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Modeling of Viral Dynamics after Liver Transplantation in Patients with Chronic Hepatitis B and D

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***Abstract*—Viral kinetic models have become an important tool for understanding the main biological processes behind the dynamics of chronic viral diseases and optimizing effectiveness of anti-viral therapy. We analyzed the dynamics of hepatitis B and D co-infection (HBV/HDV) and the pharmacokinetics/pharmacodynamics of the reinfection prophylaxis with polyclonal antibodies after liver transplantation. Therefore we developed a mechanistic model consisting of a system of ordinary differential equations. This model was fitted by analyzing the kinetics of HBV/HDV viremia after liver transplantation in patient data and correlated with the reinfection prophylaxis dosing schemes. The results suggest that this modeling approach may help to optimize reinfection prophylaxis.**

***Keywords*-Infectious diseases; hepatitis B and D; viral dynamics; PK/PD**

I. Introduction

Hepatitis B is an infectious disease of the liver caused by the hepatitis B virus. Although vaccination is possible nowadays, hepatitis B is still a major concern in global health. Approximately 2 billion people have been infected with the hepatitis B virus (HBV) ([3], [4]) and it is estimated that 350-400 million people are chronic carriers of HBV [5]. Persistent hepatitis B infection comprises a high risk for liver cirrhosis or hepatocellular carcinoma [3]. In these cases liver transplantation often remains the only therapy option.

A. Hepatitis B Virus

The hepatitis B virus is a DNA virus that belongs to the family Hepadnaviridae. It replicates in the liver by utilization of an RNA-mediate and reverse transcription. The produced virus is secreted into serum, where it might infect hepatocytes or be detected by the immune system and degraded. The virus itself is non-cytopathic, but apoptosis of infected hepatocytes might be induced by immune response (especially CTL-response). A viral protein of particular clinical significance is the hepatitis B surface Antigen (HBsAg), the envelope of the hepatitis B virus. HBsAg particles (lacking of virus DNA) are produced in excess by infected hepatocytes: the ratio of HBsAg to complete virus particles in serum is approximately 1000-10000:1.
Hepatitis B surface antibodies (anti-HBs) are directed to the hepatitis B surface antigen and may prevent the entry of the virus by binding and neutralizing circulating virions [9].

B. Delta Hepatitis

Delta hepatitis is considered as the most severe form of chronic viral hepatitis frequently leading to end-stage liver disease and hepatocellular carcinoma. It is caused by the Hepatitis D virus (HDV), a single-stranded RNA genom which depends on the hepatitis B virus surface antigen for complete replication and transmission. Therefore, HDV infection only occurs in HBsAg-positive individuals either as acute co-infection or as

superinfection in patients with chronic hepatitis B [7].

C. Liver Transplantation

Liver transplantation (LTX) remains the only therapy option for patients with end-stage liver disease due to chronic hepatitis B virus (HBV) infection or hepatitis B and D (HBV/HDV) co-infection. To prevent reinfection of the graft, caused by circulating virions, Hepatitis B immune globuline (HBIg) and HBV polymerase inhibitors are administered. Hepatitis B immune globuline (HBIg) is a blood plasma product containing polyclonal antibodies (anti-HBs) against HBsAg. This protection by anti-HBs in the liver transplant setting, however, is not sterile: HBV DNA is detectable in the new liver even in cases with effective prophylaxis. Lamivudine inhibits the production of hepatitis B virions, but neither the production nor release of HBsAg particles nor Delta virions.

With the introduction of HBIg and HBV polymerase inhibitors as standard prophylaxis, the risk for a reinfection has decreased from approximately 80% to less than 10%. Despite these progresses there does not exist any rational basis for HBIg doses schedules up to now, typically HBIg is given during the anhepatic phase, followed by daily infusions at a fixed dose until HBsAg is negative. There is large interest to optimize/individualize HBIg treatment schedules, since high doses of antibodies can be a burden for the patient and HBIg is very expensive [6].

II. Modeling of Virus Dynamics in Hepatitis

Models for hepatitis B virus dynamics are mostly derived from the basic model for hepatitis C, introduced by Neumann et al. [10]:

$$\begin{aligned}\frac{dV}{dt} &= pI(t) - cV(t)\\ \frac{dI}{dt} &= \beta T(t)V(t) - \delta I(t)\\ \frac{dT}{dt} &= \lambda - \beta T(t)V(t) - dT(t)\end{aligned}$$

In this model the uninfected cell population is denoted by T, infected cells by I and free virus particles in serum by V. Uninfected cells T are assumed to be produced at a constant rate λ and to die at a rate d. Free virus particles V are produced at a rate p proportional to I and are removed from the system at a rate c. Target cells T are infected at a rate β proportional to TV. Infected cells I are killed by the immune system at a rate δ.

The effect of antiviral therapy may be modeled by partial blocking of release of virions (hence $(1-\epsilon)p$, $0 < \epsilon < 1$) and/or partial blocking of infection of hepatocytes ($(1-\eta)\beta$, $0 < \eta < 1$).

There exist several extensions of this basic model. For example, Dahari et al. introduced proliferation of (uninfected and infected) hepatocytes and a curing rate of infected liver cells, which allows modeling of complex decline profiles [11].

De Sousa et al. proposed a model for chronic HBV/HDV co-infection [12]: the basic model was extended by including compartments for circulating Delta virions, HDV-mono-infected and HBV/HDV co-infected liver cells. Forde modeled the dynamics of chronic HBV/HDV co-infection under consideration of the patients immune response (HBV- and HDV-specific CTL-response, not published).

For the setting of liver transplantation only few models exist for hepatitis C ([13], [14]). Since in hepatitis C an infection of the liver graft is unavoidable with current treatments and extrahepatic compartments might play a significant role, these models may not be transferred to the case of HBV/HDV- or HBV-induced liver transplantation.

Neumann et al. examined the effect of a single dose of monoclonal anti-HBs in patients with chronic hepatitis B [15]. He assumed that anti-HBs not only acts by neutralizing circulating HBsAg and virions, but also may enter hepatocytes and reduce the release of virions and HBsAg particles.

III. Dynamics after liver transplantation

We propose that the dynamics after liver transplantation can be described as shown in Figure 1: HBIg (i.e. anti-HBs particles) is injected intravenously and immediately available. Anti-HBs are cleared by metabolism at a constant rate. Due to binding to circulating HBsAg particles, and hepatitis B virions, we have an accelerated clearing of anti-HBs, HBsAg, HBV, and HDV. We assume that formed immune complexes dissociate with a certain probability.

At the time of transplantation, we assume that all hepatocytes are uninfected and susceptible. Free virions infect hepatocytes of the graft at a constant rate. Since the replication cycle for HBV takes 1-2 days [19], we introduce two different kinds of compartments of infected cells, one, that does not secrete virus and HBsAg particles yet and a compartment of mature infected cells, that does.

Our model is based on the basic model by Neumann et al. [10], a standard one-compartment PK-model, and on the delay differention equation model for HBV by Gourley et al. [18]. The corresponding ODE system is

decribed as below:

$$\begin{aligned}
\frac{dA(t)}{dt} &= \frac{d(t)}{V_d(t)} - k_{e1}A(t) - kA(t)(H(t) + V_1(t) + \\
&\quad V_2(t)) + k_D\overline{AH}(t) + k_D\overline{AV_1}(t) + k_D\overline{AV_2}(t) \\
\frac{dH(t)}{dt} &= p_{H1}I_1(t) + p_{H12}I_{12}(t) - cA(t)H(t) \\
&\quad - \delta_H H(t) + c_D\overline{AH}(t) \\
\frac{dV_1(t)}{dt} &= p_1 I_1(t) + p_{12}I_{12}(t) - cA(t)V_1(t) - \delta_1 V_1(t) \\
&\quad + c_D\overline{AV_1}(t) \\
\frac{dV_2(t)}{dt} &= p_2 I_{12}(t) - cA(t)V_2(t) - \delta_2 V_2(t) + c_D\overline{AV_2}(t) \\
\frac{d\overline{AH}(t)}{dt} &= cA(t)H(t) - c_D\overline{AH}(t) - \delta_{AH}\overline{AH}(t) \\
\frac{d\overline{AV_1}(t)}{dt} &= cA(t)V_1(t) - c_D\overline{AV_1}(t) - \delta_{AV_1}\overline{AV_1}(t) \\
\frac{d\overline{AV_2}(t)}{dt} &= cA(t)V_2(t) - c_D\overline{AV_2}(t) - \delta_{AV_2}\overline{AV_2}(t) \\
\frac{dT(t)}{dt} &= \lambda - \delta T(t) - \beta_1 V_1(t)T(t) - \beta_2 V_2(t)T(t) \\
\frac{E_1(t)}{dt} &= \beta_1 V_1(t)T(t) - \delta E_1(t) - \beta_2 V_2(t)E_1(t) \\
&\quad - e^{-\delta\tau}\beta_1 V_1(t-\tau)T(t-\tau) \\
\frac{E_2(t)}{dt} &= \beta_2 V_2(t)T(t) - \delta E_2(t) - \beta_1 V_1(t)E_2(t) \\
\frac{E_{12}(t)}{dt} &= \beta_2 V_2(t)E_1(t) + \beta_1 V_1(t)E_2(t) \\
&\quad + \beta_2 V_2(t)I_1(t) - \delta E_{12}(t) \\
&\quad - e^{-\delta\tau}(\beta_2 V_2(t-\tau)E_1(t-\tau) \\
&\quad + \beta_1 V_1(t-\tau)E_2(t-\tau) \\
&\quad + \beta_2 V_2(t-\tau)I_1(t-\tau)) \\
\frac{dI_1(t)}{dt} &= e^{-\delta\tau}\beta V_1(t-\tau)T(t-\tau) - \delta_{I_1} I_1(t) \\
&\quad - \beta_2 V_2(t)I_1(t) \\
\frac{dI_{12}(t)}{dt} &= e^{-\delta\tau}(\beta_2 V_2(t-\tau)E_1(t-\tau) \\
&\quad + \beta_1 V_1(t-\tau)E_2(t-\tau) \\
&\quad + \beta_2 V_2(t-\tau)I_1(t-\tau)) - \delta_{I_{12}}
\end{aligned}$$

where $A(t)$, the level of anti-HBs in serum,
$H(t)$, HBsAg level in serum,
$V_1(t)$, HBV DNA in serum,
$V_2(t)$, HDV RNA in serum,
$\overline{AH}(t)$, anti-HBs-HBsAg immune complexes,
$\overline{AV_1}(t)$, anti-HBs-HBV immune complexes,
$\overline{AV_2}(t)$, anti-HBs-HDV immune complexes,
$T(t)$, target cells,

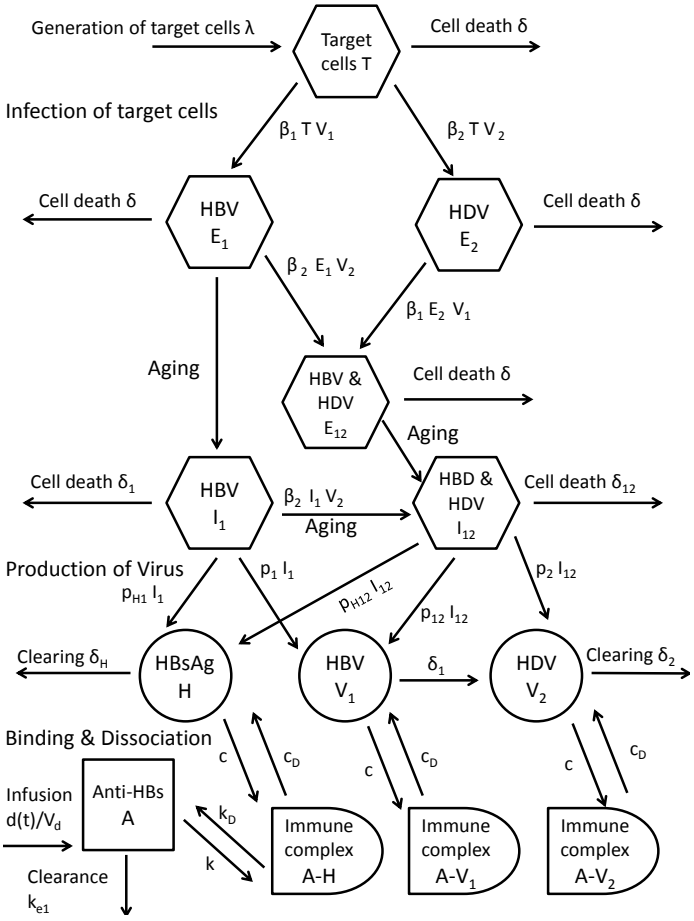

Fig. 1. The model of the main mechanism during treatment with anti-HBs after LTX.

$E_1(t)$, HBV mono-infected cells not replicating yet,
$E_2(t)$, HDV mono-infected cells (cannot replicate),
$E_{12}(t)$, HBV/HDV co-infected cells not replicating yet,
$I_1(t)$, replicating HBV mono-infected cells,
and $I_{12}(t)$, replicating cells co-infected with HBV/HDV.
The compartments are described as follows:

A. Anti-HBs A

Anti-HBs A is assumed to be administered intravenously with complete and immediate bioavailability. To model the pharmacokinetics of anti-HBs we use a standard one-compartment intravenous infusion model. We assume a zero order infusion rate constant $d(t) > 0$ during time intervals $[T_i^{start}, T_i^{stop}], i = 1, \ldots, n$ and $d(t) = 0$ for $t \notin [T_i^{start}, T_i^{stop}], i = 1, \ldots, n$, and a constant volume of distribution V_d. The loss of anti-HBs A due to metabolism is modeled as a first order elimination with a constant rate k_{e1}, corresponding to the half-life of $\log(2)/k_{e1}$ of HBIg in immunosuppressed patients [[17], [16]]. The additional loss of anti-HBs caused by binding

of anti-HBs to circulating HBsAg particles, hepatitis B virions, and Delta virions is modeled with a constant rate k proportional to V_1, and H. Since the formation of immune complexes $\overline{AH}$, $\overline{AV_1}$, and $\overline{AV_2}$ is a reversible reaction, we introduce immune complex compartments $\overline{AH}$, $\overline{AV_1}$, and $\overline{AV_2}$ and a dissociation rate k_D.

B. HBsAg H, HBV DNA V_1, and HDV RNA V_2

HBsAg particles H are produced at constant rates p_{H1} and p_{H12} proportional to the number of infected cells I_1 and I_{12}, eliminated at a constant rate δ_H and are bound to anti-HBs A at a constant rate c proportional to A. The dissociation rate of HBsAg is calculated as $c_D = \frac{ck_D}{k}$.
The HBV and HDV compartments V_1 and V_2 are described analogously, except that Delta virions are exclusively produced in co-infected cells.

C. Immune Complexes $\overline{AH}$, and $\overline{AV_1}$, and $\overline{AV_2}$

The immune complex compartment $\overline{AH}$ is characterized by a constant association rate k proportional to A and H, a constant dissociation rate k_D proportional to $\overline{AH}$, and a constant clearing rate δ_{AH}.
The immune complex compartments $\overline{AV_1}$ and $\overline{AV_2}$ are described analogously.

D. Target cCells T

Target cells are infected by hepatitis B and Delta virions V_1 and V_2 at constant rates β_1 and β_2 proportional to T, V_1, and V_2, die at a constant rate δ and are produced at a constant rate λ.

E. Infected Cells E_1, E_2, E_{12}, I_1 and I_{12}

Since the replication cycle of HBV takes 1-2 days [19], for the Delta virus we assume the same length, we incorporate a delay in our model: we employ the age structured model after McKendrick-Forster, as it was introduced for the setting of chronic hepatitis B infection by Gourley et al. [18]. Target cells T infected with HBV V_1 begin after τ units of time to secrete virions. Cells mono-infected with HDV E_2 are not able to produce Delta virions (due to the lack of the helper virus), in case they are superinfected with HBV, they begin after τ units of time to secrete HBsAg H, HBV V_1, and HDV V_2. Note, that Delta virus may decrease the production rates of HBV and HBsAg severely in co-infected cells. Infected cells not secreting virus yet E_1, E_2, and E_{12} die at the constant rate δ. We use the same death rate δ as for the target cells T, because we assume these cells are not recognized by the immune system before they start to secrete virions. If a mono-infected cell E_1 is superinfected with the Delta virus, we assume it will start to secrete HBsAg H, HBV V_1, and HDV V_2 after τ units of time and neglect a possible release of HBV and HBsAg particles beforehand.
The increase in the number of mature infected cells I_1 and I_{12} is proportional to the number of cells that have been infected before τ units of time and the number of free virus at the time $t-\tau$. Mature infected cells I_1 and I_{12} die at constant rates δ_{I_1} and $\delta_{I_{12}}$.

IV. Simple Variant of the Model

Since a reinfection with HBV or HBV/HDV after liver transplantation can be successfully prevented in most cases nowadays (the risk is less than 10% in HBV mono-infected patients, in HDV/HBV even smaller), we assume that the amount of hepatocytes that will be infected after transplantation is rather small and may be neglected. Hence, we propose a simplified variant of our model that focus on the clearance of HBV, HDV and HBsAg and the dose-effect relationship of anti-HBs and HBsAg/HBV/HDV and does neither include liver cell nor immune complex compartments:

$$\begin{aligned}
\frac{dA(t)}{dt} &= \frac{d(t)}{V_d} - k_{e1}A(t) - kA(H(t) + V_1(t) + V_2(t)) \\
\frac{dH(t)}{dt} &= -c_H A(t)H(t) - \delta_H H(t) \\
\frac{dV_1(t)}{dt} &= -c_1 A(t)V_1(t) - \delta_1 V_1(t) \\
\frac{dV_2(t)}{dt} &= -c_2 A(t)V_2(t) - \delta_2 V_2(t)
\end{aligned}$$

Note, that due to different methods of quantification for HBsAg, HBV DNA, and HDV RNA, we consider different binding rates c_H, c_1, and c_2 here.

A. Application of the Simple Model

To analyze the dynamics after liver transplantation and to evaluate our model assumptions, we fitted the simplified model to data on co-infected patients that underwent liver transplantation at Hannover Medical School between 1994-2009. Viral load (HBV and HDV), HBsAg and HBIg (anti-HBs) were measured serially before and after liver transplantation. Since in most cases HBV DNA was negative or below the limit of detection at the time of liver transplantion we only analyzed the kinetics of HDV RNA, HBsAg and anti-HBs.
Note that a previous analysis of this data with a different pharmacokinetics was published in Journal of Hepatology [2].

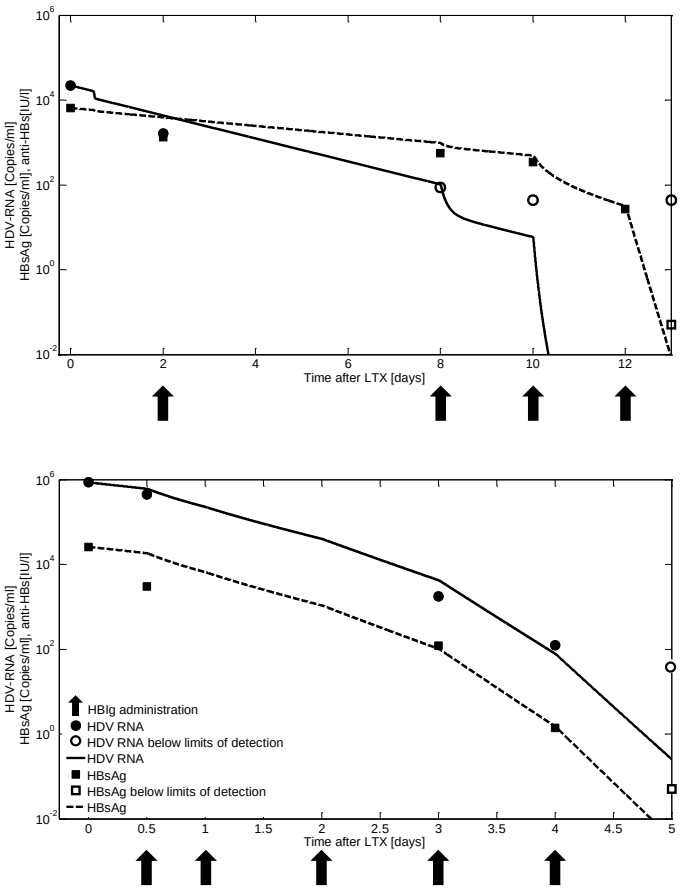

Fig. 2. Fitting results of representative patients.

1) Parameter Fitting: The parameter k_{e1} was fixed to 0.028, δ_2 and δ_H were fixed to 0.69. The parameters c_2, c_H, V_d and k were estimated individually.
The algorithms were implemented in MATLAB (MATLAB 7.10.0, Mathworks Inc, Natick, MA, USA) using a stiff differential equation solver (ode23s, based on a modified Rosenbrock formula of order 2) and nonlinear optimization routines (fminsearch, based on the Nelder-Mead Simplex Method). Hereby a maximum likelihood approach was used for non-linear fitting of the model function; values below the limit of detection were considered as random variables following a normal distribution.

B. Results

We observed a strong correlation between HDV and HBsAg decline, anti-HBs increase and HBIg dose rates. Despite the high interpatient variation we observed an overall similar kinetic pattern with a nearly parallel decline of HDV RNA and HBsAg (Figure 2). The decline of HBsAg and HDV RNA seems to be determined almost exclusively by anti-HBs administration: in cases of intermittent HBIg administration, the decline was delayed. This was also reflected in our modeling approach, as there were no systematic deviations from the model fit.

V. Conclusion

We showed that it is possible to model the dynamics of HBV/HDV-infected patients after liver transplantation with the simplified model without taking reinfection into account. The strong correlation between HDV and HBsAg decline, anti-HBs increase and HBIg dose rates which is also displayed by our model suggest that this approach may help to individualize and optimize HBIg dosing schemes in patients undergoing HBV/HDV- or HBV-indicated liver transplantation. Currently HBIg is mostly given at a fixed daily dose until HBsAg level becomes negative.
The next step is to simulate reinfections after liver transplantation by means of our general model and further variants. For example it might be important to include resistance mutations, because resistance mutations caused by Lamivudine therapy might lead to reduced antigenity of HBsAg and hence resistence to HBIg [8]. By means of these extended models which take reinfection into account, the factors which indicate an upcoming (chronic) reinfection shall be specified by Monte Carlo filtering and the necessary HBIg dose rate to successfully prevent reinfection shall be quantified.

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