11.92)	23.65 (9.07)	25.00 (11.81)	8.57 (5.28)	7.41 (3.95)	11.57 (4.44)
CV (%)	51	39	47	62	53	38
C_{max} (mg/L)	3.59 (1.57)	3.67 (1.49)	3.91 (1.79)	1.46 (0.86)	1.13 (0.58)	1.85 (0.77)
CV (%)	44	41	46	59	51	42
C_{min} (mg/L)	0.82 (0.55)	0.84 (0.42)	0.78 (0.47)	0.28 (0.19)	0.29 (0.20)	0.31 (0.14)
CV (%)	67	50	60	68	69	45
Half-life (h)	3.72 (1.12)	3.90 (0.93)	3.79 (1.27)	3.48 (0.80)	4.45 (1.75)	3.68 (1.03)
CV (%)	30	24	34	23	39	28
T_{max} (h)	3.65 (1.29)	3.36 (1.25)	3.89 (1.91)	3.59 (1.89)	3.70 (1.65)	2.98 (2.09)
CV (%)	35	37	49	53	45	70
CL/F (L/h)	48 (27)	45 (30)	55 (57)	13 (6.3)	13 (6.3)	8.8 (3.9)
CV (%)	57	67	104	49	47	45

All parameters are expressed as mean (±SD); P-values calculated with the paired samples t-test; AUC, area under the curve; C_{max}, maximum plasma concentration; C_{min}, trough concentration at 12h; GA, gestational age; PP, postpartum; T_{max}, time of maximum concentration.

Table 3. Within patient comparison of pharmacokinetic parameters

	GA week 20	GA week 33	P-value	GA week 33	Week 6 PP	P-value
Patients, n	**15**	**15**		**9**	**9**	
Saquinavir						
$AUC_{0\text{-}12h}$ (h·mg/L)	23.35 (12.33)	24.40 (10.27)	0.710	20.63 (9.47)	25.00 (11.81)	0.379
C_{max} (mg/L)	3.53 (1.61)	3.70 (1.73)	0.667	3.12 (1.54)	3.91 (1.79)	0.319
C_{min} (mg/L)	0.83 (0.57)	0.92 (0.48)	0.490	0.79 (0.43)	0.78 (0.47)	0.935
Half-life (h)	3.75 (1.15)	3.76 (0.80)	0.951	3.95 (0.97)	3.79 (1.27)	0.814
T_{max} (h)	3.69 (1.33)	3.66 (1.60)	0.915	3.41 (1.88)	3.89 (1.91)	0.519
CL/F (L/h)	48 (28)	45 (33)	0.672	54 (39)	55 (57)	0.987
Ritonavir						
$AUC_{0\text{-}12h}$ (h·mg/L)	8.40 (5.43)	7.87 (4.80)	0.321	6.73 (1.95)	11.57 (4.44)	0.030
C_{max} (mg/L)	1.44 (0.89)	1.23 (0.65)	0.147	1.03 (0.35)	1.85 (0.77)	0.021
C_{min} (mg/L)	0.28 (0.19)	0.30 (0.23)	0.320	0.30 (0.22)	0.31 (0.14)	0.920
Half-life (h)	3.49 (0.83)	3.86 (1.24)	0.146	3.94 (0.96)	3.68 (1.03)	0.557
T_{max} (h)	3.70 (1.90)	3.76 (2.00)	0.923	4.04 (1.82)	2.98 (2.09)	0.066
CL/F (L/h)	13 (6.4)	13 (4.7)	0.704	13 (4.4)	8.8 (3.9)	0.091

All parameters are expressed as mean (±SD); P-values calculated with the paired samples t-test; AUC, area under the curve; C_{max}, maximum plasma concentration; C_{min}, trough concentration at 12h; GA, gestational age; PP, postpartum; T_{max}, time of maximum concentration.

respectively, had undetectable viral load. None of the patients showed a virological failure according to our definition.

Discussion

In this study, we evaluated the pharmacokinetics of the new 500 mg tablet formulation of saquinavir when boosted with 100 mg of ritonavir in pregnant HIV-1-infected patients, at different time points along the gestation period (week 20 and week 33) and after delivery (6 weeks postpartum). For all time points, the saquinavir exposure was adequate and no sub-therapeutic concentrations, defined as < 0.1 mg/L, were recorded. No difference was found between the different time points, suggesting that pregnancy had no effect on the saquinavir concentrations. The ritonavir exposure seemed more affected during pregnancy than the saquinavir concentrations.

PI disposition is thought to be most affected during the third trimester of pregnancy. In this phase, close to delivery, viral suppression is considered important in order to reduce

mother-to-child transmission to a minimum. Subtherapeutic concentrations in this trimester may have a negative effect on drug antiviral efficacy. In this study, we collected 31 curves for the third trimester (week 33) in order to generate a reliable mean estimate of saquinavir exposure, accounting for the large inter-variability in its pharmacokinetics. Although the variability was large, none of the patients had concentrations <0.1 mg/L. The cut off of 0.1 mg/L is currently considered the most accurate, although other studies suggested other cut-offs in the past, or could not prove the correlation. To our knowledge this is the first extensive pharmacokinetic study in pregnant women with the tablet formulation of saquinavir. Earlier studies with saquinavir in pregnant women were either performed with the unboosted soft gel capsule (no longer available)[16] or the boosted 200 mg hard gel capsules (HGC).[13] One study with the new tablet formulation reported only the C_{min}.[17] In the study of Acosta *et al*.[13] the AUC antepartum was 29 h·mg/L with a 800/100 mg twice-daily (HGC) dosing in 13 patients, which is somewhat higher than the 23.7 h.mg/L we found in our study. In this study, breakfast with a higher fat percentage was used at the days of pharmacokinetic monitoring, this could explain the higher exposure to saquinavir. Bittner *et al*.[18] reported a bioequivalence study comparing the HGC with the new tablet formulation in healthy volunteers. An $AUC_{0\text{-}inf}$ of 26.8 h·mg/L saquinavir was reported for the tablet. An AUC of 18.8 mg/L was calculated (n=11) in another study in the non-pregnant population using the new tablet formulation.[19] This reduced exposure could possibly be explained by gender differences (only one female was included). It has been suggested that some antiretrovirals, saquinavir in particular, are affected by sex differences.[20] The postpartum concentrations found in our study are expected to be representative for the concentrations of the non-pregnant female population. The $AUC_{0\text{-}12}$ determined in our study is similar to AUCs found in other studies.

Our within-patient comparison between the ante- and postpartum curves did not show a difference in saquinavir exposure. This is consistent with the finding of Acosta *et al*. [13]; however, in that study, a trend toward lower saquinavir exposure during the gestational period was observed. It must be noted that our postpartum sample size may be too small to be conclusive on the precise effect of pregnancy on the saquinavir concentrations. However, saquinavir seems less sensitive to the physiological changes during pregnancy than other PIs such as indinavir, nelfinavir and lopinavir, for which, even with small sampled studies significant differences were found ante- and postpartum. Nelfinavir is the most extensively studied PI during pregnancy, due to its frequent use in pregnant women over the past years. All pharmacokinetic studies reported a decreased exposure to nelfinavir during pregnancy, which is more pronounced towards the end of the gestation.[21,22] Owing to safety and efficacy concerns, nelfinavir is currently relatively contra-indicated in pregnancy and in most guidelines replaced by lopinavir/ritonavir. However, one may question if lopinavir/ritonavir should be the PI of choice during pregnancy. In Stek *et al*.[23] a comparison within 12 patients on lopinavir/ritonavir was made ante- and postpartum. A significant reduction in total exposure of approx-

imately 33% during pregnancy was observed. Based on this and other studies, an increase of the lopinavir/ritonavir dose when no therapeutic drug monitoring is available is suggested. The same is true for (unboosted) indinavir with a decrease of 68% in its exposure. For atazanavir, the results of two small studies are conflicting.[24,25]
The differences between the individual PIs are difficult to explain considering they all use the same metabolic pathway, CYP3A4, which is known to play a major role in the changes of drug disposition during gestation.
In our study we found a significant reduction of 36% for ritonavir exposure during pregnancy, which did not affect the saquinavir concentrations accordingly. Kilby *et al.*[26] showed in healthy volunteers that lower concentrations of ritonavir do not necessarily generate lower concentrations of saquinavir. This relative independence of ritonavir exposure could explain why saquinavir plasma concentrations are more stable during pregnancy compared to other PIs, despite the reduced exposure of its booster ritonavir. The role of the ritonavir reduction in combination with PIs in pregnancy is currently difficult to address as most studies do not report the ritonavir concentrations. Additional studies looking into this mechanism are warranted before strong conclusions can be drawn.
During our study we recorded 3 cases of severe hepatotoxicity, out of which one had hepatitis C; consistent with another study of saquinavir use in pregnancy.[27] However, the risk of developing severe hepatotoxicity is similar to non-pregnant patients starting boosted saquinavir.[28] Moreover, pregnancy in itself is an additional risk factor for developing hepatotoxicity.[29] Larger studies should be conducted for the different PIs to assess if there is additional risk of hepatotoxicity when saquinavir is used during pregnancy.

The antiviral potency was adequate although the sample size was too small and the group too heterogeneous to be conclusive. Currently, the use of PIs is recommended and considered safe and effective during pregnancy
On the basis of this study, the use of boosted saquinavir tablet in a saquinavir/r 1,000/100 mg twice-daily regimen can be recommended to be used during pregnancy. This treatment generates adequate saquinavir concentrations throughout the course of pregnancy and has a solid safety and antiviral efficacy profile.

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4

Atazanavir Exposure is Effective during Pregnancy, Regardless of Tenofovir Use

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Abstract

Background: We studied the effect of pregnancy on atazanavir pharmacokinetics in presence and absence of tenofovir.

Methods: This was a non-randomized, open-label, multicentre phase IV study in HIV-infected pregnant women recruited from European HIV treatment centres. HIV-infected pregnant women treated with boosted atazanavir (300/100mg or 400/100mg atazanavir/ritonavir) as part of their combination antiretroviral therapy (cART) were included in the study. 24h pharmacokinetic curves were recorded in the third trimester and postpartum. Collection of a cord blood and maternal sample at delivery was optional.

Results: 31 patients were included in the analysis, 21/31 patients used tenofovir as part of cART. Median (range) gestational age at delivery was 39 weeks (36-42). Approaching delivery 81% (25 patients) had an HIV viral load <50 copies/mL, all <1,000 copies/mL. Least squares means ratios (90% CI) of atazanavir pharmacokinetic parameters third trimester/postpartum were: 0.66 (0.57-0.75) for $AUC_{0\text{-}24h}$, 0.70 (0.61-0.80) for C_{max} and 0.59 (0.48-0.72) for C_{24h}. No statistical difference in pharmacokinetic parameters was found between patients using tenofovir versus no tenofovir. None of the patients showed atazanavir concentrations <0.15 mg/L (target for treatment-naive patients). One baby had a congenital abnormality, which was not likely to be related to atazanavir/ritonavir use. None of the children were HIV-infected.

Conclusions: Despite 34% lower atazanavir exposure during pregnancy, atazanavir/ritonavir 300/100mg once daily generates effective concentrations for protease inhibitor (PI)-naive patients, even if co-administered with tenofovir. For treatment-experienced patients (with relevant PI resistance mutations) therapeutic drug monitoring of atazanavir should be considered to adapt the atazanavir/ritonavir dose on an individual basis.

Introduction

The risk of mother-to-child transmission (MTCT) of HIV has been reduced by the introduction of combination antiretroviral therapy (cART), reducing the risk from 15-40% in the absence of therapy to <2%.[1] Since 2012 Department of Health and Human Services (DHHS) perinatal guidelines have classified ritonavir-boosted atazanavir (atazanavir/r) as one of the preferred protease inhibitors (PIs) to be used during pregnancy.[2] The European AIDS Clinical Society guidelines[3] as well as the British HIV Association guidelines for the management of HIV infection in pregnant women report boosted lopinavir, saquinavir as well as atazanavir as compounds effective as the third agent in cART in pregnancy.[4]

In the US, PIs are the most common third class of drugs (combined with NRTIs) used in pregnancy: up to 86% in 2009. In 2009, atazanavir/r use during pregnancy was much lower compared to lopinavir/r use (20% versus 55%).[5] The use of atazanavir/r during pregnancy may increase because atazanavir/r has been classified as one of the preferred PIs to be used in pregnancy. Moreover, atazanavir is classified as FDA Pregnancy Category B indicating that animal reproduction studies failed to demonstrate risk to the foetus; whereas lopinavir/r is classified as FDA Pregnancy Category C, indicating that animal reproduction studies have shown an adverse effect on the foetus.[2]

Sufficient numbers of first trimester exposures to atazanavir (n=746 up to July 2012) have been monitored by the Antiretroviral Pregnancy Register to have power to detect at least a twofold increase in risk of overall birth defects, but no such increases have been detected to date. A subset of the registry (all cases using atazanavir during pregnancy from 2002 onwards) was analysed for possible birth defects.[6] No pattern of birth defects suggestive of a common aetiology was observed.

During pregnancy human physiology alters, potentially affecting the pharmacokinetics of drugs. These changes include: decreased gastric emptying and motility, increased gastric pH, increased total body water and plasma volume, increased hepatic blood flow, alteration of cytochrome P450 activity, increased cardiac output, increased glomerular filtration and decreased protein binding. In most cases resulting in lower exposure of medication during pregnancy,[7-9] this effect seems to be rather strong for boosted PIs.[10, 11]

Between 2005 and 2012 several studies were performed investigating the pharmacokinetics of atazanavir/r during pregnancy. A systematic review of these studies was recently published.[12] Pharmacokinetic studies as well as studies on safety and efficacy were reported, including one study with an increased dose during the third trimester (400/100mg once daily atazanavir/r). Most studies report lower exposure during pregnancy, with area under the curve (AUC) 21% (geometric mean ratio [GMR]) lower in the second and 21-33% lower in the third trimester of pregnancy. When combined with tenofovir disoproxil fumarate (TDF) in cART the decrease in exposure was even

more pronounced (34%).[13] However, not all pharmacokinetic studies performed during pregnancy showed decreased exposure: Ripamonti *et al.* reported an AUC GMR (90% CI) of 0.93 (0.79-1.08; third trimester versus postpartum).[14]
Two intensive pharmacokinetic studies investigated an atazanavir dose increase in the third trimester of pregnancy. The increased dose (400/100mg once daily) resulted in therapeutic concentrations in both studies, but also a doubling of maternal grade 3-4 hyperbilirubinaemia in one study.[15,16]
The atazanavir product characteristics state that as atazanavir/r 300/100mg once daily may not give sufficient exposure during the second and third trimester, therapeutic drug monitoring is recommended and dose increase if necessary. If TDF or an H2-receptor antagonist is needed, a dose increase to atazanavir/r 400/100mg once daily with therapeutic drug monitoring may be considered. Atazanavir/r should not be used by pregnant women also using TDF and an H2-receptor antagonist.[17] TDF use may play a bigger role in pregnancy, because tenofovir/emtricitabine is considered a preferred NRTI backbone during pregnancy according to the recent changes in DHHS perinatal guidelines.[2]
Given that information on atazanavir pharmacokinetics during pregnancy and after pregnancy was not consistent, TDF was reported to decrease atazanavir levels during pregnancy to a greater extent, and atazanavir and TDF are considered preferred agents to be used during pregnancy, we studied the effect of pregnancy on atazanavir pharmacokinetics in presence and absence of TDF.

Methods

This was a non-randomized, open-label, multicentre phase IV study in HIV-infected pregnant women recruited from HIV treatment centres in Europe (PANNA network: www.pannastudy.com). The PANNA network is a European network of hospitals collecting pharmacokinetic curves of several antiretrovirals (ARVs) during pregnancy in a prospective study. In total 17 hospitals are involved in the network; data in this publication were collected between February 2010 and May 2013.
The study was conducted in compliance with the principles of the Declaration of Helsinki. Informed consent was obtained from each participant before entering the study. The study was approved by the medical ethical committee from each individual centre involved and by the national authorities if applicable. The study was registered at ClinicalTrials.gov under number NCT00825929.
Here we describe the pharmacokinetics of atazanavir/r in pregnancy compared to postpartum, with a focus on concomitant use of TDF. Patient eligibility included being HIV-infected, pregnant, at least 18 years of age at screening and treated with a cART regimen containing atazanavir for at least 2 weeks before the day of first pharmacokinetic curve evaluation (in the third trimester of pregnancy). Patients were excluded if they

had a past medical history or current condition that might interfere with drug absorption, distribution, metabolism or excretion or presented with grade III/IV anaemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL) at screening.

Safety assessments and viral load

Blood samples for safety assessments and viral load were taken at screening and visits for pharmacokinetic blood sampling. Patients were asked for adverse events at each visit. Birth weight, congenital abnormalities and HIV status of the infants were collected.

Pharmacokinetic blood sampling

Pharmacokinetic curves (samples were taken pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24h post-medication intake) were recorded during the third trimester (preferably at week 33) and at least 2 weeks postpartum (preferably 4-6 weeks postpartum). At delivery (if possible) a cord blood sample was taken and at the same time a blood sample from the mother was taken. A standard breakfast (650kCal; 30g fat) was served prior to (observed) dosing on the pharmacokinetic days.

Analytical and pharmacokinetic methods

Concentrations of atazanavir and ritonavir in plasma were analyzed by use of a validated (ultra) high-performance liquid chromatography method for the simultaneous quantitative determination of several HIV PIs in human plasma.[18] Lower limit of quantification (LLOQ) was 0.090 mg/L for atazanavir and 0.045 mg/L for ritonavir. The assays were externally validated through ACTG and KKGT.[19,20]

Pharmacokinetic parameters were determined using a non-compartmental model in WinNonlin/Phoenix version 6.3 [Pharsight Corporation, Mountain View, CA, USA]. Concentrations below LLOQ occurring before the first measurable concentration were set to zero, the first <LLOQ concentration at the end of the curve was set to ½ LLOQ, subsequent concentrations were left empty. Area under the curve over a dosing interval ($AUC_{0\text{-}24h}$) using the trapezoidal rule, trough concentration (C_{24h}) defined as the sample taken at time point 24hr (or extrapolated from the last available concentration, using lambda, if the sample was missing), maximum concentration (C_{max}), elimination half-life (T_{half}), time of maximum concentration (T_{max}) and apparent clearance (CL/F, being the dose/AUC_{tau}) were determined per individual curve.

Statistical analysis data handling

Patients from whom a pharmacokinetic curve was taken during pregnancy were included in demographic, safety analyses and descriptive statistics of the pharmacokinetic parameters. Demographic data were summarized with descriptive statistics. Quantitative data were compared between patients with and without TDF co-treatment using the Mann-Whitney U test, categorical data were compared using the chi-square test. Pharmacokinetic parameters are reported as geometric means with 95% intervals (for

300/100mg atazanavir/r dose only). Least squares means ratios (LSMRs) and 90% confidence intervals (CI) of $AUC_{0\text{-}24h}$, C_{max}, C_{24h}, CL/F and T_{half} of third trimester versus postpartum were calculated. For geometric mean calculations patients using 400/100mg atazanavir/r were excluded, for the LSMR calculations these patients were included. To indicate whether the pharmacokinetic parameters during pregnancy differed statistically significantly from the postpartum parameters, a mixed models test (using SPSS) was performed on the natural log (ln)-transformed parameters. Pharmacokinetic parameters (ln-transformed) with and without TDF co-treatment were compared by an independent t-test. The non-parametric test for independent samples: Mann-Whitney U was used to determine a difference in pharmacokinetic parameters for patients with and without a detectable viral load around delivery. Cord blood/maternal blood concentration ratios were determined and described.

Results

Thirty-six patients receiving atazanavir/r during pregnancy were enrolled in the study from 13 different sites from the PANNA network. Four of these patients dropped out before the first pharmacokinetic curve was taken: three delivered before the intensive sampling pharmacokinetic day (the gestational age [GA] was 28 weeks, 33 weeks and 40 weeks) and one withdrew consent. For one patient all plasma concentrations (for both atazanavir and ritonavir) were below LLOQ, indicating that she was not adherent to therapy and in that intake of medication was apparently not supervised. These five patients were excluded from the analysis.

The characteristics and pregnancy outcome of the remaining patients (n=31) are presented in Table 1. Fourteen patients were White, 16 Black and 1 of mixed race. At the time of conception, 11 (35%) of the patients were treatment-naive and 13 patients used atazanavir/r and continued during pregnancy. The majority of the patients used atazanavir/r 300/100mg once daily (94%), 2 patients used atazanavir/r 400/100mg once daily. Twenty-one out of 31 patients used TDF. Other NRTIs taken were: emtricitabine (n=20), lamivudine (n=10), zidovudine (n=6) and abacavir (n=4). One patient also used raltegravir. Non-ARV concomitant substances possibly influencing atazanavir exposure was marijuana used by one patient. No statistical differences in subject characteristics were observed between patients with TDF and without TDF in their backbone therapy (Table 1).

A total of 31 curves were collected in the third trimester (median 35 weeks GA) and 26 curves postpartum (median 6 weeks postpartum). Five patients did not have a postpartum curve for the following reasons: withdrawn consent (n=1); lost to follow-up (n=2); changed medication (n=1); plasma atazanavir levels <LLOQ (n=1) probably due to non-adherence.

Table 1. Subject characteristics

	All patients (n=31)	Patients on ATV/r 300/100mg with TDF (n=19)	Patients on ATV/r 300/100mg without TDF (n=10)	P-value
Median age at delivery, years (range)	32 (18-44)	32 (20-42)	34 (24-44)	0.154[a]
Race/ethinicity				
White, n (%)	14 (45%)	7 (37%)	7 (70%)	0.147[b]
Black, n (%)	16 (52%)	12 (63%)	3 (30%)	
Asian, n (%)	0	0		
Other, n (%)	1 (3%)	0	0	
Smoking, n (%)	6 (19%)	2 (11%)	4 (40%)	0.098[b]
Alcohol use, n (%)	2 (6%)	1 (5%)	1 (10%)	0.579[b]
Drug use, n (%)	3 (10%)	0	3 (30%)	
Treatment-naive pregnancy start, n (%)	10 (32%)	5 (26%)	5 (50%)	0.259[b]
Median ARV treatment duration before pregnancy (weeks)	259 (3-561)	223 (3-561)	306 (23-519)	0.405[a]
Conception on atazanavir, n (%)	13 (42%)	10 (53%)	3 (30%)	
Start atazanavir per trimester, n (%)				
First trimester	1 (3%)	-	1 (10%)	
Second trimester	13 (42%)	8 (42%)	4 (40%)	
Third trimester	4 (13 %)	1 (5 %)	2 (20 %)	
atazanavir/r 300/100mg QD, n (%)	29 (94%)	-	-	
Concomitant antiretrovirals				
NRTI	21 (68%) tenofovir; 20 (65%) emtricitabine; 10 (32%) lamivudine; 6 zidovudine (19%); 4 abacavir (13%)	-	-	

Table 1. - continued Subject characteristics

	All patients (n=31)	Patients on ATV/r 300/100mg with TDF (n=19)	Patients on ATV/r 300/100mg without TDF (n=10)	P-value
Raltegravir	1 (3%)	1 (5%)	0	
Median age at delivery, years (range)	32 (18-44)	32 (20-42)	34 (24-44)	0.154[a]
Third trimester (n=31)				
Median gestational age, weeks (range)	35 (28-38)	35 (32-38)	35 (28-37)	0.493[a]
Median weight, kg (range)	78 (56-139)	77.5 (55.5-136)	80.1 (63-139)	0.565[a]
HIV-RNA undetectable <50 copies/ml, n (%)	25 (81%) / <200: 29 (94%)	13 (68%) / 17 (89%)	10 (100%) / 10 (100%)	0.141[b]
Median CD4+ T-cell count, cells/µL (range)	555 (196-1333)	508 (196-1333)	648 (360-1170)	0.350[a]
Postpartum (n=25)				
Median time after delivery, weeks (range)	6 (3-10)	6 (3-10)[d]	6 (3-7)[e]	0.414[a]
Median weight, kg (range)	72 (51-126)	71 (51-89)	73 (56-126)	0.713[a]
HIV-RNA undetectable <50 copies/ml, n (%)	21 (81%) / <200: 24 (92%) / unk: 1 (4%)	14 (82%) / 16 (94%)	7 (88%) / 1 unk (12%)	0.407[b]
Median CD-4+ T-cell count, cells/µL (range)	653 (150-1020)	620 (150-940)	689 (346-1020)	0.816[a]
Pregnancy outcomes				
Median gestational age, weeks (range)	39 (36-42)	39 (36-42)	39 (36-41)	0.626[a]
Caesarian section, n (%)	18 (69%); 2 unknown	13 (76%); 1 unk	5 (63%); 1 unk	
Median birth weight, g (range)	3195 (2230-4350)	3260 (2290-4350)	3145 (2710-3500)	0.404[a]
Infant HIV DNA PCR status				
Negative, n (%)	28 (90%)	18 (95%)	8 (80%)	
Unknown, n (%)	3 (10%)	1 (5%)	2 (20%)	

[a] Mann-Whitney U test ; [b] chi-square test ; [c] 2 patients on 400/100mg ATV/r once daily ; [d] n=17; [e] n=8 ARV, antiretroviral TDF, tenofovir disoproxil fumarate

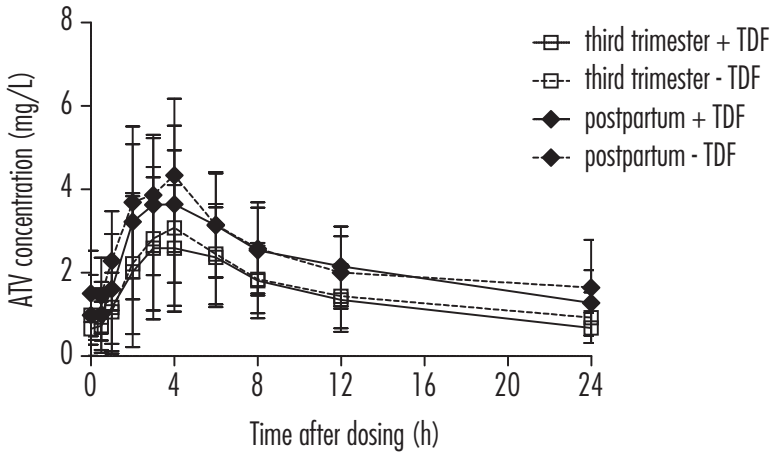

Figure 1. Mean atazanavir concentration-time profiles
Mean (±SD) concentration versus time curves for HIV-infected pregnant women using 300/100mg ritonavir boosted atazanavir (atazanavir/r) once daily during the third trimester and postpartum. Solid lines represent patients using tenofovir disoproxil fumarate (TDF) and dashed lines represent subjects not using TDF.

Pharmacokinetics

Mean plasma concentration-time profiles of atazanavir 300/100mg once daily with separate lines for TDF use and non-TDF use are presented in Figure 1. Summary statistics of the pharmacokinetic parameters are listed in Tables 2 and 3.

The $AUC_{0\text{-}24h}$, C_{max}, C_{24h} for atazanavir were respectively 34%, 30% and 41% lower during pregnancy compared with postpartum (intra-subject comparison) and 55%, 58% and 53% lower for ritonavir, respectively. For both compounds the steady-state apparent clearance (CLss/F) was increased during pregnancy (53% and 124% for atazanavir and ritonavir, respectively) and T_{half} tended to be shorter in the third trimester. No statistical difference in atazanavir $AUC_{0\text{-}24h}$, C_{max}, C_{24h}, T_{half} or CLss/F was found between patients (atazanavir 300/100mg only) using TDF versus no TDF in the third trimester or postpartum ($p>0.15$ for all parameter; Table 3). Geometric mean (95% CI) atazanavir $AUC_{0\text{-}24h}$ in the third trimester was 32.1 (21.1-48.7) h·mg/L without TDF and 28.8 (22.2-37.4) h·mg/L with TDF and atazanavir C_{24h} was 0.58 (0.32-1.05) mg/L without and 0.44 (0.31-0.62) mg/L with TDF co-treatment. Postpartum geometric mean (95% CI) atazanavir $AUC_{0\text{-}24h}$ was 49.2 (34.7-69.8) h·mg/L without TDF and 46.1 (36.2-58.6) h·mg/L with TDF and atazanavir C_{24h} was 0.90 (0.47-1.71) mg/L without and 0.89 (0.59-1.32) mg/L with TDF.

None of the patients had atazanavir concentrations below 0.15 mg/L (target for treatment-naive patients) in the third trimester or postpartum. For three patients extrapolated third trimester C_{24h} concentrations would have been below 0.15 mg/L (i.e. 0.130, 0.135 and 0.139 mg/L). The pre-dose concentrations of these patients were well above 0.15

Table 2. Pharmacokinetic parameters 300/100mg atazanavir/r once daily

	Third Trimester[a]	Postpartum[a]	LSM Ratio (90% CI)[b] Third Trimester/Postpartum	p-value[c]
Atazanavir	**(n=29)**	**(n=25)**		
$AUC_{0\text{-}24h}$ (h·mg/L)	29.9 (24.3-36.8)	47.0 (39.1-56.6)	0.66 (0.57-0.75)	<0.001
C_{max} (mg/L)	2.92 (2.36-3.61)	4.29 (3.67-5.02)	0.70 (0.61-0.80)	<0.001
T_{max} (h)	3 (0-6)	3 (1-7.9)		
$C_{predose}$ (mg/L)	0.60 (0.46-0.79)	0.92 (0.69-1.23)		
C_{24h} (mg/L)	0.48 (0.36-0.65)	0.89 (0.65-1.22)	0.59 (0.48-0.72)	<0.001
T_{half} (h)	10 (9-12)	12 (10-15)	0.87 (0.76-1.00)	0.109
CL_{ss}/F (L/h)	10 (8-12)	6 (5-8)	1.53 (1.34-1.75)	<0.001
Ritonavir	**(n=29)**	**(n=25)**		
$AUC_{0\text{-}24h}$ (mg·L/h)	5.01 (4.09-6.15)	11.68 (9.46-14.42)	0.45 (0.37-0.53)	
C_{max} (mg/L)	0.59 (0.46-0.77)	1.50 (1.22-1.86)	0.42 (0.34-0.51)	
T_{max} (h)	4.0 (0.00-8.00)	4 (0-7.9)		
C_{24h} (mg/L)	0.036[d] (0.028-0.045)	0.08[e] (0.060-.012)	0.47 (0.36-0.62)	
T_{half} (h)	5 (4-6)	5 (5-6)	0.90 (0.78-1.04)	
CL_{ss}/F (L/h)	20 (16-24)	9 (7-11)	2.24 (1.88-2.68)	

[a] Geometric mean (95% confidence interval); except for Tmax: median (min-max); [b] LSM (Least squares mean) ratio includes one patient using 400/100mg atazanavir/r; [c] mixed model analysis; [d] 16 below LLOQ, taken as 1/2 LOQ, i.e. 0.0225 mg/L; [e] 5 below LLOQ, taken as 1/2 LOQ, i.e. 0.0225 mg/L

$AUC_{0\text{-}24h}$, area under the curve over a dosing interval; CLss/F, apparent steady-state clearance; C_{max}, maximum concentration; $C_{predose}$, predose concentration; C_{24h}, trough concentration defined as the sample taken at time point 24 h (or extrapolated from the last available concentration, using lambda, if the sample was missing); T_{half}, elimination half-life.

mg/L, 2 of these patients used TDF concomitantly.

Eighteen umbilical cord blood (CB) samples were collected with matching maternal blood samples. The median time between the reported last dose and delivery was 12h (range 2-27h); the median time between CB sample and maternal sample was 3 minutes (0-345 min). In five cord blood samples, atazanavir concentrations were undetectable; in one case the time between maternal and cord blood sample was 10h, this sample was excluded from descriptive statistics. The median (range) ratio of CB/maternal blood was 0.20 (0.06-3.05; n=12) for atazanavir. For ritonavir all CB samples were <LLOQ.

Table 3. Pharmacokinetic parameters 300/100mg atazanavir/r in presence and absence of TDF

	Third Trimester plus TDF[a]	Third Trimester without TDF[a]	P value[b]	Postpartum plus TDF	Postpartum without TDF	P value[b]	Third Trimester / Postpartum plus TDF[c]	Third Trimester / Postpartum without TDF[c]
Atazanavir	**(n=19)**	**(n=10)**		**(n=17)**	**(n=8)**			
$AUC_{0\text{-}24h}$ (h·mg/L)	28.8 (22.2-37.4)	32.08 (21.1-48.7)	0.624	46.1 (36.2-58.6)	49.2 (34.7-69.8)	0.735	0.65 (0.55-0.78)	0.66 (0.53-0.83)
C_{max} (mg/L)	2.92 (2.21-3.84)	2.93 (1.95-4.40)	0.985	4.17 (3.42-5.08)	4.58 (3.31-6.34)	0.570	0.72 (0.60-0.86)	0.65 (0.52-0.82)
C_{24h} (mg/L)	0.44 (0.31-0.62)	0.58 (0.32-1.05)	0.351	0.89 (0.59-1.32)	0.90 (0.47-1.71)	0.972	0.57 (0.43-0.75)	0.64 (0.47-0.87)
T_{half} (h)	9 (8-11)	12 (8-17)	0.181	12 (10-16)	12 (8-18)	0.768	0.82 (0.68-0.97)	1.00 (0.80-1.26)

[a] Geometric mean (95% confidence interval); except for Tmax: median (min-max); [b] independent t-test on ln-transformed parameters; [c] LSM (Least squares mean) ratio includes one patient using 400/100mg atazanavir/r
$AUC_{0\text{-}24h}$, area under the curve over a dosing interval; C_{max}, maximum concentration; C_{24h}, trough concentration defined as the sample taken at time point 24 h (or extrapolated from the last available concentration, using lambda, if the sample was missing); TDF, tenofovir disoproxil fumerate; T_{half}, elimination half-life.

Efficacy and safety

HIV viral load close to delivery (median 34 weeks GA) was undetectable in 25 (out of 31) women and detectable in 6 women: 68, 100, 120, 162, 290 and 402 copies/mL. One patient started cART in the second trimester, the other patients were on cART at conception. All of these patients used atazanavir/r 300/100mg once daily and TDF concomitantly.

The average GA at delivery was 39 (range 36-42) weeks. Two children were born pre-term at 36 weeks and 36 weeks and 3 days. Birth weight of 26 children was reported, 2 had a low birth weight (<2500 g), whereas 5 out of 26 were small for gestational age (reference 10th percentile of birth weight for GA by gender, US, 1991). Twenty-eight children were tested HIV-negative (PCR DNA after delivery) and the status of 3 children was unknown. One child had a congenital diaphragmatic hernia resulting in respiratory failure, septic shock and death. A relationship with the ARV medication used could not be ruled out, however the closure of the pleuroperitoneal canal in the developing embryo occurs at approximately week 8 of pregnancy and the patient started antiretroviral therapy in week 18 of her pregnancy (atazanavir/r even later at week 21). Furthermore, this patient also used methadone since 2000 (also during this pregnancy). Five patients developed a serious adverse event (SAE). One was the congenital abnormality described above. Five hospital admissions occurred (3 patients) for several reasons: a patient thought the baby was not moving (the baby was born without

problems); early contractions at 31.3 weeks GA (baby was born at GA 38.6 weeks) and urinary tract infection; tonic/clonic seizure and headache after delivery. All patients recovered. The final SAE was in a patient who had postnatal uterus agony, coagulapathy and postpartum haemorrhage, which prolonged hospital admission. She recovered within one week. The local investigators judged these SAEs not to be related to the cART given. Nine other patients reported adverse events, all were grade 1 or 2 and not or unlikely related to the cART given. No clinically relevant laboratory abnormalities were reported.

Pharmacokinetic – efficacy relationship

HIV viral loads were detectable for 6 patients around delivery. The Mann-Whitney U test did not reveal significant differences in third trimester atazanavir pharmacokinetic parameters ($AUC_{0\text{-}24h}$, Cmax, C_{24h}, T_{half} or CLss/F) compared to the pharmacokinetic parameters of patients with an undetectable viral load around delivery (Table 3). One patient had an extrapolated C_{24}h <0.15 mg/L, with a pre-dose concentration of 0.54 mg/L. Twenty-eight children were tested HIV-negative (PCR DNA after delivery), the status of 3 children was unknown.

Discussion

In this study we evaluated the pharmacokinetics of atazanavir/r in the third trimester of pregnancy in absence and presence of TDF in 31 pregnant HIV-infected patients.

In the third trimester of pregnancy a decrease in atazanavir $AUC_{0\text{-}24h}$, C_{max} and C_{24h} (34%, 30% and 41%, respectively) was observed as well as a marked decrease in ritonavir $AUC_{0\text{-}24h}$, C_{max} and C_{24h} (55%, 58% and 53%, respectively). Clearance (CLss/F) was markedly increased during pregnancy for both compounds (atazanavir 53% and ritonavir 124%).

Four previous studies report intensive pharmacokinetics of atazanavir during pregnancy.[13,15,16,21] Our findings are in line with other groups reporting a decrease in exposure (atazanavir AUC during 300/100mg atazanavir treatment) of 21%,[15] 30% without TDF or 34% with TDF[13] in the third trimester of pregnancy versus postpartum or historical controls. None of the studies reported a significant decrease of C_{24h} concentrations during pregnancy, whereas we did find a statistically significant decrease. Despite this significant decrease of atazanavir C_{24h}, no concentrations below the target trough concentration of 0.15 mg/L were measured, indicating sufficient exposure for PI-naive patients.

For six patients a detectable HIV viral load was reported around delivery, although all were <1,000 copies/mL, this might be of concern. Five of these patients were treatment experienced and all of them used atazanavir/r 300/100mg once daily and TDF. Although all of these patients used TDF concomitantly, no statistically significant

difference in pharmacokinetic parameters in the third trimester was observed for these patients compared with the patients with an undetectable HIV viral load and none showed atazanavir concentrations <0.15 mg/L. None of the children of the patients with a detectable viral load were HIV-infected.

In contrast to data reported by Mirochnick *et al.*,[13] our study showed no statistical difference in $AUC_{0\text{-}24h}$, C_{max} or C_{24h} for patients (atazanavir 300/100mg only) using TDF versus no TDF (in the third trimester or postpartum). Although the atazanavir product characteristics[17] and Taburet *et al.*[22] report decreased exposure to atazanavir if combined with TDF, some other studies do not find this effect (for which a mechanism has not been discovered).[23,24]

Furthermore, most studies investigating the pharmacokinetics of atazanavir during pregnancy[13,15,16] report the postpartum exposure to be unexpectedly higher compared to the non-pregnant population. Atazanavir C_{max} and AUCs were found to be approximately 26-40% higher, and C_{trough} concentrations even twofold higher during the postpartum period than those observed historically in HIV-infected, non-pregnant patients. This finding is not confirmed in our study, as the postpartum atazanavir $AUC_{0\text{-}24h}$ of 47.0 h·mg/L and C_{max} of 4.29 mg/L observed in this study (with and without TDF combined) is in line with historical data: atazanavir $AUC_{0\text{-}24h}$ of 44.2 h·mg/L and C_{max} of 4.47 mg/L.[17]

The cord blood/maternal blood concentration ratio we found for atazanavir (0.20) is in line with the CB/M ratios reported in literature: ranging from 0.13-0.20.[25]

We did not analyse bilirubin concentrations in the mother, nor in the child. As this is a known side effect of atazanavir, this would have been interesting for safety purposes. However, as the bilirubin concentrations seem to be correlated to high plasma atazanavir concentrations, and the plasma atazanavir concentrations postpartum were in the "normal range" and during pregnancy even lower, we do not think this is a major concern.

The mechanism behind the decreased exposure during pregnancy remains unclear, as C_{max} is decreased (indicating decreased absorption and/or increased volume of distribution) and the elimination (T_{half}) seems to be faster during pregnancy. As this study was performed under steady-state conditions, the half-life is difficult to determine accurately. We determined total atazanavir concentrations in plasma, not the unbound concentrations. During pregnancy protein binding is decreased, resulting in a higher free fraction, possibly (partly) compensating for the lower concentrations during pregnancy.

Despite 34% lower atazanavir exposure during pregnancy, 300/100mg atazanavir/r seems to generate effective concentrations for PI-naive patients, even if co-administered with TDF. For treatment-experienced patients (with relevant PI resistance mutations) therapeutic drug monitoring of atazanavir should be considered to adapt the atazanavir/r dose on an individual basis.

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5

The Pharmacokinetics of Total and Unbound Darunavir in HIV-1 Infected Pregnant Women

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Abstract

Objective: To describe the pharmacokinetics of darunavir in HIV-infected pregnant women in the third trimester and postpartum.

Patients and Methods: This was a non-randomized, open-label, multicentre, phase IV study in HIV-infected pregnant women recruited from HIV treatment centres in Europe. HIV-infected pregnant women treated with darunavir/r (800/100mg once daily or 600/100mg twice daily) as part of their combination antiretroviral therapy were included. Pharmacokinetic curves were recorded in the third trimester and postpartum. A cord blood sample and maternal sample were collected.

Results: Twenty-four women were included in the analysis (darunavir/r 600/100mg twice daily (n=6); 800/100mg once daily (n=17); and 600/100mg once daily (n=1)). Geometric mean ratios of third trimester versus postpartum (90% confidence interval) were 0.78 (0.60-1.00) for total darunavir $AUC_{0\text{-}tau}$ after 600/100mg twice-daily dosing and 0.67 (0.56-0.82) for total darunavir $AUC_{0\text{-}tau}$ after 800/100mg once-daily dosing. The unbound fraction of darunavir was not different during pregnancy (12%) compared with postpartum (10%). The median (range) ratio of darunavir cord blood/maternal blood was 0.13 (0.08-0.35). Viral load close to delivery was <300 copies/mL in all but two patients. All children were tested HIV-negative and no congenital abnormalities were reported.

Conclusions: Darunavir AUC and C_{max} are substantially decreased in pregnancy for both darunavir/r regimens. This decrease in exposure did not result in mother-to-child transmission. For antiretroviral-naive patients, who are adherent, take darunavir with food and are not using concomitant medication reducing darunavir concentrations, 800/100mg darunavir/r once daily is adequate in pregnancy. For all other patients 600/100mg of darunavir/r twice daily is recommended during pregnancy.

Introduction

Combination antiretroviral therapy (cART) has been shown to be a highly effective strategy for preventing mother-to-child transmission (MTCT) of HIV, reducing the risk from 15-40% to <2%.[1,2] Boosted darunavir is a preferred agent for antiretroviral-naive adult patients in a dose of 800/100mg once daily for patients without mutations associated with resistance to protease inhibitors (PIs). For patients with evidence of limited protease resistance-associated mutations, the 600/100mg twice-daily dose should be used.[3] Darunavir exposure-response data are not sufficient to recommend a minimum trough concentration (C_{trough}). However, the EC_{50} are 0.055mg/L for wild type virus,[4] and 0.55 mg/L for resistant virus[5] with an EC_{90} of 0.2mg/L for wild type virus. These targets are frequently used for therapeutic drug monitoring purposes.

Department of Health and Human Services (DHHS) perinatal guidelines classify ritonavir-boosted darunavir as an alternative agent to be used during pregnancy. The DHHS guidelines as well as the British HIV Association guidelines for the management of HIV infection in pregnant women 2012 recommend the 600/100mg twice-daily dose to be used during pregnancy,[6,7] because 800/100mg darunavir/r once daily during pregnancy leads to reduced trough levels, whereas trough levels after the 600/100mg twice-daily dose seem to be more in line with exposure in non-pregnant adults. During pregnancy human physiology alters, potentially affecting the pharmacokinetics of drugs.[8,9] These changes mostly result in lower exposure of medication during pregnancy, which has been reported for total darunavir, with decreases in $AUC_{0\text{-}tau}$ and C_{trough} ranging from 17-31%,[10-13] and with a less pronounced decrease in active, unbound darunavir (7-8%[10] and 24%[13]), due to changes in protein binding.

Zorilla *et al.* studied the pharmacokinetics of 600/100mg of darunavir/r twice daily in pregnancy in a limited number of patients (n=11) and conclude that, because the change in unbound (active) darunavir was not clinically significant, no dose adjustment is required for pregnant women receiving darunavir/r 600/100mg twice daily.[10] Capparelli *et al.* presented preliminary results of darunavir/r (600mg twice daily and 800/100mg once daily) pharmacokinetics in pregnancy. They concluded that twice-daily dosing should be used during pregnancy as the C_{trough} of total darunavir was low in the patients on darunavir/r 800/100mg once daily: 1.25 (0.15-2.49) mg/L.[11] A 20% reduction of total darunavir C_{trough} in the third compared with the first trimester was also reported by Courbon *et al.* Some women did not meet the 0.55 mg/L target for treating HIV with resistance-associated mutations. The conclusion was that 600/100mg of darunavir/r twice daily results in better exposure during pregnancy and can be suggested.[12]

Curran *et al.* presented the total and unbound darunavir AUCs during pregnancy and postpartum of five patients taking 800/100mg of darunavir/r once daily. None of the patients had a C_{trough} below the IC_{50} for wild type virus.[13] Crauwels *et al.* also reported total and unbound darunavir AUCs of 16 patients using this once-daily dose in

pregnancy. In the third trimester of pregnancy an AUC decrease of 35% total darunavir and 20% of unbound darunavir was observed. Unbound darunavir was >10-fold above the wild-type EC_{50}, high viral suppression rates were maintained during pregnancy and no MTCT was reported.[14] Both studies concluded that dose adjustment to 600/100mg of darunavir/r twice daily in pregnancy would not be necessary.
Safety of darunavir use in pregnancy is summarized in the Antiretroviral Pregnancy Registry (APRegistry). Darunavir is a pregnancy category C drug according to FDA guidelines, which is defined as: "animal reproduction studies have shown an adverse effect on the foetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks." The APRegistry contains sufficient numbers of pregnancy outcomes for first trimester exposures to darunavir to rule out at least a 2-fold increase in risk of overall birth defects (n=212) compared with the reference population. The percentage of defects/live births was 2.4%, which is not different from CDC's birth defects surveillance system: i.i. 2.72%.[15]
cART might be associated with an increased rate of pre-term delivery (<37 weeks gestational age (GA)).[16,17] Some studies report higher rates of pre-term birth when cART contains protease inhibitors, but these results are not consistent.[16,18] Furthermore, no strong association was found between antiretroviral use during pregnancy and small for GA.[18]
Because the information on pharmacokinetic changes during pregnancy is limited for darunavir (especially for the 800/100mg once-daily dose) and the use of protease inhibitors during pregnancy is increasing in high- and middle-income countries, we studied the effect of pregnancy on darunavir total and unbound plasma concentrations.

Patients and methods

This was a non-randomized, open-label, multicentre, phase IV study in HIV-infected pregnant women recruited from HIV treatment centres in Europe (PANNA network: www.pannastudy.com). The PANNA Network is a European network of 19 hospitals collecting pharmacokinetic curves of several antiretrovirals during pregnancy in a prospective study. Data in this publication were collected by nine hospitals between October 2009 and January 2014.

The study was conducted in compliance with the principles of the "Declaration of Helsinki". Informed consent was obtained from each participant before entering the study. The study was approved by the Medical Ethics Committee from each individual centre involved and by the national authorities if applicable. The study is registered at ClinicalTrials.gov under number NCT00825929.
Patient eligibility included being HIV-infected, pregnant, at least 18 years of age at

screening and treated with a cART regimen containing darunavir for at least two weeks prior to the first pharmacokinetic curve evaluation (in the third trimester of pregnancy). Patients were excluded if they had a past medical history or current condition that might interfere with drug absorption, distribution, metabolism or excretion or presented with grade III/IV anaemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL) at screening.

Safety assessments and viral load

Inclusion screening consisted of: medical history, physical examination, serum biochemistry, haematology and qualitative urinalysis, HIV-1 RNA load and CD4+ T cell count determination. Analyses for safety assessments were performed by local laboratories. Blood samples for safety assessments were further taken at the visits for pharmacokinetic blood sampling. Patients were asked for adverse events at each visit. Birth weight, congenital abnormalities and HIV-status of the infants were collected.

Pharmacokinetic blood sampling

A 12 or 24 hour pharmacokinetic curve was obtained after at least 2 weeks of darunavir treatment during the third trimester (preferably at week 33) and at least 2 weeks postpartum (preferably 4-6 weeks postpartum). At delivery (where possible) a cord blood sample and a contemporary maternal blood sample were collected. Total concentrations of darunavir in plasma were analyzed in all samples. Unbound darunavir concentrations were analyzed in two samples per curve (1 sample with low and 1 sample with high total darunavir concentrations). Samples were analyzed by the laboratory of the Pharmacy of the Radboud university medical center.

A standard breakfast (650kCal; 30g fat) commenced prior to (observed) dosing on the pharmacokinetic days. A 6 mL venous blood sample was collected into heparin tubes just before drug intake (pre-dose) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and (if applicable) 24h post-medication intake on both pharmacokinetic study days. Plasma was separated and stored at <-18°C until shipment on dry ice to the central laboratory for analysis.

Analytical and pharmacokinetic methods

Concentrations of total darunavir and ritonavir in plasma were analyzed by use of a validated (ultra)HPLC method for the simultaneous quantitative determination of several HIV protease inhibitors in human plasma.[19] The lower limit of quantification (LLOQ) was 0.10 mg/L for darunavir and 0.045 mg/L for ritonavir. The assays were externally validated through ACTG and KKGT.[20,21]

Protein-bound and -unbound darunavir was separated using an ultrafiltration method (using pre-washed Centifree-30K protein binding filters, Amicon). The unbound fraction was analysed using the same UPLC method as described above with an LLOQ of 0.015 mg/L (range 0.015-1.5 mg/L). Unbound concentrations were assessed in a sample with a low (total) darunavir concentration and a high (total) darunavir concentration per pharmacokinetic curve, if the sample volume was sufficient for the analysis.

Pharmacokinetic parameters were determined using a non-compartmental model in WinNonlin version 6.3 (Pharsight Corporation, CA, USA). Area under the curve over a dosing interval (AUC_{tau}, $AUC_{0\text{-}12h}$/$AUC_{0\text{-}24h}$) using the trapezoidal rule, trough concentration (C_{12h}/C_{24h}) defined as the sample taken at time point 12hr or 24hr, maximum concentration (C_{max}), elimination half life (T_{half}), time of maximum concentration (T_{max}) and apparent clearance (CL/F, being the dose/AUC_{tau}) were determined per individual curve.

Statistical analysis data handling

Patients for whom a curve was taken during pregnancy were included in demographic, safety analyses and descriptive statistics of the pharmacokinetic parameters. Geometric mean ratios (GMR) were calculated for the patients with a curve in the third trimester and a postpartum curve. Pharmacokinetic parameters are reported as geometric means with 95% intervals. GMRs of AUC_{tau}, C_{max}, $C_{12h/24h}$, CL/F and T_{half} of third trimester versus postpartum were calculated for both dosing regimens separately using a mixed-effects model in WinNonlin/Phoenix. Unbound plasma concentrations and the percentage unbound were determined, and mean percentage unbound darunavir was calculated for the third trimester and postpartum. Cord blood/maternal blood concentration ratios were determined and described.

Results

Twenty-four patients receiving darunavir during pregnancy were enrolled in the study. Six patients used 600/100mg of darunavir/r twice daily and 17 used 800/100mg of darunavir/r once daily, one patient used 600/100mg darunavir/r once daily (due to previous hepatotoxicity and high darunavir concentrations on 800/100mg once daily the dose had been reduced by the treating physician, liver function normalised after dose reduction). Characteristics of the patients as well as pregnancy outcome summarized for the total group and per dosing regimen are depicted in Table 1. Approximately 50% of the patients were white; smoking, alcohol use and drug use only appeared in the 800/100mg once daily regimen group. The age at delivery was similar between both regimens (median age was 31 and 33 years, respectively). Most patients (75%) conceived whilst taking cART; however, only 25% were on darunavir at conception. If not used prior to conception, darunavir was started mainly during the first and second trimester, only three patients (13%) started darunavir in the third trimester, of which only one patient was antiretroviral treatment-naive. Darunavir/r and two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) was used by nine (50%) of the patients on 800/100mg of darunavir/r once daily and by one patient on 600/100mg of darunavir/r twice daily. Most other patients used at least four antiretroviral drugs, mainly two NRTIs, darunavir/r and raltegravir or maraviroc. One patient used 800/100mg of darunavir/r

Table 1. Patient characteristics

Screening	All n=24	600/100mg DRV/r BID n=6	800/100mg DRV/r QD n=18[a]
Age at delivery, years (range)	30 (20-44)	31 (24-41)	33 (20-44)
Caucasian race/ Black race, n (%)	11 (46%)/ 13 (54%)	2 (33%)/ 4 (66%)	9 (50%)/ 9 (50%)
Smoking, n (%)	4 (17%)	0 (0%)	4 (22%)
Alcohol, n (%)	2 (8%)	0 (0%)	2 (11%)
Drugs, n (%)	1 (4%)	0 (0%)	1 (6%)
Treatment-naive at start pregnancy, n (%)	6 (25%)	1 (17%)	5 (28%)
ARV treatment duration before pregnancy, median weeks (range)	235 (28-813)	107 (28-127)	384 (60-813)
Conception on darunavir, n (%)	6 (25%)	2 (33%)	4 (22%)
Start darunavir per trimester, n (%)	6 (25%) 1st trimester	2 (33%) 1st trimester	4 (22%) 1st trimester
	9 (38%) 2nd trimester	1 (17%) 2nd trimester	8 (44%) 2nd trimester
	3 (13%) 3rd trimester	1 (17%) 3rd trimester	2 (11%) 3rd trimester
Concomitant ARV			
NRTI, n (%)		3 (50%) zidovudine; 2 (33%) tenofovir	13 (72%) tenofovir; 11 (61%) emtricitabine
		1 (17%) emtricitabine; 2 (33%) lamivudine	2 (11%) lamivudine
Integrase inhibitor, n (%)		2 (33%) raltegravir	6 (33%) raltegravir
Fusion/entry inhibitor, n (%)		2 (33%) maraviroc; 1 (17%) enfuvirtide	2 (11%) maraviroc
NNRTI, n (%)		1 (17%) etravirine	
Third trimester			
Gestinational age, weeks (range)	34 (31-37)	33 (31-36)	34 (32-37)
Weight, kg (range)	80 (65-117)	80 (70-103)	80 (65-117)
Delivery			
Gestational age, weeks (range)	38 (36-41)	38 (37-39)	38 (36-41)
Vaginal delivery, n (%)	6 (25%)	2 (33%)	4 (22%)
Undetectable viral load <50 copies/mL, n (%)[b]	16 (67%), unk 1 (4%)	3 (50%)	13 (72%), unk 1 (6%)
CD4+ T cell count, cells/mm^3 (range)[b]	479 (82-1196), unk2	330 (82-837)	591 (151-1196), unk 2
Pregnancy outcomes			
Birth weight, g (range)	3090 (2060-3718)	3260 (2470-3700)	3075 (2060-3718)
Small for gestational age, n (%)[c]	6 (25%)	1 (17%)	5 (28%)
HIV infected childs, n	0	0	0

Table 1. - continued Patient characteristics

Screening	All n=24	600/100mg DRV/r BID n=6	800/100mg DRV/r QD n=18[a]
Postpartum	**All n =18**	**600/100mg DRV/r BID n=6**	**800/100mg DRV/r QD n=12[a]**
Time after delivery, weeks (range)	5 (3-14)	5 (4-9)	5 (4-13)
Weight, kg (range)	76 (62-109)	76 (63-109)	76 (62-100)
Undetectable viral load <50 copies/mL, n (%)	12 (67%)	3 (50%)	9 (75%)
CD4+ T cell count, cells/mm^3, median (range)	445 (93-1279), unk 6	385 (93-716)	496 (149-1279), unk 6

ARV, antiretroviral; BID, twice daily; QD, once daily; DRV/r: darunavir/ritonavir, NRTI; nucleoside reverse transcriptase inhibitor, NNRTI: nonnucleoside reverse transcriptase inhibitor [a]: Subject using DRV/r 600/100mg QD was included in 800/100mg QD analysis. [b]: Undetectable viral load and CD4+ count measured at delivery in 7 subjects. For the other subjects (n=17) the viral load and CD4+ count from the third trimester were used. The median time between sampling third trimester and delivery, weeks (range) was 4 (0-6) [c]: Small for gestational age (SGA) was determined as 10th percentile of birth-weight-for-age, based on US population-based reference for SGA

as monotherapy. No other concomitant medication was used which could potentially influence darunavir or ritonavir exposure.

A total of 21 evaluable darunavir curves were collected in the third trimester (five for the twice-daily regimen and 16 for the once-daily regimen) and 13 evaluable postpartum curves (five for the twice-daily regimen and eight for the once-daily regimen). Reasons for non-evaluable curves in the third trimester were: one patient did not have measurable darunavir concentrations in the samples (twice-daily regimen), probably due to non-adherence, and was excluded from pharmacokinetic analysis (third trimester and postpartum); one patient on the once-daily regimen took the darunavir/r medication at night, a 12-24h curve was collected (this patient was only partly included in the analysis); one patient on once-daily regimen used 600/100mg of darunavir/r once daily and was only included in the GMR calculations. Additionally six patients did not have a postpartum curve: five withdrew consent and one was lost to follow-up. One patient had only a postpartum curve collected until 4 hours after dosing and C_{max} was not observed, and one patient did not have measurable concentrations in the postpartum curve. For some patients the 12h or 24h sample was not collected: one patient on 600/100mg of darunavir/r twice daily in the third trimester; four patients on 800/100mg of darunavir/r once daily in the third trimester and two patients on 800/100mg of darunavir/r once daily postpartum. Curves were taken at median (range) 34 (31-37) weeks GA for the third trimester and median (range) 5 (3-14) weeks postpartum.

Pharmacokinetics

The mean plasma concentration-time profiles of darunavir in the third trimester and postpartum are presented by regimen in Figure 1; summary statistics of the PK parameters are listed in Table 2 for darunavir and in supplementary Table 1 for ritonavir.

AUC_{tau} was 22% and 34% lower in the third trimester for 600/100mg twice daily and 800/100mg once daily, respectively. For the twice-daily regimen the 90% CI of the GMR ranged from 60-100% and for the once-daily dose this was 56-82% (intra-subject comparison). C_{max} was 24% and 22% lower during pregnancy and C_{12h}/C_{24h} was 11% and 42% lower for twice-daily and once-daily regimen, respectively. Mean protein-free fraction of darunavir (95% CI) was 12% (11-13%, 44 samples of 19 patients) in the third trimester and 10% (9-11%, 30 samples of 14 patients) postpartum.

For the 800/100mg once-daily regimen one patient (8%) had documented darunavir concentrations <0.55 mg/L (EC_{50} for resistant virus), but >0.055 mg/L (EC_{50} for wild-type virus) in the third trimester. None of the patients on 600/100mg of darunavir/r twice daily had observed concentrations below this target. In Figure 2 individual AUC_{tau} and

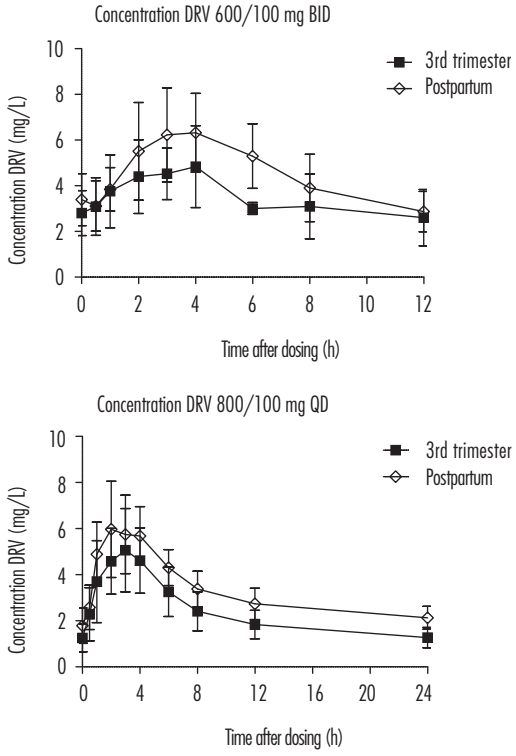

Figure 1. Mean concentration-time profiles

Darunavir (DRV) concentrations at steady-state after both the twice-daily (BID) as well as once-daily (QD) dosing regimen.

Table 2. Darunavir pharmacokinetic parameters

	GM (95% CI) Third Trimester	GM (95% CI) Postpartum	GM Ratio (90% CI) Third Trimester / Postpartum
Darunavir 600/100mg BID	**(n=5)**	**(n=5)**	
$AUC_{0\text{-}12h}$ (h·mg/L)	41.0 (27.2-61.8)	52.8 (38.2-73.1)	0.78 (0.60-1.00)
C_{max} (mg/L)	4.98 (3.17-7.82)	6.5 (4.36-9.70)	0.76 (0.53-1.11)
T_{max} (h)	4 (2-6)	3 (2-4)	
$C_{predose}$ (mg/L)	2.68 (1.76-4.08)	3.26 (2.21-4.81)	0.82 (0.70-0.96)
C_{12h} (mg/L)	2.41 (1.20-4.86)[b]	2.76 (1.84-4.13)	0.89 (0.58-1.36)
t_{half} (h)	8.5 (6-12)	7.6 (4.2-13.7)	1.12 (0.79-1.59)
CL_{ss}/F (L/h)	15 (10-22)	11 (8-16)	1.28 (1.00-1.66)
Darunavir 800/100mg QD	**(n=16)**	**(n=8)**	
$AUC_{0\text{-}24h}$ (h·mg/L)	52.9 (44.5-63.0)	76.2 (64.9-89.5)	0.67 (0.56-0.82)[a]
C_{max} (mg/L)	5.30 (4.49-6.25)	6.75 (5.86-7.77)	0.78 (0.65-0.95)[a]
T_{max} (h)	3 (1.5-6)	2.5 (1-7)	
$C_{predose}$ (mg/L)	1.21 (0.92-1.61)	1.62 (1.06-2.48)	0.77 (0.54-1.10)[a]
C_{24h} (mg/L)	1.14 (0.90-1.46)[c]	2.05 (1.65-2.55)[d]	0.58 (0.44-0.78)[a]
t_{half} (h)	13 (9-18)	21 (13-34)	0.59 (0.40-0.87)[a]
CL_{ss}/F (L/h)	15 (13-18)	10.5 (9-12)	1.50 (1.24-1.81)[a]

[a] GMR includes one patient using 600/100mg QD [b] 1 value (20%) was missing, no sample taken at 12h after dosing
[c] 4 values (25%) were missing, no sample taken at 24h after dosing [d] 2 values (25%) were missing, no sample taken at 24h after dosing

C_{12h}/C_{24h} results during pregnancy and postpartum are depicted.
Ritonavir exposure (AUC_{tau}) was markedly decreased during pregnancy: 26% and 41% for twice-daily and once-daily regimen, C_{max} was even more affected: 37% and 41% lower for the twice-daily and once-daily regimen, respectively (Table 3).

Eleven umbilical cord blood samples were collected with matching maternal blood samples. The median time between the reported last dose and cord blood sampling was 9.1h (range 1.4-19.0h); the median time between cord blood sample and maternal sample was 10 minutes (0-79 min). Darunavir concentrations were below the LLOQ in three cord blood samples (with matching maternal samples ranging from 0.18-0.79

Table 3. Ritonavir pharmacokinetic parameters

	GM (95% CI) Third Trimester	GM (95% CI) Postpartum	GM Ratio (90% CI) Third Trimester / Postpartum
Darunavir 600/100mg BID	**(n=5)**	**(n=5)**	
$AUC_{0\text{-}12h}$ (h·mg/L)	4.67 (3.00-7.28)	6.32 (4.54-8.79)	0.74 (0.65-0.84)
C_{max} (mg/L)	0.61 (0.38-0.98)	0.96 (0.58-1.60)	0.63 (0.53-0.76)
T_{max} (h)	4 (3-8)	4 (2-6)	
C_{12h} (mg/L)	0.22 (0.11-0.41)	0.30 (0.18-0.49)	0.72 (0.56-0.92)
t_{half} (h)	5.5 (4-7)	5 (4-6)	1.17 (0.95-1.43)
CL_{ss}/F (L/h)	21 (14-33)	16 (11-22)	1.35 (1.18-1.55)
Darunavir 800/100mg QD	**(n=16)**	**(n=8)**	
$AUC_{0\text{-}24h}$ (h·mg/L)	3.11 (2.19-4.42)	5.16 (3.60-7.42)	0.59 (0.42-0.83)[a]
C_{max} (mg/L)	0.31 (0.23-0.44)	0.56 (0.37-0.83)	0.59 (0.42-0.82)[a]
T_{max} (h)	4 (1.5-8)	4 (3-7)	
C_{24h} (mg/L)	0.03 (0.02-0.04)[b]	0.041 (0.03-0.06)[c]	0.72 (0.47-1.10)[a]
t_{half} (h)	6 (5-7)	6 (5-8)	1.00 (0.75-1.33)[a]
CL_{ss}/F (L/h)	32 (23-46)	19 (13-28)	1.70 (1.20-2.41)[a]

[a] GMR includes one patient using 600/100mg QD; [b] 13 (81%) below LLOQ, taken as 1/2 LOQ, i.e. 0.0225 mg/L; [c] 3 (38%) below LLOQ, taken as 1/2 LOQ, i.e. 0.0225 mg/L

mg/L). Ritonavir concentrations were below LLOQ in all cord blood samples. The median (range) ratio of darunavir cord blood/maternal blood was 0.13 (0.08-0.35; n=8).

Efficacy and safety

The median GA at delivery was 38 (range, 36-41) weeks. All children were tested HIV-negative (PCR DNA after delivery) and no congenital abnormalities were reported. One infant (4%) was born between 36 and 37 weeks GA. Four babies had a low birth weight (<2,500 gram: 2,060-2,470 gram). Six children (25%) were small for GA (reference 10th percentile of birth weight for GA by gender, US, 1991).[22]

One (maternal) serious adverse event (SAE) was reported: one patient on darunavir 800/100mg once-daily was hospitalized for 3 days (starting at 34 weeks and 4 days GA) because of suspicion of pre-eclampsia, which was not confirmed. The patient was released from the hospital and delivered at 37 weeks and 2 days GA. This SAE was judged not to be related to darunavir/r treatment. Seven patients reported adverse events, all but 1 were grade 1 or 2 and not or unlikely related to the cART given. Severe anaemia (due to haemorrhagic delivery) was reported for one subject; she recovered and this adverse event was not related to darunavir/r.

HIV viral load close to delivery was undetectable (<50 copies/mL) in the majority (67%) of the women. However, a detectable viral load was seen in 3/6 women on the twice-daily regimen (242, 272 and 1,610 copies/mL) and 4/18 women on once-daily regimen (74; 121; 144; 28,711 copies/mL) around delivery.

Pharmacokinetic – Efficacy relationship

As reported above, HIV viral loads were detectable (>50 copies/mL) for 7 patients around delivery. See Table 4 for detailed information of these patients. The two patients with the high viral load (i.e. 1,610 and 28,711 copies/mL respectively) were probably

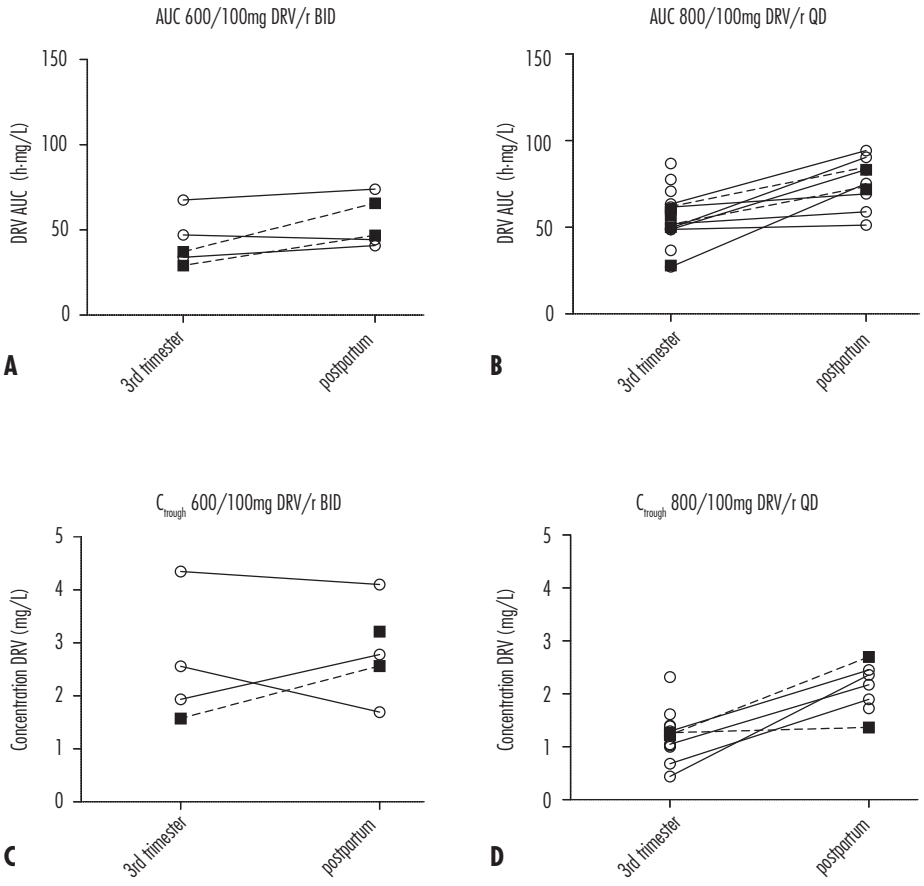

Figure 2. Individual darunavir AUC and C_{trough} levels
Open circle and continuous lines: undetectable viral load around delivery (<50 copies/mL) filled squares and broken lines: detectable viral load around delivery (>50 copies/mL) DRV/r=darunavir/ritonavir; BID = twice daily; QD = once daily

not adherent; they did not have detectable darunavir concentrations in the plasma during the third trimester visit and/or postpartum. All other patients with detectable viral load around delivery reported to have been adherent to therapy during that time period. In Figure 2 individual AUCs and C_{trough} levels are presented for patients with and without detectable viral load. For patients reported to be adherent, darunavir AUC and C_{trough} were similar between patients with or without detectable viral load for the once-daily regimen. For the twice-daily regimen the C_{trough} and AUCs show a trend towards lower concentration and exposure for the patients with a detectable viral load in the third trimester of pregnancy.

Table 4. Detailed information patients with detectable viral load around delivery

					darunavir concentrations		
DRV	cART	cART use at pregancy start	Viral load around delivery (copies/mL)	Viral load postpartum (copies/mL)	3rd trim predose (mg/L)	3rd trim 12h or 24h (mg/L)	postpar-tum 12h or 24h (mg/L)
600/100mg BID	lamivudine + zidovudine + tenofovir	Yes	525	74	1.8	1.6	2.565
600/100mg BID	etravirine + fuzeon	Yes	1,610 (<50 at 3rd trim visit)	16,300	<LLOQ	<LLOQ	<LLOQ
600/100mg BID	lamivudine + zidovudine, raltegravir added after 3rd trim visit	Yes	242	99	2.9	No sample	3.216
800/100mg QD	tenofovir + emtricitabine	Yes	121	112	1.136	1.263	1.365
800/100mg QD	raltegravir	Yes	74	Undet <50c/mL	1.324	1.222	2.699
800/100mg QD	tenofovir + emtricitabine + raltegravir	No, start in 2nd trimester	144		2.252	No sample	Not done
800/100mg QD	tenofovir + emtricitabine	Yes	28711	5673	<LLOQ	<LLOQ	Not done

Discussion

In this paper, we describe the pharmacokinetics of darunavir/r in 24 pregnant HIV-infected patients (6 on 600/100mg darunavir/r twice daily and 18 on darunavir/r once daily), in the third trimester of pregnancy and after delivery. In the third trimester of pregnancy a decrease in total darunavir AUC_{tau}, C_{max} and $C_{12h/24h}$ was observed for both treatment regimens. Darunavir free fraction was similar during pregnancy and postpartum. For one patient on 800/100mg darunavir/r once daily (8%) darunavir a C_{trough} below the EC_{50} for resistant virus (0.55mg/L) was reported in the third trimester. Transplacental passage of darunavir was low: 0.13 (median ratio of darunavir cord blood/maternal blood), and ritonavir was undetectable in all cord blood samples. Darunavir was well tolerated during pregnancy, none of the children was tested HIV-positive and no congenital abnormalities were reported. The median GA at delivery was 38 weeks, with a range of 36-41 weeks. Six children (25%) were small for GA (reference 10th percentile of birth weight for GA by gender, US, 1991),[22] which is higher than observed in the US for children born from HIV-infected women (7.3%).[18]

The darunavir postpartum curves reported in this study were comparable to the reference pharmacokinetic curves reported in literature for both the 600/100mg twice-daily dose[5,23] and the 800/100mg once-daily dose,[4,5,23] indicating that the postpartum curves can be used as reference for the normal, non-pregnant, situation.

The decreased exposure (22%) for darunavir/r 600/100mg twice daily in the third trimester of pregnancy was in line with the 18% and 26% decrease in AUC observed by Zorrilla *et al.* and Capparelli *et al.*[10,11] However, in this study, we did not find a difference in fraction unbound between the pregnant and non-pregnant situation, whereas Zorrilla *et al.* described a slightly higher fraction unbound during pregnancy, resulting in a less pronounced decrease in unbound darunavir in pregnancy (appr. 7% decrease in AUC).[10]

In this study, for darunavir/r 800/100mg once daily a 33% lower AUC_{tau} was observed in the third trimester compared with postpartum. This is also in line with previously reported data by Curran *et al.* and Crauwels *et al.* (31% and 35% decrease of total darunavir AUC)[13,14] and slightly more pronounced than reported by Capparelli *et al.* (24% decrease in AUC).[11] The darunavir pharmacokinetic parameters we describe tend to be marginally lower compared with both other studies describing pharmacokinetics in pregnancy after 800/100mg of darunavir/r once daily.[11,13] Darunavir C_{trough} in the third trimester after 800/100mg of darunavir/r once daily reported by Courbon *et al.*[12] (1.08 mg/L) was in line with the levels observed in our study (1.14 mg/L).

In this study, for one patient a C_{24h} concentration below the EC_{50} for resistant virus (but above the EC_{50} for wild type virus) was reported in the third trimester (800/100mg of darunavir/r once daily). This patient had an undetectable viral load around delivery. For

the twice-daily dosing regimen the C_{12h} concentrations seem lower in the third trimester for patients with a detectable viral load around delivery; however, the C_{12h} concentrations were well above the EC_{50} for resistant virus for the adherent patients. The number of patients is too low to draw conclusions. A clear relationship between low darunavir plasma concentrations and detectable viral load could not be demonstrated for these dosing regimens.

A limitation of the study is that we determined the unbound darunavir concentrations in only a limited number of samples and not for the entire curve, because not enough plasma was available to perform the analyses. Another limitation is the fact that we did not collect genotyping data; therefore information on possible protease inhibitor resistance is not known.

Physiological changes possibly causing lower exposure to darunavir and ritonavir during pregnancy are reduced intestinal motility, a larger plasma volume, increased hepatic blood flow, decreased protein binding and induced hepatic enzymes. The C_{max} for both compounds was approximately 20-60% lower in the third trimester, which potentially is caused by a larger plasma volume, possibly decreased gastrointestinal absorption and/or slower gastro intestinal transit time. For the 800/100mg once-daily dose, the darunavir half-life was shorter during pregnancy, indicating a faster elimination (possibly due to enzyme induction), which could lead to lower trough levels, especially in case of (accidental) prolonged (>24h) dosing intervals. It should be noted that half-life calculations are less reliable because we only cover a limited period of the terminal half-life in this dosing period, especially for the twice-daily dosing regimen. Next to the physiological changes, the reduced ritonavir concentrations might imply less boosting, resulting in lower darunavir concentrations (and faster elimination). Ritonavir exposure was approximately 26% (twice daily) and 41% (once daily) lower during pregnancy. This is in line with the ritonavir AUC decrease reported for 800/100mg of darunavir/r once daily[14] and 600mg/100mg of darunavir/r twice daily[10] during pregnancy and also similar to results when ritonavir was combined with atazanavir (once daily).[24,25] Assuming dose-linear pharmacokinetics for ritonavir, this decrease would be similar to a 50mg dose. In a study in healthy volunteers steady state 800/50mg of darunavir/r once daily revealed that the darunavir AUC was only approximately 13% lower compared to 100mg boosting. Darunavir C_{max} was not affected but the largest effect was seen on C_{min}: 32% lower for the 50mg ritonavir dose. Furthermore, ritonavir concentrations decreased more than dose-linear in that study.[26] Although less boosting due to lower ritonavir concentrations probably only partly explains the decreased exposure during pregnancy, it might explain the more prominent decrease in exposure to darunavir in the 800/100mg once daily regimen compared to the 600/100mg twice daily regimen.

In conclusion, darunavir AUC and C_{max} are substantially decreased in pregnancy (22-34%) for both 600/100mg of darunavir/r twice daily and 800/100mg of darunavir/r once daily. This decrease in exposure did not result in mother-to-child-transmission.

For antiretroviral-naive patients, who are adherent, take darunavir with food and are not using concomitant medication reducing darunavir concentrations, 800/100mg of darunavir/r once daily is adequate in pregnancy. For all other patients, 600/100mg of darunavir/r twice daily is recommended during pregnancy.

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6

The Pharmacokinetics, Safety and Efficacy of Tenofovir and Emtricitabine in HIV-1 Infected Pregnant Women

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Abstract

Objective: To describe the pharmacokinetics of tenofovir and emtricitabine in the third trimester of pregnant HIV-infected women and at postpartum.

Design: A nonrandomized, open-label, multicentre phase IV study in HIV-infected pregnant women recruited from HIV treatment centres in Europe.

Methods: HIV-infected pregnant women treated with the nucleotide/nucleoside analogue reverse transcriptase inhibitors (NRTIs) tenofovir disoproxil fumarate (300mg; equivalent to 245mg tenofovir disoproxil and/or emtricitabine (200mg) were included in the study. Twenty-four-hour pharmacokinetic curves were recorded in the 3rd trimester (preferably week 33) and postpartum (preferably week 4-6). Collection of a cord blood sample and maternal sample at delivery was optional. Pharmacokinetic parameters were calculated using WinNonlin software version 5.3. Statistical analysis was conducted using SPSS version 16.0.

Results: Thirty-four women were included in the analysis. Geometric mean ratios of 3rd trimester vs. postpartum [90% confidence interval (CI)] were 0.77 (0.71-0.83) for tenofovir $AUC_{0\text{-}24h}$; 0.81 (0.68-0.96) for tenofovir C_{max} and 0.79 (0.70-0.90) for tenofovir C_{24h}, and 0.75 (0.68-0.82) for emtricitabine $AUC_{0\text{-}24h}$; 0.87 (0.77-0.99) for emtricitabine C_{max} and 0.77 (0.52-1.12) for emtricitabine C_{24h}. The viral load close to delivery was less than 200 copies/mL in all but one patient, the average gestational age at delivery was 38 weeks. All children were tested HIV negative and no congenital abnormalities were reported.

Conclusions: Although pharmacokinetic exposure of the NRTIs tenofovir disoproxil and emtricitabine during pregnancy is approximately 25% lower, this was not associated with virological failure in this study and did not result in mother-to-child transmission.

Introduction

In 2010, approximately 17.5 million women were infected with HIV, most of who were of child bearing age.[1] It is estimated that 39% of the European women infected with HIV have a desire for childbearing in the future, which is comparable to HIV-uninfected women.[2] This has also been reported for women infected with HIV in the USA[3] and South Africa.[4]

Combination antiretroviral therapy (cART) has been shown to be a highly effective strategy for preventing mother-to-child transmission (PMTCT) of HIV, reducing the risk from 15-40% to less than 2%.[5] The US Department of Health and Human Services (DHHS) guidelines recommend the inclusion of one or more nucleoside analogue reverse transcriptase inhibitors (NRTIs) with good transplacental passage in the cART regimen, when feasible.[6] The most commonly used NRTIs are zidovudine (ZDV) and lamivudine (3TC), mainly because of the vastly greater clinical experience with these compounds during pregnancy. However, an overview of antiretroviral prescribing during pregnancy between 1995 and 2009 showed an increase of tenofovir disoproxil/emtricitabine use to approximately 30%, whereas ZDV/3TC use during pregnancy decreased from approximately 90% to 70%.[7] This reflects the recommendations for first-line NRTI back-bone (tenofovir disoproxil/emtricitabine combination) in non-pregnant adults.[8] All four NRTIs cross the placenta well.[9-14] The current summary of product characteristics of Truvada®[15] states that its use may be considered during pregnancy, if necessary. Safety issues on the use of antiretrovirals during pregnancy concern exposure of the mother, influence on pregnancy duration and teratogenicity. cART use (especially protease inhibitor based) during pregnancy has been reported to be associated with an increased rate of pre-term delivery (<37 weeks gestational age (GA)) in European studies.[16] Most North American studies have not shown this association.[17,18]

The antiretroviral pregnancy registry interim report (up to 31 Jan 2012) did not detect a two-fold increase in risk of overall birth defects: the prevalence of birth defects of both tenofovir disoproxil and emtricitabine was 2.3%,[19] compared with 2.1% prevalence of major birth defects in the European general population.[20] Two individual cases of pyelectasis in children born from mothers receiving tenofovir disoproxil-containing therapy during pregnancy have been described.[21] In a macaque model, perinatal exposure to very high doses of tenofovir disoproxil resulted in bone toxicity in some offspring.[22] This has not yet been reported in humans,[23,24] nor in another macaque model.[25] In studies with emtricitabine during pregnancy, no emtricitabine-related congenital anomalies were reported [26,27] and emtricitabine animal studies do not indicate reproductive toxicity.[28]

Human physiology alters during pregnancy, potentially affecting the pharmacokinetics

of drugs,[29-31] mostly resulting in lower exposure of medication during pregnancy.

Pharmacokinetic parameters of chronic exposure to tenofovir (TFV) during pregnancy have been presented as abstracts at conferences only. It was concluded that exposure during pregnancy is lower, but with area under the curves (AUCs) not below the 10th percentile of nonpregnant patients (2 mg·h/L) for most women.[10] In studies of single-dose tenofovir disoproxil given for HIV PMTCT at onset of labour, doses of 600mg and 900mg tenofovir disoproxil, which are higher that for chronic administration (300mg), have been used.[14,27] For the 600mg dose, plasma concentrations were similar to those observed after chronic administration of 300mg tenofovir disoproxil fumarate in non-pregnant adults[12,14] and with an initial dose of 600mg tenofovir disoproxil fumarate in nonpregnant adults.[11] A population study of 186 women (of whom 46 were pregnant), with a sparse sampling method, showed 39% higher apparent clearance of tenofovir in the pregnant women.[32]

Pharmacokinetic parameters of chronic exposure to emtricitabine have been reported as lower during pregnancy, but the magnitude of the decrease appears to be small, 10%[33] to 18%.[26] When 400mg is administered at labour initiation, the plasma concentrations appear higher than after chronic administration of 200mg emtricitabine in non-pregnant adults.[34]

As information on pharmacokinetic changes during pregnancy is limited (especially for chronic use during pregnancy) and the use of tenofovir disoproxil and emtricitabine during pregnancy is increasing, we studied the effect of pregnancy on tenofovir and emtricitabine pharmacokinetics.

Methods

This was a nonrandomized, open-label, multicentre phase IV study in HIV-infected pregnant women recruited from HIV treatment centres in Europe (PANNA network: www.pannastudy.com). The PANNA network is a European network of hospitals collecting pharmacokinetic curves of several antiretroviral drugs during pregnancy in a prospective study. In total, 17 hospitals are involved in the network, data in this publication were collected by 10 hospitals between November 2008 and January 2012.

The study was conducted in compliance with the principles of the "Declaration of Helsinki". Informed consent was obtained from each participant before entering the study. The study was approved by the medical ethical committee from each individual centre involved and by the national authorities if applicable. The study is registered at ClinicalTrials.gov under number NCT00825929.

Patient eligibility included being HIV infected, pregnant, at least 18 years of age at screening and treated with a cART regimen containing tenofovir disoproxil and/or emtricitabine for at least 2 weeks before the day of first pharmacokinetic curve evaluation (in the third trimester of pregnancy). Patients were excluded if they had a past medical history or current condition that might interfere with drug absorption, distribution, metabolism or excretion (such as renal failure or hepatic failure) or presented with grade III/IV anaemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL) at screening.

Safety assessments and viral load

Inclusion screening consisted of: medical history, physical examination, serum biochemistry, haematology and qualitative urinalysis, HIV-1 RNA load and CD4 T cell counts. Analyses for safety assessments were performed by local laboratories. Blood samples for safety assessments were further taken at the visits for pharmacokinetic blood sampling and at delivery (if they delivered at the hospital). Patients were asked for adverse events at each visit, the DAIDS toxicity table (2004) was used to grade the reported adverse events. The HIV-status of the infants was collected.

Pharmacokinetic blood sampling

A 24-hour pharmacokinetic curve was recorded after at least two weeks of tenofovir disoproxil and/or emtricitabine treatment during the third trimester (preferably at week 33) and at least 2 weeks postpartum (preferably 4-6 weeks postpartum). At delivery (if possible) a cord blood sample and a blood sample from the mother were taken. Concentrations of tenofovir and emtricitabine in plasma were analyzed by the laboratory of the Pharmacy of the Radboud University Nijmegen Medical Centre.
A standard breakfast (650kCal; 30g fat) was served prior to (observed) dosing on the pharmacokinetic days. Six mL of blood was collected just before drug intake (predose) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24h after medication intake (10 samples) at all pharmacokinetic study days. Plasma was separated and stored at -18°C or lower until shipment on dry ice to the central laboratory for analysis.

Analytical and pharmacokinetic methods

Concentrations of tenofovir and emtricitabine in plasma were analysed by use of a validated reversed phase high-pressure liquid chromatography (HPLC) method with fluorescence detection.

Sample preparation for tenofovir consisted of a liquid-liquid extraction. The solution was injected onto a SymmetryShield RP 18 column (3.5 mm, 150 x 4.6 mm). The flow rate was set at 1.0 mL/min and tenofovir was detected by use of a fluorescence detector ($L_{excitation}$=232 nm, $L_{emission}$=420 nm). Tenofovir lower limit of quantification (LOQ) was 0.015 mg/L. The linear calibration ranges in plasma were 0.015-1.5 mg/L.

Sample preparation for emtricitabine consisted of a solid-phase extraction. 190 µL of the solution was injected onto an Atlantis CP18 column (5 mm, 150 x 4.6 mm). The flow rate was set at 1.0 mL/min. Emtricitabine was detected by use of a fluorescence detector ($L_{excitation}$=244 nm, $L_{emission}$=356 nm). Emtricitabine LOQ was 0.030 mg/L. The linear calibration ranges in plasma were 0.03-5.0 mg/L. The assays were externally validated through ACTG.[35]

Pharmacokinetic parameters were determined using a noncompartmental model in WinNonlin version 5.3 (Pharsight Corporation, Sunnyvale, California, USA). Area under the curve ($AUC_{0\text{-}24h}$) using the trapezoidal rule, the trough concentration (C_{24h}) defined as the sample taken at time point 24hr (or extrapolated if the sample was missing), maximum concentration (C_{max}), elimination half-life (T_{half}), time of maximum concentration (T_{max}) and apparent clearance (CL/F, being the dose/$AUC_{0\text{-}24h}$) were determined per individual curve.

Statistical analysis data handling

Patients for whom a curve was taken during pregnancy were included in demographic, safety analyses and descriptive statistics of the pharmacokinetic parameters. Geometric mean ratios (GMRs) with 90% confidence intervals (CIs) were calculated for the patients with a curve in the third trimester and a postpartum curve. Pharmacokinetic parameters are reported as geometric means with 95% CIs. GMRs of $AUC_{0\text{-}24h}$, C_{max}, C_{24h}, CL/F and T_{half} of third trimester vs. postpartum were calculated. To indicate whether the pharmacokinetic parameters during pregnancy differed statistically significantly from the postpartum parameters a paired t-test was performed on the log-transformed parameters. Cord blood/maternal blood concentration ratios were determined and described.

Results

Thirty-four patients receiving tenofovir disoproxil and/or emtricitabine during pregnancy from 10 different sites from the PANNA network were enrolled in the study. The characteristics of the patients and pregnancy outcome are depicted in Table 1. Sixteen patients were white, 17 black and 1 was of mixed race. Eleven (32%) of the patients were treatment naive at conception and 23 were already on cART before pregnancy. Thirty-one out of 34 patients used Truvada®. Other NRTIs used were: zidovudine (n=2) and lamivudine (n=1). Twenty-four of the patients were on a boosted protease inhibitor based cART; six were on a nonnucleoside reverse transcriptase inhibitor (NNRTI) based cART; four were on the integrase inhibitor raltegravir; one was on raltegravir+protease inhibitor and one was on maraviroc+protease inhibitor. No other concomitant medication was used which could possibly influence tenofovir or emtricitabine exposure.

Table 1. Subject characteristics

	median (range) or n (%)
Age at delivery [years, median (range)]	32 (19-44)
Race/ethnicity [n (%)]	
White	16 (47%)
Black	17 (50%)
Other	1 (3%)
Smoking [n (%)]	7 (21%)
Alcohol use [n (%)]	4 (12%)
Truvada® use [n (%)]	31 (91%)
Treatment naive at pregnancy start [n (%)]	11 (32%)
ARV treatment duration before pregnancy [months, median (range)]	50 (2-135)
Concomitant antiretrovirals [n (%)]	
Protease inhibitors	24 (71%)
	11 atazanavir/r; 10 darunavir/r; 2 lopinavir/r; 2 saquinavir/r; 1 fosamprenavir/r
NNRTI	6 (18%)
	4 nevirapine; 2 efavirenz
Raltegravir	2 (6%)
Rategravir + PI	1 (3%)
Maraviroc + PI	1 (3%)
Third trimester (n=34)	
Gestational age [weeks, median (range)]	33 (28-38)
Weight [kg, median (range)]	75 (49-123)
HIV-RNA undetectable <50 [n (%)]	28 (83%) / <200: 33 (97%)
CD4 cell count [cells/µL, median (range)]	545 (120-1333)
Creatinine concentration [µmol/L, median (range)]	54 (33-71)
Creatinine clearance (Cockcroft) [mL/min, median (range)]	171 (110-292)
Post partum (n=28)	
Time after delivery [weeks, median (range)]	5 (3-9)
Weight [kg, median (range)]	70 (43-114)
HIV-RNA undetectable <50 [n (%)]	23 (82%) / <200: 28 (100%)
CD4 cell count [cells/µL, median (range)]	588 (130-1210)
Creatinine concentration [µmol/L, median (range)]	67 (50-86)
Creatinine clearance(Cockcroft) [mL/min,median (range)]	124 (82-190)

Table 1. - continued Subject characteristics

	median (range) or n (%)
Pregnancy outcomes	
Gestational age [weeks, median (range)]	38 (36-41)
Caesarian section [n (%)]	20 (65%): 3 unknown
Birth weight [g, median (range)]	3070 (2190-4350)
Infant VL undetectable [n (%)]*	34 (100%)

ARV, antiretroviral; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; VL, viral load
*HIV DNA polymerase chain reaction (PCR) test.

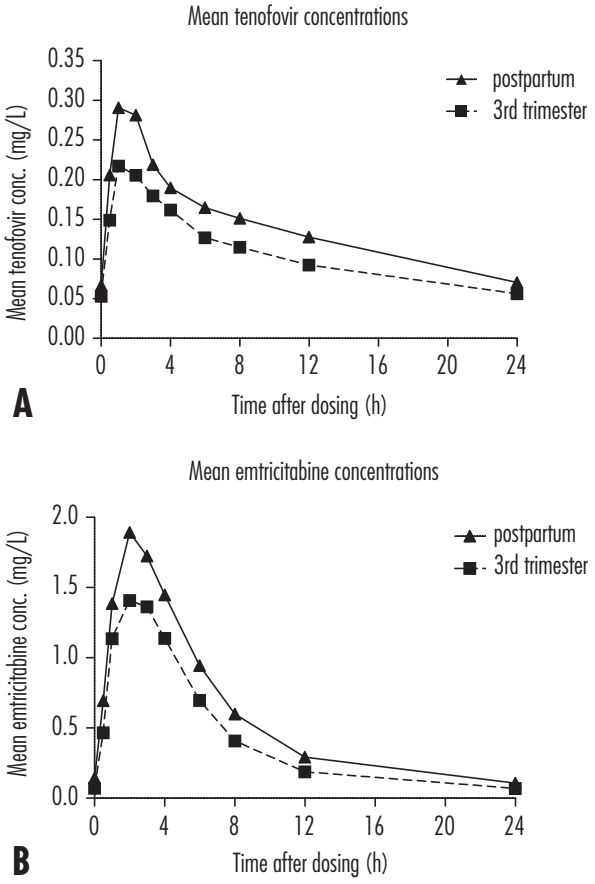

Figure 1. Mean concentration-time profiles

A total of 34 tenofovir curves were collected in the third trimester (median 33 weeks GA) and 27 curves postpartum (median 5 weeks postpartum). For emtricitabine, a total of 27 curves were collected in the third trimester and 24 postpartum. For four patients who had been treated with tenofovir disoproxil and emtricitabine insufficient plasma remained to determine the emtricitabine concentrations. Seven patients did not have a postpartum curve due to several reasons: withdrawn consent (n=2), lost to follow-up (n=3), changed medication (n=1) and insufficient plasma for analysis (n=1).

Pharmacokinetics

The mean plasma concentration-time profiles of tenofovir and emtricitabine in the third trimester and postpartum are presented in Figure 1; summary statistics of the pharmacokinetic parameters are listed in Table 2. The $AUC_{0\text{-}24h}$, C_{max}, C_{24h} of tenofovir were, respectively, 23, 19 and 21% lower during pregnancy compared with postpartum (intra-subject comparison). For emtricitabine, the $AUC_{0\text{-}24h}$, C_{max}, C_{24h} were 25, 13 and 23%

Table 2. Pharmacokinetic parameters

	Third Trimester*	Postpartum*	GM Ratio (90% CI) Third Trimester / Postpartum	P-value**
Tenofovir	**(n=34)**	**(n=27)**	**(n=27)**	
$AUC_{0\text{-}24h}$ (mg·L/h)	2.46 (2.23-2.66)	3.17 (2.86-3.52)	0.77 (0.71-0.83)	<0.001
C_{max} (mg/L)	0.28 (0.24-0.31)	0.33 (0.29-0.39)	0.81 (0.74-0.89)	0.001
T_{max} (h)	1.0 (0.5-4.0)	1.1 (0.5-4.0)		
$C_{predose}$ (mg/L)	0.049 (0.043-0.056)	0.060 (0.050-0.073)	0.81 (0.68-0.96)	
C_{24h} (mg/L)	0.052 (0.047-0.059)	0.066 (0.058-0.076)	0.79 (0.70-0.90)	0.003
$T_{1/2}$ (h)	15 (14-16)	15 (13-17)	1.00 (0.87-1.15)	0.987
CLss/F (L/h)	55 (51-61)	43 (39-48)	1.30 (1.20-1.40)	<0.001
Emtricitabine	**(n=27)**	**(n=24)**	**(n=24)**	
$AUC_{0\text{-}24h}$ (mg·L/h)	9.56 (8.99-10.48)	13.0 (11.8-14.3)	0.75 (0.68-0.82)	<0.001
C_{max} (mg/L)	1.79 (1.57-1.99)	2.02 (1.78-2.30)	0.87 (0.77-0.99)	0.048
T_{max} (h)	2.0 (0.5-4.0)	2.0 (1.0-6.0)		
$C_{predose}$ (mg/L)	0.057 (0.051-0.084)	0.115 (0.088-0.150)	0.57 (0.44-0.73)	
C_{24h} (mg/L)	0.052 (0.043-0.073)	0.073 (0.054-0.098)	0.77 (0.52-1.12)	0.232
$T_{1/2}$ (h)	6 (5-7)	6 (5-6)	1.05 (0.91-1.21)	0.570
CLss/F (L/h)	21 (19-22)	15 (14-17)	1.34 (1.22-1.47)	<0.001

AUC, area under the curve; CI, confidence interval; Gm, geometric mean

* Geometric mean (95% confidence interval); except for T_{max}: median (minimum-maximum)

** paired t-test on log-transformed data

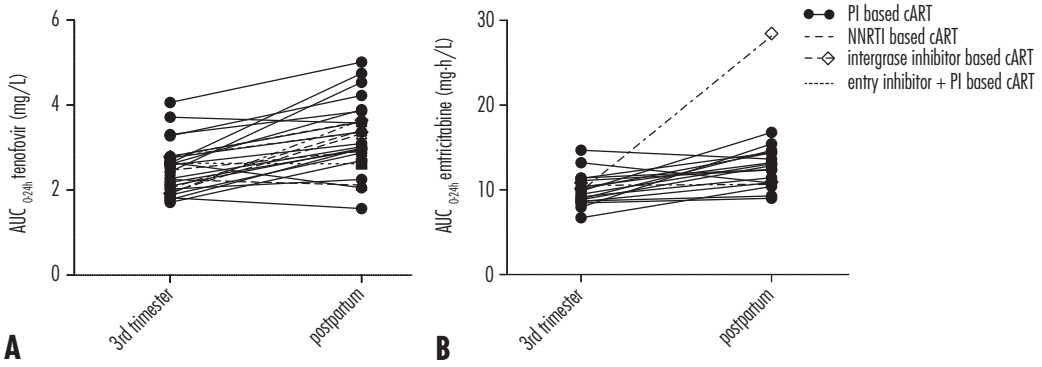

Figure 2. Individual area under the curve plot

lower, respectively. For both compounds the CLss/F is increased during pregnancy (30 and 34% increased for tenofovir and emtricitabine, respectively), whereas the T_{half} was not affected. The paired samples t-test revealed a significant difference for tenofovir and emtricitabine $AUC_{0\text{-}24h}$, C_{max} and CLss/F as well as tenofovir C_{24h} between the third trimester and postpartum.

In Figure 2 the individual $AUC_{0\text{-}24h}$ for tenofovir and emtricitabine during the third trimester and postpartum are depicted, a subdivision was made for the concomitant use of NNRTI, protease inhibitor and/or integrase inhibitor. No difference between the different cART regimens was observed for tenofovir or emtricitabine exposure.

Sixteen umbilical cord blood (CB) samples were collected with matching maternal blood samples. In one cord blood sample (and the matching maternal sample), emtricitabine/tenofovir concentrations were undetectable. The median time between the reported last dose and delivery was 8.5h (range 0-32h) and the median time between cord blood sample and maternal sample was 3 minutes (0-75 min). The median (range) ratio of cord blood/maternal blood was 0.82 (0.64-1.10; n=14) for tenofovir and 1.63 (0.46-1.82; n=10) for emtricitabine.

Efficacy and safety

HIV viral load close to delivery (median 34 weeks gestational age) was detectable in seven women (72- 272 copies/mL). The average gestational age at delivery was 38 (range 36-41) weeks. All children were tested HIV-negative and no congenital abnormalities were reported. Four of the infants (12%) were born between 36 and 37 weeks gestational age. Three babies had a low birth weight (<2,500 gram).

Three patients developed a serious adverse event (SAE). One patient had a hospital admission because she thought the baby was not moving, the baby was born without problems (36.5 weeks gestational age); one patient had a transfusion with packed cells to treat anaemia 24h postpartum, anaemia was attributed to blood loss during/

after delivery; and one patient had a postnatal uterus atony, coagulation problems and massive blood loss. All patients recovered. These SAEs were judged by the local investigator not to be related to the cART given. Nine other patients reported adverse events, all were grade 1 or 2 and not or unlikely related to the cART given. These adverse events included: back pain; oesophagus pain; inflammation right eye; urinary tract infection; common cold; gestational diabetes; blood loss during pregnancy; vomiting and nausea; anemia (2); bronchitis; infection to caesarean section wound; 400mL blood loss at vaginal delivery and coryza.

Creatinine concentrations and Glomerular Filtration Rate (GFR; using the Cockroft-Gault formula) were determined in the third trimester and postpartum (see Table 1). During pregnancy creatinine concentrations were lower (median 54 versus 67 µmol/L) and estimated GFR higher (171 mL/min versus 124 mL/min) compared to postpartum.

Pharmacokinetic – Efficacy relationship

HIV viral loads were detectable (>50 copies/mL) for seven patients around delivery. Five out of the seven patients with a detectable viral load were on cART before pregnancy (one on NNRTI-based cART and four on protease inhibitor-based cART) and two started treatment during pregnancy (protease inhibitor-based cART); the treatment duration was 24 and 28 weeks at delivery for these two patients. Adherence was checked by asking whether the patients had been taking their medication according to prescription for the last 2 weeks before the measurement. All patients reported to have been adherent to therapy during that time period.

Third trimester tenofovir, geometric means (95% CI) for $AUC_{0\text{-}24h}$ were 2.39 (2.17-2.64) mg·h/L and 2.72 (2.03-3.65) mg·h/L for patients with undetectable and detectable viral loads around delivery, respectively. For third trimester emtricitabine, geometric means (95% CI) for $AUC_{0\text{-}24h}$ were 9.41 (8.66-10.2) mg·h/L (patients with undetectable viral load) and 10.08 (7.83-13.0) mg·h/L (patients with detectable viral load) respectively. In Figure 3, the individual tenofovir and emtricitabine $AUC_{0\text{-}24h}$, C_{max} and C_{24h} are depicted for patients who had a detectable viral load around delivery compared with the patients who had an undetectable viral load around delivery. For all these parameters, the values are comparable between these two groups.

Discussion

In this study we evaluated the pharmacokinetics of tenofovir and emtricitabine, in the majority of cases combined in Truvada®, in 34 pregnant HIV-infected patients, at the third trimester of pregnancy and after delivery. In the third trimester of pregnancy, a decrease in tenofovir $AUC_{0\text{-}24h}$, C_{max} and C_{24h} (23%, 19% and 21% respectively) was

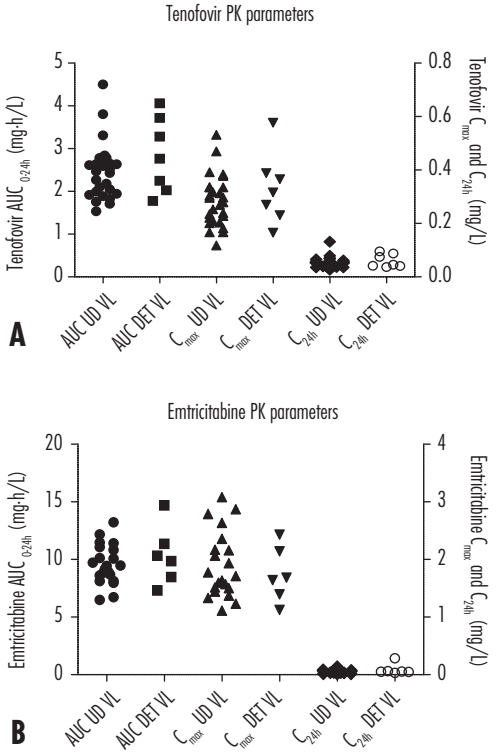

Figure 3. Comparison of individual pharmacokinetic parameters for patients with and without detectable viral load around delivery; UD: undetectable viral load; DET: detectable viral load.

observed as well as a decrease in emtricitabine $AUC_{0\text{-}24h}$, C_{max} and C_{24h} (25%, 13% and 23% respectively). The clearance (CLss/F) was markedly increased during pregnancy for both compounds (tenofovir 30% and emtricitabine 34%).

Tenofovir and emtricitabine are mainly excreted unchanged in urine, indicating that renal clearance is the major route of elimination. It is known that renal clearance is increased during pregnancy,[36] in line with our findings that estimated creatinine clearance increased during pregnancy by around 40%. Although possibly influencing the decreased exposure during pregnancy found in this study, this was not translated into shorter half-life of tenofovir and emtricitabine. The half-life was determined during a dosing interval, which is possibly not a correct estimate, because the last sample was taken 24h after dosing, this means that the entire elimination phase was not covered.

Other physiological changes during pregnancy are reduced intestinal motility, increased

gastric pH, a larger plasma volume, increased hepatic blood flow, decreased protein binding and induced hepatic enzymes. The C_{max} decreased by 19% for tenofovir and 13% for emtricitabine, potentially implying the influence of larger plasma volume and possibly decreased gastrointestinal absorption. However, the absorption was not delayed, as T_{max} was similar during and after pregnancy for both compounds.

Protease inhibitors are known to increase tenofovir concentrations.[37] In this study, the tenofovir $AUC_{0\text{-}24h}$ for patients on an NNRTI regimen are similar to these on a protease inhibitor regimen. Possible explanations for this finding could be: a decrease in boosting effect during pregnancy because of the lower exposure to protease inhibitors during pregnancy;[9,38,39] furthermore the number of patients using a nonprotease inhibitor regimen in this study was low (only 18%), reducing the power to detect a difference.

There is no efficacy threshold level for tenofovir or emtricitabine blood concentrations. In previous studies with tenofovir disoproxil, a threshold of 2 mg·h/L for $AUC_{0\text{-}24h}$[6] (being the 10TH percentile of non-pregnant controls) was used and an $AUC_{0\text{-}24h}$ threshold for emtricitabine of at least 7 mg·h/L (≤ 30% reduction from the normal controls).[26] Using these thresholds, the study showed that 26% of the patients receiving tenofovir disoproxil did not meet the threshold in the third trimester compared with only 4% of the patients in the postpartum period. For the patients on emtricitabine only 4% did not meet the threshold in the third trimester compared with 0% postpartum. One of 9 of patients with tenofovir $AUC_{0\text{-}24h}$ below the threshold had a detectable viral load around delivery, compared with six of 25 with $AUC_{0\text{-}24h}$ above the threshold. This finding indicates that in this study, tenofovir $AUC_{0\text{-}24h}$ below the 10TH percentile of nonpregnant controls was not associated with virological failure of the mother and did not result in mother-to-child transmission.

The reference tenofovir AUC in nonpregnant adults is 3.324 mg·h/L with a C_{max} of 0.326 mg/L, a C_{min} of 0.064 mg/L and a T_{half} of 12-18h.[40-42] The reference emtricitabine AUC is 10.0 mg*h/L with a C_{max} of 1.86 mg/L, a C_{min} of 0.09 mg/L and a T_{half} of 10h.[28] The tenofovir and emtricitabine postpartum pharmacokinetic parameters found in this study are in line with reference values reported in the summary product characteristics. This implies that pharmacokinetic parameters recorded 5 weeks after delivery can be used as reference values for the nonpregnant situation, that is, the pregnancy induced physiological changes were not present anymore.

The decreased emtricitabine AUC and C_{24h} we observed in this study is in line with the decrease reported by Stek *et al*.[26] However, we also observed a decrease in emtricitabine C_{max}, which was not observed earlier.

For other NRTIs (zidovudine, lamivudine, didanosine and abacavir), pharmacokinetic

studies during pregnancy also reported decreased exposure, without a need for dose alteration.[43-46]

For both compounds placenta passage is good, concentrations in the cord blood are somewhat lower for tenofovir and approximately similar to the concentrations of the mother for emtricitabine. This is in line with the findings of other NRTIs.[47] In this review, cord blood/maternal ratios for both compounds ranged from 0.60 to 1.6 (with an outlier of 6.0 for tenofovir).

HIV viral load was undetectable (<50 copies/mL) for 79% patients around delivery, and less than 200 copies/mL for 97% of the patients. A possible explanation for the detectable viral load could be shorter treatment duration in these patients. The shortest treatment duration in these patients was 24 weeks, which should be sufficient to suppress the viral load, although both patients were on a protease inhibitor based cART.[48, 49] The third trimester exposure to tenofovir and emtricitabine was not lower in patients with a detectable viral load (n=7) compared with the patients who had an undetectable viral load around delivery.

None of the babies had a detectable HIV viral load and no congenital abnormalities were reported. The adverse events observed in this study were judged not be related to the antiretroviral drugs taken, but mainly to pregnancy. Although the number of patients in this study is limited, the safety information collected is extensive (safety laboratory and adverse events were collected at each visit). The safety information from this study suggests that the use of Truvada® during pregnancy seems to be safe. This reflects the safety reporting on tenofovir use during pregnancy.[23,24,50]

None of the available formula for GFR is accurate during pregnancy: the Cockroft-Gault (we used) overestimates the GFR during pregnancy, because the increase in weight is an increase in body water and fat but not in body muscle mass.[36] The only reliable measure for GFR is creatinine clearance by 24h urine collection, but this information was not collected in this study.

One of the strengths of this study is that it includes several antiretroviral drugs in one study protocol. Patients using these antiretroviral drugs as part of their cART can be included and their treatment is not adapted for the study. Many patients use more than one antiretroviral drug from the list of medication to be investigated. Another advantage of the study draws from the PANNA network itself and is the variation in European and non-European ethnicities available for investigation: approximately 50% white Europeans and 50% black patients were included.

A limitation of the study is that no pharmacokinetic curve was collected in the second

trimester. We focussed on the third trimester as drug disposition of antiretroviral drugs is thought to be most affected during this period, because of the prominent physiological changes present. Furthermore, in the phase close to delivery, maximum viral suppression and antiretroviral effectiveness is considered important in order to minimise MTCT. Sub-therapeutic concentrations in late pregnancy may have a negative effect on antiviral efficacy. This is also a reason for assessing drug exposure during the third trimester of pregnancy.

In conclusion, although pharmacokinetic exposure of the NRTIs tenofovir disoproxil and emtricitabine during pregnancy is approximately 25% lower, this was not associated with virological failure in this study and did not result in mother-to-child transmission.

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Raltegravir in HIV-1 Infected Pregnant Women: Pharmacokinetics, Safety and Efficacy

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Abstract

Background: The use of raltegravir in HIV-infected pregnant women is important in the prevention of mother-to-child HIV transmission (MTCT), especially in circumstances when a rapid decline of HIV RNA viral load is warranted or when preferred antiretroviral agents cannot be used. Physiological changes during pregnancy can reduce antiretroviral drug exposure. We studied the effect of pregnancy on the pharmacokinetics of raltegravir and its safety and efficacy in HIV-infected pregnant women.

Methods: An open-label, multi-centre, phase IV study in HIV-infected pregnant women receiving raltegravir 400 mg twice daily, was performed (PANNA Network). Steady-state pharmacokinetic profiles were obtained in the third trimester and postpartum along with cord and maternal delivery concentrations. Safety and virological efficacy were evaluated.

Results: Twenty-two patients were included of which 68% started raltegravir during pregnancy. Approaching delivery, 86% of the patients had an undetectable viral load (<50 copies/mL). None of the children were HIV-infected. Exposure to raltegravir was highly variable. Overall AUC and C_{12h} plasma concentrations in the third trimester were on average 29% and 36% lower compared to postpartum: Geometric mean ratios (90% confidence interval) were 0.71 (0.53-0.96) for $AUC_{0\text{-}12h}$ and 0.64 (0.34-1.22) for C_{12h}. The median (IQR) ratio of raltegravir cord/maternal blood was 1.21 (1.02-2.17; n=9).

Conclusions: Raltegravir was well tolerated during pregnancy. The pharmacokinetics of raltegravir showed extensive variability. The observed mean decrease in exposure to raltegravir during third trimester compared to postpartum is not considered to be of clinical importance. Raltegravir can be used in standard dosages in HIV-infected pregnant women.

Introduction

An estimated 1.4 million pregnant women infected with HIV give birth annually worldwide, of which the majority live in Sub-Saharan Africa.[1] Mother-to-child HIV transmission (MTCT) is the most common route of HIV-infection among infants and children. Each day, approximately 1,000 infants acquire HIV due to MTCT during pregnancy, delivery or breastfeeding.

Combination antiretroviral therapy (cART) is the standard of care for the prevention of perinatal transmission. The main goal of cART is maximal suppression of HIV replication. Its implementation together with other effective interventions has led to dramatic declines in the number of perinatally HIV-infected children from 15-40% to <2%. Absent or delayed prenatal care, acute primary infection in late pregnancy, and the continued increase in incidence of HIV infection in women of childbearing age are among the most important obstacles to fully eliminate perinatal transmission in the United States and other resource-rich countries.[2]

In current US and European treatment guidelines for HIV-1 infection in pregnancy, preferred combined antiretroviral agents include two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) in combination with the protease inhibitors (PIs) lopinavir or atazanavir boosted with ritonavir or the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine. Regimens including the HIV-1 integrase inhibitor raltegravir can be considered for use in special circumstances because information on the pharmacokinetics and the safety of raltegravir in pregnancy is limited. Examples of these special circumstances could be pregnant women who present late in care (>28 weeks gestational age) or whose HIV RNA load is not undetectable at third trimester.[2-4] HIV integrase inhibitors such as raltegravir have demonstrated to rapidly reduce HIV RNA load with shorter times to achieve virological suppression compared to agents from other drug classes.[5] Case reports and small case series suggest that raltegravir could play an important role when a rapid decline in maternal plasma HIV RNA is needed to prevent MTCT during delivery or as an alternative antiretroviral drug in complex treatment-experienced HIV-infected pregnant women.[6-17] In a pilot study including 28 pregnant HIV-infected women, which was presented as abstract at a conference, the use of raltegravir seemed safe in both women and infants.[18]

Pregnancy is associated with considerable physiological changes such as changes in gastrointestinal, hepatic, and renal function as well as alterations in the expression and activity of transport proteins and metabolic enzymes. Pregnancy may influence the pharmacokinetic profile of antiretroviral agents and lead to decreased drug exposure. Suboptimal drug exposure can result in HIV RNA rebound, the selection of resistant virus and an increased risk of HIV-1 transmission to the infant.[19,20] Published information on the pharmacokinetics of raltegravir during pregnancy is limited.[21,22] Watts *et al.* describe a 50% reduction in median exposure to raltegravir during pregnancy compared with

postpartum and a large variability in raltegravir pharmacokinetics. The authors report that 92% of women had an HIV RNA load of <400 copies/mL at delivery and none of the infants were confirmed to be infected. Additional well-controlled studies are needed to confirm that raltegravir can be used safely in this special patient population. We studied the effect of pregnancy on the pharmacokinetics of raltegravir and its safety and efficacy in pregnant HIV-infected women.

Methods

Study design and participants

This multi-centre, phase IV study was designed as a non-randomised, open-label trial in HIV-infected pregnant women and coordinated by the PANNA network study group. The PANNA network is a European network of 19 hospitals in seven countries with the primary aim to collect pharmacokinetic data during pregnancy on antiretroviral agents for which no or limited data are available (www.pannastudy.com). We enrolled HIV-infected pregnant women (aged at least 18 years) who were on a cART regimen containing raltegravir 400 mg twice daily. Patients were eligible for inclusion if they were on raltegravir treatment for at least two weeks prior to the first pharmacokinetic assessment in the third trimester of pregnancy. Exclusion criteria were a medical history or current condition that might interfere with drug absorption, distribution, metabolism or excretion (such as renal failure or hepatic failure), and grade III/IV anaemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL). The study was conducted in compliance with the principles of the "Declaration of Helsinki". Informed consent was obtained from each participant before undergoing any protocol-specified procedures. The study was approved by the appropriate medical ethical committee of each centre and by the national authorities where applicable. The trial is registered at ClinicalTrials.gov, number NCT00825929.

Procedures

Inclusion screening consisted of clinical evaluations (medical history and physical examination) and laboratory assays (serum biochemistry, haematology, qualitative urinalysis, HIV-1 RNA load and CD4 T cell count). Blood samples for safety and efficacy assessments were obtained on pharmacokinetic sampling days and analysed at local laboratories. Adverse events were recorded at each visit and graded according to the Division of AIDS (DAIDS) toxicity table (2004). Infant birth weight, gestational age at birth, congenital abnormalities and HIV infection status were collected. Safety outcomes were maternal adverse events and congenital abnormalities. Efficacy outcomes were an undetectable HIV RNA load (<50 copies/mL) measured at or prior to delivery, and infant HIV infection status measured by HIV DNA Polymerase Chain Reaction test.
Pharmacokinetic assessment took place in the third trimester (approximately at week

33) and at least two weeks postpartum (approximately 4-6 weeks postpartum). Blood samples for pharmacokinetic assessment were collected during a 12-hour period at 0 (pre-dose), 0.5, 1, 2, 3, 4, 6, 8, 12 hours after observed intake of 400 mg of raltegravir after a standard breakfast (650kCal; 30g fat). Where possible umbilical cord blood (CB) and matching maternal blood samples were obtained at delivery to assess placental transfer. Plasma was separated and stored at ≤-18°C until shipment on dry ice to the laboratory of the Pharmacy of the Radboud university medical center (Nijmegen, The Netherlands). Concentrations of raltegravir in plasma were analyzed using validated reversed phase high-pressure liquid chromatography (HPLC) with fluorescence detection. The linear calibration ranges in plasma were 0.014-10.0 mg/L with a lower limit of quantification (LLOQ) of 0.014 mg/L. The raltegravir assay was externally validated through the International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma as well as by the Proficiency Testing program of the ACTG/IMPAACT group.[16, 23]

Statistical analysis

Pharmacokinetic parameters were determined using a non-compartmental model in WinNonlin/Phoenix version 6.3 (Pharsight Corporation, CA, USA). Based on the individual plasma concentration-time data, the following pharmacokinetic parameters of raltegravir were determined: the area under the plasma concentration-time curve from 0 to 12 hours after intake using the trapezoidal rule ($AUC_{0\text{-}12h}$), the trough concentration (C_{12h}) defined as the sample taken at 12 hours, the maximum plasma concentration of the drug (C_{max}), the time to reach C_{max} (T_{max}), the apparent volume of distribution (V/F), the apparent oral clearance being the dose divided by $AUC_{0\text{-}12h}$ (CL_{ss}/F) and the apparent elimination half life (T_{half}). Patients from whom a curve was taken during pregnancy were included in demographic, safety analyses and descriptive statistics of the pharmacokinetic parameters. Pharmacokinetic parameters are reported as geometric means with 95% confidence intervals (CI). We calculated geometric means ratios (GMRs) and 90% CI of raltegravir pharmacokinetic parameters of third trimester versus postpartum using a mixed effects model in WinNonlin/Phoenix. Cord blood/maternal blood plasma concentration ratios were determined and described.

Results

Twenty-two HIV-infected pregnant women receiving raltegravir 400 mg twice daily were enrolled in 10 European hospitals during 2010 to April 2014. The characteristics of the study population are presented in Table 1. Four patients (18%) were diagnosed with HIV after conception at 12, 16, 18 and 23 weeks of gestational age respectively. Of the 18 pregnant women who were already aware of their HIV-positive status, 14 were on cART at the time of conception with a median duration of approximately five

Table 1. Patient characteristics and pregnancy outcomes

Characteristics (n=22)	
Age at delivery (years)	33 (29-36)
Race/ethinicity	
White	9 (41%)
Black	12 (55%)
Other	1 (5%)
Smoking	0 (0%)
Alcohol use	0 (0%)
Drug use	0 (0%)
ARV treatment at start of pregnancy	14 (64%)
Median ARV treatment duration before pregnancy (weeks)	257 (110-440)
Start raltgravir	
Before conception	7 (32%)
1st trimester	2 (9%)
2nd trimester	6 (27%)
3rd trimester	7 (32%)
Concomitant ARVs	
NRTI	15 (68%) [11 (50%) tenofovir + emtricitabine; 3 (14%) tenofovir; 1 (5%) zidovudine + lamivudine]
Protease inhibitors•	13 (59%) [8 (36%) DRV/r; 3 (14%) ATV/r; 2 (9%) LPV/r]
NNRTI	2 (9%) etravirine
Entry inhibitor	2 (9%) maraviroc
Third trimester (n=22)	
Gestational age (weeks)	33 (32-35)
Weight (kg)	73 (67-79)
HIV RNA detectable >50 copies/mL	3 (14%) [74 copies/mL; 144 copies/mL; 242 copies/mL]
CD-4 count (copies/uL)#	622 (240-756)
Delivery (n=22)	
Gestational age (weeks)	38 (38-39)
Caesarian section#	11 (52%)
HIV RNA detectable closest to delivery >50 copies/mL	3 (14%) [144 copies/mL; 242 copies/mL; 290 copies/mL]
Time between HIV RNA measurement and delivery (weeks)	3 (0-4)

Table 1. - continued Patient characteristics and pregnancy outcomes

Characteristics (n=22)	
Postpartum (n=18)	
Time after delivery (weeks)	5 (4-6)
Weight (kg)	64 (59-72)
HIV RNA detectable >50 copies/mL†	2 (12%) [99 copies/mL; 650 copies/mL]
CD-4 count (cells/uL)	585 (266-806)
Pregnancy outcomes	
Birth weight (grams) (n=22)	3115 (2628-3360)
Small for gestational age‡	3 (14%)
Infant HIV DNA PCR test negative	22 (100%)

Data are n(%) or median and interquartile range (IQR)
ARV, antiretroviral; ATV/r, atazanavir/ritonavir; DRV/r, darunavir/ritonavir; LPV/r, lopinavir/ritonavir; (N)NRTI, (non)nucleoside reverse transcriptase inhibitor; PCR, polymerase chain reaction.
• One subject stopped DRV/r before delivery; Available for: #21 patients, † 17 patients
‡ Small for gestational age was determind as below the 10th percentile of the fetal-infant growth chart by Fenton .[25]

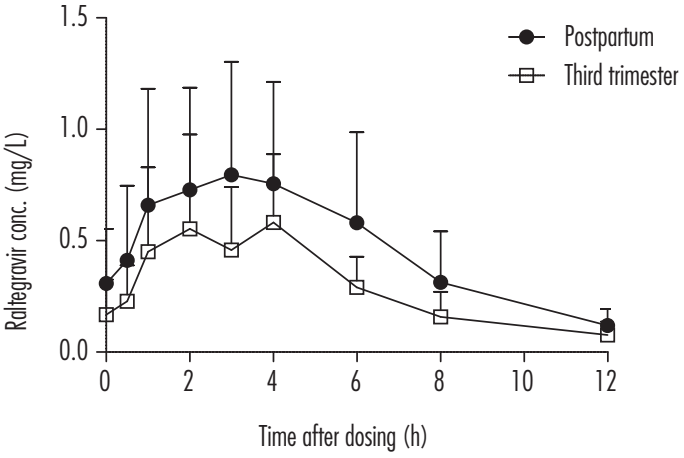

Figure 1. Geometric mean (+upper 95% confidence interval) raltegravir concentration-time profiles during the third trimester of pregnancy (open squares) and postpartum (filled circles).

Table 2. Pharmacokinetic parameters raltegravir during third trimester of pregnancy and postpartum

	Third trimester[a] (n=21)		Postpartum[a] (n=18)		GM ratio (90% CI) of third trimester: postpartum (n=17)	
$AUC_{0\text{-}12h}$ (h·mg/L)	5.00	(3.56-7.01)	7.11	(4.91-10.30)	0.71	(0.53-0.96)
C_{max} (mg/L)	1.43	(0.93-2.22)	1.76	(1.10-2.80)	0.82	(0.55-1.23)
T_{max} (h)	1.98	(0-11.3)	2.03	(0-7.97)		
C_{12h} (mg/L)	0.077	(0.043-0.137)	0.120	(0.074-0.193)	0.64	(0.34-1.22)
t_{half} (h)	2.55[b]	(1.88-3.45)	2.53[c]	(1.91-3.36)	1.04	(0.73-1.47)
CL_{ss}/F (L/h)	80.1	(57.0-112)	56.2	(38.8-81.4)	1.41	(1.04-1.90)
V/F (L)	311[b]	(159-607)	205[c]	(115-367)	1.24	(0.67-2.27)

AUC, area under the curve; CI, confidence interval; GM, geometric mean.
[a]All values are GM (95% CI) except for Tmax [median (minimum–maximum)].
[b]Available for 15 patients; [c]Available for 14 patients.

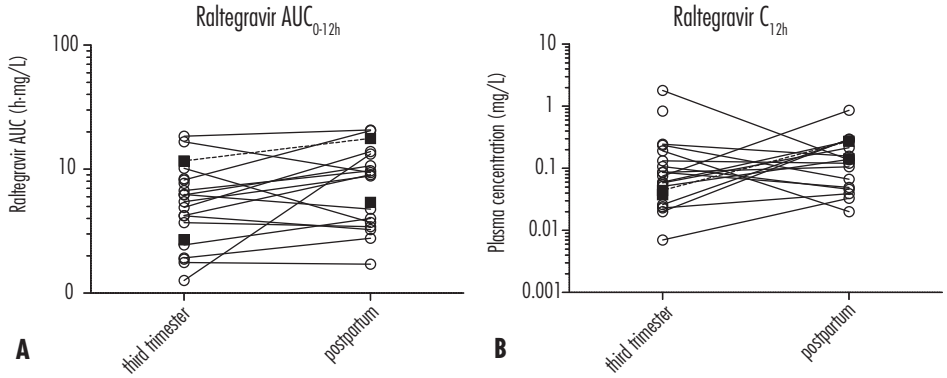

Figure 2. Individual raltegravir $AUC_{0\text{-}12h}$ (A) and C_{12h} (B) parameters during the third trimester of pregnancy and postpartum.
Symbols: Filled square ■ is a detectable (≥50 copies/mL) and an open circle ○ is an undetectable (<50 copies/mL) HIV RNA load close to delivery.

years (257 weeks). Seven patients (32%) were using raltegravir 400 mg twice daily prior to conception. If not used prior to conception raltegravir was started mainly during the second (27%) and third (32%) trimester of the pregnancy. Only two patients (9%) started a raltegravir-based regimen during the first trimester, of which one patient was still unaware of her pregnancy at that time. Various indications for raltegravir in this special patient population were presented: raltegravir was started either as part of the first cART

regimen to obtain a rapid decline in HIV RNA viral load with raltegravir as 4th agent; or added to the current regimen to optimize or intensify treatment in patients with a detectable viral load; or used as alternative to a preferred antiretroviral agent due to side effects (gastro-intestinal or hyperbilirubinemia). Concomitant HIV and non-HIV medication which could possibly influence raltegravir exposure was the use of ritonavir boosted atazanavir in three patients, the use of acid reducing agents (ranitidine 150 mg twice daily or sodium alginate as needed) in two patients, the use of a calcium carbonate supplement in two patients, and the use of a magnesium supplement in one patient. All potential drug-interacting agents were used during both pharmacokinetic assessments.

Pharmacokinetic assessment in third trimester took place at a median (IQR) gestational age of 33 (32-35) weeks. A total of 21 evaluable raltegravir pharmacokinetic curves were obtained. One pharmacokinetic profile sampling was stopped at 3 hours at the volunteer's request and these plasma concentrations could only be partly included in the analysis. Pharmacokinetic assessment postpartum took place at a median (IQR) of 5 (4-6) weeks after delivery and resulted in 18 evaluable pharmacokinetic postpartum curves. Four patients did not have a postpartum curve because they withdrew consent. The mean plasma concentration-time profile of raltegravir in the third trimester and postpartum are presented in Figure 1 and summary statistics of the pharmacokinetic parameters are listed in Table 2.

Exposure to raltegravir, which is expressed as $AUC_{0\text{-}12h}$, was 29% lower in the third trimester compared to postpartum by intrasubject comparison. C_{max} and C_{12h} were on average 18% and 36% lower during pregnancy. The apparent elimination half-life of raltegravir did not appear to be influenced by pregnancy. One patient in the third trimester (and none postpartum) had a C_{12h} plasma concentration below the suggested threshold of 0.020 mg/L which was associated with failure to achieve an undetectable HIV RNA load in treatment-naive patients in the QDMRK study.[24] Raltegravir pharmacokinetics was highly variable which is best seen in the large 90% confidence intervals around the GMR in Table 2 and graphically in Figure 2. Figure 2 shows the individual changes in $AUC_{0\text{-}12h}$ (A) and C_{12h} (B) of raltegravir in the third trimester of the pregnancy compared with postpartum. Although a mean decrease in raltegravir exposure (29%) and C_{12h} plasma concentrations (36%) in the third trimester was observed, considerable variation in the amount and direction of the effect is seen as well as variation between individual patients. Eleven out of 17 patients with complete paired pharmacokinetic curves (65%), showed a decrease in raltegravir exposure in third trimester compared with postpartum.

Nine umbilical cord blood (CB) samples were collected with matching maternal blood samples. The median (IQR) time between the reported last dose and CB sampling if available was 10 h (7-11 h); the median (IQR) time between CB sample and maternal sample was 0 minutes (0-4 min). The median (IQR) ratio of raltegravir CB/maternal blood was 1.21 (1.02-2.17; n=9).

No congenital abnormalities were reported. Five patients reported a total of ten adverse

events which were considered not or unlikely to be related to the cART given. Seven events were grade 1 or 2. Grade 3 neuropatic pain was reported as a serious adverse event not related to the use of raltegravir. Other grade 3 adverse events were severe anaemia due to haemorrhagic delivery and varicella lesions.
Twenty-two infants were born and they were all tested HIV-negative. Three infants (14%) were small for gestational age (below 10th percentile of fetal-infant growth chart by Fenton) [25], which is higher than observed in the US for children born from HIV-infected women (7.3%).[26] Other pregnancy outcomes are shown in Table 1 as well as the results of the maternal HIV RNA viral load measurements. In summary 3/22 patients (14%) failed to achieve an undetectable HIV RNA viral load (<50 copies/mL) close to delivery (144, 242 and 290 copies/mL) when measured a median (IQR) of 3 (0-4) weeks before delivery. The patient with a C_{12h} level below the threshold of 0.020 mg/L in third trimester had an HIV RNA viral load of 74 copies/mL measured in third trimester and an undetectable viral load on the day of delivery. Adherence, based on self-reporting, was good in all patients.

Discussion

In this study we evaluated the effect of pregnancy on the pharmacokinetics of raltegravir and its safety and efficacy in 22 HIV-infected women. In the third trimester of pregnancy systemic exposure (AUC) to raltegravir was on average 29% lower compared with postpartum. However, pharmacokinetics of raltegravir was highly variable and exposure was not consistently decreased in third trimester compared with postpartum. Of the 17 women with paired pharmacokinetic curves six (35%) had a higher $AUC_{0\text{-}12h}$ in the third trimester. A similar effect of pregnancy on C_{12h} plasma levels was observed, leading to an average decrease of 36% of the plasma levels seen postpartum. The magnitude of the observed effect is not considered to be of clinical importance. Similar effects of drug-interacting agents on the pharmacokinetics of raltegravir are described in the product information leaflet without special recommendation to adjust the dosage of raltegravir.[27] Viral suppression was good in our population with an HIV RNA load <400 copies/mL in all women and <50 copies/mL in 84% of women prior to delivery. The women (14%) who failed to have an undetectable viral load prior to delivery had adequate C_{12h} levels in third trimester. Only one patient had a C_{12h} level below 0.020 mg/L in third trimester, which is considered to be too low for adequate virological response in treatment-naive patients.[24] She had an undetectable viral load on the day of delivery.
The decrease in AUC (29%) in third trimester compared with postpartum was in line with the observations in a previous study with intensive pharmacokinetics of raltegravir during pregnancy from Watts *et al.*.[21] They describe a more pronounced decrease of approximately 50% in AUC in the third trimester compared with postpartum. Given

the high rate of viral suppression at delivery and the lack of a clear pharmacokinetic/pharmacodynamic relationship in non-pregnant adults, the authors suggest that a higher dose of raltegravir is not necessary during pregnancy. Watts *et al.* reported a median AUC of 5.4 h·mg/L (n=41) in third trimester, which is comparable to the geometric mean AUC in third trimester (5.00 h·mg/L) found in our study. The postpartum median AUC reported by Watts *et al.* was higher than the AUC we found: 11.6 h·mg/L (n=38) measured 3-14 weeks postpartum versus 7.11 h·mg/L. This difference probably causes the more pronounced decrease between third trimester and postpartum found by Watts *et al.*. Raltegravir C_{12h} levels in third trimester were comparable: 0.064 mg/L reported by Watts et al. and 0.077 mg/L in our study. The postpartum curves in our study are consistent with intensive pharmacokinetic profiles in non-pregnant HIV-infected patients in the twice-daily treatment arm of the QDMRK study.[24] Geometric mean AUC and C_{12h} (n=20) of raltegravir are 5.84 h·mg/L and 0.114 mg/L respectively in the QDMRK study compared to 7.11 h·mg/L and 0.120 mg/L postpartum (n=18) in our study. This would suggest that the pharmacokinetic parameters collected at a median of 5 weeks postpartum in our study can be used as reference for the non-pregnant situation. Patient characteristics, drug-drug interactions, time of postpartum pharmacokinetic assessment and the large inter-subject variability in raltegravir pharmacokinetics could have contributed to the differences in pharmacokinetic parameters of raltegravir postpartum between Watts *et al.* and our study. The large inter-subject and intra-subject variability in raltegravir pharmacokinetics observed in our study is well recognised by others in non-pregnant populations.[28,29]

There are many physiological changes during pregnancy that could alter distribution, metabolism and clearance of antiretroviral drugs used in pregnancy.[19,20] During pregnancy the apparent volume of distribution increases with subsequent decreases in peak plasma concentrations, which was observed in our study as well. Alterations in drug elimination clearance during pregnancy can affect steady-state concentrations. Raltegravir is primarily metabolized by uridine diphosphate glucuronosyltransferase (UGT)1A1. The potential effect of pregnancy on UGT1A1 activity has been evaluated and is believed to be increased during pregnancy.[19,30] Jeong *et al.* suggest that the induction of UGT1A1 expression by rising progesterone levels in pregnant women may be responsible for the increase in clearance of UGT1A1 substrates.[31] This hypothesis is not supported by our study in which the apparent elimination half-life of raltegravir in the third trimester was similar to postpartum.

Raltegravir was well tolerated during pregnancy and all of the children were tested HIV-negative. Only nine babies were exposed to raltegravir during the first trimester, with no birth defects reported. To assess prevalence rates of birth defects in infants exposed to raltegravir compared to non-exposed infants, more experience of raltegravir in human pregnancy is needed. Placental transfer of raltegravir is efficient with a median raltegravir CB/maternal plasma ratio of 1.21 in agreement with previous reports.[12,13,21,32,33] Unfortunately the collection of neonatal blood samples to describe the washout phar-

macokinetics and safety of in utero exposure to raltegravir was not part of this study. UGT1A1 neonatal enzyme activity is still immature after birth and leads to prolonged elimination of raltegravir post-delivery. In newborns whose mothers were exposed to raltegravir during pregnancy raltegravir is slowly metabolized with an elimination half life that is highly variable.[9,12,33]

In conclusion, raltegravir was well tolerated during pregnancy in our study population. Raltegravir pharmacokinetics showed extensive inter- and intra-individual variability. Our findings show a mean decrease in exposure to raltegravir during third trimester compared with postpartum which is not considered to be of clinical importance. Raltegravir in combination with other antiretroviral agents was effective in preventing MTCT by reducing and/or maintaining HIV RNA viral load at an undetectable (<50 copies/mL) or low level (<400 copies/mL). Our data support the use of raltegravir in standard dosages in HIV-infected pregnant women for the prevention of MTCT.

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Pharmacokinetics, Safety and Transplacental Passage of Rilpivirine in Pregnancy: Two Cases

Authors

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Introduction

Generally, no information is available about new drugs during pregnancy owing to exclusion of pregnant women from clinical trials. For the new antiretroviral drug rilpivirine no safety or pharmacokinetic information during pregnancy is available to date.[1] Rilpivirine is a once-daily dosed nonnucleoside reverse transcriptase inhibitor, available as a single 25mg tablet (Edurant®) and also coformulated with tenofovir and emtricitabine (Eviplera®) to accomplish a one tablet/day regimen. Rilpivirine is indicated for the treatment of HIV type 1 infection in combination antiretroviral for treatment (cART)-naive adult patients with a viral load of 100,000 HIV-1 RNA copies/mL or less.[2]
A European network studies pharmacokinetics and transplacental passage of newly developed antiretrovirals during pregnancy (PANNA, http://www.pannastudy.com/ ClinicalTrials.gov NCT00825929). It is a non-randomized, open-label, multicentre phase IV study, see Colbers *et al.*[3] for the methods used. Here, we describe two cases of rilpivirine use during pregnancy from the PANNA study.

Case one

Case 1 was a 19-year-old black woman, abstinent from nicotine and alcohol use, was diagnosed with HIV in 2011, when she immediately started Eviplera®. Conception occurred after approximately 14 weeks of Eviplera® use. When pregnancy was established, Eviplera® was interrupted (week 5 gestational age). This was restarted in week 25 gestational age (viral load 3543 copies/mL) and continued for the rest of the pregnancy and after delivery.
In week 32 gestational age a pharmacokinetic curve was recorded (Figure 1A): $AUC_{0\text{-}24h}$ was 1.25 mg·h/L; C_{max} was 0.07 mg/L, C_{0h} was 0.04 mg/L, t_{half} was 30h. Viral load was undetectable (<50 copies/mL). At week 38 gestational age, she was admitted to the hospital because of irregular contractions. This hospital admission was reported as SAE, judged not to be related to Eviplera® use.
Vaginal delivery took place at 39 weeks and 5 days gestational age; viral load was undetectable. A healthy girl (no congenital abnormalities), 3620gr, 53cm, head circumference 34cm, APGAR score of 10 after 1, 5, 10 min, was born. An HIV DNA PCR test 2 weeks after delivery was negative.
At delivery, a cord blood (rilpivirine 0.016 mg/L) sample and maternal sample (rilpivirine 0.021 mg/L) were collected 16 hours after the last maternal rilpivirine intake. The cord blood/maternal blood ratio was 0.74.
Forty-five days after delivery, a postpartum pharmacokinetic curve was collected, which represents the normal situation. $AUC_{0\text{-}24h}$ was 1.79 mg·h/L; C_{max} was 0.11 mg/L, C_{0h} was 0.07 mg/L, t_{half} was 43h.

Case two

Case 2 was a 24-year-old white woman, smoking more than 10 cigarettes/day, no alcohol use, was diagnosed with HIV in 2010. In September 2011, she started cART: atazanavir/ritonavir 300/100mg daily (q.d.) and zidovudine/lamivudine (Combivir®); in August 2012, she switched to Eviplera®. At conception she was using Eviplera® for 10 weeks.

At week 32 gestational age a pharmacokinetic curve was taken (see Figure 1B): $AUC_{0\text{-}24h}$ was 1.42 mg·h/L; C_{max} was 0.14 mg/L, C_{0h} was 0.04 mg/L and t_{half} was 33h. Viral load was undetectable.

Nonelective Caesarian section (due to non-progressing dilatation) was performed at 38 weeks and 5 days gestational age. Two weeks before delivery, viral load was 77 copies/mL; 4 weeks after delivery viral load was undetectable again. A healthy girl, 2945gr, 51.5cm, head circumference 35.8cm, APGAR score after 1 and 5 min: 8 and 10, was born. The infant was tested for HIV 1 day and 18 days after birth and the tests were negative (DNA PCR).

Thirty-six days after delivery, a postpartum pharmacokinetic curve was collected: $AUC_{0\text{-}24h}$ was 2.49 mg·h/L; C_{max} was 0.15 mg/L, C_{0h} was 0.09 mg/L and t_{half} was 48h.

Discussion

Exposure ($AUC_{0\text{-}24h}$) during pregnancy was 30-43% lower during pregnancy (comparable to the decrease seen for protease inhibitors). Postpartum $AUC_{0\text{-}24h}$ of these two cases is in line with the mean steady state $AUC_{0\text{-}24h}$ in patients (2.397 mg·h/L).[4] The lower exposure during pregnancy is possibly driven by a shorter rilpivirine half-life; however, accurate determination of the half-life under steady-state conditions is difficult. A suggested rilpivirine target trough concentration is 0.040 mg/L, derived from the exposure-response relationship in phase III studies.[5] For both cases the C_{trough} concentrations in the 3rd trimester and the maternal sample at delivery were 0.040 mg/L or less, indicating subtherapeutic exposure during pregnancy. However, no mother-to-child transmission of HIV was observed, but should be confirmed by at least 4 months of age. A limitation is that no unbound rilpivirine concentrations were determined: lower protein binding during pregnancy might (partly) compensate for lower total concentrations. No major safety issues were reported for these two cases.

In our case, rilpivirine moderately crosses the placenta and exposure during pregnancy is decreased by approximately 30-43%. More data regarding rilpivirine in pregnancy are needed to confirm these first findings, but therapeutic drug monitoring for rilpivirine during pregnancy is strongly recommended.

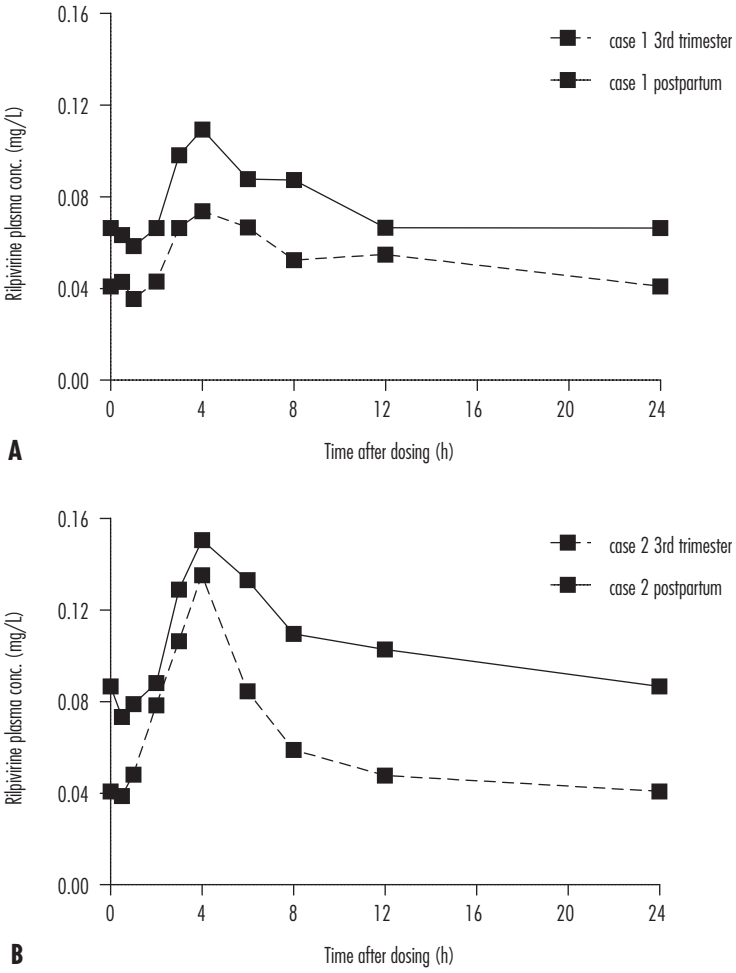

Figure 1. Individual rilpivirine plasma concentrations during and after pregnancy
For C_{24h} the C_{0h} was used because at t=24h no sample was taken.

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Maraviroc Pharmacokinetics in HIV-1 Infected Pregnant Women

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Abstract

Objective: To describe the pharmacokinetics of maraviroc in HIV-infected women during pregnancy and postpartum.

Methods: HIV infected pregnant women receiving maraviroc as part of clinical care had intensive steady-state 12-hour pharmacokinetic profiles performed during the 3rd trimester and at least 2 weeks after delivery. Cord blood samples and matching maternal blood samples were taken at delivery. The data were collected in two different studies: P1026 (US) and PANNA (Europe). Pharmacokinetic parameters were calculated.

Results: Eighteen women were included in the analysis. Most women received 150mg maraviroc twice daily with a protease inhibitor (12; 67%), two (11%) received 300mg maraviroc twice daily without protease inhibitor, and four (22%) had an alternative regimen. Geometric mean ratios (90% confidence interval) of third trimester versus postpartum maraviroc were 0.72 (0.60-0.88) for AUC_{tau} and 0.70 (0.58-0.85) for maraviroc C_{max}. Only one patient showed C_{trough} concentrations below the suggested target of 50ng/mL, both during pregnancy and postpartum. The median (range) ratio of maraviroc cord blood/maternal blood was 0.33 (0.03-0.56). Viral load close to delivery was less than 50 copies/mL in 13 participants (76%). All children were tested HIV negative at testing.

Conclusions: Overall maraviroc exposure during pregnancy was decreased, with a reduction in AUC and C_{max} of around 30%. C_{trough} was reduced by 15% but exceeded the minimum C_{trough} target concentration. Therefore, the standard adult dose seems to be sufficient in pregnancy.

Introduction

It is estimated that worldwide 16 million women were living with HIV in 2013, with around 1.3 million of them giving birth.[1,2] HIV infected pregnant women may receive antiretrovirals both to protect their own health and to reduce the risk of mother to child transmission (MTCT) of HIV.[3] Combination antiretroviral therapy (cART) has been shown to be a highly effective strategy for prevention of MTCT of HIV, reducing the risk from 15-40% to less than 2%.[3]
Maraviroc is an antagonist to C-C chemokine receptor type 5 (CCR5), which plays an important role in blocking HIV-1 entry into susceptible cells.[4] It is effective for treatment of CCR5-tropic HIV-1 as part of cART therapy. The standard recommended dose for maraviroc therapy in adults or adolescents is 300mg twice daily (BID), unless co-administered with a boosted protease inhibitor, in which case the dose is reduced to 150mg BID.[5] There are no data available describing maraviroc pharmacokinetics and safety when used during pregnancy, and the U.S. Department of Health and Human Services (DHHS) perinatal guidelines include no recommendations regarding maraviroc therapy or dosing regimens during pregnancy.[6]

Pregnancy is associated with a myriad of physical changes that affect the pharmacokinetics of drugs,[7,8] mostly resulting in a reduction in drug exposure during pregnancy.[9] Decreased antiretroviral concentrations may lead to inadequate viral suppression and/or development of antiretroviral resistance, and may increase the risk of MTCT in HIV-infected pregnant women.[3, 10] Data describing maraviroc pharmacokinetics in pregnancy have not been published.

Specific safety issues of maraviroc during pregnancy include the influence of maraviroc on pregnancy duration and fetal development. Maraviroc is assigned to the FDA Pregnancy Category B, as animal reproduction studies failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women. Reports of safety of maraviroc during human pregnancy are limited. The most recent Antiretroviral Pregnancy Registry interim report (through 31 July 2014) includes only a few patients who received maraviroc, who did not exhibit any birth defects with exposure to maraviroc in the first trimester (n=16) or second and third trimesters (n=6).[11] No data about the influence of maraviroc on pregnancy duration are available.

Current available data on transfer of maraviroc across the human placenta are limited to one case report and data from an *ex vivo* placenta perfusion study both indicating minor placental transfer.[12,13] Fetal antiretroviral exposure via placental transfer may provide pre-exposure prophylaxis of the fetus and newborn against HIV infection, possibly contributing to prevention of perinatal transmission of HIV, but also may result in drug related

fetal teratogenicity and/or toxicity.[6]
Overall, available data are too limited to make grounded recommendations regarding maraviroc use and dosing regimens during pregnancy, highlighting the exclusion of pregnant women from clinical trials during the development of new drugs, mainly due to concerns of potential risks to the fetus.[14] As a result, HIV-infected pregnant women are currently receiving maraviroc as part of clinical care in the absence of pregnancy specific safety and pharmacokinetic data. Two protocols, the 'International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Network' P1026s protocol and the 'Study on pharmacokinetics of newly developed antiretroviral agents in HIV-infected pregnant women (PANNA) Network', have been developed to study the safety and pharmacokinetics during pregnancy of antiretroviral drugs used for clinical care that lack safety and pharmacokinetic data obtained during pregnancy. In this report, these networks have collaborated to describe the pharmacokinetics, transplacental transfer, and safety of maraviroc in pregnant HIV-infected women.

Methods

The data presented in this study were collected in two studies. Both were non-randomized, open-label, parallel-group, multi-center phase-IV studies in HIV-infected pregnant women. PANNA recruited patients from HIV treatment centers in Europe. IMPAACT recruited patients from sites in the Americas. Here, we report data of pregnant HIV-infected patients treated with maraviroc as part of their cART.

The studies were conducted in compliance with the principles of the "Declaration of Helsinki". Informed consent was obtained from each participant before entering the studies. The studies were approved by the medical ethical committee from each individual centre involved and by the national authorities if applicable. The studies were registered at ClinicalTrials.gov under the numbers NCT00825929 and NCT00042289.

Patient eligibility

Patient eligibility included being HIV-infected, pregnant, at least 18 years of age at screening and treated with a cART regimen containing maraviroc as prescribed for clinical care for at least 2 weeks before the day of first pharmacokinetic evaluation. Subjects continued to take their prescribed medications throughout pregnancy. Women continued on study until the completion of postpartum pharmacokinetic sampling. Patients were excluded if they had a past medical history or current condition that might interfere with drug absorption, distribution, metabolism or excretion (such as renal failure or hepatic failure) or presented with grade III/IV anaemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL) at screening (PANNA specific) or multiple pregnancy (IMPAACT specific).

Safety assessments and viral load

Inclusion screening consisted of: medical history, physical examination, serum biochemistry and hematology, HIV-1 RNA load determination and CD4+ T cell count. Analyses for safety assessments were performed by local laboratories. Blood samples for safety assessments were further taken at the visits for pharmacokinetic blood sampling and at delivery. Patients were asked for adverse events at each visit and serum biochemistry, hematology, HIV-1 RNA load and CD4+ T cell count. The HIV-status of the infants was assessed.

Pharmacokinetic blood sampling

The 12 or 24 hour intensive pharmacokinetics were performed in the third trimester between 30 and 36 weeks of gestation. At least 2 weeks postpartum (preferably 4-6 weeks postpartum) pharmacokinetic sampling was repeated. At delivery (if possible) a cord blood and a maternal blood sample were taken. Evaluation at visits included sampling pre-dose and at 1, 2, 4, 6, 8 and 12 hours post medication intake at all pharmacokinetic study days. Subjects in the PANNA study also had samples collected 0.5 and 3 hours after dosing, and subjects receiving once a day dosing had samples collected 24 hours post dosing. Plasma was separated and stored at < -18°C until shipment on dry ice to the central laboratory for analysis. Information collected included the time of the two prior doses of maraviroc and maternal weight.

Analytical methods

Concentrations of maraviroc in plasma were analyzed by two centers.

The PANNA samples were analyzed at the Ottawa Hospital Research Institute, Ottawa, Ontario, Canada and IMPAACT samples were analyzed at the Pediatric Clinical Pharmacology Laboratory at the University of California, San Diego. Both laboratories used a validated liquid chromatography mass spectrometry/mass spectrometry (LC/MS/MS) method. The lower limits of quantification were 5 ng/mL (PANNA) and 3.9 ng/mL (IMPAACT). The linear calibration ranges in plasma were from 5 to 1000 ng/mL (PANNA) and 3.9 to 2000 ng/mL (IMPAACT). Both pharmacology laboratories participate in the AIDS Clinical Trial Group (ACTG), United States, pharmacology quality control (precision testing) program, which performs standardized interlaboratory testing twice a year.[15]

Pharmacokinetic assessments

Pharmacokinetic parameters were determined using a non-compartmental model in WinNonlin version 6.3 (Pharsight Corporation, CA, USA). Area under the curve ($AUC_{0\text{-}tau}$) using the trapezoidal rule, the trough concentration (C_{last}) defined as the sample taken at time point 12hr or 24hr, average plasma concentration (C_{avg}), maximum concentration (C_{max}), elimination half life (T_{half}), time of maximum concentration (T_{max}), apparent

clearance (CL/F, being the dose/$AUC_{0\text{-}tau}$) and apparent volume of distribution (Vd/F, being (CL/F)/k_{el}) were determined per individual curve.

Statistical analysis data handling

Patients with evaluable pharmacokinetic data during pregnancy were included in demographic, safety analyses and descriptive statistics of the pharmacokinetic parameters. pharmacokinetic parameters are reported as geometric means with 95% intervals for the 150mg maraviroc BID dose with a protease inhibitor. The pharmacokinetic parameters for the other dosing regimens were reported for each individual, because the data were too limited to be described statistically. Geometric mean ratios (GMR) with 90% confidence intervals for individual third trimester to postpartum parameters were determined for all pharmacokinetic parameters by combining data from all dosing regimens, provided that the same maraviroc dose was used during and after pregnancy. A paired t-test on the ln-transformed pharmacokinetic parameters was performed to compare third trimester data with postpartum.

The Mann-Whitney U test was used to compare $AUC_{0\text{-}tau}$ ratios between studies (PANNA versus IMPAACT) to test for between-study differences. Cord blood/maternal blood concentration ratios were determined and described.

Results

Eighteen HIV-infected pregnant women (IMPAACT 11; PANNA 7) completed third-trimester sampling between 31 and 38 weeks of gestation, and 14 completed sampling between 4 and 15 weeks postpartum. Four subjects withdrew from the study and did not have postpartum sampling performed.

The characteristics of the women and their pregnancy outcomes are depicted in Table 1. Twelve women (67%) were on 150mg maraviroc BID with a protease inhibitor, two (11%) were on 300mg maraviroc BID without a protease inhibitor, two were on 300mg maraviroc once daily (QD) with a protease inhibitor, two were on 300mg maraviroc BID with a protease inhibitor. The protease inhibitors used were darunavir/ritonavir (14 women), lopinavir/ritonavir (1 woman) and atazanavir/ritonavir (1 woman). Ten patients also received raltegravir. Eleven women started maraviroc treatment before pregnancy, six patients started during pregnancy (two in the first trimester, four in the second trimester), and the start date was not known for one patient. None of the women used other medication possibly interacting with maraviroc.

Pharmacokinetics

The mean plasma concentration-time profiles of maraviroc for the 150mg BID with a protease inhibitor regimen in the third trimester and postpartum are presented in Figure 1; summary statistics for the pharmacokinetic parameters for the 150mg maraviroc BID

Table 1. Subject characteristics

	median (range) or n (%)
Number of patients included	18 (IMPAACT 11/PANNA 7)
Age at delivery (years)	25 (20-41)
Race/ethnicity	
Black, non-hispanic (n (%))	9 (50%)
Hispanic (n (%))	6 (33%)
White (n (%))	2 (11%)
Other, more than one race (n (%))	1 (6%)
Smoking (n (%))	7 (21%)
Alcohol use (N (%))	4 (12%)
Maraviroc regimen	
150mg maraviroc BID + PI	12 (67%)
300mg maraviroc BID – PI	2 (11%)
300mg maraviroc QD + PI	2 (11%)
300mg maraviroc BID + PI	2 (11%)
NRTIs used	Combivir® (4, 22%); Truvada® (3; 17%); tenofovir DF alone (2, 11%); lamivudine alone (1, 6%); abacavir alone (1, 6%); zidovudine alone (1, 6%); NRTI-free regimen (6, 33%)
Other ARVs used	Raltegravir (10, 56%); etravirine (1, 6%)+PI; enfuvirtide (1, 6%)
Start MVC during pregnancy n(%)	6/17 (35%), 1 unknown
First trimester	2 (12%
Second trimester	4 (24%)
Third trimester (n=18)	
Gestational age (weeks)	34 (31-38)
Weight (kg)	75 (48-128)
HIV-RNA undetectable <50 (n (%))	13 (72%)
HIV-RNA <400 (n (%))	15 (83%)
CD-4 count (cells/mm^3)	481 (66-1030)
Post partum (n=14)	
Time after delivery (weeks)	7 (4-15)
Weight (kg)	73 (56-121)
HIV-RNA undetectable <50 (n (%))	11 (73%) 3 missing
HIV-RNA <400 (n (%))	14 (93%), 3 missing
CD-4 count (cells/ mm^3)	521 (66-1465)

Table 1. - continued Subject characteristics

	median (range) or n (%)
Pregnancy outcomes (n=18)	
Gestational age (weeks)	39 (37-41)
Caesarian section	12 (67%), 1 unknown
Maternal HIV-RNA undetectable <50 (n (%))	13 (76%), 1 missing
Maternal HIV-RNA undetectable <400 (n (%))	14 (82%), 1 missing
Birth weight (grams)	3215 (2350-3750)
Infant uninfected (n (%))	18 (100%)

PI: Protease inhibitor

with protease inhibitor dosing regimen are listed in Table 2. Individual pharmacokinetic parameters for the other maraviroc treatment regimens can also be found in Table 2. Figure 2 presents the individual $AUC_{0\text{-}tau}$ ratios (third trimester/postpartum) for the different maraviroc treatment regimens. Individual ratios did not indicate obvious differences between these regimens. When the ratio between pregnancy and postpartum pharmacokinetic parameters for all subjects (separate regimens were pooled together) were compared, significant reductions in $AUC_{0\text{-}tau}$ (28%; p=0.008) and C_{max} (30%; p=0.007) were observed (Table 3). C_{last} was reduced by 15% (p=0.096) and t_{half} was marginally increased (4%, p=0.407). $AUC_{0\text{-}tau}$ ratios did not show significant differences

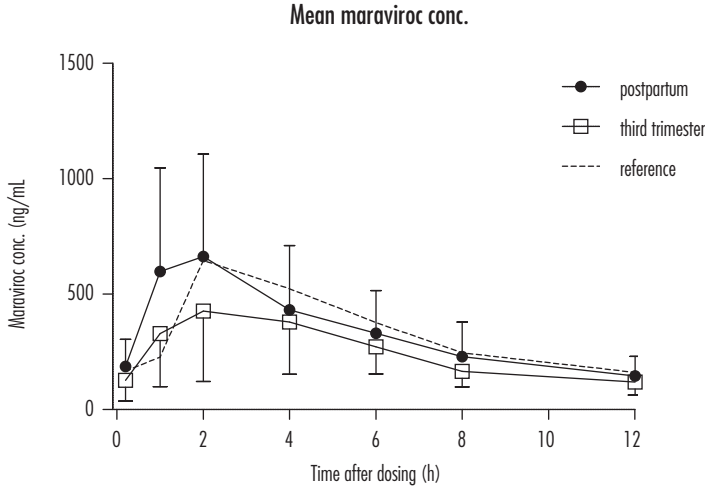

Figure 1. Maraviroc (150mg BID + PI) mean concentration-time profiles in pregnancy and postpartum
Mean +- SD maraviroc concentrations at steady state measured in the third trimester of pregnancy (square) and postpartum (filled circle), reference concentrations (dotted line) from Kakuda *et al.*[17]

Table 2. Pharmacokinetic parameters maraviroc during pregnancy and postpartum

	3rd trimester, n=12			postpartum, n = 10		
150 mg BID + PI						
	GM	95%CI lower	95%CI upper	GM	95%CI low	95%CI upper
AUC_{tau} (ng·mL/h)	2717	2038	3622	3645	2429	5469
C_{max} (ng/mL)	448	318	632	647	408	1025
T_{max} (h)	3.09	1	6	2.03	0.98	4
$T_{1/2}$ (h)	5.7	4.2	7.7	5.5	4.1	7.5
C_{last} (ng/mL)	108	81	145	128	89	184
Cl/F_{ss} (L/h)	55	41	74	44	28	69
Vd/F_{ss} (L)	456	293	711	352	181	683
C_{avg} (ng/mL)	226	170	302	304	202	456
300 mg BID no PI						
	indiv 1	indiv 2		indiv 1	Indiv 2	
AUC_{tau} (ng·mL/h)	911	1601		995	NA	
C_{max} (ng/mL)	339	349		368	NA	
T_{max} (h)	1.92	6.03		0.83	NA	
$T_{1/2}$ (h)	8.3	3.3		12.0	NA	
C_{last} (ng/mL)	30	91		33	NA	
Cl/F_{ss} (L/h)	329	187		301	NA	
Vd/F_{ss} (L)	3934	890		5227	NA	
C_{avg} (ng/mL)	76	133		83	NA	
300 mg QD + PI						
	indiv 3	indiv 4		indiv 3	indiv 4	
AUC_{tau} (ng·mL/h)	5548	10289		7368	12990	
C_{max} (ng/mL)	906	1173		835	1796	
T_{max} (h)	3.00	2.05		2.08	3.00	
$T_{1/2}$ (h)	7.9	7.2		7.9	5.1	
C_{last} (ng/mL)	56	115		74	90	
Cl/F_{ss} (L/h)	54	29		41	23	
Vd/F_{ss} (L)	620	303		466	170	
C_{avg} (ng/mL)	231	429		307	541	

Table 2. - continued Pharmacokinetic parameters maraviroc during pregnancy and postpartum

	3rd trimester, n=12		postpartum, n = 10	
300 mg BID + PI				
	indiv 5	indiv 6	indiv 5	indiv 6
AUC_{tau} (ng·mL/h)	8400	5784	15447	NA
C_{max} (ng/mL)	1143	1246	2161	NA
T_{max} (h)	4.00	2.08	4.00	NA
$T_{1/2}$ (h)	5.1	4.0	3.7	NA
C_{last} (ng/mL)	314	136	482	NA
Cl/F_{ss} (L/h)	36	26	19	NA
Vd/F_{ss} (L)	264	148	104	NA
C_{avg} (ng/mL)	700	482	1287	NA

* Tmax: median + range; GM: geometric mean; CI: confidence interval; BID: twice daily; QD: once daily; PI: protease inhibitor; NA: not available; AUC, area under the curve; C_{max}, maximum concentration; T_{max}, time of maximum concentration; C_{last}, concentration at last time point (12h or 24h post dosing); $T_{1/2}$, half-life; CL, apparent clearance; Vd, apparent volume of distribution

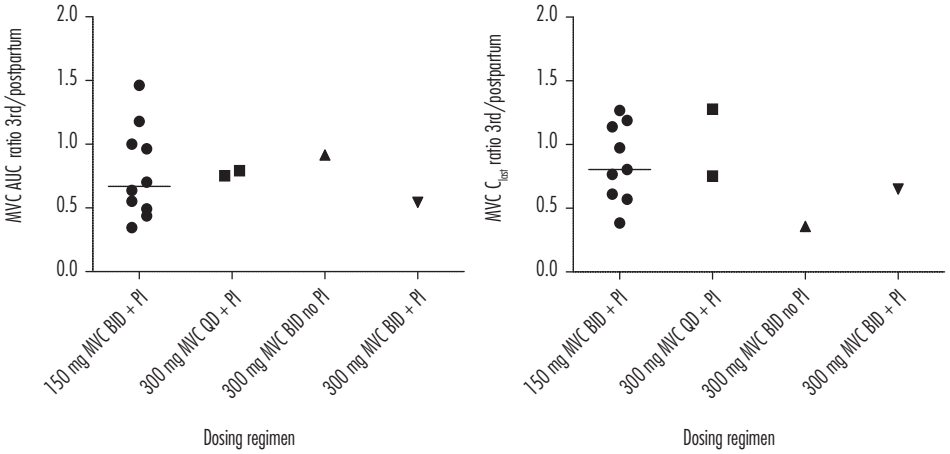

Figure 2. Individual maraviroc AUC and C_{last} ratios (3rd trimester of pregnancy/postpartum) per dosing regimen
Individual maraviroc AUC and C_{last} ratios in the third trimester of pregnancy/postpartum for each dosing regimen used. For the 150mg twice daily dose in combination with a protease inhibitor the median was reported (line). MVC = maraviroc; BID = twice daily; PI = protease inhibitor; QD = once daily

Table 3. Geometric mean ratios for maraviroc pharmacokinetics: 3rd trimester versus postpartum (n=14)

Parameter	Ratio (%)	90% CI Low	90% CI Upper	P-value
AUC_{tau}	72	60 -	88	0.008
C_{max}	70	58 -	85	0.007
$T_{1/2}$	104	86 -	127	0.407
C_{last}	85	72 -	101	0.096
CL_{ss}/F	131	105 -	164	0.043
V_d/F	139	95 -	204	0.116
C_{avg}	72	60	88	0.008

P-value from paired samples t-test on ln-transformed parameters.
CI, confidence interval; AUC, area under the curve; C_{max}, maximum concentration; $T_{1/2}$, half-life; CL, apparent clearance; Vd, apparent volume of distribution;

between women from the PANNA study and the IMPAACT studies (p=0.755), indicating no between-study differences, although statistical power to exclude a difference between the cohorts was limited.

All women but one had maraviroc concentrations both during pregnancy and postpartum above the suggested minimum target trough concentrations for ART-experienced patients with resistant HIV-1 strains of 50 ng/mL.[5] Furthermore, all patients had a C_{avg} above the suggested threshold of 75 ng/mL.[16] The patient with the sub-therapeutic C_{trough} showed a C_{avg} just above the threshold (76 and 83 ng/mL). The woman whose trough concentration did not exceed the target C_{trough} received maraviroc 300mg BID without a protease inhibitor. Her third trimester C_{12h} concentration was 29.7ng/mL and postpartum 33.4ng/mL. Predose concentrations were 24.6 ng/mL (third trimester) and 24.2ng/mL (postpartum) respectively.
Ten umbilical cord blood (CB) samples were collected with matching maternal blood samples. The median time between the reported last dose and delivery was 10h (range 2-16h); the median time between CB sample and maternal sample was 2.5 minutes (0-79 min). The median (range) of the concentrations found in the maternal and CB samples were 222 (26-597) ng/mL and 52 (4-209) ng/mL, respectively. The median (range) ratio of CB/maternal blood was 0.33 (0.03-0.56).

Pregnancy outcome and safety

The median gestational age at delivery was 39 (range: 37-41) weeks and median birth weight was 3215 (range: 2350-3750) grams. One baby was low birth weight, weighing 2350 grams after delivery at 37.9 weeks GA. All children were tested negative for HIV. Two congenital abnormalities were reported: congenital pulmonary airway malforma-

tion (or cystic adenomatoid malformation), for which the relationship to maternal ARV use could not be judged; sacral dimple, assessed as not related to maternal ARV use.

Two mothers developed a serious adverse event (SAE). One had a three day hospital admission due to haemoptysis at 38 weeks gestation, and was diagnosed with a respiratory tract infection. Another mother was admitted to a psychiatric hospital. These SAEs were assessed as not related to maraviroc administration. Two grade 3 or 4 laboratory events were reported: abnormal glucose and abnormal potassium level 3. HIV viral loads were detectable (>50 copies/mL) for four patients around delivery: 55, 2106, 3547 and 5110 copies/mL. These patients used maraviroc 150mg BID with a protease inhibitor (darunavir/r) and etravirine (n=2) and/or raltegravir (n=3), enfuvirtide (n=1) and an NRTI backbone (n=3). Three out of these four patients had relatively low maraviroc exposure ($AUC_{0\text{-}tau}$) and C_{last} in the third trimester of pregnancy. However, three patients with an even lower $AUC_{0\text{-}tau}$ and C_{last} did not show a detectable viral load.

Discussion

We describe the pharmacokinetics of maraviroc in 18 pregnant HIV-infected patients during the third trimester of pregnancy and after delivery. The data were collected by two networks: PANNA (Europe) and IMPAACT (US and Argentina). Overall, maraviroc concentrations were reduced in the third trimester of pregnancy, with reductions in AUC_{tau} (28%), C_{max} (30%) and C_{last} (15%). Transplacental passage of maraviroc was low, with a median cord blood to maternal delivery ratio of 0.33.

In our population of mainly cART-experienced women, 76% had undetectable HIV RNA levels at delivery and none of the children tested HIV positive. Maraviroc was well tolerated during pregnancy and infant outcomes were good. The median gestational age at delivery was 39 weeks, with no preterm births.

The maraviroc postpartum pharmacokinetic profiles with 150mg maraviroc BID with a protease inhibitor observed in this study were similar to those reported in the literature for non-pregnant adults (mixed sex).[17] The postpartum curves for the other dosing regimens: 300mg BID without a protease inhibitor, or 300mg QD with a protease inhibitor were also consistent with the non-pregnant reference pharmacokinetic profiles.[18] Most patients used 150mg maraviroc BID with a protease inhibitor and the individual ratios of the patients using other regimens fell within the range of ratios reported for this regimen. Consistent with previous assessments of antiretroviral pharmacokinetics in pregnancy there was substantial inter patient variability. Because the number of patients using alternative regimes was low, we could not directly compare the effect of pregnancy on the different regimens.

Only one subject showed maraviroc trough concentrations below the suggested minimum target concentration for ART-experienced patients with resistant HIV-1 strains of 50 ng/

mL, both during pregnancy and postpartum.[5] She was using the 300mg BID regimen without a protease inhibitor, but with tenofovir and raltegravir. Plasma concentrations of tenofovir were also very low on both occasions, whereas raltegravir exposure was not abnormal (data not shown). She reported to have been adherent for at least the week prior to pharmacokinetic assessment and she did not use other medication concomitantly. The below target trough concentrations of maraviroc in this subject during both pregnancy and postpartum suggests that the low troughs were patient specific and not caused by pregnancy. Despite these low levels she had an undetectable viral load in the third trimester and postpartum, which is in line with a recent study in which no significant relationship between maraviroc exposure and antiviral response was found.[19]
Four subjects had a detectable viral load around delivery, the maraviroc exposure was relatively low in the third trimester in three of these patients, but three other patients showed even lower exposure. Therefore, a relationship between exposure and having a detectable viral load could not be demonstrated. The four subjects with a detectable viral load were patients with a complicated treatment regimen (including at least three classes of antiretrovirals) and long treatment histories, indicating that these patients were difficult to treat.

Maraviroc is a CYP450 3A4 substrate and exposure is increased when taken together with the strong CYP3A4 inhibitor ritonavir, leading to the recommendation that maraviroc doses should be decreased when administered along with a boosted protease inhibitor regimen. A limitation of our study is the heterogeneous population and different doses and antiretroviral regimens used, a consequence of the opportunistic design of both studies. The population studied by PANNA and IMPAACT mainly received the recommended adult dose of 150mg maraviroc BID with a protease inhibitor (67%) or 300mg maraviroc BID without a protease inhibitor (11%) However, our subjects received other doses as well, including 300mg maraviroc once daily with a protease inhibitor (11%) and 300mg maraviroc twice daily with a protease inhibitor (11%).
We observed a decrease of around 30% in AUC, average and peak concentration in the third trimester of pregnancy. No other data on the influence of human pregnancy on maraviroc pharmacokinetics have been published or presented. A study in rhesus macaques reported unchanged maraviroc pharmacokinetics of a single dose intrapartum compared to non-pregnant animals.[20] However, this study is of limited value as it is difficult to compare data from non-human primates with human data and to extrapolate exposure with a single dose to chronic exposure.
The decrease in exposure to maraviroc observed in our subjects during pregnancy is of similar magnitude to the decrease seen with the protease inhibitors atazanavir, darunavir and lopinavir.[21-23] Several metabolism related mechanisms could explain the lower maraviroc exposure in pregnancy: increased CYP450 3A4 activity in the gut and liver leading to decreased gastro-intestinal absorption due to increased gut metabolism and increased hepatic clearance, and/or to less boosting by ritonavir associated with

lower ritonavir exposure in pregnancy. Unfortunately, paired third trimester and postpartum data are available for only a single woman not using a protease inhibitor. The ratio of the maraviroc AUC_{tau} third trimester/postpartum was 0.92 for this patient, while the ratios of the maraviroc AUC_{tau} third trimester/postpartum for patients concomitantly using a protease inhibitor ranged from 0.35-1.46. Therefore, we cannot conclude that less boosting in pregnancy is the major cause of the lower maraviroc exposure. Other mechanisms may also explain the lower exposure, including reduced intestinal motility, a larger plasma volume and increased hepatic blood flow.

Placenta passage of maraviroc is limited, with a median cord blood to maternal blood ratio of 0.33. This is consistent with the previously ratio of 0.37 in a single case report[12] and higher than that reported in an *ex vivo* placenta perfusion model. ABC efflux transporters may possibly play a role resulting in limiting maraviroc transfer across the placenta to the fetus.[13]

In conclusion, although overall maraviroc exposure is 28-30% lower during pregnancy, C_{trough} was reduced to a lesser extent. All except one of the subjects met the target trough concentration during pregnancy for antiretroviral resistant HIV-1, suggesting that the standard adult dose seems to be sufficient in pregnancy.

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Physiologically-Based Modelling of Darunavir/Ritonavir Pharmacokinetics during Pregnancy

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Abstract

Pregnant women are usually excluded from clinical trials. Physiologically-based-pharmacokinetic (PBPK) modelling may provide a method to predict pharmacokinetics in pregnant women, without having to perform extensive *in vivo* clinical trials. Here, we used mechanistic modelling to delineate the potential impact of drug transporters on darunavir pharmacokinetics and identify current knowledge gaps that limit the accurate PBPK modelling of darunavir/ritonavir (darunavir/r) exposure in pregnancy. Simcyp (v13.2) was used for PBPK modelling, using physicochemical and *in vitro* pharmacokinetic parameters of darunavir and ritonavir from literature. K_m and V_{max} for CYP3A4-mediated darunavir biotransformation and inhibition by ritonavir were determined experimentally. Sensitivity analyses were used to assess the contribution of hepatocyte influx and efflux transporters. Simulations were compared to previously published clinical pharmacokinetic data. We found that the well-stirred-liver-model overestimated darunavir exposure substantially. A permeability-limited-hepatic-model, including hepatic-uptake and -efflux transporters and efficient enterohepatic circulation, resulted in an acceptable description of darunavir/r exposure. For the 600/100mg darunavir/r twice-daily dose and the 800/100mg once-daily dose, peak and total exposure at steady state were estimated within 2-fold range of reported data. The model predicted a decrease in AUC of 27% and 41%, which is in the range of the observed decrease during pregnancy of 17-22% and 33% for the twice-daily and the once-daily dose, respectively. In conclusion: our data support a clinically relevant role of hepatic transporters in darunavir pharmacokinetics. The described model successfully approximated ritonavir-boosting and the decrease in darunavir exposure during pregnancy. Future *in vitro* experiments should generate quantitative kinetic data of passive and transporter-mediated darunavir handling by hepatocytes and intestinal epithelium.

Introduction

Pregnant women are generally excluded from clinical trials during the development phase of new drugs, for ethical and safety reasons.[1-3] However, medication use during pregnancy is not uncommon.[4] Most information regarding safety, pharmacokinetics and placental transfer is collected after drugs are available on the market. This implicates that pregnant women often use medication, while the treating physicians have no knowledge of the systemic drug exposure, nor placental transfer of the drug and hence the safety for the unborn child. Vice versa, medication in pregnant women may be withheld by physicians because they lack this critical information, and clinical benefit is missed. It would be a tremendous advantage to use *in silico* modelling for the prediction of maternal and foetal exposure of (new) drugs possibly being used during pregnancy. Such models may be particularly relevant in the treatment of HIV. To prevent mother-to-child transmission, pregnant HIV-infected women should use combination antiretroviral treatment (cART).[5,6] Pregnancy-induced reductions in maternal exposure to these drugs may lead to sub-therapeutic levels, eventually leading to virological failure and/or resistance, thus requiring dose adjustment.[7] Moreover, clinical drug-drug interaction trials are not performed in this patient population, leaving physicians unaware of the possible impact of concomitantly administered interacting drugs in the pregnant patient population.

Physiologically based pharmacokinetic (PBPK) modelling is a mechanistic approach to predict absorption, distribution, metabolism and excretion (ADME) of drugs, based on anatomy and physiology of the human body, physicochemical properties of the drug as well as *in vitro* pharmacokinetic data on the transport and biotransformation of the drug. In this way, the handling of a drug by the body can be simulated, taking molecular processes as a starting point. This contrasts with population or compartmental pharmacokinetic methods, which are based on empirical models that take clinical pharmacokinetic data as a starting point. As such, PBPK modelling provides a potentially relevant approach to predict the effect of pregnancy itself on drug pharmacokinetics. During pregnancy, numerous changes in physiology are known to occur, which affect pharmacokinetic parameters like volume of distribution, plasma protein binding and metabolic clearance of drugs.[8-10] In addition, PBPK modelling would in principle allow quantitative assessment of the potential impact of concomitantly prescribed interacting drugs on drug exposure. This is particularly useful in HIV-infected pregnant women, who typically use a cocktail of drugs. In fact, in these patients drug-drug interactions are intentionally employed to optimize treatment. For example, ritonavir, a strong cytochrome P450 3A4 (CYP3A4) inhibitor, is used to boost (increase the exposure) of antiretroviral drugs that are metabolized by CYP3A4. Finally, PBPK modelling provides an effective manner to integrate the currently available mechanistic drug disposition data and obtain a better understanding of the pharmacokinetic behaviour of a compound.

Recently, Simcyp has developed a PBPK modelling platform that includes physiological changes in relation to duration of pregnancy (p-PBPK model).[11,12] Exposure to drugs during pregnancy at any gestational age may be predicted, permitted that *in vitro* pharmacokinetic data of all relevant processes to drug disposition are available.[11] To study the feasibility of such a p-PBPK modelling approach for adequate prediction of maternal drug pharmacokinetics during pregnancy, we now focused on the antiretroviral compound darunavir. Darunavir is an HIV-protease inhibitor (PI), always co-administered with ritonavir to boost darunavir plasma concentrations.

We developed and evaluated a mechanistic model to predict darunavir exposure of the common treatment regimens used in pregnancy, namely 600/100mg darunavir/r twice daily and 800/100mg darunavir/r once daily. These efforts also aimed to elucidate whether the required mechanistic *in vitro* pharmacokinetic parameters to incorporate in such a model are currently available and identify current knowledge gaps that limit the accurate PBPK modelling of darunavir/r exposure, thereby generating new hypotheses and hence directions for future studies.

Methods

PBPK modelling platform

For this study we used the Simcyp Population-based Pharmacokinetic Simulator version 13 release 2 (Simcyp Limited, a Certara company, Sheffield, UK) as PBPK platform. Details on the algorithms to calculate *in vivo* pharmacokinetic parameters, a description of the Simcyp *in vitro–in vivo* extrapolation (IVIVE) methods, the structure of the physiological model and a description of the differential equations used, have been published before.[13-16] Simulations were performed using the Simcyp virtual populations of healthy volunteers and pregnant women. For each simulation, number of patients, gender, age range and gestational age of the virtual population were matched with clinical data sets used for validation.

Physicochemical and in vitro pharmacokinetic parameters of darunavir

Physicochemical properties (molecular weight, log P) and blood and protein binding properties of darunavir were obtained from literature (see Table 1). Holmstock *et al.*[17] studied the permeability of darunavir using Caco-2 monolayers in absence and presence of ritonavir. In presence of ritonavir the intestinal permeability of darunavir in mice was reported to be 2.7-fold increased, as a result of the strong P-glycoprotein inhibitory effect of ritonavir.[17] This was in line with studies reporting similar increases in darunavir permeabilities in Caco-2 monolayers in the presence of P-glycoprotein (P-gp) inhibitors.[18] Therefore, when modelling the pharmacokinetics of darunavir alone, we used a reported *in vitro* Caco-2 permeability of $7 \cdot 10^{-6}$ cm s^{-1} for darunavir alone and $18.6 \cdot 10^{-6}$ cm s^{-1}

when simulating combined darunavir/r administration. Darunavir is mainly eliminated by hepatic metabolism, almost exclusively by CYP3A4, without a significant contribution of renal elimination.[19] We therefore did not include a renal clearance component for the unchanged drug in our simulations. To determine K_m and V_{max} for CYP3A4-mediated darunavir metabolism, we performed *in vitro* tests using human liver microsomes (HLM) and baculosomes overexpressing human recombinant CYP3A4. See the corresponding paragraph below for a more detailed description of these studies.

For darunavir simulations the Simcyp full PBPK distribution model was applied, which makes use of a number of time-based differential equations in order to simulate the concentrations in various organ compartments: the blood (plasma), adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, pancreas, skin and spleen, with addition of the fetoplacental compartment during pregnancy. Inter-individual variability is introduced through tissue volume prediction, based on known relations between these and easy-to-measure parameters such as age, sex, weight and height. Initially, we used a well-stirred liver model without hepatic basolateral uptake, canalicular excretion or intestinal re-absorption of darunavir. In subsequent steps, modelling with a permeability-limited liver model was performed, exploring various degrees of hepatic uptake and efflux clearance, while also addressing the impact of various efficiencies of intestinal re-uptake of drug that was excreted unchanged into bile. Modelling results were validated against literature, as indicated in the PBPK workflow section below, as well as in the results section.

Physicochemical, in vitro and clinical pharmacokinetic parameters of ritonavir

The ritonavir model used to simulate boosting was a semi-mechanistic pharmacokinetic model, based on both *in vitro* as well as clinical pharmacokinetic data and physicochemical parameters. Absorption was based on first-order kinetics (ka=0.24/h and fa=1) and Vss=0.41 L/kg, in line with data that has been derived from clinical studies, and as included in the existing Simcyp compound library.[20] Oral ritonavir clearance of the 100mg boosting dose has been reported in healthy volunteers to be 16 L/h.[21] For the pregnant situation we increased the oral clearance to 20 L/h, as derived from clinical observations.[22,23] The interaction potential of ritonavir with other drugs was based on competitive inhibition of CYP2C9, CYP2D6 and CYP3A4 (see Table 2), of which CYP3A4 competitive inhibition potential is particularly important to simulate darunavir boosting. We performed *in vitro* tests in HLM to corroborate and if necessary update the IC_{50} of ritonavir on CYP3A4-mediated darunavir biotransformation (see below). In addition to competitive inhibition, Fahmi *et al.*[24] and Kaspera *et al.*[25] described that ritonavir also displays mechanism-based inhibition of CYP3A4. In addition to a competitive inhibition, we also included mechanism-based inhibition in the model. Kaspera *et al.* reported a K_{app} (concentration of mechanism-based inhibitor associated with half maximal inactivation rate) of 0.1 μM and a K_{inact} (rate of enzyme inactivation) of 0.32/h, respectively.[25] Inductive properties of ritonavir on CYP3A4

have also been described and were added to the model based on Kirby *et al.*.[26] As we also explored a potential role of hepatic uptake and efflux transport in the pharmacokinetics of darunavir, we included reported *in vitro* inhibitory potencies of ritonavir on the following relevant transport proteins: P-gp (hepatic canalicular efflux), Organic Anion Transporting Polypeptide 1B1 (OATP1B1, *SLCO1B1*) and OATP1B3 (*SLCO1B3*), which are both hepatic basolateral influx transporters.[27,28]

In vitro testing

Enzyme kinetics

A range of darunavir concentrations (1-25 µM) was incubated with HLM in presence and absence of NADPH. To avoid depletion of NADPH during the incubations a regenerating system was employed. Each incubation mixture (250 µL) contained HLM (0.25 mg/mL), components of the NADPH-regenerating system (1 mM NADP, 3 mM $MgCl_2$, 1 IU/L glucose-6-phosphate dehydrogenase, 10 mM glucose-6-phosphate) and darunavir (1-25 µM) in phosphate buffer (pH=7.4). After 10 minutes pre-incubation of buffer containing HLM and darunavir at 37 °C, the reaction was started by adding the NADPH-regenerating system. The mixture was subsequently incubated for 30 min at 37 °C, a time interval over which darunavir biotransformation was linear (data not shown). Parallel incubations were performed in the absence of NADPH to correct for loss of darunavir (substrate), unrelated to CYP3A4-mediated biotransformation. The reaction was stopped by adding 500 µL of ice-cold methanol, after which the samples were homogenized and left on ice for at least 10 minutes. The samples were then centrifuged at 10,000 rpm for 2 minutes. Darunavir biotransformation was assessed by measuring disappearance of the substrate from the incubation buffer (also see paragraph on kinetic analysis). An aliquot of 500 µL of the supernatant was used for determination of darunavir concentrations by HPLC, a method adapted from the protocol described by Droste *et al.*[29] (LLOQ of 50nM or 0.027 mg/L). The same protocol was used to analyse the results obtained from the incubations with baculosomes over-expressing human recombinant CYP450 3A4 (20 pmol/mL), over a darunavir concentration range of 1-10 µM.

Michaelis-Menten kinetic analysis

The rate of darunavir conversion by HLM and baculosome incubations was calculated by subtracting the amount of darunavir remaining in the preparation at the end of the incubation period in the presence of NADPH from the amount of darunavir that remained after the incubation in the absence of NADPH. Data were expressed as $pmol.min^{-1}.mg^{-1}$ microsomal protein (HLM) or $pmol.min^{-1}.pmol^{-1}$ CYP3A4 (baculosomes) and plotted against measured initial darunavir concentrations (µM). Data were analysed

by nonlimear regression analysis according to the Michaelis–Menten equation using Graphpad Prism 5 (version 5.02, GraphPad Software Inc) according to:

$V = (V_{max} \cdot [darunavir])/(K_m + [darunavir])$

where V_{max} is the maximum bioconversion rate of the enzyme and K_m (μM) is the Michaelis constant, defined as the reaction concentration required to reach half of V_{max}.

Inhibitory effect of ritonavir on darunavir biotransformation

Incubations with HLM were performed as described above, in the presence of 1 μM darunavir (substrate) and ritonavir (inhibitor) at concentrations ranging from 0.01-0.3 μM. The concentration of ritonavir causing half maximal inhibition of CYP3A4-mediated darunavir biotransformation (IC_{50}) was obtained by plotting the normalized darunavir bioconversion rates (% of control) against log [ritonavir] (M). The IC_{50} was estimated by fitting a one binding site inhibition model with a variable slope to the data, using Graphpad. Derived IC_{50} values from three separate experiments each performed in duplicate were subsequently converted to a K_i value (binding affinity of the inhibitor) using the Cheng-Prusoff equation (IC_{50}/ ((1+ [darunavir] /$K_{m,\ darunavir}$)).

PBPK workflow and statistical analysis

We first simulated darunavir kinetics in healthy volunteers, when administered as a single dose without ritonavir. We then verified the interactions of darunavir with ritonavir, first for a single dose of darunavir, after Sekar *et al.* and Rittweger *et al.*[19,30] and then for the steady-state situation for the clinical dosing regimens 600/100mg darunavir/r twice-daily[31,32] and 800/100mg once-daily.[33,34] Before applying the ritonavir semi-mechanistic model in the darunavir PBPK modelling approach, ritonavir model performance was validated against data available from interaction studies between ritonavir and midazolam (a probe substrate for CYP3A4).[26,35] Acceptance criteria were defined as follows: both geometric mean C_{max} and AUC should not deviate more than 2-fold from the observed pharmacokinetic parameters, as is commonly applied in assessing PBPK-model performance. After modelling of darunavir/r exposure in healthy volunteers in single as well as multiple dose regimens, we proceeded to modelling darunavir/r pharmacokinetics during pregnancy. Simulations of twice daily 600/100 and once daily 800/100 darunavir/r were performed during the second and third trimester (the same gestational age as reported in published clinical sudies). For the non-pregnant situation, simulations were performed using the healthy volunteer population (100% females, matched for age where reported). An independent samples t-test (SPSS 20) was performed on the log-transformed AUCs comparing the pregnant situation with the non-pregnant situation (per gestational age), resulting in geometric mean ratios (pregnant:non-pregnant) and 90% confidence intervals. Finally, we performed simulations with higher darunavir/r dosages: 900/100mg darunavir/r once daily,

as well as 800/100mg and 900/100mg darunavir/r twice daily during pregnancy (third trimester), to predict which dose would compensate for the observed decrease in exposure. These dosages were chosen because darunavir is available as 300, 400, 600 or 800mg film-coated tablets for oral use.

Comparison of physiological parameters of the Simcyp pregnant population with a real-life HIV-infected pregnant population

Within a clinical study coordinated by our institution (PANNA study; www.pannastudy.com), several physiological parameters (haematocrit, serum creatinine, α1-acid glycoprotein and albumin concentrations) were collected during pregnancy and after delivery, next to the primary aim of the study to collect pharmacokinetic parameters of antiretroviral drugs. We compared these physiological parameters within the real-life HIV-infected pregnant population collected in PANNA, with those used to define the Simcyp virtual healthy pregnant population as describe by Abduljalil *et al.*[10] Mean ± SD values for the respective physiological parameters were calculated (99 HIV-infected pregnant patients) at the different gestational ages, ± 2 weeks of the gestational ages reported by Abduljalil *et al.*[10] Data were compared with the mean values summarized by Abduljalil to investigate if there were any differences between HIV-infected and non-HIV infected pregnant women. An independent samples t-test was used to analyse the data (SPSS 20).

Results

In vitro metabolic clearance of darunavir

Literature search yielded values for a substantial number of mechanistic pharmacokinetic parameters related to absorption and distribution of darunavir (Table 1). However, quantitative data on *in vitro* darunavir metabolic clearance by CYP enzymes (K_m and V_{max} values) were not available in peer-reviewed literature (we found only a conference abstract). As it is known from literature that darunavir is a CYP3A4 substrate, we determined the *in vitro* metabolic K_m and V_{max} using HLM.

The rate of conversion of darunavir in HLMs was found to be linear up to 30 min (data not shown). Enzyme kinetics was therefore determined at a protein concentration of 0.25 mg/mL over a 30 minute time period. We found that darunavir bioconversion was saturable with increasing concentrations (Figure 1), characterized by a K_m (95% CI) of 1.1 (0.3-1.8) µM and V_{max} (95% CI) of 180 (150-210) $pmol.min^{-1}.mg^{-1}$ protein. The K_m value derived from the experiments performed in baculosome CYP3A4-overexpression system was 0.80 (0.17-1.43) µM ($CL_{int, met}$ [intrinsic metabolic clearance] of 2.25 µl $min^{-1}pmol^{-1}$ CYP3A4) which, with respect to K_m, is in line with results obtained with the HLM.

Table 1. Physico-chemical and in vitro pharmacokinetic parameters of darunavir

	Parameter	Value	References
Physicochemical	Molecular weight	548 g/mol	Pubchem[52] ; http://www.antimicrobe.org/d94.asp (Feb 2015)[53] ; http://www.drugbank.ca/drugs/db01264 (Feb 2015)[54]
	Log Po:w	1.8	
	Compound type	Weak Base	
	pKa (strongest basic) pKa (strongest acidic)	2.39 13.59	http://www.drugbank.ca/drugs/db01264 (Feb 2015)[54]
	Physiological charge	0	
	Blood to plasma ratio	0.64	EMA scientific discussion[19,55]
	Fraction unbound in plasma	0.06	EMA scientific discussion[19,55]
	Main plasma binding protein	α1-acid glycoprotein (AGP)	EMA scientific discussion[19,55]
Absorption	Absorption model	Advanced Absorption and Dissolution Model (ADAM)	
	Permeability predicted via	Caco-2	
	Apical pH : Basolateral pH	6.5 : 7.4	
	Activity	Passive & Active	
	Papp Caco-2 (10E-06 cm/s)	7 (absence of ritonavir) 18.9 (presence of ritonavir)	Holmstock *et al.* 2012[17] Holmstock *et al.* 2012[17]
	Scalar	1	
	Dosage form	Immediate release, dissolution over 2 hour period (fed condition)	
	Available for reabsorption after biliary excretion	Sensitivity analysis 0-100%, 80% was optimal	
Distribution	Distribution Model	Full PBPK Model	
	Predicted V_{ss} (L/h)	1.23	Prediction method by Rodgers *et al.*[56]
	Kp scalar	6	Empirically determined[19,55]

Table 1. - continued Physico-chemical and in vitro pharmacokinetic parameters of darunavir

	Parameter	Value	References
Metabolism/ elimination	Clearance Type	Enzyme Kinetics	
	Test system	HLM	
	Enzyme kinetics CYP3A4	V_{max} = 181 pmol/min/mg microsomal protein K_m = 1.1 µM Protein concentration = 0.25 mg/mL $F_{u,}$ mic = 0.96 (pH 7.4)	Determined Determined Determined Calculated
Transport	Passive difussion clearance	0.1 µL/min/million cells	Simcyp default value
	Basolateral hepatocyte uptake, $CL_{int,T}$	Sensitivity analysis 0-500 µL/min/million cells; 100 µL/min/million cells used in final model	
	Canalicular hepatocyte efflux (P-gp) $CL_{int,T}$	Sensitivity analysis 0-500 µL/min/million cells; 100 µL/min/million cells used in final model	

* Prediction method 2 = after Rodgers *et al.*[56]
V_{max}: maximum rate of metabolite formation (pmol/min/mg microsomal protein)
K_m: Michaelis-Menten constant (substrate concentration at ½ V_{max}) µM
$CL_{int,T}$: *In vitro* transporter-mediated intrinsic clearance
$F_u$mic: fraction of unbound drug in the *in vitro* microsomal incubation (calculated)

Simulation of darunavir pharmacokinetics without ritonavir

We simulated the concentration-time curve of a single oral 600mg dose of darunavir in plasma with the well-stirred liver model, hence assuming passive partitioning of drug from plasma into liver tissue, instantaneous homogeneous distribution across liver mass, and CYP3A4-mediated metabolic clearance as the only relevant clearance mechanism. As can be seen in Figure 2, the simulations overestimated the total exposure of darunavir when compared with the clinical data presented by Sekar *et al.* and Rittweger *et al.*.[19, 30]

Inclusion of hepatic influx and efflux transport via sensitivity analyses

According to the general workflow of PBPK modelling and simulation described by Ke *et al.*,[12] we refined the model by taking clues from *in vitro* pharmacological studies as a starting point. Visual inspection of the curves indicated that the simulated absorption phase was in line with the observed data. Therefore, we explored whether other clearance mechanisms may contribute to darunavir plasma clearance. It is known that darunavir interacts with basolateral OATP1B1, 2B1, 1B3 and canalicular P-gp hepatocyte membrane transporters.[18,28,36] Therefore, we investigated whether inclusion of a

Table 2. Physico-chemical and in vitro pharmacokinetic parameters of ritonavir

	Parameter	Value	References
Physicochemical	Molecular weight	720.95 g/mol	Pubchem[57]
	Log Po:w	4.3	Simcyp compound library
	Compound type	Monoprotic Base	
	pKa	2	Simcyp compound library
	Blood to plasma ratio	0.587	Simcyp compound library
	Fraction unbound in plasma	0.02	http://www.drugbank.ca/drugs/DB00503 [58]
	Main plasma binding protein	α1-acid glycoprotein (AGP)	
Absorption	Absorption model	First-order absorption	
	Fraction absorbed	1	Simcyp compound library
	Ka (1/h)	0.24	Simcyp compound library
Distribution	Distribution Model	Minimal PBPK Model	
	Vss (L/kg)	0.41	Simcyp compound library
Metabolism/elimination	Clearance Type	*In vivo* clearance	
	CL_{po} (L/h)	16, non pregnant; 20 pregnant	Product characteristics Norvir,[21] Colbers *et al.*[22]
	CL_r (L/h)	0.32	
Interaction CYP	CYP2C9 Ki (μM)	4 (fumic 0.29)	
	CYP2D6 Ki (μM)	10 (fumic 0.29)	
	CYP3A4 Ki (μM)	0.03 (fumic 0.976)	determined
	CYP3A4 Kapp (μM)	0.1 (fumic 0.91)	Kaspera *et al.* 2014[25]
	CYP3A4 Kinact (1/h)	0.32 (fumic 0.91)	Kaspera *et al.* 2014[25]
Interaction transp	ABCB1 (P-gp) Ki (μM)	0.2 (fuinc 0.233)	Drewe 1999[27]
	Pooled basoleteral uptake Ki (μM)	2.5 (fuinc 1)	Annaert 2010[28]

* Prediction method 2 = after Rodgers *et al.*[56]
Ki: concentration of inhibitor that supports half maximal inhibition
Fumic: fraction of unbound drug in the *in vitro* microsomal incubation (calculated)
K_{app}: concentration of mechanism-based inhibitor associated with half maximal inactivation rate
K_{inact}: inactivation rate of the enzyme
Fuinc: fraction of unbound drug in the *in vitro* hepatocyte incubation

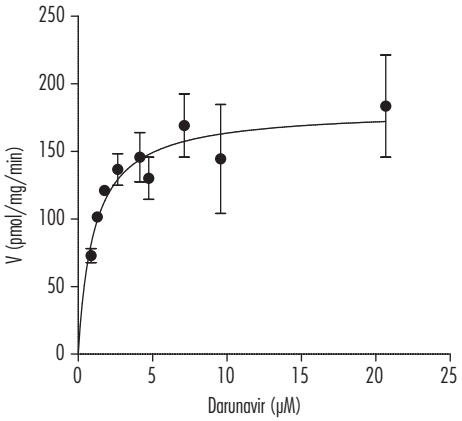

Figure 1. Rate of darunavir disappearance from human liver microsomal incubations at increasing substrate concentrations. Data represent a typical experiment performed in duplicate, mean ± range (min/max) are presented. A Michaelis-Menten equation was fitted to the data as described in the materials and methods section. Calculated K_m and V_{max} are 1.1 μM and 180 pmol.min^{-1}.mg^{-1} microsomal protein.

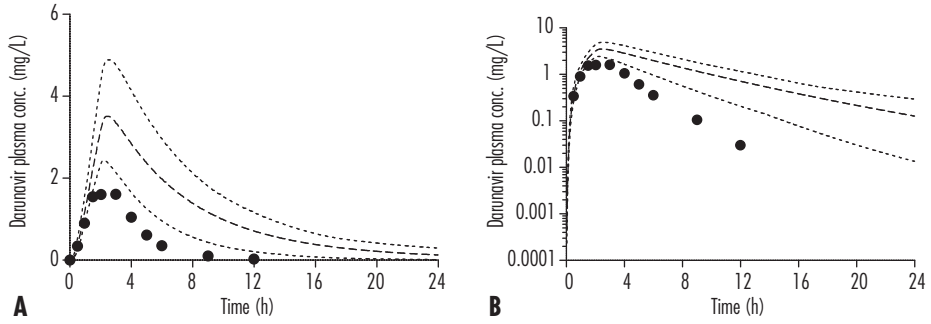

Figure 2. Simulation of darunavir plasma concentrations after a single oral dose of 600mg, using the well-stirred liver model on linear **(A)** and semi-logarithmic **(B)** scale. The closed circles represent the observed concentrations,[19] the dashed line represents the simulated mean concentrations, and the dotted lines represent the associated 95% confidence intervals of the simulated concentrations. Simulated plasma concentration data were derived from 8 healthy subjects (3 female, 5 male), aged 27-37 years, matching the subjects in the reported trial as much as possible.

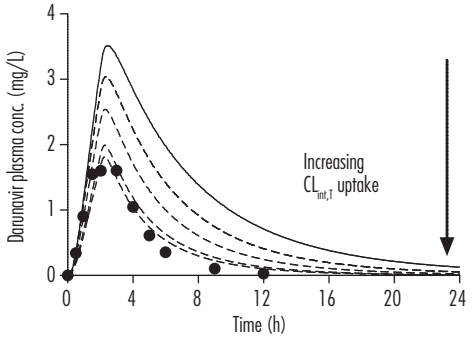

Figure 3. Darunavir single dose (600mg oral), simulation of increasing $CL_{int,T}$ uptake values using the permeability-limited liver model. The closed circles represent the observed concentrations,[19] the solid line reflects the data obtained with the well-stirred liver model while the dashed lines represent simulated mean concentrations applying the permeability-limited liver model with varying $CLi_{nt,T}$ uptake values: 100, 200, 300, 400 and 500 μL/min/million hepatocytes.

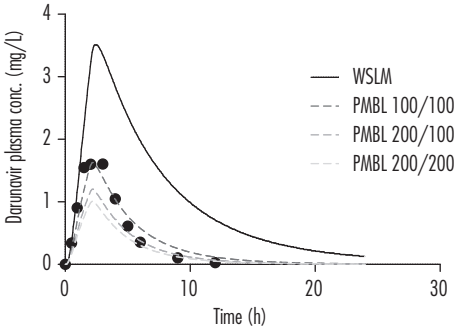

Figure 4. Darunavir single dose (600mg oral) simulation results when assuming combined influx and efflux processes taking place using the permeability-limited liver model (PMBL). The closed circles represent the observed concentrations,[19] the solid line reflects the data obtained with the well-stirred liver model (WSLM), while the dashed lines represent simulated mean concentrations applying the permeability-limited liver model with different $CL_{int,T\ uptake}/CL_{int,T\ efflux}$ values, as indicated in the graph.

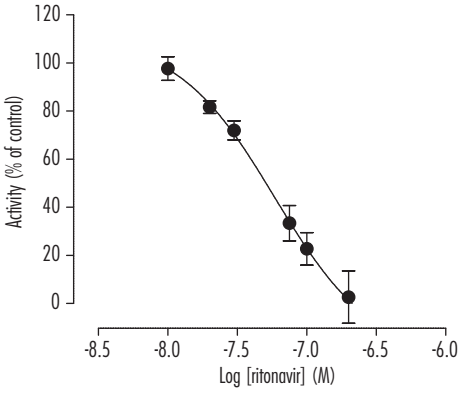

Figure 5. Inhibition of darunavir metabolism in human liver microsomal preparations by ritonavir. Data represent the mean ± SEM of three separate experiments, each performed in duplicate. A one-site binding model with variable slope was fitted to the data in order to estimate ritonavir IC_{50}. The calculated IC_{50} was 0.06 µM.

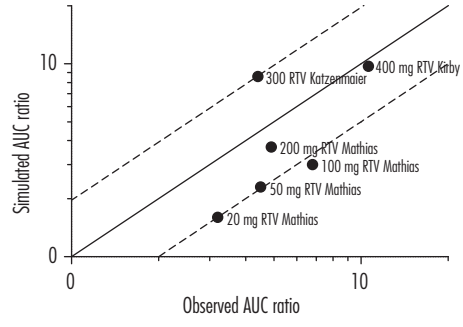

Figure 6. Scatter plot representing the AUC ratio ($AUC_{with\ inhibitor}/AUC_{without}$) of simulated and clinical drug-interaction studies between midazolam and different ritonavir doses, as reported by Katzenmaier *et al.*, Kirby *et al.* and Mathias *et al.*.[26, 35, 37] Dashed lines represent a two-fold difference from the observed values. AUC: area under the plasma concentration-time curve, RTV: ritonavir

Table 3. $CL_{int,T}$ influx and efflux values reported in literature

Compound	CL_{int} uptake transport (µL min^{-1} million $cells^{-1}$)	CL_{int} efflux transport (µL min^{-1} million $cells^{-1}$)	Reference
Bosentan	17.9		Ménochet *et al.*
	9.1	7.4	Jones *et al.*
Cerivastatin	9.6 (2.7)	6.2 (1.8)	Jones *et al.*
Fluvastatin	45 (21)	17	Jones *et al.*
Pitavastatin	40.7		Ménochet *et al.*
Pravastatin	2.77		Ménochet *et al.*
	1.8	1.2	Jones *et al.*
Repaglinide	**79.0**		Ménochet *et al.*
	30 (16)	0	Jones *et al.*
Rosuvastatin	9.21		Ménochet *et al.*
	9.3 (2.6)	1.5 (0.088)	Jones *et al.*
Telmisartan	**95.2**		Ménochet *et al.*
Valsartan	2.88		Ménochet *et al.*
	2.1 (0.48)	**96**	Jones *et al.*

Jones *et al.*[43] presented *in vitro* parameters estimated from sandwich culture human hepatocyte parameters at a single substrate concentration, mean from multiple replicates of one to two donors. Ménochet *et al.*[44] performed uptake kinetics in cryopreserved human hepatocytes at seven concentrations, the uptake parameters were estimated using a mechanistic two-compartment model. In bold: parameters reported close to simulation estimates.

basolateral influx and a canalicular efflux component in the model could improve simulations. In Figure 3, the effect was examined of an increased intrinsic uptake clearance on model fit. In Figure 4, it can be seen that inclusion of a hepatocyte cannalicular efflux component next to a basolateral uptake step, allows for better model fits at lower intrinsic transport clearance values ($CL_{int,T}$, µL.min^{-1}·million $cells^{-1}$).

In vitro transport parameters (K_m, V_{max} or $CL_{int,T}$) for darunavir in human hepatocytes or recombinant overexpression systems of drug transporters were not available in literature. We therefore compared the estimated $CL_{int,T}$ values derived from our simulations with measured values of other hepatocyte uptake and efflux transporter substrates. In Table 3, it can be seen that the required $CL_{int,T}$ values are of pharmacological relevance, when combined hepatocellular influx and efflux processes take place.

Inhibitory effect of ritonavir on CYP3A4

Darunavir is, however, always administered together with ritonavir. Ritonavir inhibits CYP3A4-mediated darunavir metabolism, hence boosting its plasma exposure. In order to simulate this effect on a mechanistic level, the ritonavir IC_{50} for CYP3A4-mediated darunavir metabolism is required. As can be seen in Figure 5, ritonavir completely

inhibited darunavir bioconversion, with an IC_{50} of 0.06 (0.04-0.10) μM, which was equivalent to a mean Ki value of 0.03 μM. The Ki value was included in the ritonavir model, together with other parameters listed in Table 2. Performance of the ritonavir PBPK model was then validated in simulations with midazolam, a probe substrate for CYP3A4. We simulated the interaction between several doses of ritonavir and midazolam, as described by Katzenmaier *et al.*[37], Kirby *et al.*[26] and Mathias *et al.*[35] In Figure 6 the results of these simulations are presented, demonstrating that the model could predict the increase in midazolam exposure within a 2-fold difference of reported clinical C_{max} and AUC ratio's.

Simulation of combined darunavir/r administration

Next, simulations of the interaction of darunavir with ritonavir were performed. First, the influence of ritonavir multiple doses (start 2 days prior to darunavir administration until day 4) on a single dose darunavir (600mg, given on day 3) was simulated and the data were compared with those reported by Rittweger *et al.*[19] As can be seen in Figure 7, a strong boosting effect could be simulated when combining darunavir with ritonavir. However, this was only the case if it was assumed that unchanged drug excreted via the bile was readily available for enteric reabsorption. Reducing the percentage available for re-absorption to 0% abolished enterohepatic cycling and the boosting effect of ritonavir was lost. In contrast, it followed from our simulations that enterohepatic recirculation does not appear to be a major player in determining exposure of single dose darunavir without ritonavir, as inclusion of reabsorption of biliary excreted darunavir was not necessary to describe these clinical data (Figure 7).

We also assessed whether the model predicted the steady-state pharmacokinetics of relevant clinical dosage regimens of 600/100mg darunavir/r twice daily and 800/100mg darunavir/r once daily. Simulations were compared with two studies for each regimen.[32-34,38] For this situation, darunavir/r pharmacokinetics could also be simulated succesfully with indicated $CL_{int,T}$ uptake of 100 μL/min/million cells, $CL_{int,T}$ efflux of 100 μL/min/million cells and efficient enteric reuptake (80%) values in place. The simulated geometric mean darunavir AUCs and C_{max} values were within a factor 2 from the observed parameters (Figure 8).

Simulation of darunavir and ritonavir in pregnancy

With the same model, simulations were performed in pregnancy, for both regimens at steady-state, during the second and third trimester, which could be compared with available literature data. The results are depicted in Figures 9 and 10. It can be seen that that the prediction of the shape of the curves for both the non-pregnant as the pregnant situation was good. For the 800/100mg darunavir/r once-daily dosing regimen the exposure, however, was somewhat underestimated. In Table 4 the simulated pharmacokinetic parameters are compared with the observed parameters from several studies.[22,39] Pharmacokinetic parameter values as well as their decrease during pregnancy

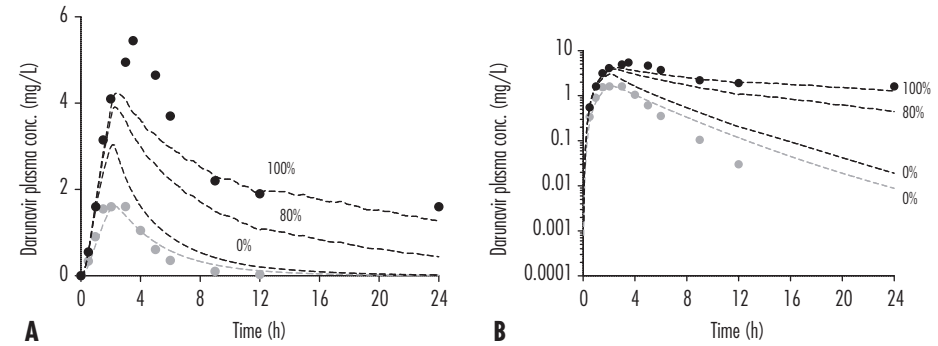

Figure 7. Linear **(A)** and semi-logarithmic **(B)** plasma concentration-time curves for a single oral dose of darunavir (600mg) either after 2 days pre-treatment and in combination with 100 mg ritonavir twice daily (black), or as a single oral dose of 600 mg darunavir alone (grey). A permeability-limited liver model was simulated, with combined influx and efflux processes taking place, both at a clearance rate of 100 µl/min/million cells. For the SD darunavir/r simulations, response of the model to varying percentages of biliary excreted unchanged drug available for enteric re-absorption are indicated (reflecting a maximum degree of enteric hepatic recirculation possible). The closed circles represent the observed concentrations.[19]

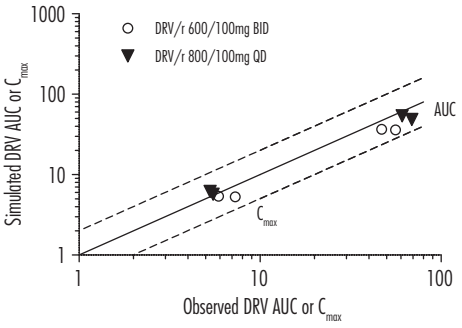

Figure 8. Scatter plot representing the AUC and C_{max} from simulated and clinical darunavir/r trials at steady-state. For the 600/100mg darunavir/r twice-daily dose two studies by Sekar *et al.*[31, 32] were used and for the 800/100mg darunavir/r once-daily dose results reported by Boffito *et al.*[33] and Kakuda *et al.*[34] were used. Dashed lines represent a two-fold difference from the observed values. AUC: area under the plasma concentration-time curve, C_{max}: maximum concentration, DRV: darunavir.

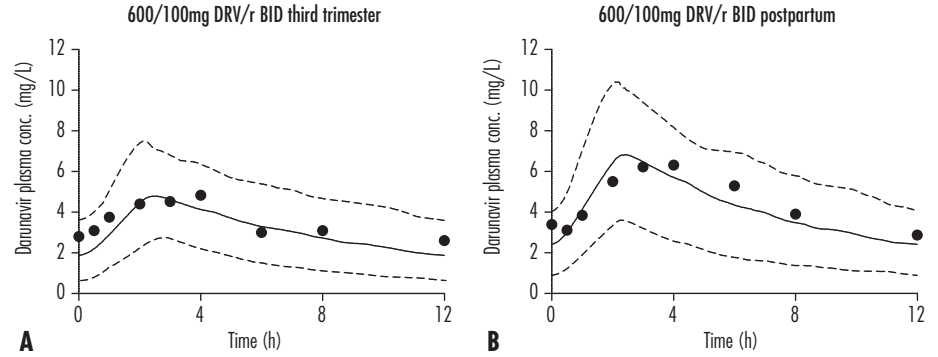

Figure 9. Simulation of darunavir plasma concentrations at steady-state (14 days of treatment) after BID 600/100mg darunavir/r in **(A)** the third trimester of pregnancy (gestational week 36, n=11, age 20-35 years) and **(B)** postpartum/non-pregnant (n=11, age 20-35 years). The closed circles represent the observed concentrations,[39] the solid line represents the simulated mean concentrations, the dashed lines represent the 95% confidence interval of the simulated concentrations.

Table 4. Darunavir pharmacokinetic parameters in pregnancy: simulated versus observed

Reference	2nd trimester	3rd trimester	postpartum	GM ratio 2nd/pp (90%CI)	GM ratio 3rd/pp (90%CI)
600/100mg darunavir/ritonavir BID					
AUC (mg·h/L)					
Zorrilla[39]	39 (SD 10)	44 (SD 16)	55 (SD 27)	0.76 (0.63-0.90)	0.83 (0.72-0.97)
Colbers[22]		41 (27-62)	53 (38-73)		0.78 (0.60-1.00)
simulation	34 (25-46)	34 (26-45)	47 (36-61)	0.73 (0.51-1.02)	0.73 (0.53-1.02)
C_{max} (mg/L)					
Zorrilla[39]	4.6 (SD 1.1)	5.1 (SD 0.5)	6.5 (SD 2.4)	0.72 (0.61-0.86)	0.81 (0.69-0.96)
Colbers[22]		5.0 (3.2-7.8)	6.5 (4.4-9.7)		0.76 (0.53-1.11)
simulation	4.7 (3.7-6.1)	4.6 (3.7-5.8)	6.6 (5.3-8.3)	0.71 (0.53-0.96)	0.70 (0.53-0.93)
800/100mg darunavir/ritonavir QD					
AUC (mg·h/L)					
Colbers[22]		53 (44-63)	76 (65-90)		0.67 (0.56-0.82)
simulation		29 (23-37)	49 (37-65)		0.59 (0.42-0.83)
C_{max} (mg/L)					
Colbers[22]		5.3 (4.5-6.2)	6.8 (5.9-7.8)		0.78 (0.65-0.95)
simulation		3.8 (3.3-4.5)	5.9 (4.7-7.5)		0.65 (0.51-0.82)

Simulations increased doses, third trimester of pregnancy					
dosing regimen	AUC simulation	nonpregnant AUC BID	nonpregnant AUC QD	GM ratio 3rd/non-pregn BID (90%CI)	GM ratio 3rd/non-pregn QD (90%CI)
800/100mg BID	44 (33-58)	47 (36-61)		0.93 (0.67-1.29)	
900/100mg BID	50 (38-66)	47 (36-61)		1.05 (0.76-1.47)	
600/100mg BID*2	69 (53-90)		49 (37-65)		1.41 (1.00-1.98)
900/100mg QD	39 (30-51)		49 (37-65)		0.80 (0.57-1.12)

600/100mg $AUC_{0\text{-}12h*2}$ to compare the AUC to an $AUC_{0\text{-}24h}$. GM, geometric mean; 2nd, second trimester of pregnancy; 3rd, third trimester of pregnancy; pp, postpartum; CI, confidence interval; BID, twice daily; AUC, area under the curve; C_{max}, maximum concentration; SD, standard deviation; QD, once daily.

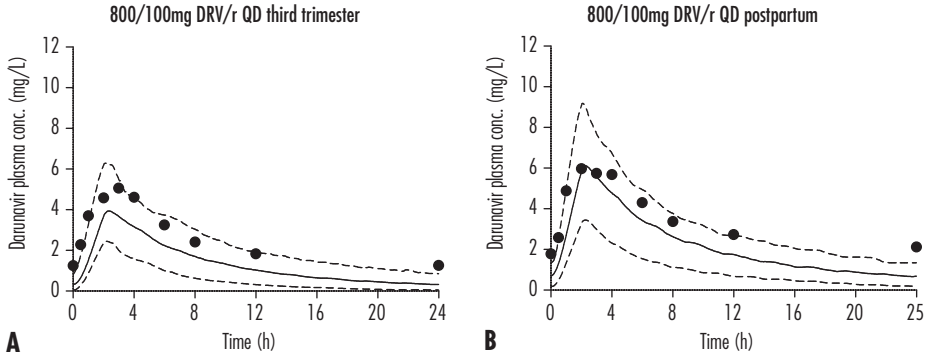

Figure 10. Simulation of darunavir plasma concentrations at steady-state, after 14 days of treatment with QD 800/100mg darunavir/r in **(A)** the third trimester of pregnancy (gestational week 34, n=16, age 20-44 years) and **(B)** postpartum/non-pregnant (n=8, age 20-44 years). The closed circles represent the observed concentrations,[22] the solid line represents the simulated mean concentrations, the dashed lines represent the 95% confidence interval of the simulated concentrations.

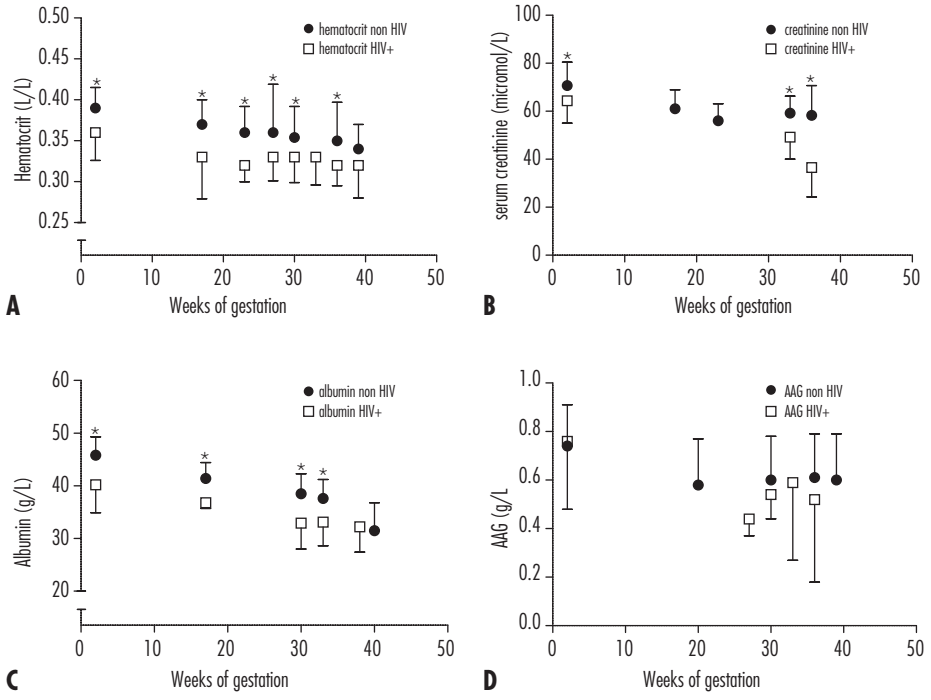

Figure 11. Physiological parameters compared for healthy (non HIV) and HIV-infected pregnant women (HIV+). Data represent mean ± SD per week of gestation for healthy women as reported[10] and HIV-infected women (from the PANNA network). *indicate a significant difference between HIV-infected and not infected women (unrelated samples t-test).

were predicted well for both dosing regimens, and remain within a factor two from the observed values. The decrease of AUC in the second and third trimester for the 600/100mg darunavir/r twice- daily dose was predicted to be 27% (ratio of 0.73) versus 24% (ratio 0.76) observed in the second trimester and 27% (ratios of 0.73) predicted versus 17-22% observed in the third trimester. For the 800/100mg darunavir/r once-daily dose, a decrease in AUC of 41% was predicted, versus an observed decrease of 33% in the third trimester.

Also the simulations with increased dosages are described in this table. The 600/100mg darunavir/r twice-daily dose in the third trimester of pregnancy is expected to result in a total daily exposure compensating (even overcompensating) for the decrease in exposure during pregnancy with the 800/100mg once-daily dose. A dose of 900/100mg darunavir/r once daily did not compensate for the decrease in exposure of the 800/100mg darunavir/r once-daily dose, as still a 20% lower exposure was observed compared with the non-pregnant situation (Table 4). A dose of 800/100mg darunavir/r twice daily was predicted to compensate for the decreased exposure during pregnancy for the twice daily regimen, and a dose of 900/100mg darunavir/r twice daily was expected to overcompensate the decreased exposure (see Table 4).

Physiological parameters in the Simcyp virtual population library versus a real-life HIV-infected pregnant population

In Figure 11 the data on hematocrit, serum creatinine, albumin and alpha-1-acid glycoprotein concentrations were compared between HIV-infected pregnant women included in the PANNA study and the summary of Abduljalil that describes the parameters used for the Simcyp p-PBPK model.[10] Hematocrit was 15% lower in weeks 36-39 of gestation in healthy pregnant women and decreased with 11% in HIV-infected pregnant women. Albumin concentrations decreased with 18% for both populations. Serum creatinine concentrations were approximately 18% lower at the end of pregnancy in healthy pregnant women, and for the HIV-infected pregnant population the serum creatinine concentration was 40% lower at the end of pregnancy compared with the postpartum period. However, alpha-1-acid glycoprotein levels, the main darunavir binding protein in plasma, did not differ between the two populations. Updating the Simcyp model with indicated parameters did also not significantly alter simulation outcomes (data not shown).

Discussion

In this study, we developed and evaluated a mechanistic model to predict darunavir exposure, both in non-pregnant as well as pregnant women. Particularly, these efforts also focussed on elucidating the availability of crucial mechanistic *in vitro* pharmacokinetic parameters that need to be incorporated in such a model. Recently, Siccardi *et*

al. published a paper on the simulation of the pharmacokinetics of several antiretrovirals with anti-depressant drugs.[40] The pharmacokinetics of darunavir/r were also briefly described in terms of a PBPK model, which only included CYP3A4-mediated metabolism as a clearance mechanism. Interestingly, a literature search (Table 1) revealed that darunavir pharmacokinetics are likely to be also subject to P-gp mediated transport. Various *in vitro* studies demonstrated an altered disposition of darunavir during co-administration or co-incubation with P-gp inhibitors, one of which is ritonavir, the drug commonly used to boost darunavir exposure *in vivo*.[17] Moreover, there is also a possible involvement of active hepatocellular uptake in the disposition of darunavir. At least in the rat, high concentrations of darunavir were found in the liver.[41] This would be in line with active hepatic uptake, while also from *in vitro* studies it is known that darunavir can interact with OATPs.[28] Therefore, we argued that an accurate PBPK model describing darunavir/r should at least take into account these active transport processes.

An accurate determination of metabolic clearance forms the basis of our conclusion that uptake and efflux transporters are indeed crucial to describe darunavir pharmacokinetic mechanistically. Therefore, we first conducted extensive studies on *in vitro* metabolic darunavir clearance. The values we found were in the same order of magnitude as the data described by Mamidi *et al.*,[42] *viz.* a K_m and V_{max} of 3.1 μM and 458 $pmol.mg^{-1}.min^{-1}$, respectively. Our *in vitro* studies yielded similar metabolic intrinsic *in vitro* clearances, i.e. 164 versus 147 $\mu l.mg^{-1}.min^{-1}$ by Mamidi *et al.* Using the baculosomal data for exposure simulation with the well-stirred liver model indeed led to similar results as obtained with our HLM data and the preliminary HLM data described by Mamidi *et al.*. [42] The consistent overestimation of exposure further underlined the need to include drug transporters. That Siccardi *et al.* were able to describe darunavir kinetics with only a metabolic clearance step, may be partly explained by the higher CYP3A4 $CL_{int,\ met}$ they used of 3.25 $\mu l.min^{-1}.pmol^{-1}$ *vs* 2.25 $\mu l.min^{-1}.pmol^{-1}$ in our study.

Initial (well-stirred liver) model performance could indeed be improved when uptake and efflux transport mechanisms were included against the background of a permeability-limited liver model. Quantitative transport kinetic data for darunavir is missing, but what is crucial in our findings is that simulation outcome improved with $CL_{int,T}$ values in the range of what is found to be pharmacologically feasible for other transport substrates.[43, 44] Nevertheless, our findings now urge for more detailed, quantitative studies on darunavir transport kinetics in cultures of hepatocytes or overexpression systems, in order to validate whether $CL_{int,T,}$ uptake and $CL_{int,T\ ,}$ efflux values are in line with the values that we propose in our simulations. These studies should also include measurements on membrane transporter abundance to allow accurate *in vitro–in vivo* extrapolations (IVIVE) of this aspect. As *in vivo* abundance of several hepatic transporters have already been reported in literature,[45] it is of particular relevance that new studies on the transporter kinetics of darunavir should also include quantification of absolute transporter expression in the *in vitro* incubation systems.

Figure 7 depicts the simulation of exposure following a single dose of darunavir alone

or single dose of darunavir combined with ritonavir. The boosting effect of ritonavir could be adequately simulated if efficient enterohepatic recycling was assumed, i.e. when 80-100% of the darunavir that was excreted unchanged via the bile was set to be available for enteric re-absorption. Enterohepatic circulation of darunavir has been suggested previously[46] but to our knowledge we are the first to capture this in a mechanistic model. However, for the unboosted darunavir dose, it was not necessary to include an efficient enterohepatic cycling step to adequately simulate exposure. In fact, upon including this process, this resulted in a slightly over-predicted exposure. This raises a mechanistic question, as it seems likely that the majority of unchanged darunavir excreted into bile should in principle be readily available for reabsorption. Indeed, biliary excreted darunavir enters the intestine in an already solubilised, highly micellar state, irrespective of ritonavir co-administration.

An alternative explanation may be that this discrepancy results from non-linearity in intestinal absorption of darunavir. At high concentrations, e.g. after ingestion of a tablet, efflux at the level of the intestine may be less likely to occur as a result of P-gp saturation, resulting in a higher intestinal permeability. At the relatively low concentrations of darunavir that reach the intestine via the bile, only a limited amount may permeate the intestinal epithelium as it could be actively excreted back into the intestinal lumen by P-gp again. This would explain why modelling with an inefficient enterohepatic cycling step provides a better fit to the clinical data obtained for administration of darunavir alone. In the presence of ritonavir, however, P-gp efflux is inhibited, and therefore efficient enterohepatic cycling takes place. Hence, it seems likely that linearity holds over a wider concentration range enabling an efficient enterohepatic circulation. We took into account the effect of ritonavir on absorption (see Table 1, Caco-2 absorption data). However, to our knowledge permeability data for darunavir in absence of ritonavir were assessed only at high concentrations, which did not allow us to correct for possible concentration dependency of absorption. Inclusion of transporters on a mechanistic level and additional measurements in Caco-2 cells covering a wider concentration range may reveal whether this effect indeed takes place.

A limitation of the ritonavir model is that physiological changes in pregnancy did not affect the ritonavir concentrations in the current model, whereas in real-life this is the case.[22, 39] We compensated for this in our simulations by adjusting the oral ritonavir clearance used for simulations in non-pregnant individuals to reported values during pregnancy. In general a 50% decrease in ritonavir exposure is described in pregnancy, which could lead to less boosting of the co-administered protease inhibitor.[22,47] However, clinical studies demonstrated that also a 50mg ritonavir dose boosted darunavir almost as powerfully as the 100mg dose.[48] Therefore, we found this aspect not crucial for our current simulation study and did not write a full mechanistic ritonavir PBPK model based on *in vitro* metabolic clearance parameters. Future studies may address this in more detail.

As can be seen in Figures 10 and 11, particularly for 800/100mg darunavir/r once daily, the model over-predicted the elimination rate. We hypothesize that a possible

explanation for discrepancies between the observed and simulated data is that the Simcyp pregnancy PBPK model is based on physiological changes described for 'healthy' pregnant women, whereas the pregnancy pharmacokinetic curves we used were taken from HIV-infected pregnant women. To determine whether these populations are different, we compared physiological parameters (hematocrit, albumin, serum creatinine and AAG) included in the Simcyp model with parameters collected in the PANNA study. Although the hematocrit and albumin concentrations were lower in the HIV-infected women, the effect of pregnancy was similar for both physiological parameters. Slightly, but significantly lower hematocrit, albumin and creatinine concentrations were observed for HIV-infected women, both in pregnancy and in non-pregnant state (postpartum for the PANNA study). Anaemia, with decreased hematocrit, is common in HIV-infected patients, as a result of the disease, or possibly caused by certain antiretroviral agents.[49] Albumin concentrations are known to be lower in HIV-infected patients, but also known to increase again after initiation of antiretroviral treatment (32mg/L pretreatment, increasing to 37.7mg/L)[50], which is in line with the serum albumin concentrations we found in the women on antiretroviral treatment. Decreased serum creatinine concentration at the end of pregnancy, pointing to possible increased GFR, is relevant for renally excreted drugs. However, for darunavir/r we do not expect an effect, as renal clearance is very low. Renal impairment is not expected to influence darunavir clearance.[41] Nevertheless, our findings are important for mechanistic pharmacokinetic modelling on other drugs, which are excreted renally, have high albumin binding, or exhibit high distribution into red blood cells.

Finally, we used the pregnancy PBPK model to predict which alternative dose levels may compensate for the decreased exposure observed in pregnancy. It is, however, questionable whether it is necessary to increase the dose during pregnancy. As lower exposure could not be correlated to virological failure in the PANNA study, the conclusion was that antiretroviral naive pregnant patients, who are adherent, take darunavir with food and are not using concomitant medication reducing darunavir concentrations, the darunavir/r 800/100mg once daily is adequate. For all other patients, darunavir/r 600/100mg twice daily is recommended during pregnancy.[22] However, in certain cases, our prediction using the PBPK model will be helpful in choosing the most optimal dose. We expect a twice-daily 900/100mg darunavir/r dose to generate higher exposure. Both alternative dosages (800/100mg twice daily and 900/100mg twice daily) are being tested in a P1026 protocol by the IMPAACT group.[51]

Conclusion

A PBPK model that takes hepatic transporter action and entero-hepatic circulation into account could adequately simulate darunavir/r pharmacokinetics for several dosage regimens and patient populations. To improve the mechanistic basis of the model, we

propose that future studies address hepatic, but also intestinal transporter-mediated darunavir disposition in more detail. The current model predicted decreased exposure during pregnancy to be compensated by the 600/100mg darunavir/r twice daily dosing for a woman who initially took the 800/100mg darunavir/r once-daily dose. For the 600/100mg twice-daily dose (used in treatment-experienced patients) the reduction in exposure can be compensated by a 800/100mg darunavir/r twice-daily dosing regimen.

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Ethical standards

The PANNA study was conducted in compliance with the principles of the "Declaration of Helsinki". Informed consent was obtained from each participant before entering the study. The study was approved by the medical ethical committee from each individual centre involved and by the national authorities if applicable. The study was registered at ClinicalTrials.gov under number NCT00825929.

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11

General Discussion

General discussion

HIV-infected women should use antiretrovirals during pregnancy to prevent the transmission of the virus from the mother to the child. The knowledge of pharmacokinetic behaviour of antiretrovirals in pregnancy is very limited. With the results described in this thesis, mainly generated by the PANNA network, we have enlarged the knowledge about the pharmacokinetic alterations in pregnancy for a selection of antiretrovirals. This is important information when selecting the optimal treatment and dosing regimen for pregnant HIV-infected women. The results of the PANNA study, reported in the public domain, have been incorporated in two clinical guidelines for the treatment of HIV-infected pregnant women: perinatal guidelines of the Department of Health and Human Services (DHHS) and the British HIV Association.[1,2] This confirms the importance of these pharmacokinetic data coming available for treating physicians.

The impact of pharmacokinetic changes in pregnancy and the clinical consequences

In the PANNA study an approximate 25% decrease in exposure (AUC) was observed in the third trimester of pregnancy for most antiretrovirals studied: tenofovir DF (23%), emtricitabine (25%), atazanavir (34%), darunavir (22-33%), saquinavir (14%), maraviroc (28%) and rilpivirine (30-40%, two cases only). For the booster ritonavir an even more dramatic decrease of 50% was observed, independent of the protease inhibitor used concomitantly: approximately 43% decrease in AUC with saquinavir; 55% decrease with atazanavir and a 26% and 41% decrease for the twice-daily regimen and the once-daily darunavir regimen respectively. For raltegravir lower exposure in pregnancy was observed but this was less clear, due to the substantial inter- and intra-person variation of raltegravir pharmacokinetic parameters.

Table 1a and 1b show an overview of geometric mean ratios of the pharmacokinetic parameters and the 90% confidence interval comparing the third trimester's situation with the postpartum situation, extracted from studies from the PANNA network and presented in this thesis. If a minimal therapeutic concentration is defined, the number of patients (%) with concentrations below this target is reported. Additionally the conclusions drawn in the separate papers are summarised in this table.

Lower AUC_{tau} and C_{max} were observed for all compounds during pregnancy, with 90% confidence intervals of the geometric mean ratio not including 1 for most compounds. This indicates that most women have a lower exposure during pregnancy, compared with the postpartum (control) situation. For most antiretrovirals described here the half-life was not affected by pregnancy: the geometric mean ratio was approximately 1. For atazanavir/ritonavir 300/100mg once daily and darunavir/ritonavir 800/100mg once daily and rilpivirine the half-life was shorter in the pregnant situation by 13-41%.

The minimum plasma concentration (C_{trough}, or C_{last}), the parameter mostly used for

Table 1a. Overview of pharmacokinetic changes of antiretrovirals in pregnancy (summary of data presented in this thesis)

Parameter	chapter	GMR third trimester/postpartum	(90%CI)	Study conclusion
Nucleoside/Nucleotide reverse transcriptase inhibitors				
Emtricitabine 200mg QD	6	n=24		
$AUC_{0\text{-}tau}$		0.75	(0.68-0.82)	Although pharmacokinetic exposure of the NRTIs TDF and FTC during pregnancy is approximately 25% lower, this was not associated with virological failure in this study and did not result in MTCT. No dose adaptation recommended.
C_{max}		0.87	(0.77-0.99)	
$C_{predose}$		0.57	(0.44-0.73)	
C_{trough}		0.77	(0.52-1.12)	
T_{half}		1.05	(0.91-1.21)	
CLss/F		1.34	(1.22-1.47)	
Vd/F		1.40	(1.28-1.53)	
n (%)<ther. threshold		NA		
Tenofovir 245mg QD	6	n=27		
$AUC_{0\text{-}tau}$		0.77	(0.71-0.83)	
C_{max}		0.81	(0.74-0.89)	
$C_{predose}$		0.81	(0.68-0.96)	
C_{trough}		0.79	(0.70-0.90)	
T_{half}		1.00	(0.87-1.15)	
CLss/F		1.3	(1.20-1.40)	
Vd/F		1.30	(1.12-1.51)	
n (%)<ther. threshold		NA		
Protease Inhibitors				
Saquinavir/r 1000/100mg BID	3	n=9 (paired data)		The standard dose of 1000/100mg BID tablet regimen can be recommended in pregnancy. This treatment generates adequate levels throughout pregnancyand has a solid safety and efficacy profile.
$AUC_{0\text{-}tau}$		0.86	(0.50-1.48)	
C_{max}		0.81	(0.48-1.35)	
C_{trough}		1.64	(0.56-1.97)	
T_{half}		1.05	(0.80-1.39)	
CLss/F		1.13	(0.65-1.95)	
n (%)<ther. threshold 0.1 mg/L		none		

Table 1a. - continued Overview of pharmacokinetic changes of antiretrovirals in pregnancy (summary of data presented in this thesis)

Parameter	chapter	GMR third trimester/postpartum	(90%CI)	Study conclusion
Atazanavir/r 300/100mg QD	4	n=26 (incl 1 patient 400/100mg QD)		Despite 34% lower atazanavir exposure during pregnancy, atazanavir/ritonavir 300/100mg once daily generates effective concentrations for PI-naive patients, even if co-administered with TDF. For treatment experienced patients (with relevant PI resistance mutations) TDM of atazanavir should be considered to adapt the atazanavir/ritonavir dose on an individual basis.
$AUC_{0\text{-}24h}$		0.66	(0.57-0.75)	
C_{max}		0.70	(0.61-0.80)	
C_{24h}		0.59	(0.48-0.72)	
T_{half}		0.87	(0.76-1.00)	
CLss/F		1.53	(1.34-1.75)	
Vd/F		1.15	(0.85-1.56)	
n (%)<ther. threshold 0.15 mg/L		none		
Darunavir/r 600/100mg BID	5	n=5		For antiretroviral-naive patients, who are adherent, take darunavir with food and are not using concomitant medication reducing darunavir concentrations, 800/100 mg of darunavir/ritonavir once daily is adequate in pregnancy. For all other patiënt 600/100 mg of darunavir/ritonavir twice daily is recommended during pregnancy.
$AUC_{0\text{-}12h}$		0.78	(0.60-1.00)	
C_{max}		0.76	(0.53-1.11)	
$C_{predose}$		0.82	(0.70-0.96)	
C_{12h}		0.89	(0.58-1.36)	
T_{half}		1.12	(0.79-1.59)	
CLss/F		1.28	(1.00-1.66)	
Vd/F		1.43	(1.11-1.84)	
n (%)<ther. threshold 0.55 mg/L		none		
Darunavir/r 800/100mg QD	5	n=9 (including 1 patient using 600/100mg QD)		
$AUC_{0\text{-}24h}$		0.67	(0.56-0.82)	
C_{max}		0.78	(0.65-0.95)	
$C_{predose}$		0.77	(0.54-1.10)	
C_{24h}		0.58	(0.44-0.78)	
t_{half}		0.59	(0.40-0.87)	
CLss/F		1.50	(1.24-1.81)	
Vd/F		0.76	(0.47-1.23)	
n (%)<ther. threshold 0.55 mg/L resistant virus		1 (8%) in third trimester		
n (%)<ther. threshold 0.055 mg/L wild type		none		

Table 1a. - continued Overview of pharmacokinetic changes of antiretrovirals in pregnancy (summary of data presented in this thesis)

Parameter	chapter	GMR third trimester/postpartum	(90%CI)	Study conclusion
Integrase inhibitor				
Raltegravir 400mg BID	7	n=17		The pharmacokinetics of raltegravir showed extensive variability. The observed mean decrease in exposure toraltegravir during third trimester compared to postpartum is not considered to be of clinical importance. Raltegravir can be used in standard dosages in HIV-infected pregnant women.
$AUC_{0\text{-}12h}$		0.71	(0.53-0.96)	
C_{max}		0.82	(0.55-1.23)	
C_{12h}		0.64	(0.34-1.22)	
T_{half}		1.04	(0.73-1.47)	
CLss/F		1.41	(1.04-1.90)	
V/F		1.24	(0.67-2.27)	
n (%)<ther. threshold of 0.020 mg/L		1 (5%) in third trimester		
Entry inhibitor				
Maraviroc	8	n=15		While overall maraviroc exposure is 28-30% lower during pregnancy, C_{trough} was reduced to a lesser extent. All but one of our patients met the target trough concentration during pregnancy and virologic responses were good, suggesting that the standard adult dose seems to be sufficient in pregnancy.
AUC_{tau}		0.72	(0.60-0.88)	
C_{max}		0.70	(0.58-0.85)	
C_{last}		0.85	(0.72-1.01)	
T_{half}		1.04	(0.86-1.27)	
CLss/F		1.31	(1.05-1.64)	
V/F		1.39	(0.95-2.04)	
n (%)<ther. threshold of 50 ng/mL		1 in third trimester and postpartum (300mg BID regimen without PI)		
Non-Nucleoside reverse transcriptase inhibitors				
Rilpivirine		case 1	case 2	More data regarding rilpvirine pharmacokinetics in pregnancy are needed. But, due to the large decrease in exposure in these two cases, TDM of rilpivirine in pregnancy is recommended.
AUC_{tau}		0.70	0.57	
C_{max}		0.64	0.93	
C_{last}		0.57	0.44	
T_{half}		0.70	0.69	
n (%)<ther. threshold of 0.040 mg/L		both cases		

Table 1b. Overview of pharmacokinetic changes of ritonavir in pregnancy (summary of data presented in this thesis)

Parameter	chapter	GMR third trimester/postpartum	(90%CI)
Ritonavir + saquinavir: twice daily 100mg	3	n=9	
$AUC_{0\text{-}tau}$		0.57	(0.39-0.82)
C_{max}		0.61	(0.41-0.89)
C_{trough}		0.88	(0.52-1.47)
CLss/F		1.64	(1.18-2.42)
Ritonavir + atazanavir: once daily 100mg	4	n=26	
$AUC_{0\text{-}tau}$		0.45	(0.37-0.53)
C_{max}		0.42	(0.42-0.51)
C_{trough}		0.47	(0.36-0.62)
T_{half}		0.90	(0.78-1.04)
CLss/F		2.24	(1.88-2.68)
Ritonavir + darunavir: twice daily 100mg	5	n=5	
$AUC_{0\text{-}tau}$		0.74	(0.65-0.84)
C_{max}		0.63	(0.53-0.76)
C_{trough}		0.72	(0.56-0.92)
T_{half}		1.17	(0.95-1.43)
CLss/F		1.35	(1.18-1.55)
Ritonavir + darunavir: twice daily 100mg	5	n=9	
$AUC_{0\text{-}tau}$		0.59	(0.42-0.83)
C_{max}		0.59	(0.42-0.82)
C_{trough}		0.72	(0.47-1.10)
T_{half}		1.00	(0.75-1.33)
CLss/F		1.70	(1.20-2.41)

TDM purposes (the lowest concentration during the dose interval, just before the next medication intake) was decreased in pregnancy for all compounds described, except for saquinavir. For saquinavir and atazanavir no C_{trough} concentrations were observed below the advised therapeutic threshold. For darunavir one patient on the 800/100mg once-daily regimen showed a C_{trough} below the threshold for treatment experienced patients, however the concentration was above the threshold for treatment-naive patients. The 800/100mg once daily dose is not recommended in patients with relevant protease inhibitor mutations. In that case the 600/100mg twice-daily dose should be given and in this group neither we, nor another group[3] found values below this limit. One patient had a C_{trough} below the target for raltegravir in the third trimester of pregnancy, at this visit she had a viral load of 74 copies/mL, which declined to undetectable at delivery. For maraviroc one patient had C_{trough} concentrations below the target during pregnancy and

postpartum. She used the 300mg twice-daily dose without protease inhibitor, which in her case might result in sub-therapeutic exposure. Both women using rilpivirine in pregnancy showed C_{trough} levels below the target, although we only described two cases, we would suggest that TDM should be performed during pregnancy for this compound.

Special attention should be given to the important decrease of ritonavir exposure during pregnancy (Table 1b). Ritonavir is used to increase the plasma concentrations of other protease inhibitors by its strong inhibition of Cytochrome P450 3A4. This decrease could result in a diminished boosting effect on the protease inhibitors and contribute to the lower exposure to these agents in pregnancy.

Taken together, these data suggest an overall trend of lower drug concentrations occurring during pregnancy, with a proportion of women having sub-therapeutic plasma concentrations, which occasionally was associated with suboptimal virological response. Fortunately, no MTCT was observed in the population studied. Another consequence of sub-therapeutic levels of antiretroviral agents in presence of the virus is development of resistance. The PANNA network was not designed to detect resistance in the period after delivery, this should be studied in larger groups of patients.

Possible mechanisms behind the pharmacokinetic alterations in pregnancy

The pregnancy induced physiological changes causing the lower exposure to antiretrovirals seem most likely to be the increased plasma volume, decreased absorption, and increased hepatic clearance due to enzyme induction, or increased liver blood flow.

The increased blood volume (increases by 40–50% up till week 32 of pregnancy)[4] can result in lower C_{trough} and C_{max}, and a lower AUC, and higher volume of distribution.[5] This is in line with the Vd/F changes observed for most compounds, see Table 1. Also reduced absorption can lead to lower C_{max} and AUC; we cannot confirm or rule out this mechanism. Further, we did not observe a shorter half-life for most compounds during pregnancy. The only compounds showing faster elimination were darunavir with the once-daily dosing regimen and rilpivirine (only 2 cases studied). The mechanism behind this faster metabolism/excretion can be by increased activity of CYP3A4. It is thought that this increased activity is regulated by progesterone activation of the pregnane X receptor (PXR) receptor, leading to upregulation of the CYP3A4 hepatic enzyme.[5] A reason for not being able to detect a difference in half-life for the other compounds is the fact that we cannot accurately determine the elimination half-life because of the limited sampling time after dosing. Within PANNA we collect information over a dosing interval only in the steady-state situation.

For the protease inhibitors, less boosting by ritonavir can also be considered as a contributing factor. Ritonavir exposure dramatically decreases during pregnancy: the AUC is approximately 50% lower (see Table 1b). However, for the protease inhibitors described in this thesis a 50mg ritonavir dose boosts almost as powerfully as the 100mg dose. This was observed for darunavir,[6] 1,500/50mg versus 1,500/100mg saquinavir/r in

Thai individuals,[7] and 300/50mg atazanavir/r in healthy volunteers.[8]

Within PANNA two renally excreted drugs were studied: tenofovir and emtricitabine. Because it is known that renal clearance is increased during pregnancy,[4,5] this was explored in our study (chapter 6). The estimated creatinine clearance was increased during pregnancy by around 40%. Although possibly influencing the decreased exposure during pregnancy found in this study, this was not translated into shorter half-life of tenofovir and emtricitabine. The reason could also be the limited sampling period, due to which the terminal elimination half-life cannot be assessed accurately.

Another mechanism possibly changing pharmacokinetics is decreased protein binding in pregnancy. In the PANNA study albumin concentrations and alpha-1-acid glycoprotein (AAG) concentrations (if possible) were determined during pregnancy and postpartum. In chapter 10 we summarize these findings. A decrease of approximately 18% for albumin concentrations was observed in pregnancy and a decrease of approximately 32% for AAG (only limited number of observations). However, this did not result in higher free fraction of darunavir in pregnancy. The measured free fraction was 10% postpartum and 12% in the third trimester.

Role of therapeutic drug monitoring

It is recognized that a general dose of medication can result in variable levels of exposure in individual patients: some patients can experience toxicity because of high drug levels and for others the general dosage might be sub-therapeutic. By determining individual exposure in clinical practice (usually by collecting a blood sample just before the next dose of medication is taken), we are able to individualize treatment. This approach is called therapeutic drug monitoring (TDM). TDM can also play a role in assessing adherence to medication, when treatment fails. TDM is not recommended in all cases and for all drugs. Requirements for TDM to be useful are: listed in Table 2.[9]
Most antiretrovirals meet more than two of these criteria: 1, 2, 3, 5 and 7. In the management of HIV, TDM of antiretrovirals can, together with viral load measurement,

Table 2. Requirements for therapeutic drug monitoring

Good relationship between concentration and clinical response
Good relationship between concentration and toxicity
Narrow therapeutic index
Poor relationship between dose and concentration
Significant pharmacokinetic variability
Difficulty in monitoring therapeutic effect
Available drug assay

CD4 T-cell counts and genotyping for resistance, improve therapy. The advantage of combining TDM with clinical parameters is the possibility to adjust dosing, and hence exposure, early in treatment which may prevent or delay resistance development. Using viral load alone as marker for effective therapy has the disadvantage that drug resistance may already have developed by the time HIV replication is observed.[10] For NRTIs TDM is not a useful tool, because the NRTIs are pro-drugs and the plasma concentrations have not (yet) been correlated successfully to viral outcome. For protease inhibitors and NNRTIs this relation has been established, which results in minimal effective trough levels, as stated in treatment guidelines for adults.[11] These minimal concentrations are based on *in vitro* (IC50/IC95 adjusted for protein binding) and *in vivo* (EC50/EC95) literature data.

There is consensus that TDM should not be used for all patients on antiretroviral treatment. But it is indicated for some circumstances or patient groups with changes or variability in drug levels: when possibly pharmacokinetic interacting drugs are used; renal or hepatic insufficiency; gastrointestinal disturbances; less than recommended food intake; children; or pregnancy.

Pregnancy induces many physiological changes, influencing the absorption, distribution, metabolism and excretion of drugs, mostly leading to lower exposure to drugs in pregnancy.[4] These changes generate a great variability of pharmacokinetics in pregnant women, making prediction of individual alterations in pregnant patients difficult, which supports the use of TDM to avoid underdosing and subtherapeutic drug concentrations during pregnancy. A concern to address as well, is the fact that the physiological changes evolve over gestation, so, changes in exposure, and dose adjustment requirements, could be different for the first, second or third trimester.

The use of TDM in pregnancy has been evaluated by several groups. Weinberg *et al.*[12] found that 44% of the patients in the study showed protease inhibitor concentrations below the target in the third trimester of pregnancy (mainly nelfinavir and lopinavir) and 44% of the women had a viral load >50 copies/mL at delivery. However, there was no correlation between low virologic response and protease inhibitor concentrations below the target. Roustit *et al.* (antiretroviral treatment specific)[13] and Matsui (general)[9] reviewed the use and impact of the TDM in pregnancy. According to Matsui it is questionable whether the target concentrations derived from "non-pregnant" adults can be extrapolated to the pregnant population. For antiretroviral medication we believe that the target concentration is probably the same, as the concentrations required to inhibit viral enzymes/processes are not changed in the pregnant situation. A factor to take into account is the correction for protein binding applied to define the target concentration. Because protein binding decreases in pregnancy, the target could possibly be lower. Roustit *et al.* conclude that despite the uncertainty of the clinical consequences of the lower exposure to several antiretrovirals, TDM for nevirapine, nelfinavir, saquinavir, indinavir and lopinavir is advisable. For atazanavir, TDM seems not to be necessary

and for the (in that period of time) newer antiretrovirals they conclude that more data are needed.[13] For two protease inhibitors: boosted lopinavir and boosted atazanavir dedicated papers were published where TDM in pregnancy has been investigated. Else *et al.*[14] showed that during pregnancy the number of patients with atazanavir levels below the target (0.15 mg/L, 6-8%) was similar to the number postpartum (7%). In this study 21% of the patients had a viral load >50 copies/mL in the third trimester of pregnancy. Low atazanavir exposure during pregnancy did not appear to be detrimental to virological control. They advise careful monitoring during pregnancy, and to increase the dose from 300 to 400mg once daily if necessary.[14]

Lambert *et al.*[15] reported lopinavir TDM in 43 patients during pregnancy. Six (13%) patients had lopinavir concentrations below 1.0 mg/L, of which only one had a detectable viral load (209 copies/mL) during the third trimester of pregnancy. In total 39% of the patients showed a detectable viral load during pregnancy. Therefore, a correlation between subtherapeutic exposure and detectable viral load could not be concluded. A retrospective study performed in the UK on TDM of lopinavir in pregnancy in 73 women showed that as a result of TDM the dose was adjusted in 10% of the patients and 11% of the patients (with low lopinavir levels) had adherence reviews. However, also in this study, TDM was not associated with virological outcome. They conclude that TDM can play an important role in the clinical management of HIV-positive pregnant women.[16]

We have also performed a retrospective analysis on lopinavir TDM in pregnancy using the Athena cohort (The Netherlands)(unpublished data). Patients taking lopinavir for >4 weeks during pregnancy were selected from the Athena cohort (2004-2011). 243 pregnant patients using lopinavir were included, 76% started lopinavir during pregnancy. 153/243 (63%) had TDM during pregnancy (increasing from 26% in 2004 to approximately 69% from 2006 onwards); a total of 300 evaluable lopinavir TDM samples were collected from 131 patients using lopinavir 400/100mg twice daily. The majority (71%) of samples was taken in the third trimester. 11/131 (8%) of the patients showed at least one sample below the target concentration for naive patients (1 mg/L), in 8 of these patients TDM was repeated resulting in therapeutic levels in 7/8 patients. In 1 of the 3 patients without repeat sample the lopinavir concentration was below detection limit, an indication for non-adherence. 47/243 (19%) of all patients had an HIV viral load >50 copies/mL at delivery. 2/11 (18%) of the patients with at least one sample below the target concentration for naive patients had a detectable viral load at delivery vs. 17/120 (14%) of patients with concentrations above the target (p=0.718), which is in line with the virological response reported by other groups.

These data indicate that treating physicians in The Netherlands are aware of the possible effect of pregnancy on pharmacokinetics of protease inhibitors, because in 63% of the cases TDM was performed. These data were not published, because we were confronted with possible errors in the data when we investigated the actions taken in case of sub-therapeutic levels in a pregnant woman. We found that in most cases the lopinavir dose had not been increased, which was not consistent with the information

the treating physician had on file. This shows that it is important to be cautious using cohort data when the number of patients to draw conclusions from is very low. In this case it concerned 11 patients, with important consequences when one or two entries in the database are not correct, without judging the quality of the entire database. The reason for the discrepancy is usually not clear: lack of clarity of patient notes or error in data entry in the database.

Instead of performing TDM on an individual basis, which is not readily available for every hospital, another option would be to increase the dose for all patients during pregnancy for certain compounds. Increased doses in pregnancy have been explored for lopinavir/r (500/133 mg twice daily and 600/150 mg twice daily[17-20] and atazanavir/r (also because it is frequently given with tenofovir, possibly reducing atazanavir exposure).[21] An increased dose of 600/150 mg lopinavir/r compensates for the lower exposure during pregnancy.[18,20] A randomized trial studied safety and efficacy of lopinavir/r 600/150 mg twice daily compared with 400/100 mg twice daily.[19] No difference in virological response (viral load at delivery) for women with a baseline viral load<50 copies/mL was observed, but the viral suppression of the increased dose was better in patients with a higher baseline viral load. Maternal adverse effects were not more prominent in the higher dose, nor preterm births or lower birth weight.

The high dose atazanavir/ritonavir of 400/100 mg once daily compensated for the exposure loss during pregnancy in the third trimester and should be considered during the second trimester, especially if given in combination with tenofovir.[21]

The number of studies (and the number of patients in the studies) is low. In addition, it is prudent to avoid higher doses than necessary as foetal drug exposure is related to maternal concentration. Therefore, if TDM is available, TDM during pregnancy seems a better option in case of doubt.

TDM can be a tool to optimize the dose for an individual pregnant patient; however, for most antiretroviral agents it seems not necessary to perform standard TDM during pregnancy. For the compounds investigated in this thesis we suggest that TDM during pregnancy can help to optimize treatment of HIV-infected women during pregnancy for atazanavir (for treatment-experienced patients) and rilpivirine (because we do not know much about this compound and the first results were consistent and made us alert). Another antiretroviral agent for which TDM adds to the treatment of pregnant HIV-infected women is lopinavir, because during pregnancy 13-44% of the women experience subtherapeutic levels. Although this has not been correlated to virologic failure or mother-to-child transmission of the virus, it seems safe to perform TDM in the second and third trimester of pregnancy and increase the dose if necessary.

Detectable viral load and exposure to antiretrovirals

The decrease in exposure to antiretrovirals observed in our studies was not very large and, more importantly, could not be linked to virological failure in our study. Most patients had therapeutic levels during pregnancy except for four patients: one on darunavir, one on maraviroc and two on rilpivirine. Despite the overall achievement of therapeutic drug concentrations, a considerable percentage of the women had a detectable viral load at, or around delivery (in the PANNA study approximately 20-25%), see Table 3 for a summary.

The percentage of women with detectable viral load (>50 copies/mL) at or around delivery despite cART use is in line with what has been reported by other groups.[22-26] A large American cohort study reported 13% of cART naive women at conception (pregnancy start) to have a detectable viral load (>400 copies/mL) at delivery. They found the following factors to be related: timing of cART initiation and consistent use during pregnancy. Approximately 24% of the women starting cART in the third trimester had a detectable viral load at delivery. Also social factors, as ethnicity and education,

Table 3. Viral load around delivery

	VL detectable around delivery	Viral load	Median GA	Relation with ARV exposure
Saquinavir 1000/100 mg BID	1/30 (3%)	189 copies/mL	Around delivery	SQV C_{min}: 0.781 mg/L at 33 weeks
Atazanavir	6/31 (19%)	68, 100, 120, 162, 290 and 402	34 weeks (median)	No stat significant difference
Tenofovir DF/ emtricitabine	7 (21%) /<200: 1 (3%)	72-272 copies/mL	34 weeks (median)	Comparable tenofovir and emtricitabine exposure as patients with undet VL
Darunavir	7/21 (29%)	3/6 BID: 242, 272 and 1610 copies/ mL; 4/18 on QD 74, 121, 144 and 28711 copies/mL	35 weeks (median)	2 non-adherent patients; for QD regimen no relation/BID maybe lower
Raltegravir	3/22 (14%)	144, 242, 290 copies/mL	35 weeks (median)	All had adequate C_{12h} levels in third trimester
Rilpivirine	1/2 (50%)	VL 77 copies/mL	36 weeks and 5 days	C_{0h} at 32 weeks 0.04 mg/L
Maraviroc	4/17 (24%)	<400: 2 (18%)		

These entries may contain the same patients, as these are data from the PANNA study and patients could use more than one compound of the described ARVs.

were associated with a detectable viral load at delivery.[25] Read *et al.*[27] found a detectable viral load (>50 copies/mL) around delivery in 23% of the women, who were treatment naive at pregnancy start. Starting treatment early was associated with successful treatment especially when pre-treatment RNA viral load was above 10,000 copies/mL.

A cohort study of the UK and Ireland showed that 20% of the cART treated pregnant women had a detectable viral load at delivery (>50 copies/mL) in the period 2007-2011.[23] They found a mother-to-child transmission rate of 0.1% for women with viral loads of less than 50 copies/mL around delivery, whereas the rate was 1.2% among women with viral loads between 51 and 999 copies/mL. This stresses the importance of achieving an undetectable viral load around delivery. Furthermore they found a steep decrease in mother-to-child transmission probability with each additional week of cART, up till 15 weeks of cART use during pregnancy. This decrease was more marked for patients with a higher baseline viral load > 30,000 copies/mL.

In 2008 a French group published the results from their cohort. They found an overall MTCT rate of 1.3% for women on cART during pregnancy. The rate was 0.4% in term births and when maternal HIV RNA was below 50 copies/mL at delivery. The rate of mother-to-child transmission increased with viral load, short duration of antiretroviral therapy, female gender and severe premature delivery. The type of antiretroviral therapy was not associated with transmission.[26] An Italian cohort showed results in line with these data: 25% of the pregnant patients on cART had a detectable viral load (>50 copies/mL) in the period 2010–2013, in this cohort no MTCT took place (n=169 pregnancies).[24]

The Monitoring Report 2013 of the HIV monitoring in The Netherlands reported a percentage of women with a detectable viral load around delivery between 14 and 24 (between 2005 and 2011). They indicate that factors associated with a detectable viral load at delivery are: low CD4 T-cell counts and high HIV RNA levels at baseline. Women starting cART during pregnancy are more likely to have a detectable viral load around delivery than women who started cART before they became pregnant.[22]

Overall, the percentage of women with a detectable viral load around delivery is above 10%, for patients in cART, which is suggested to be the aim of WHO target for 2020 (90–90–90 - An ambitious treatment target to help end the AIDS epidemic, issued in 2014).

The underlying mechanism for the relatively high percentage of women with a detectable viral load around delivery is still unclear. In the individual studies described in this thesis we could not relate lower exposure to the individual antiretrovirals to increased viral load. The number of patients in each article are low, but this number increases when we analyse the complete data-set within the PANNA study (n=125 now). It would also be possible to take into account whether patient were treatment-naive at start of pregnancy, or treatment-experienced, as well as initial viral load for the first group of patients.

We plan to analyse the full PANNA dataset, possibly determining factors related to sub-optimal virologic response.

Placenta passage

The ability of the compounds to pass the placenta barrier was explored, this transport was quantified by relating the cord blood concentrations at delivery to the maternal plasma concentrations at the same time point. Cord blood/maternal ratios were 1.63 for emtricitabine, 1.21 for raltegravir, 0.82 for tenofovir, 0.33 for maraviroc, 0.20 for atazanavir, and 0.15 for darunavir. This is in line with what has been reported in the recent review of Else *et al.*[28] For rilpivirine a ratio of 0.74 was observed, which was the first human *in vivo* information on placenta passage published in the public domain. See Figure 1 for an overview.

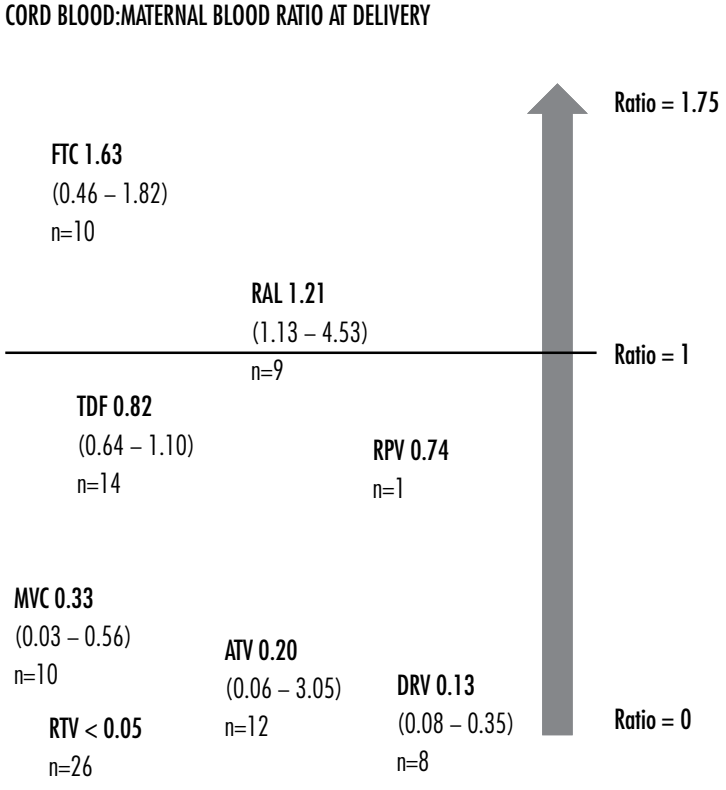

Figure 1. Cord blood-maternal blood ratios
FTC = emtricitabine; RAL = raltegravir; TDF=tenofovir; RPV=rilpivirine; MVC=maraviroc; ATV=atazanavir; DRV=darunavir; RTV=ritonavir

Ritonavir is the only compound investigated in this thesis which was not detectable in most cord blood samples. A total of 26 cord blood samples only 1 had a detectable ritonavir concentration, with a cord blood/maternal ratio of 0.05 (0.05mg/L in cord blood and 1.06mg/L in maternal blood). Twelve samples had ritonavir concentrations below the lower limit of quantification (LLOQ) with detectable maternal concentrations (ranging from 0.058-0.416mg/L) at the same time point. For the remaining 13 samples both cord blood and maternal samples were below LLOQ. Ritonavir seems hardly to reach the foetus during pregnancy. Overall, ritonavir is administered in a low dose (100mg) as a pharmacological booster. During pregnancy ritonavir exposure reduced 2-fold; therefore, concentrations in the maternal circulation are low. From our data a ratio (CB/maternal blood) of 0.05 is suggested.[28] This very low placenta passage could be due to affinity to transporters such as P-gp, which transport compounds from the syncytiotrophoblast into the maternal circulation.[29] This finding is also in line with a human placental perfusion model, indicating that the clearance index of ritonavir was very low, with little accumulation in the foetal compartment and no accumulation in placental tissue.[30] Other groups also found transplacental passage of ritonavir (n=6) to be minimal.[31] On the other hand, in a study of plasma and hair drug concentration in 51 mother-infant pairs in Uganda receiving ritonavir-boosted-lopinavir-based therapy during pregnancy and breastfeeding, infant plasma levels at delivery and hair levels at age 12 weeks suggested in utero transfer of ritonavir: 2% of infants had detectable plasma ritonavir concentrations at birth and the mean infant-to-maternal-hair ritonavir concentration at 12 weeks postpartum was 0.47. However, transfer during breastfeeding was not observed, with no infant having detectable ritonavir plasma levels at 12 weeks.[32]

Knowledge of placenta transfer properties of antiretroviral drugs is important to be able to predict whether a drug will reach the foetus during pregnancy and possibly cause teratogenic effects. Then again, treatment guidelines recommend a cART regimen with at least one antiretroviral drug which passes the placenta, in order to protect the baby from infection with HIV. The highest chance of vertical transmission of the virus occurs during labour. When a child has antiretrovirals 'on board' at that time point, this minimizes the chance of infection. This approach has been translated to 'adult' pre-exposure prophylaxis (PrEP): HIV infection can be prevented in people who do not have HIV, but who are at substantial risk of infection, by taking tenofovir DF and emtricitabine combined in a pill every day. These medicines can work to keep the virus from establishing a permanent infection when someone is exposed to HIV through sex or injection drug use.

Because until now information regarding human placenta passage of drugs only comes available after the drug has reached the market, it would be convenient to be able to predict whether a new compound will possibly pass the placenta barrier. There are several possibilities, amongst which the placenta perfusion model. This model is currently being used by the department of pharmacology and toxicology of the Radboud uni-

versity medical center in cooperation with our research group. The placenta transfer of antiretroviral compounds studied in PANNA is being tested. The results can be verified with the human *in vivo* results from PANNA. Because many aspects of the placenta barrier have to be taken into account, for example transporter abundance, activity and changes of activity over time of gestation, this is a field to be explored to support the development of an in silico model for the prediction of placental passage of drugs and foetal exposure.

The choice of an antiretroviral regimen in pregnancy, product information and guidelines

Factors to be considered when choosing a regimen for a pregnant HIV-infected woman include co-morbidities, convenience, adverse effects, drug interactions, resistance testing results, pharmacokinetics, and knowledge of safety for the mother and the child and experience with use in pregnancy.
Product characteristics of antiretrovirals are very conservative in most cases. In Table 4 an overview is given of the recommendations on use in pregnancy of the different compounds. Only lamivudine, zidovudine and lopinavir are allowed to be used during pregnancy, tenofovir DF and atazanavir use may be considered and for nevirapine the recommendation is left for the treating doctor. For most compounds the product characteristics state that the medication should be used during pregnancy only if the potential benefit justifies the potential risk to the foetus. Furthermore, information concerning pharmacokinetic changes in pregnancy is only mentioned for lamivudine (no change), zidovudine (no change), lopinavir (lower exposure, but not leading to adaptation of the dose), atazanavir (lower exposure, with dose increase for specific groups). Data extracted from the product information as published on the European Medicines Agency website.[33]

More information on treatment and dosing recommendations can be found in specific guidelines: WHO, EACS and DHHS.[1,34,35] The recommendations in these guidelines, however, differ.
The WHO guideline focuses on the low- and middle income settings. The guideline from 2013 in which they recommend to test all pregnant women for HIV at the first antenatal visit included in the routine package, but also to repeat this test in the third trimester of pregnancy. The WHO guideline recommends that all pregnant and breastfeeding women with HIV should initiate triple ARVs (ART), which should be maintained at least for the duration of mother-to-child transmission risk. Women meeting treatment eligibility criteria (amongst others CD4 T cell count <500 cells/mm^3) should continue lifelong ART. The first line regimen contains tenofovir DF/emtricitabine (or lamivudine) and efavirenz,

Table 4. SPC text recommendations for use in pregnancy

	PK information	Recommendation for use in pregnancy
Nucleoside/nucleotide reverse transcriptase inhibitors (NRTI)		
Abacavir	No PK information	Not recommended for use during pregnancy.
Didanosine	No PK information	Not recommended for use during pregnancy.
Emtricitabine	No PK information	Not usually used unless absolutely necessary.
Lamivudine	PK in late pregnancy were similar to non-pregnant women	Benefit of PMTCT is greater than the risk of having side effects. Talk to your doctor about the risks and benefits.
Stavudine	No PK information	Not recommended for use during pregnancy.
Tenofovir	No PK information	The use of tenofovir disoproxil fumarate may be considered during pregnancy, if necessary.
Zidovudine	PK in late pregnancy were similar to non-pregnant women	Benefit of PMTCT is greater than the risk of having side effects. Talk to your doctor about the risks and benefits.
Non-nucleoside reverse transcriptase inhibitors (NNRTI)		
Nevirapine	No PK information	Currently available data on pregnant women indicate no malformative or foeto/ neonatal toxicity.
Efavirenz	No PK information	Women should not get pregnant during treatment with Stocrin and for 12 weeks thereafter.
Etravirine	No PK information	Not recommended for use during pregnancy, unless specifically directed by the doctor.
Rilpivirine	No PK information	Not recommended for use during pregnancy, unless specifically directed by the doctor.
Protease inhibitors		
Atazanavir	Extensive PK information leading to dose recommendations	May be considered during pregnancy only if the potential benefit justifies the potential risk.
Fosamprenavir	No PK information	Not recommended for use during pregnancy.
Indinavir	No PK information	Not recommended in HIV-infected pregnant patients.
Darunavir	No PK information	PREZISTA co-administered with cobicistat or low dose ritonavir should be used during pregnancy only if the potential benefit justifies the potential risk.
Lopinavir	Extensive PK information, leading to dose recommendations	Lopinavir can be used during pregnancy if clinically needed. No dose adjustment is required, once daily dosing not recommended.
Saquinavir	No PK information	Only if the potential benefit justifies the potential risk to the foetus.
Tipranavir	No PK information	Only if the potential benefit justifies the potential risk to the foetus.

Table 4. - continued SPC text recommendations for use in pregnancy

	PK information	Recommendation for use in pregnancy
Boosters, CYP3A4 inhibitors		
Ritonavir	No PK information	Only if the potential benefit justifies the potential risk to the foetus.
Cobicistat	No PK information	Should not be used during pregnancy unless the clinical condition of the woman requires treatment with cobicistat co-administered with atazanavir or darunavir.
Integrase inhibitors		
Dolutegravir	No PK information	Should be used during pregnancy only if the expected benefit justifies the potential risk to the foetus
Elvitegravir	No PK information	Should not be used during pregnancy unless the clinical condition of the woman requires treatment with elvitegravir.
Raltegravir	No PK information	Should not be used during pregnancy.
Entry inhibitors (CCR5 binding)		
Maraviroc	No PK information	Should be used during pregnancy only if the potential benefit justifies the potential risk to the foetus.
Fusion inhibitors		
Enfuvirtide	No PK information	Should be used during pregnancy only if the potential benefit justifies the potential risk to the foetus.

PK: pharmacokinetic

also for pregnant women in the first trimester of pregnancy and women of childbearing age, the same regimen as for non-pregnant adults. There are some safety concerns about efavirenz: reproduction toxicology studies in primates suggested increased number of birth defects. Next to that, case reports and retrospective clinical data reported neural tube defects among humans,[36] which led to a concern about efavirenz use in the first trimester of pregnancy (or even in non-pregnant women of childbearing potential). The WHO issued a "technical update on treatment optimization concerning the use of efavirenz during pregnancy: a public health perspective"[37] comparing safety, tolerability, efficacy and costs of efavirenz treatment with nevirapine in pregnancy. In this document they conclude that efavirenz exposure in early pregnancy has not resulted in increased birth defects; new evidence suggests that efavirenz is clinically superior to nevirapine in terms of long-term viral suppression; the costs of efavirenz decreased and has as advantage that it is available in a once-daily fixed-dose combination tablet.[37] A recent systematic review and a meta-analysis (with data of 2026 pregnancies on efavirenz) did not find an increase in overall birth defects and no elevated birth defects for efavirenz compared with other ARV exposure in pregnancy[38] and a prevalence for neural tube defect of 0.05%, which is comparable to estimates of 0.02-0.2% in the general population in the USA. A study in Ugandan HIV-infected pregnant women

compared lopinavir/r with efavirenz based cART.[39] They found that virologic suppression (HIV-1 RNA <400 copies/mL) at delivery was higher for efavirenz (98% versus 86%) and the women on efavirenz experienced less diarrhoea and nausea.[39]
Furthermore, neural tube defects are induced in the first 5-6 weeks of pregnancy and in most cases; the woman is unaware of her pregnancy at that timepoint. Although the WHO Guidelines Development Group emphasized that better data on birth defects are needed, it felt confident that this potential low risk should be balanced against the programmatic advantages and the clinical benefit of efavirenz in preventing HIV infection in infants and for the mother's health. The need for monitoring of safety of efavirenz, emtricitabine and tenofovir in pregnancy, as these compounds are more widely used after implementation of the new WHO guidelines is stressed by experts in the field.[40] Pregnancy does not seem to change efavirenz exposure.[41]

EACS and DHHS guidelines still recommend avoidance of efavirenz use during conception and early pregnancy. The EACS guideline recommends to start cART early in the second trimester with cART in the same regimen as non-pregnant patients for treatment-naive HIV-infected pregnant women, but avoiding efavirenz in the first 8 weeks and not starting nevirapine (NVP) in pregnancy (continuation is possible). Preferred protease inhibitors are: lopinavir/r, atazanavir/r, saquinavir/r with an option to add raltegravir to the triple therapy if the viral load is not undetectable in the third trimester.[35] The DHHS guideline recommends 2 NRTIs and a boosted protease inhibitor or an NNRTI. They indicate preferable ARVs in all classes. Preferred NRTIs are: abacavir/lamivudine, tenofovir DF/emtricitabine or lamivudine, zidovudine/lamivudine; preferred protease inhibitor: atazanavir/r, lopinavir/r; preferred NNRTI: efavirenz, if started after week 8 of gestation. Alternative ARVs are darunavir/r, saquinavir/r, nevirapine and raltegravir. The DHHS guidelines identify the alteration in pharmacokinetics as a factor to be considered when choosing a treatment regimen in pregnancy. They state that pharmacokinetic changes in pregnancy may lead to lower plasma levels of drugs and necessitate increased dosages, more frequent dosing, or boosting, especially of protease inhibitors.[1]

For atazanavir and lopinavir, pharmacokinetic changes during pregnancy and the consequences thereof have been reported in the SPC text as well. For atazanavir these recommendations are in line with the recommendations given in the guidelines: unboosted atazanavir is not recommended during pregnancy.[1] Use of an increased dose (400/100mg atazanavir/r once daily with food) from the second trimester onwards results in plasma concentrations equivalent to those in non-pregnant adults on standard dosing. Although some experts recommend increased atazanavir dosing in all women during the second and third trimesters, the package insert recommends increased atazanavir dosing only for ARV-experienced pregnant women in the second and third trimesters also receiving either tenofovir DF or an H2-receptor antagonist. For

lopinavir, however, the label information states that pregnant women without documented lopinavir associated resistance mutations can use the 400/100mg lopinavir/r twice-daily dose during pregnancy and in the postpartum period. The DHHS guideline, in contrast, suggests to increase the dose to 600/150mg lopinavir/r twice daily from the second trimester onwards.[1]
Protease inhibitor based regimens were most frequently used during pregnancy from 1996 onwards in the US.[42] Townsend *et al.*[23] reported 72% of the treatments during pregnancy to contain protease inhibitors, 23% to contain NNRTIs and 4% a combination of a protease inhibitor and an NNRTI, the remaining patients were on a triple NRTI regime. These data were collected between 2007 and 2011 in the UK and Ireland. D'Armnio Monforte *et al.* reported the actual ARVs used during pregnancy from 1997-2013 in an Italian cohort. Lopinavir was the most popular protease inhibitor (28%), atazanavir the second with 16%. The most frequently used NNRTI was nevirapine (16%). Lamivudine and zidovudine were the most frequently used NRTIs (69% and 63% respectively), with tenofovir DF and emtricitabine as second combination (32 and 25%).[24] Griner *et al.* reported that in 2009 boosted lopinavir was also the most frequently used protease inhibitor during pregnancy in the US.[42]

In summary: the treatment guidelines are not in line with each other concerning the preferred treatment of HIV-infected pregnant women. With respect to efavirenz use, they even contradict each other: WHO guidelines recommend this compound as first line; the guidelines used in the developed world recommend not to use efavirenz during conception and early pregnancy. Because of the possible teratogenicity and possible effects on brain development, which can only be detected on the long term in children exposed in utero, efavirenz does not seem to be the best choice for treatment during pregnancy when other options are available.
The DHHS and EACS guidelines are in line recommending boosted protease inhibitors, with the exception of saquinavir/r, which is not used very frequently. Applying dose increases for lopinavir and atazanavir is suggested by the DHHS guidelines, however, it remains questionable whether this should be done in every pregnancy, considering also the wide inter-subject variability and potential for an increase in maternal adverse events with some dose increases. If TDM is available, it is preferable to dose increase on an individual basis. The option of EACS to add raltegravir in late pregnancy in case of suboptimal virologic suppression seems a good idea, because in the prevention of mother-to-child transmission suppression of the virus in the mother is essential.

Is there a need for regulatory guidelines for trials in pregnant women?

During the development process of a new drug generally pregnant women, and even women of childbearing potential, are excluded from clinical trials. The reasons for exclusion of pregnant women from clinical trials are primarily the safety concerns for both the mother and the foetus (possible teratogenicity). Institutional review boards are reticent to approve trials in pregnant women, for ethical reasons mentioned above. Other reasons mentioned include a lack of financial incentives for investigation in pregnant women and the absence of a mandate for such studies to be conducted prior to approval by the regulatory authorities.[43] Therefore, when a drug reaches the market, no information on safety or pharmacokinetics in pregnant women is available, nor whether the compound passes the placental barrier and reaches the foetus during pregnancy. It is regarded not to be ethical to expose pregnant women (and the unborn child) to new medication, as the consequences may be dramatic. However, when a drug reaches the market pregnant women might be exposed to the drug, without knowing the consequences. For this reason regulatory authorities require pharmaceutical industry to set up a registry collecting safety data of exposure to the drug during pregnancy.[44,45] Pharmacokinetic data in pregnancy are generally not collected by the pharmaceutical industry. Most post marketing studies investigating pharmacokinetic changes of drugs during pregnancy are set up by independent, academic groups. Two examples are the IMPAACT group and the PANNA network, on which data this thesis is based. An advantage for independent groups to study pharmacokinetics of for example antiretrovirals is that these groups can include "all" compounds in their study. The pharmaceutical industry is only interested in the compound they market, therefore, performing a study like this is (relatively) much more expensive for industry.

Voices rise for development of a regulatory guidance to perform pharmacokinetic and safety studies in pregnancy for compounds very probably going to be used in pregnancy.[43] The timing of such a study would be after completion of the reproductive toxicity studies, which could be planned earlier in the development of the drug. Another option to study the behaviour of the compound in pregnancy is to not exclude pregnant women from clinical studies. For example: if a woman becomes pregnant participating in a clinical trial with a new drug, at this moment they will drop-out immediately from the study and study drug is discontinued as soon as possible. The pregnancy and outcome will be followed-up for safety reasons. If a pharmaceutical company has a study protocol in place for collection of information in pregnancy (pharmacokinetics, efficacy, and safety) a woman could enter this study when she becomes pregnant. The ethical feasibility of this approach depends on the alternative medication available for the disease in question. But, if there is no alternative, this would be an option.

When deciding if performing studies in pregnant women is ethical, one should consider whether it is ethical NOT to perform studies in pregnant women when a drug will be used during pregnancy when it reaches the market.

Alternative options for extensive in vivo studies

Post marketing studies as described above are expensive to perform. It would be convenient to be able to predict alterations in pharmacokinetics in pregnancy in a computer model.

Population pharmacokinetic models can help to predict exposure of pregnant women to antiretrovirals. Population pharmacokinetics identifies factors which can explain (correlate to) the variability in drug concentrations in the various individuals within the treated population. Examples of the factors possibly correlated to the drug concentrations are: body weight, metabolic functions, plasma protein concentrations, disease, co-medication use or, in our case: pregnancy. To build such models, real life data are necessary, although the models can be developed using only sparse samples. The results from the PANNA study can be used to develop population pharmacokinetic models for different antiretrovirals. The first model has been developed based on the data from PANNA and more models will be developed in the future.

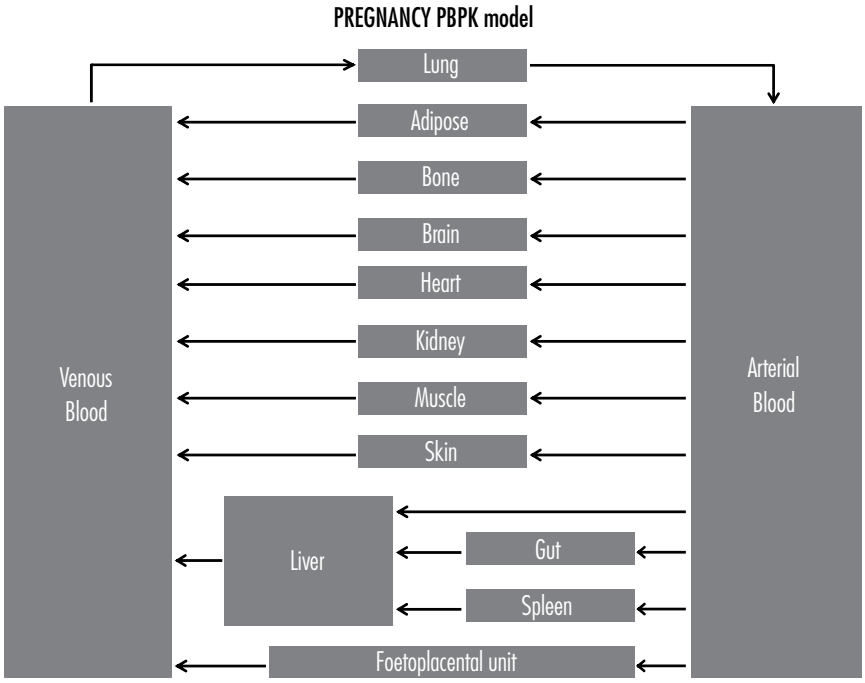

Figure 2. Pregnancy PBPK model (Simcyp)[46]

An *in silico* model which does not necessarily need input from clinical studies is the physiologically-based-pharmacokinetic (PBPK) model. PBPK modelling is a mathematical technique to predict absorption, distribution, metabolism and excretion (ADME) of for example medication, based on physicochemical properties and *in vitro* biotransformation. The model consists of different compartments corresponding to the different organs and tissues, connected by blood or lymph flows. Recently, a PBPK model including physiological changes in relation to duration of pregnancy (pregnancy PBPK model) has been developed by Simcyp, see Figure 2.

The pregnancy PBPK model can be used to predict exposure to drugs during pregnancy at any gestational age, and eventually predict exposure of increased doses of medication, and indicate the gestational age at which a dose increase should be suggested. The foetoplacental unit (combination of foetus, placenta, amniotic fluid, membranes and umbilical cord) is included as a perfusion-limited compartment running in parallel with the other maternal compartments.
In chapter 10 we describe a first exploration of the pregnancy PBPK model (p-PBPK) with an antiretroviral drug, in this case darunavir/r. This model predicts the maternal exposure at steady state (including the interaction with ritonavir) fairly well. Both the AUC and C_{max} in pregnancy and postpartum are comparable with the observed data and the decrease of exposure during pregnancy is predicted acceptably well. A major shortcoming of the available p-PBPK model is that the exposure of the foetus cannot be assessed. Furthermore, for the development of a valid model for a drug to be able to predict human exposure requires much *in vitro* data, often not published in the public domain, or published without some essential information needed for the model (protein concentrations in the matrix for example). Using a PBPK model can on the other hand help to determine gaps in the knowledge concerning the exact behaviour of a compound in the body.
We plan to develop PBPK models for other compounds for which data are available from the PANNA network and predict the exposure in pregnancy using the p-PBPK model. According to our knowledge the p-PBPK model has not yet (or not extensively) been validated for renally excreted compounds, or UGT substrates. Furthermore, we plan to expand the model with a valid placenta-foetal compartment, see the "placenta passage" section of this discussion.

Conclusion, a personal perspective for treatment recommendations during pregnancy

The aim of this thesis was to describe pharmacokinetic alterations of antiretrovirals during pregnancy, and indicate efficacy, safety and cord blood/maternal ratios.
In the patients described in this thesis, despite some of them having sub-therapeutic drug concentrations, this, fortunately, did result in mother-to-child transmission of the virus. There are several factors to consider when a relationship between drug exposure and mother-to-child transmission is being made: women are treated with combination antiretroviral therapy, consisting of at least three compounds. This means that the compound measured in this study is not the only active antiretroviral drug used. Furthermore, in >50% of the cases delivery was done by caesarean section, which is known to decrease the chance of mother-to-child transmission.[47,48]
For most antiretrovirals reported in this thesis the exposure is approximately 25% lower during pregnancy. Only 4 patients showed C_{trough} levels below the target for efficacy. The pregnancy induced physiological changes causing the lower exposure to antiretrovirals seem most likely to be the increased plasma volume, decreased absorption, and increased hepatic clearance due to enzyme induction, or increased liver blood flow.
For tenofovir DF, emtricitabine, saquinavir, darunavir, raltegravir and maraviroc we concluded that the dose does not have to be adapted during pregnancy. For atazanavir (for treatment-experienced patients) and rilpivirine we suggest that TDM during pregnancy can help to optimize treatment of HIV-infected women during pregnancy.
Twenty percent of the patients had a detectable viral load (>50 copies/mL) around delivery, which did not lead to mother-to-child transmission. The viral loads around delivery were mostly below 500 copies/mL (84%) and maximally 28 711 copies/mL. In most cases the mode of delivery was caesarean section, further reducing the chance of mother-to-child transmission. One congenital abnormality was reported in the PANNA study, a relationship with ARV use could not be ruled out, but because the patient also used methadone during pregnancy, and no ARVs during the first weeks after conception were used, the relationship to ARV use is questionable.
The underlying mechanism for the relatively high percentage of women with a detectable viral load around delivery is still unclear. Lower exposure to antiretrovirals could not be related to increased viral load.
Placenta passage for emtricitabine and raltegravir is good, with a cord blood/maternal blood ratio >1; moderate for tenofovir DF and rilpivirine (ratio between 0.5 and 1) and not very good for maraviroc, atazanavir and darunavir and almost non-existent for ritonavir.
We were able to predict maternal darunavir concentrations in the second and third trimester using a pregnancy PBPK model. This tool can be applied to predict the maternal

exposure for new drugs prior to coming to the market. Further development of the foetal unit is needed to also predict placenta transfer of new drugs.
What is the optimal choice of antiretroviral treatment during pregnancy? This was not a research question of the PANNA network, but here I will pose my opinion below.
From our work we can confirm that emtricitabine and tenofovir tenofovir DF pass the placenta well, seem to be generally safe and can be a good choice as back-bone of the treatment during pregnancy. The child will be protected also by these agents in case of blood-blood contact during partum.
When a choice for a protease inhibitor is being made, atazanavir/r once daily, or saquinavir/r or darunavir/r twice daily seem to be the most robust choice, more robust from a pharmacokinetic perspective than lopinavir/r (twice daily).
Raltegravir or maraviroc are also an acceptable option, but safety data during pregnancy are scarce. Adding raltegravir to a triple regimen when the viral load is still high at the end of pregnancy seems a good choice, also protecting the baby during delivery. For rilpivirine we have not yet collected enough data to draw conclusions.
My personal opinion is that efavirenz should be avoided in the first trimester of pregnancy for safety reasons and because other options are available in the Western world.

Data generated by the PANNA network help to decide on the optimal treatment for pregnant HIV infected women, and make treating physicians aware of the influence of pregnancy on drug exposure. It is important to continue this network and add new antiretroviral agents to the list of medication to be investigated in pregnancy. This approach can also be used for other agents used in pregnancy.

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Appendix

Summary

HIV (human immunodeficiency virus) is the virus that causes AIDS. The blood, semen, vaginal fluid and breast milk of HIV-infected persons contains the virus. If these infected body fluids come into contact with the blood stream (for example wounds) or mucous membranes, the infection can be transmitted. The virus binds to specific immune cells (CD4 T lymphocytes, CD4-cells). It is through these cells that the virus replicates and even destroys the cells over time. The immune system weakens; the body has problems fighting other infections and eventually the lethal disease AIDS can develop.
In 2013 a total of 35 million people were infected with HIV worldwide, 3.2 million of them were children. The majority of the HIV infected people live in Sub-Saharan Africa (70%) and 50% of the patients is female. Approximately 17,750 people were infected with HIV in 2014 in The Netherlands; most of the patients in The Netherlands (80%) are male.
HIV cannot (yet) be cured, and no vaccine is available. Medication can suppress the replication of the virus. These medicines affect the different steps of the life cycle of the virus. HIV patients need to take at least three of these medicines daily. By the use of these medicines, the amount if HIV in the body is reduced, and the immune system is strengthened.
This thesis describes the use of HIV medication by HIV infected pregnant women. HIV can be transmitted from mother to child, especially during delivery, when blood-blood contact can take place. To prevent infection of the child, the mother needs to use HIV medication during pregnancy. The amount of virus particles in blood and other body fluids is reduced by the treatment, which decreases the chance of infection during blood-blood contact dramatically. Without treatment 25-40% of the children would be infected during pregnancy or delivery; treatment reduces this percentage to <2%. HIV medication can reach the unborn child during pregnancy and could be harmful for the health and development of the child. On the other hand, a positive aspect is that the child already has medication in the body at the moment of possible contact with the HIV virus of the mother. This additionally protects against infection.
I investigate the concentrations of HIV medication during pregnancy in the blood of the mother; we call this exposure to HIV medication. The human body removes possible toxic compounds during pregnancy, to protect the child. The body recognizes HIV medication as possibly dangerous compounds and often eliminates the medication faster. Apart from that, the weight of pregnant women increases and the total body volume is higher, as a result of which concentrations of the medication can be lower during pregnancy. For efficacy of HIV medication it is important that sufficient medication is present in the body. When the concentrations are too low, this may lead to therapeutic failure and resistance against the HIV medication.
To perform this study, investigating the exposure to HIV medication (antiretroviral med-

ication) in pregnancy, we have set up a network of hospitals in Europe, because the number of HIV-infected pregnant women in The Netherlands was too low to perform such a study. This so called PANNA network – both the network and the study carry this name – includes 21 hospitals in the Netherlands, United Kingdom, Germany, Belgium, Spain, Italy and Ireland.

In cooperation with the PANNA investigators, I have composed a list of antiretrovirals for which, according to our opinion, none or insufficient knowledge was available of the effect of pregnancy on exposure (blood concentrations) to these medicines. To study this, we have included pregnant women, using antiretrovirals, and recorded a pharmacokinetic curve in the third trimester of pregnancy. The pharmacokinetic curve consisted of collection of blood samples at 10 time points after medication intake. Four to six weeks after delivery, the women returned to the study site to have another pharmacokinetic curve recorded. This curve served as control (non-pregnant) situation. We compared the exposure during pregnancy with the "non-pregnant" exposure, a within-subject comparison. Besides that, we also explored whether effective blood concentrations were reached.

We also wanted to know whether the compounds passed the placenta barrier, and hence reached the unborn child. For this purpose we collected a cord blood sample during delivery and determined the concentration of the medication in this sample.

The objective of the study, as described in my thesis, was to describe pharmacokinetic alterations of specified antiretroviral agents during pregnancy, and to indicate efficacy, safety and cord blood/maternal ratios.

Chapter 2 of the thesis is a review of the studies investigating the pharmacology of antiretrovirals in pregnancy, published in 2012. Both, studies with intensive sampling (collection of a pharmacokinetic curve), as well as (random) sparse sampling studies, were reported. Furthermore, for some antiretrovirals, placenta passage was described. Most of the agents investigated in 2012 showed a decrease in exposure during pregnancy. In spite of that, the need for dose increase during pregnancy remains ambiguous.

In the subsequent seven chapters the effect of pregnancy is described on pharmacokinetics of three protease inhibitors; two NRTIS; one NNRTI; one integrase inhibitor and one entry inhibitor.

The first agent, saquinavir, is discussed in chapter 3. The study was performed prior to initiation of the PANNA-network. However, the design of the study was approximately identical. In the saquinavir study, a curve was taken in the second trimester – if this was possible – in addition to the third trimester curve. Pharmacokinetic curves were collected from 37 women using 1000/100mg saquinavir/ritonavir twice daily during pregnancy (16 in the second trimester, 31 in the third trimester and 9 postpartum). No significant difference was found in saquinavir exposure during pregnancy compared with postpartum. The variance in results was very large in this study. No sub-therapeutic saquinavir concentrations (<0.1 mg/L) were observed during pregnancy or postpartum. This led to the conclusion that saquinavir exposure in the new tablet formulation generates

adequate saquinavir concentrations throughout the course of pregnancy and is safe to use; therefore, no dose adjustment during pregnancy is needed.
The following chapters describe antiretroviral agents investigated in the PANNA protocol. Atazanavir/r results of 31 patients are depicted in chapter 4. Apart from the effect of pregnancy on the exposure to atazanavir, we also investigated whether concomitant use of tenofovir DF had an additional effect because tenofovir DF possibly reduces atazanavir concentrations. Twenty one out of 31 patients used tenofovir DF as NRTI. Atazanavir exposure was 34% lower during the third trimester, C_{max} was 30% lower and C_{24h} was 41% lower, compared with postpartum. No statistical difference in pharmacokinetic parameters was found between patients using tenofovir versus no tenofovir. None of the patients showed atazanavir concentrations <0.15 mg/L (target for treatment-naive patients). Atazanavir showed a moderate placenta passage. The cord blood/mother blood-ratio was 0.20 (n=12). The children were born after a median (range) gestational age of 39 weeks (36-42). Approaching delivery 81% (25 patients) had an HIV viral load <50 copies/mL, all <1,000 copies/mL. One baby had a congenital abnormality, which was not likely to be related to atazanavir/ritonavir use. None of the children were HIV-infected. Our conclusion was: atazanavir/ritonavir 300/100mg once daily generates effective concentrations for protease inhibitor (PI)-naive patients, even if co-administered with tenofovir. For treatment-experienced patients (with relevant PI resistance mutations) therapeutic drug monitoring of atazanavir should be considered to adapt the atazanavir/ritonavir dose on an individual basis.
Concerning darunavir/r results of 24 patients (600/100mg twice daily (n=6); 800/100mg once daily (n=17); and 600/100mg once daily (n=1)) are described in chapter 5. Darunavir exposure was 22% decreased in pregnant patients using darunavir/r 600/100mg twice daily and 33% decreased in patients using darunavir/r 800/100mg once daily. We also analysed free darunavir concentrations in a subset of plasma samples, both of the third trimester as well as the postpartum curve. The unbound fraction of darunavir was not different during pregnancy (12%) compared with postpartum (10%). The median (range) ratio of darunavir cord blood/maternal blood was 0.13 (0.08-0.35). The children were born after a median (range) gestational age of 38 weeks (36-41). Close to delivery 67% of the patients showed a viral load <50 copies/mL and 88% had <300 copies/mL. All children were tested HIV-negative and no congenital abnormalities were reported. We concluded that darunavir AUC and C_{max} are substantially decreased in pregnancy for both darunavir/r regimens. This decrease in exposure did not result in mother-to-child transmission. For antiretroviral-naive patients, who are adherent, take darunavir with food and are not using concomitant medication reducing darunavir concentrations, 800/100mg darunavir/r once daily is adequate in pregnancy. For all other patients 600/100mg of darunavir/r twice daily is recommended during pregnancy.
In chapter 6 we describe the effect of pregnancy on the NRTIs tenofovir DF and emtricitabine. We included 34 pregnant women in the analysis. The majority used the

combination tablet Truvada® (n=31, 91%). Tenofovir exposure (AUC) was 23% lower and emtricitabine exposure was 25% lower during pregnancy. Both agents pass the placenta well. The cord blood/maternal blood ratio was 1.63 for emtricitabine and 0.82 for tenofovir. Most patients (97%) had a viral load less than 200 copies/ml around delivery, 83% had less than 50 copies/mL. The median (range) gestational age was 38 (36-41) weeks at delivery. All children were tested HIV-negative and no congenital abnormalities were reported. Tenofovir DF and emtricitabine can be used in the standard dose during pregnancy.

Raltegravir exposure in pregnancy is described in chapter 7. Twenty-two patients were included of which 68% started raltegravir during pregnancy. Overall AUC and C_{12h} plasma concentrations in third trimester were on average 29% and 36% lower compared with postpartum. Raltegravir readily crosses the placenta, with a median (IQR) ratio of raltegravir cord/maternal blood of 1.21 (1.02-2.17; n=9) and was well tolerated during pregnancy. Approaching delivery 86% of the patients had an undetectable viral load (<50 copies/mL). None of the children were HIV-infected. Exposure to raltegravir was highly variable. The observed mean decrease in exposure to raltegravir during third trimester compared to postpartum is not considered to be of clinical importance. Raltegravir can be used in standard dosages in HIV-infected pregnant women.

Chapter 8 is a description of two cases of rilpivirine use during pregnancy. Because nothing had been published on the effect pregnancy on rilpivirine pharmacokinetics or placenta passage, we reported the first two patients using rilpivirine in the PANNA study. Rilpivirine crosses the placenta moderately (cord blood/maternal blood ratio of 0.74). Rilpivirine exposure during pregnancy was decreased by approximately 30-43%. Both patients showed low trough concentrations, even lower than the target concentration of 0.040 mg/L. We strongly recommended therapeutic drug monitoring for rilpivirine during pregnancy.

Chapter 9 on the effect of pregnancy on maraviroc exposure is written in collaboration with IMPAACT. IMPAACT is an American research group, performing studies like the PANNA study in the United States of America, South America and Thailand. We combined the results regarding maraviroc to be able to report data from a substantial amount of patients. In total 18 patients were included in the analysis (IMPAACT 11; PANNA 7). Most women received 150mg maraviroc twice daily with a protease inhibitor (12; 67%), two (11%) received 300mg maraviroc twice daily without protease inhibitor, and four (22%) had an alternative regimen. Maraviroc exposure was 28% lower during pregnancy, with a 30% decreased C_{max}. Only one patient showed C_{trough} concentrations below the suggested target of 50 ng/mL, both during pregnancy and postpartum. Maraviroc passes the placenta moderately: the median (range) ratio of maraviroc cord blood/maternal blood was 0.33 (0.03-0.56).

The median (range) gestational age ate delivery was 39 (37-41) weeks. Viral load close to delivery was less than 50 copies/mL in 13 participants (76%). All children were tested HIV-negative. Despite a reduction in exposure, the standard dose of maraviroc can be

used during pregnancy.
Figure 1 and 2 summarize the effect of pregnancy on AUC (exposure) and C_{trough} (trough concentration) on several agents, depicted as geometric mean ratio and the 90% confidence interval.

The development of a physiologically-based-pharmacokinetic (PBPK) model of darunavir is described in chapter 10. A PBPK-model is a computer model, which can be used to simulate exposure of an agent in blood and also exposure in several organs, based on physicochemical properties of the drug and the physiology of the human body. Physiological changes, appearing in pregnancy, can be included in the model. This makes it

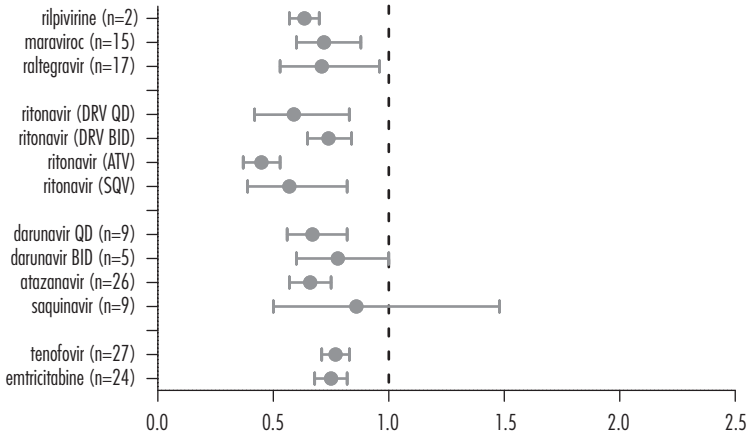


Figure 1. Geometric mean ratio of AUC (pregnancy/postpartum) and 90% confidence interval

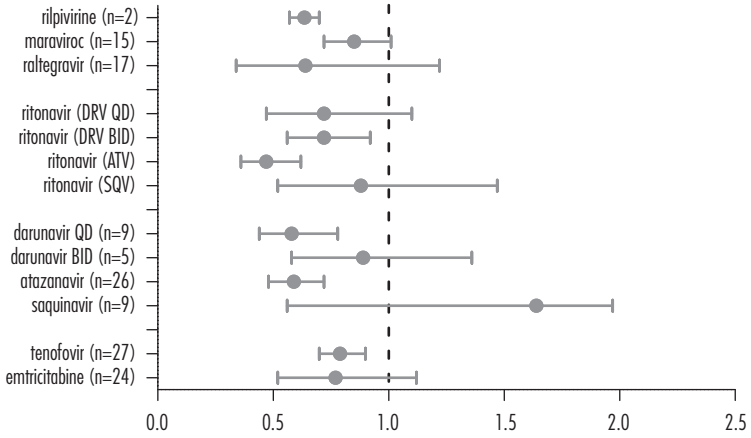


Figure 2. Geometric mean ratio of C_{trough} (pregnancy/postpartum) and 90% confidence interval

possible to predict exposure to drugs during pregnancy, without performing extensive *in vivo* studies in pregnancy. This is a major advantage of such a computer model.
We built a model that describes the exposure of both standard doses of darunavir/r (600/100mg twice daily and 800/100mg once daily) well for both healthy subjects as in pregnancy. The model was built using Simcyp (v13.2), a PBPK model platform.
The physicochemical and *in vitro* pharmacokinetic parameters of darunavir and ritonavir were derived from literature. K_m and V_{max} for CYP3A4-mediated darunavir biotransformation and inhibition by ritonavir were determined experimentally. We discovered that it was not possible to generate an acceptable model for darunavir without inclusion of a role for transporters. Sensitivity analyses were used to assess the contribution of hepatocyte uptake and efflux transporters.
For darunavir alone and also for the darunavir-ritonavir interaction, peak and total exposure at steady state were estimated within 2-fold range of reported data. The model predicted a decrease in AUC of 27% and 41%, which is in the range of the observed (literature and PANNA data) decrease during pregnancy of 17-22% and 33% for the twice-daily and the once-daily dose, respectively. In conclusion: our data support a clinically relevant role of hepatic transporters in darunavir pharmacokinetics. The described model successfully approximated ritonavir-boosting and the decrease in darunavir exposure during pregnancy. Future *in vitro* experiments should generate quantitative kinetic data of passive and transporter-mediated darunavir handling by hepatocytes and intestinal epithelium.
In the General Discussion (Chapter 11) I place the previous chapters in a broader perspective and I summarize the results and compare the results with each other. First I treat the impact of pharmacokinetic alterations in pregnancy, the possible mechanisms behind this and the clinical consequences.
During pregnancy, the exposure was decreased by approximately 25% for most antiretrovirals described in this thesis. In total only four patients showed trough concentrations below the target concentration for effectivity. Possible mechanisms behind lower plasma concentrations in pregnancy are: increased plasma volume, decreased absorption and increased hepatic clearance due to induction of liver enzymes and/or increased liver blood flow.
In the patients described in this thesis, despite some of them having sub-therapeutic drug concentrations this, fortunately, did result in mother-to-child transmission of the virus. There are several factors to consider when a relationship between drug exposure and mother-to-child transmission is being made: women are treated with combination antiretroviral therapy, consisting of at least three compounds. This means that the compound measured in this study is not the only active antiretroviral drug used. Furthermore, in >50% of the cases delivery was done by caesarean section, which is known to decrease the chance of mother-to-child transmission.
For tenofovir DF, emtricitabine, saquinavir, darunavir, raltegravir and maraviroc we concluded that the dose does not have to be adapted during pregnancy, provided that

other factors, possibly decreasing exposure are absent. For atazanavir (for treatment-experienced patients) and rilpivirine we suggest that TDM during pregnancy can help to optimize treatment of HIV-infected women during pregnancy.
Subsequently, we described the effectiveness of treatments, by providing an overview of the viral load of the pregnant women close to delivery. Twenty percent of the patients had a detectable viral load (>50 copies/mL) around delivery, which did not lead to mother-to-child transmission. The viral loads around delivery were mostly below 500 copies/mL (84%) and maximally 28711 copies/mL. The underlying mechanism for the relatively high percentage of women with a detectable viral load around delivery is still unclear. Lower exposure to antiretrovirals could not be related to increased viral load in our studies.
Placenta passage of the antiretrovirals is summarized in Figure 1 of the General Discussion. Placenta passage for emtricitabine and raltegravir is good, with a cord blood/maternal blood ratio >1; moderate for tenofovir DF and rilpivirine (ratio between 0.5 and 1) and not very good for maraviroc, atazanavir and darunavir and almost non-existent for ritonavir.
We were able to predict maternal darunavir concentrations in the second and third trimester using a pregnancy PBPK model. This tool can be applied to predict the maternal exposure for new drugs prior to coming to the market. Further development of the foetal unit is needed to also predict placenta transfer of new drugs. It would be enrichment if placenta passage could reliably be predicted with a computer model. We showed that the effect of pregnancy on maternal exposure can be simulated, but to assess the exposure of the unborn child during pregnancy needs extension of the model. An acceptable computer model for assessment of placenta passage is not yet available.

Further, I discuss the lack of clinical trials in pregnant females and the exclusion of pregnant women from clinical trials in general. It is regarded not to be ethical to use new (not yet registered) medication during pregnancy, because of possible teratogenicity. When a woman becomes pregnant during a clinical trial with a new agent, the treatment is stopped immediately and the woman is excluded from the trial. If the pregnancy is pursued, the woman is followed-up and the outcome of the pregnancy is reported. When agents are registered and marketed, these will (possibly) be used during pregnancy, under uncontrolled circumstances. Pharmacovigilance guidelines from regulatory agencies (EMA and FDA) require pharmaceutical industry to set up a registry collecting safety data of exposure to the drug during pregnancy.
In the General Discussion I suggest to include pregnant women in clinical trials in an earlier stage for agents which will, almost certainly, be used in pregnancy, like antiretrovirals. Besides that, I propose not to exclude women who become pregnant during a clinical trial immediately, but to give them the opportunity to continue with the trial medication in a sub-study to investigate safety and pharmacokinetics. This can only be done when the reproductive toxicity studies are completed. Next to that, a computer model,

like a PBPK-model, could predict the pharmacokinetics during pregnancy.
Although it was not a research question of the PANNA network, I discuss the optimal choice of antiretroviral treatment during pregnancy. From our work we can confirm that emtricitabine and tenofovir DF pass the placenta well, seem to be generally safe and can be a good choice as back-bone of the treatment during pregnancy. The child will be protected also by these agents in case of blood-blood contact during partum. When a choice for a protease inhibitor is being made, atazanavir/r once daily, or saquinavir/r or darunavir/r twice daily seem to be the most robust options. Raltegravir or maraviroc are also an acceptable option, but safety data during pregnancy are scarce. Adding raltegravir to a triple regimen when the viral load is still high at the end of pregnancy seems a good choice, also protecting the baby during delivery. For rilpivirine we have not yet collected enough data to draw conclusions, the same is true for the new agents that became recently available. My personal opinion is that efavirenz should be avoided in the first trimester of pregnancy for safety reasons and because other options are available in the Western world.
I can conclude that data generated by the PANNA network can help to decide on the optimal treatment for pregnant HIV infected women, as is confirmed by incorporation of the results of the study in international guidlines, and make treating physicians aware of the influence of pregnancy on drug exposure. It is important to continue this network and add new antiretroviral agents to the list of medication to be investigated in pregnancy.

Samenvatting

Hiv (humaan immunodeficiëntie virus) is het virus dat AIDS veroorzaakt. Het virus is bij hiv-besmette personen aanwezig in onder andere bloed, sperma, vaginaal vocht en moedermelk. Als deze besmette lichaamsvloeistoffen in aanraking komen met de bloedbaan (bijvoorbeeld wondjes) of slijmvliezen kan de infectie worden overgedragen. Het virus bindt zich aan bepaalde afweercellen (CD4 T lymfocyten, CD4-cellen). Via deze cellen vermenigvuldigt het virus zich en het breekt deze cellen na verloop van tijd af. Daardoor vermindert de afweer, kan het lichaam moeilijker met andere infecties omgaan en kan uiteindelijk de dodelijke ziekte AIDS ontstaan.
In 2013 waren wereldwijd in totaal 35 miljoen mensen besmet met hiv, waarvan 3,2 miljoen kinderen. Het grootste deel van deze mensen leeft in Sub-Sahara Afrika (70%) en 50% van de patiënten is vrouw. In Nederland waren in 2014 ca. 17.750 mensen besmet met hiv; het merendeel (80%) van deze patiënten in Nederland is man.
Hiv kan (nog) niet worden genezen en er is ook geen vaccin beschikbaar. Er zijn wel medicijnen die de vermenigvuldiging van het virus remmen. Deze medicijnen grijpen aan op verschillende stappen van de levenscyclus van het virus. Hiv-patiënten moeten dagelijks tenminste drie van deze middelen gebruiken. Door deze medicijnen neemt het aantal virusdeeltjes af en verbetert het immuunsysteem.
Dit proefschrift gaat over het gebruik van anti-hiv-medicijnen door zwangere hiv-geïnfecteerde vrouwen. Hiv kan worden overgedragen van moeder op kind, vooral tijdens de bevalling als er bloed-bloedcontact plaatsvindt. Om te voorkomen dat het kind besmet wordt, moet de moeder tijdens de zwangerschap hiv-remmers gebruiken. Het aantal virusdeeltjes in het bloed en andere lichaamsvloeistoffen daalt door de behandeling, waardoor de kans op besmetting tijdens bloed-bloedcontact drastisch wordt verlaagd. Zonder behandeling zou 25-40% van de kinderen besmet raken tijdens de zwangerschap/geboorte; met behandeling daalt dit percentage tot <2%. Deze hiv-remmers kunnen tijdens de zwangerschap in het ongeboren kind terechtkomen en schadelijk zijn voor de ontwikkeling van het kind. Een positief aspect is dat het kind al medicijnen in het lichaam heeft als het mogelijk in contact komt met het hiv-virus van de moeder. Dat geeft extra bescherming tegen besmetting.
Ik onderzoek de concentraties van de hiv-remmers tijdens de zwangerschap in het bloed van de moeder, zogenaamde blootstelling aan hiv-remmers. Tijdens de zwangerschap verwijdert het lichaam mogelijk schadelijke stoffen om het kind te beschermen. Het lichaam ziet de hiv-remmers ook als schadelijke stoffen en breekt ze vaak sneller af. Daarnaast worden zwangere vrouwen zwaarder en het totale lichaamsvolume wordt groter waardoor de concentraties van de middelen gedurende de zwangerschap lager kunnen zijn. Voor werkzaamheid van hiv-remmers moet er wel genoeg in het lichaam aanwezig zijn. Te lage concentraties kunnen leiden tot falen van de therapie en tot resistentie tegen de hiv-remmers.

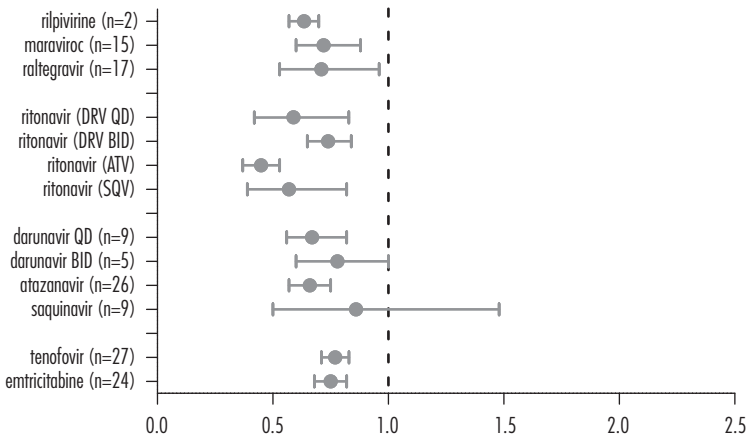
Om dit onderzoek naar de blootstelling aan hiv-remmers in de zwangeschap uit te voeren, hebben we een netwerk van ziekenhuizen verspreid over Europa opgezet, omdat er in Nederland te weinig hiv-geïnfecteerde zwangere vrouwen zijn om een dergelijk onderzoek te doen. Dit zogenoemde PANNA-netwerk – zowel het onderzoek als het netwerk heet zo – bestaat op dit moment (juli 2015) uit 21 ziekenhuizen in Nederland, het Verenigd Koninkrijk, Duitsland, België, Spanje, Italië en Ierland.
Samen met de PANNA-onderzoekers heb ik een lijst van medicijnen opgesteld waarvan we vonden dat er nog geen, of onvoldoende, informatie beschikbaar was over het effect van zwangerschap op de blootstelling (concentraties in het bloed) aan die medicijnen. Om dat uit te zoeken, hebben we bij zwangere vrouwen die hiv-remmers gebruikten tijdens het derde trimester van de zwangerschap een farmacokinetische curve afgenomen door bloed te prikken op 10 tijdstippen na inname van de medicatie. Deze vrouwen kwamen 4-6 weken na de bevalling weer terug om nog een farmacokinetische curve af te laten nemen, deze curve gebruikten we als controlesituatie. We hebben de blootstelling in de 'zwangere situatie' vergeleken met de 'niet-zwangere situatie', een vergelijking binnen personen. Daarnaast hebben we ook onderzocht of de bloedconcentraties hoog genoeg waren om werkzaam te zijn.
We wilden ook graag weten of de middelen de placenta kunnen passeren en dus in het kind terecht komen. Dat hebben we onderzocht door tijdens de bevalling navelstrengbloed af te nemen en de geneesmiddelconcentraties daarin te bepalen.
Het onderzoek, als beschreven in mijn proefschrift, had als doel het beschrijven van farmacokinetische veranderingen van bepaalde antiretrovirale middelen (hiv-remmers) in de zwangerschap. Daarnaast onderzocht ik of de middelen veilig en effectief waren en of ze de placenta passeren.
Hoofdstuk 2 van het proefschrift is een samenvatting van de farmacologische onderzoeken naar antiretrovirale middelen in de zwangerschap die in 2012 waren gepubliceerd. Er zijn zowel onderzoeken met intensieve sampling (afname van farmacokinetische curves) als onderzoeken waarbij zo nu en dan een bloedmonster is afgenomen (random sparse sampling) gerapporteerd, daarnaast was voor een aantal middelen ook de placentapassage beschreven. Voor de meeste stoffen die in 2012 waren onderzocht, is een daling van de blootstelling geobserveerd in de zwangerschap. De noodzaak van een dosisverhoging tijdens de zwangerschap blijft echter onduidelijk.
In de volgende zeven hoofdstukken wordt het effect van zwangerschap op de farmacokinetiek van drie proteaseremmers besproken: twee NRTIs, een NNRTI, een integraseremmer en een entryremmer.
Het eerste middel, saquinavir, wordt besproken in hoofdstuk 3. Het onderzoek ernaar is uitgevoerd voordat het PANNA-netwerk was opgezet, maar de opzet van het onderzoek was ongeveer gelijk. In het onderzoek naar de werking van saquinavir is naast een curve in het derde trimester ook een farmacokinetische curve afgenomen in het tweede trimester van de zwangerschap – indien dit mogelijk was. Van 37 vrouwen die tijdens de zwangerschap 1000/100mg saquinavir/ritonavir tweemaal daags ge-

bruikten, zijn farmacokinetische curves verzameld (16 in het tweede trimester, 31 in het derde trimester en 9 postpartum). Geen significant is verschil gevonden in blootstelling aan saquinavir tijdens de zwangerschap ten opzichte van postpartum. De spreiding in de resultaten was wel erg groot. Er zijn geen subtherapeutische saquinavir-concentraties (<0,1 mg/L) gemeten tijdens de zwangerschap of postpartum. De conclusie van dit onderzoek was dan ook dat saquinavir-blootstelling (van de nieuwe tablet) voldoende hoog is tijdens de zwangerschap en dat saquinavir in de zwangerschap veilig kan worden gebruikt. Er is geen dosisaanpassing nodig.
De volgende hoofdstukken beschrijven antiretrovirale middelen die onderzocht zijn in het PANNA-protocol.
Voor atazanavir/r beschrijven we de resultaten van 31 patiënten in hoofdstuk 4. We onderzochten naast het effect van zwangerschap op de blootstelling van atazanavir ook of er een verschil was tussen patiënten die tenofovir als comedicatie gebruikten, omdat tenofovir atazanavir-concentraties mogelijk verlaagt. Eenentwintig van de 31 patiënten gebruikten tenofovir als NRTI. De blootstelling aan atazanavir was 34% lager in het derde trimester, C_{max} was 30% lager en C_{24h} was 41% lager in vergelijking met postpartum. Er is geen significant verschil gevonden in farmacokinetische parameters tussen patiënten die wel en geen tenofovir als comedicatie gebruikten. Er zijn geen atazanavir-concentraties gemeten onder 0,15mg/L (de streefwaarde voor nog niet behandelde patiënten). Atazanavir passeert de placenta matig. De navelstrengbloed/moederbloed-ratio was 0,20 (n=12). De kinderen werden geboren na een mediane (range) zwangerschapsduur van 39 weken (36-42). Rond de bevalling had 81% van de patiënten een viruslast <50 kopieën/mL en 100% <1.000 kopieën/mL. Er is één aangeboren afwijking gezien bij een van de baby's, deze was waarschijnlijk niet gerelateerd aan atazanavir-gebruik. Geen van de kinderen was hiv-geïnfecteerd. We concludeerden dat atazanavir/r (300/100mg eenmaal daags) tijdens de zwangerschap voldoende blootstelling geeft, zelfs in combinatie met tenofovir. Voor patiënten die behandeling-naïef zijn (zonder relevante mutaties die relevant zijn voor proteaseremmers) hoeft de dosis in de zwangerschap niet te worden aangepast. Voor patiënten die voorbehandeld zijn en/of relevante mutaties hebben, kan therapeutische drug monitoring worden overwogen en kan de dosis op individuele basis worden aangepast.
Voor darunavir/r zijn in hoofdstuk 5 de resultaten beschreven van 24 patiënten (darunavir/r 600/100mg tweemaal daags (n=6); 800/100mg eenmaal daags (n=17); en 600/100mg eenmaal daags (n=1)). De blootstelling aan darunavir was 22% lager in zwangere patiënten die darunavir/r 600/100mg tweemaal daags gebruikten en 33% lager in patiënten die darunavir/r 800/100mg eenmaal daags gebruikten. We hebben ook de vrije concentraties darunavir gemeten in een aantal plasmamonsters van zowel de derde-trimestercurve als postpartum. De ongebonden fractie was in de zwangerschap (12%) ongeveer gelijk aan postpartum (10%). De mediane navelstrengbloed/moederbloed-ratio was 0,13. De kinderen werden geboren na een mediane (range) zwangerschapsduur van 38 weken (36-41). Rond de bevalling had 67% van

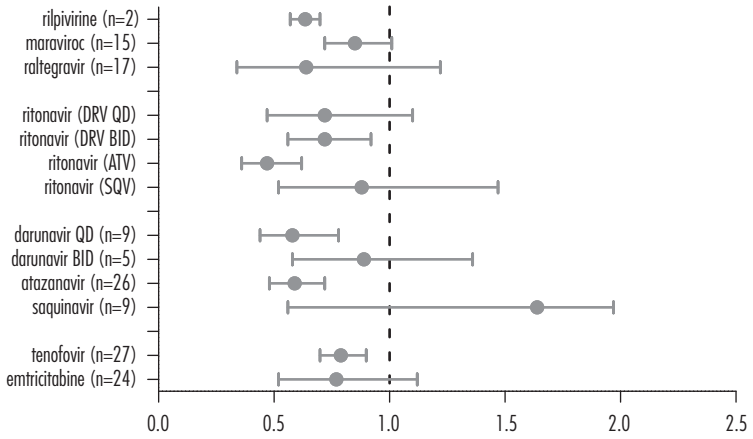
de patiënten een viruslast <50 kopieën/mL en 88% <300 kopieën/mL. Er heeft geen overdracht van hiv op het kind plaatsgevonden en er zijn geen aangeboren afwijkingen gerapporteerd. De conclusie was dat de daling van darunavir AUC en C_{max} in de zwangerschap niet leidde tot transmissie van het hiv-virus naar het kind. De dosis van 800/100mg eenmaal daags darunavir/r is voldoende voor patiënten die niet voorbehandeld zijn. Zij moeten darunavir met eten innemen en geen comedicatie gebruiken die de darunavir-concentraties kan verlagen. Voor alle andere patiënten wordt de 600/100mg dosering tweemaal daags tijdens de zwangerschap aangeraden.
In hoofdstuk 6 beschrijven we het effect van zwangerschap op de NRTIs tenofovir en emtricitabine. In totaal waren 34 zwangere vrouwen opgenomen in de analyse. De meeste vrouwen gebruikten de combinatietablet Truvada® (n=31, 91%). Tenofovir-blootstelling (AUC) was 23% lager en emtricitabine-blootstelling was 25% lager in de zwangerschap. Beide middelen passeren de placenta goed. De navelstrengbloed/moederbloed-ratio was 1,63 voor emtricitabine en 0,82 voor tenofovir. De meeste patiënten (97%) hadden minder dan 200 virusdeeltjes/mL rondom de bevalling, 83% had minder dan 50 virusdeeltjes/mL. De mediane (range) zwangerschapsduur was 38 (36-41) weken op het moment van bevalling. Geen van de kinderen was met hiv besmet en er zijn geen aangeboren afwijkingen gezien. Tenofovir en emtricitabine kunnen tijdens de zwangerschap in de gangbare dosering worden gebruikt.
De blootstelling aan raltegravir in de zwangerschap is beschreven in hoofdstuk 7. We hebben 22 patiënten geïncludeerd, waarvan 68% tijdens de zwangerschap met raltegravir-gebruik startte. Blootstelling aan raltegravir was erg variabel, de blootstelling (AUC) en C_{12h} waren 29% en 36% lager in het derde trimester. Raltegravir passeert de placenta goed met een navelstrengbloed/moederbloed-ratio van 1,21 en werd goed verdragen tijdens de zwangerschap. Rond de bevalling had 86% van de patiënten een ondetecteerbare viruslast (<50 kopieën/mL). De zwangerschapsduur was 38 (38-39) weken. Er heeft geen overdracht van het virus plaatsgevonden. De daling in blootstelling aan raltegravir tijdens de zwangerschap is als niet klinisch relevant beoordeeld. Daarom kan raltegravir tijdens de zwangerschap in de standaarddosering worden gebruikt.
Hoofdstuk 8 is een beschrijving van twee casussen van rilpivirine-gebruik in de zwangerschap. Er was nog niets bekend over het effect van zwangerschap op rilpivirine farmacokinetiek of placentapassage. Daarom hebben we de eerste twee patiënten die geïncludeerd zijn in het PANNA-onderzoek apart beschreven. Rilpivirine passeert de placenta matig (navelstrengbloed/moederbloed-ratio van 0,74). De blootstelling was 30-43% lager in de zwangerschap. Beide patiënten hadden een lage dalspiegel tijdens de zwangerschap, zelfs lager dan de streefwaarde van 0,040 mg/L. We raadden dan ook aan om rilpivirine spiegels te meten tijdens de zwangerschap.
Hoofdstuk 9 over het effect van zwangerschap op maraviroc-blootstelling is tot stand gekomen in samenwerking met IMPAACT. IMPAACT is een Amerikaanse groep die een soortgelijk onderzoek als PANNA uitvoert maar dan in de Verenigde Staten, Zuid-Amerika en Thailand. We hebben onze data met betrekking tot maraviroc samengevoegd om

een substantieel aantal patiënten te kunnen rapporteren. In totaal zijn 18 patiënten geïncludeerd (IMPAACT 11; PANNA 7). De meeste vrouwen gebruikten 150mg maraviroc, tweemaal daags, in combinatie met een proteaseremmer (12; 67%). Twee vrouwen (11%) gebruikten 300mg maraviroc, tweemaal daags, zonder proteaseremmer, en vier vrouwen hadden een ander regime. De blootstelling aan maraviroc was 28% lager in de zwangerschap, met een 30% lagere C_{max}. De dalspiegels waren 15% lager in de zwangerschap, maar bijna allemaal boven de streefwaarde. Maraviroc passeert de placenta matig met een navelstrengbloed/moederbloed-ratio van 0,33 (0,03-0,56). De kinderen werden geboren na een mediane zwangerschapsduur van 39 (37-41) weken. De viruslast rond de bevalling was <50 kopieën/mL in 13 patiënten (76%). Alle kinderen waren hiv-negatief. Ondanks een afname aan blootstelling kan de standaarddosering voor maraviroc gehandhaafd blijven in de zwangerschap.
In figuur 1 en 2 zijn de effecten van de zwangerschap op AUC (blootstelling) en dalspiegel (C_{trough}) van de verschillende middelen weergegeven als geometrisch gemiddelde ratio met het bijbehorende 90% betrouwbaarheidsinterval.

De ontwikkeling van een physiologically-based-pharmacokinetic (PBPK) model voor darunavir hebben we in hoofdstuk 10 beschreven. Een PBPK-model is een computermodel waarmee op basis van de fysisch-chemische en kinetische eigenschappen van een stof en de specifieke fysiologische parameters van het menselijk lichaam de blootstelling in het bloed, maar ook in verschillende organen kan worden gesimuleerd. Ook fysiologische veranderingen die tijdens de zwangerschap optreden, kunnen in het model worden meegenomen waardoor de blootstelling aan medicatie tijdens de zwangerschap kan worden voorspeld zonder uitgebreid *in vivo* onderzoek als basis.



Figuur 1. Geometrisch gemiddelde ratio's AUC (zwangerschap/postpartum) en 90% betrouwbaarheidsinterval



Figuur 2. Geometrisch gemiddelde ratio's C_{trough} (zwangerschap/postpartum) en 90% betrouwbaarheidsinterval

Dit is een groot voordeel van een dergelijk computermodel.
Wij hebben een model gebouwd dat de blootstelling van beide doseringen darunavir/ritonavir (600/100mg tweemaal daags en 800/100mg eenmaal daags) goed beschrijft voor zowel gezonde proefpersonen als in de zwangerschap. Het model is gebouwd in Simcyp (v13.2), een PBPK-modelplatform. De fysisch-chemische eigenschappen van darunavir en ritonavir zijn aan de literatuur ontleend. De K_m en V_{max} voor CYP3A4 gemedieerd metabolisme van darunavir en de inhibitie van dit metabolisme door ritonavir zijn via *in vitro* experimenten bepaald. We hebben ontdekt dat we zonder het includeren van een rol voor transporters geen goed passend model konden maken voor darunavir. Middels sensitiviteitsanalyses hebben we de contributie van opnamen en efflux transporters geschat. De schattingen van de blootstelling aan darunavir alleen en ook van darunavir/ritonavir-interactie waren vergelijkbaar met de waardes die zijn gerapporteerd in de literatuur. Het model voorspelde een afname van darunavir AUC van 27% en 41% (tweemaal daags en eenmaal daags) in de zwangerschap, wat overeenkomt met de daling die geobserveerd is in de literatuur en het PANNA-onderzoek (17-22% en 33% voor tweemaal daags en eenmaal daags darunavir/ritonavir respectievelijk). We concluderen dat ons model een klinisch relevante rol van transporters voor de beschrijving van darunavir farmacokinetiek ondersteunt. Ons model beschrijft het effect van ritonavir op darunavir goed, en ook het effect van zwangerschap op de blootstelling aan darunavir voor beide doseerregimes. Toekomstige experimenten moeten kwantitatieve gegevens genereren voor passief en actief transporter-gemedieerde verwerking van darunavir door hepatocyten en darmwandepitheel.
In de discussie (hoofdstuk 11) plaats ik de voorgaande hoofdstukken in een breder

perspectief en heb ik data samengevat en met elkaar vergeleken. Eerst behandel ik de impact van de farmacokinetische veranderingen in de zwangerschap, de mogelijke mechanismen hierachter en de klinische consequenties.
De blootstelling tijdens de zwangerschap was ongeveer 25% lager voor de meeste antiretrovirale middelen die in dit proefschrift zijn besproken. In totaal is bij slechts vier patiënten een dalwaarde gezien onder de streefwaarde voor effectiviteit. Mogelijke mechanismen voor de lagere plasmaconcentraties in de zwangerschap zijn: een groter plasmavolume, verminderde absorptie en verhoogde hepatische klaring door inductie van leverenzymen of verhoogde bloedflow door de lever.
Bij de patiënten die ik in dit proefschrift heb beschreven, heeft geen overdracht van het hiv-virus van moeder op kind plaatsgevonden, ondanks de lagere blootstelling aan hiv-remmers tijdens de zwangerschap en zelfs subtherapeutische concentraties van de antiretrovirale middelen bij een aantal patiënten. Er zijn enkele factoren die moeten worden meegenomen wanneer de relatie tussen blootstelling aan geneesmiddelen en de overdracht van hiv van moeder op kind wordt onderzocht: de vrouwen worden behandeld met combinatie antiretrovirale-therapie die bestaat uit tenminste drie middelen. Dit wil zeggen dat het geneesmiddel dat gemeten is in dit onderzoek niet het enige antiretrovirale middel is dat gebruikt is. Daarnaast is meer dan 50% van de vrouwen bevallen met een keizersnee, wat de kans op overdracht van het hiv-virus verlaagt.
Voor tenofovir DF, emtricitabine, saquinavir, darunavir, raltegravir en maraviroc hebben we geconcludeerd dat het niet nodig is de dosering tijdens de zwangerschap aan te passen, mits er geen andere factoren zijn die de blootstelling kunnen verlagen. Voor atazanavir (voorbehandelde patiënten) en rilpivirine kan TDM tijdens de zwangerschap een rol spelen om de behandeling te optimaliseren.
Vervolgens hebben we de effectiviteit van de behandelingen beschreven door een overzicht te geven van de viruslast in de zwangere vrouw rondom de bevalling. Twintig procent van de patiënten had een detecteerbare viruslast (>50 kopieën/mL) rondom de bevalling, wat in geen enkel geval heeft geleid tot overdracht van het hiv-virus. De viruslast was meestal onder de 500 kopieën/mL (bij 84%) en maximaal 28711 kopieën/mL. Het mechanisme voor het relatief hoge percentage vrouwen met een detecteerbare viruslast rondom de bevalling, wat overeenkomt met percentages die in de literatuur worden gerapporteerd, is nog altijd onduidelijk. Lagere blootstelling aan de individuele antiretrovirale middelen kon in onze onderzoeken niet worden gerelateerd aan verhoogde viruslast.
De placentapassage van de verschillende middelen is samengevat in figuur 1 van de General Discussion. Placentapassage van emtricitabine en raltegravir is goed, met een navelstrengbloed/moederbloed-ratio >1; matig voor tenofovir DF en rilpivirine (ratio tussen 0,5 and 1) en niet zo goed voor maraviroc, atazanavir en darunavir en bijna nul voor ritonavir. Het zou een mooie stap voorwaarts zijn als de placentapassage betrouwbaar kan worden voorspeld met behulp van een computermodel. We hebben

laten zien dat de verandering in maternale blootstelling tijdens de zwangerschap kan worden gesimuleerd, maar om de blootstelling in het ongeboren kind goed te schatten, moet het model verder worden ontwikkeld. Een goed computermodel voor placentapassage is vooralsnog niet voorhanden.
Ook behandel ik het gebrek aan geneesmiddelenonderzoek in zwangere vrouwen in het algemeen. Het wordt niet ethisch geacht nieuwe geneesmiddelen in de zwangerschap te gebruiken, vanwege niet uit te sluiten teratogeniteit. Ook als vrouwen in een onderzoek met een nieuw middel zwanger worden, wordt de behandeling met het middel in de meeste gevallen meteen gestaakt en wordt de vrouw geëxcludeerd. Als de zwangerschap wordt voortgezet, wordt de uitkomst van de zwangerschap wel gerapporteerd. Als de middelen op de markt komen, worden ze echter (mogelijk) wel gebruikt tijdens de zwangerschap, onder niet heel goed gecontroleerde omstandigheden. Er zijn farmacovigilantie-richtlijnen van de EMA en FDA die de farmaceutische industrie verplichten post-marketing bij te houden of hun stoffen teratogeen zijn.
In de discussie suggereer ik dat geneesmiddelenonderzoek in zwangere patiënten voor medicijnen die in de zwangerschap gebruikt gaan worden, zoals antiretrovirale middelen, eerder moet worden uitgevoerd. Daarnaast stel ik voor om vrouwen die tijdens een onderzoek met een nieuw geneesmiddel zwanger worden niet meteen te excluderen, maar hen de mogelijkheid te bieden het middel door te gebruiken en in een substudie de veiligheid en farmacokinetiek te bestuderen, mits er voldoende reproductietoxicologisch preklinisch onderzoek is uitgevoerd. Daarnaast kan met behulp van een computermodel, zoals een PBPK-model, een voorspelling worden gedaan van de veranderingen in farmacokinetiek tijdens de zwangerschap.
Het was weliswaar geen onderzoeksvraag van het PANNA-netwerk, maar toch probeer ik een optimaal behandelingsregime voor zwangere hiv-geïnfecteerde vrouwen samen te stellen. Uit ons onderzoek blijkt dat tenofovir en emtricitabine de placenta goed passeren en over het algemeen veilig waren en daarom een goede keus zijn voor een NRTI back-bone tijdens de zwangerschap. Bij mogelijk bloed-bloedcontact tijdens de bevalling dragen deze middelen bij aan het voorkomen van hiv-infectie van het kind. De meest robuuste proteaseremmers, wat farmacokinetische veranderingen betreft, zijn atazanavir/r, eenmaal daags 300/10 mg, en darunavir/r 600/100mg tweemaal daags. Raltegravir en maraviroc zijn ook een optie, maar hiervan zijn nog maar weinig veiligheidsgegevens beschikbaar. Het toevoegen van raltegravir aan de behandeling lijkt een goede optie om de viruslast snel te laten dalen tijdens de zwangerschap, indien nodig. Raltegravir passeert de placenta goed en het kind is daardoor tijdens de bevalling extra goed beschermd tegen infectie. Voor rilpivirine hebben we nog te weinig data om conclusies te trekken, dit geldt ook voor de nieuwe middelen die recent beschikbaar zijn gekomen.
Ik ben van mening dat efavirenz-gebruik in het eerste trimester van de zwangerschap zou moeten worden vermeden vanwege veiligheidsredenen, en omdat in de westerse wereld voldoende alternatieven beschikbaar zijn.

Ik kan concluderen dat de gegevens die worden gegenereerd door het PANNA-netwerk kunnen helpen een keuze te maken voor optimale behandeling van hiv-geïnfecteerde zwangere vrouwen. Dit wordt bevestigd doordat internationale richtlijnen de resultaten uit dit onderzoek hebben opgenomen. Daarnaast hoop ik dat behandelend specialisten meer bewust worden van de invloed van zwangerschap op blootstelling aan antiretrovirale middelen. Het is belangrijk dat het PANNA-netwerk blijft voortbestaan en dat nieuwe antiretrovirale middelen worden toegevoegd aan de lijst van medicatie die wordt onderzocht.

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Dankwoord

30 oktober 2007, vandaag precies acht jaar geleden is de basis gelegd voor dit promotieonderzoek aan de afdeling apotheek van het UMC St Radboud. De locatie was Cinemariënburg (Nijmegen) en aanwezigen waren dr. David Burger en ik (en andere filmgasten en cafégangers). Er is veel veranderd sindsdien: UMC St Radboud is Radboud universitair medisch centrum geworden, Cinemariënburg is gesloten en dr. David Burger is gepromoveerd tot professor David Burger.
David had mij uitgenodigd voor een informeel, oriënterend gesprek over een mogelijke functie als researchassistente in de apotheek. David en ik kenden elkaar van een aantal klinische onderzoeken die ik vanuit Farma Research BV had gecoördineerd. Naast het werk als researchassistente hebben we ook gesproken over 'een eigen onderzoek'. Dat leek mij wel leuk en spannend, en zo is het gekomen. In februari 2008 ben ik in de apotheek begonnen als researchassistente. Vrij snel zijn we het PANNA-onderzoek gaan opzetten. Ik had veel ervaring met kleinschalige onderzoeken met gezonde proefpersonen; het opzetten van een internationaal netwerk bleek andere koek. Gelukkig was er al een soortgelijk onderzoek opgestart: SARA, dat is beschreven in hoofdstuk 3 van dit proefschrift, en wat een mooie basis was voor het PANNA-onderzoek. De onderzoekers die we hebben benaderd, hadden gelukkig research-nurses of -coördinatoren in dienst die mij uitgebreid hebben geholpen met alle logistieke uitdagingen. De Europese clinical trials directive wordt namelijk in alle landen net iets anders geïnterpreteerd.
De onderzoekers bleken enthousiast en dachten heel goed mee. We bleken infectiologen, farmacologen, kinderartsen en gynaecologen aan boord te hebben. Dat was een goede mix om een mooi onderzoek op te zetten. Met enthousiasme en kennis kom je een heel eind, maar aan dit soort onderzoeken hangt ook een kostenplaatje. NEAT/Penta heeft de eerste financiële injectie gegeven en gaat daar nog steeds mee door. Daarnaast was Merck onze eerste partner uit de categorie farmaceutische industrie. Later volgden BMS, Janssen Research BV en recent ViiV Healthcare. Het hele PANNA-netwerk is erg blij met deze financiële ondersteuning.
Het PANNA-onderzoek, maar ook het SARA-onderzoek en de onderzoeken die in dit proefschrift zijn beschreven, kwamen tot stand door een intensieve, internationale samenwerking. Het is niet voor niets een netwerk. Ik kon dit niet alleen en ben veel personen dankbaar.
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A very important person for me and PANNA is Carlo Giaquinto, who believed in the

PANNA study from day-1 onwards. I remember our first encounter in a restaurant in Paris, where you promised to pay my salary as a coordinator of the PANNA study. Thank you very much for your support and for the opportunity to present the PANNA study in San Servolo at the bi-annual PENTA meeting and also for being here today and asking relevant questions.
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Curriculum vitae

Angela Colbers werd op 1 november 1970 te Kessel geboren. In 1989 behaalde zij haar gymnasiumdiploma aan de scholengemeenschap St. Ursula te Horn. Daarna volgde zij de studie biomedische gezondheidswetenschappen aan de Radboud Universiteit te Nijmegen, en in 1995 studeerde zij af in de richting 'toxicologie'. Haar interesse lag bij het geneesmiddelenonderzoek, waarmee zij gedurende haar stages in aanraking was gekomen. Na haar afstuderen heeft ze twee jaar als 'medical research associate' gewerkt bij het farmaceutische bedrijf NV Organon te Oss. Daar heeft ze fase-1-onderzoeken met CNS-medicatie opgezet en uitgevoerd. Vervolgens heeft ze van 1997–2006 als projectmanager gewerkt bij de contract-researchorganisatie Farma Research BV. In deze functie heeft ze veel geneesmiddelenonderzoeken opgezet, uitgevoerd, geanalyseerd en gerapporteerd; met name bioequivalentieonderzoeken en interactieonderzoeken, maar ook enkele fase-1-onderzoeken waarin een medicijn voor het eerst aan mensen werd gegeven. Van 2006–2008 werkte Angela als Regulatory Affairs en Project Manager van preklinisch onderzoek bij NOTOX BV te 's Hertogenbosch. Sinds februari 2008 is Angela werkzaam als research-assistant/promovendus op de afdeling Apotheek van het Radboud universitair medisch centrum te Nijmegen. Ze is projectcoördinator van het PANNA-netwerk en begeleidt promovendi en studenten bij geneesmiddelenonderzoek en ze ondersteunt senior-onderzoekers met hun onderzoeken. Het promotieonderzoek, zoals beschreven in dit proefschrift, heeft ze uitgevoerd onder begeleiding van prof. dr David Burger.

Angela Colbers was born on November 1st, 1970 in Kessel, The Netherlands. In 1989 she completed her secondary school education at the Scholengemeenschap St. Ursula in Horn, The Netherlands. She then studied biomedical health sciences at the Radboud University in Nijmegen, and graduated in 'toxicology' in 1995. She became interested in clinical studies, as she did internships on this subject. After her graduation, she worked two years as a 'medical research associate' at the pharmaceutical company NV Organon in Oss. In this role she developed and performed phase-1 studies with CNS medication. Subsequently, from 1997–2006, she worked as project manager at a contract research organisation: Farma Research BV. In this function she developed, performed, analysed and reported numerous clinical trials. It mainly concerned bioequivalence and interaction studies, but also some phase-1 trials in which medicines were given to humans for the first time. From 2006–2008 Angela worked as Regulatory Affairs and Project Manager of pre-clinical studies at NOTOX BV in 's Hertogenbosch. Since February 2008, Angela is research assistant/PhD student at the Pharmacy

of the Radboud university medical center in Nijmegen, The Netherlands. She is project coordinator of the PANNA-network and teaches and advices PhD and other students on developing and executing clinical trials and she supports senior scientists with their research. Her PhD work, as described in this thesis, was supervised by Prof. dr David Burger.

Notes