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A pharmacokinetic/viral kinetic model to evaluate treatment of chronic hepatitis C virus infection with a non-nucleoside polymerase inhibitor

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Abstract

Viral kinetic models have proven useful in characterizing treatment effectiveness during HCV therapy with interferon (IFN) as well as with direct acting antivirals (DAAs). Here we use a pharmacokinetic/viral kinetic (PK/VK) model to describe HCV RNA kinetics during treatment with sofosbuvir, a non-nucleosidic inhibitor of the HCV NS5B polymerase enzyme. Using PK data from 3 studies in healthy volunteers and PK and VK data from a phase 1 study, where sofosbuvir was administered for 3 days, we fitted a two-compartment PK model with first-order absorption and lag-time, an Emax pharmacodynamics model and a standard biphasic viral kinetic model. Sofosbuvir's EC₅₀ and Hill coefficient and the viral clearance rate were significantly different ($P=0.014$, $P<0.001$ and $P=0.004$, respectively) between patients infected with HCV subtypes 1b and 1a, leading to an increased viral load decline in patients infected with genotype 1b virus. Understanding the combined effects of PK/VK on the performance of a nonnucleoside polymerase inhibitor such as sofosbuvir could provide valuable insights into their use in combination with other DAAs as well as to optimize future therapy. Further, our work suggests that patients infected with subtype 1a would need higher doses than those infected with subtype 1b to achieve the same effectiveness. Whether this is true for other non-nucleoside polymerase inhibitors needs to be examined.

Introduction

Hepatitis C is a chronic liver disease caused by hepatitis C virus (HCV) infection (1). The disease affects nearly 185 million people worldwide (2). The objective of treatment is to obtain a sustained virologic response (SVR), defined as undetectable levels of HCV RNA in blood 24 weeks after cessation of treatment (3,4).

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Setrobuvir (also known as ANA-598 and RG7790) is a non-nucleoside NS5B inhibitor (NNI). It has shown potency and a high degree of specificity against HCV genotype 1 NS5B polymerase, leading to 73% SVR when orally administered to patients in combination with pegylated interferon and ribavirin (5). An interferon-free setrobuvir based regimes of three direct acting antivirals (DAAs) plus ribavirin has also been shown to be safe and effective in genotype 1 treatment naive patients (6). Unlike nucleoside NS5B inhibitors (NIs), which are often prodrugs that need to be activated by the host cell kinases (7), NNIs are active drugs (8), making it possible to model the pharmacokinetics (PK) and pharmacodynamics of the drug with simpler models.

Analysis of HCV viral kinetics (VK) under therapy with a direct-acting antiviral agent can be used to evaluate the DAA's effectiveness. Mathematical models have been developed to study the effect of interferon (IFN) and pegylated IFN plus ribavirin treatment (9,10) well as the effect of several DAAs (11–15), however, no model of viral kinetics in subjects treated with an NNI has been reported. In the current study, we use a combined pharmacokinetic (PK)/viral kinetic (VK) model to analyze data obtained during treatment of 27 patients with setrobuvir monotherapy. Our analysis illustrates that an NNI can be modeled in a manner similar to that of other DAA classes. Our modeling suggests that genotype 1a patients should be harder to treat with setrobuvir than genotype 1b patients and may require a higher drug dose to achieve an equal virological response. Whether such differences apply to other compounds in this class needs to be further explored.

Materials and Methods

Pharmacokinetics of setrobuvir

We used data from four studies, three with healthy volunteers (single dose, A, multiple ascending dose, B and one danoprevir interaction study in healthy volunteers C), used to assess drug pharmacokinetics and one with HCV-infected patients (ascending dose, D) to assess the virological response. Study designs are described in Table 1. See (16) for a detailed description of the studies. In total 77 subjects were included.

Viral kinetics under setrobuvir

HCV RNA levels obtained from study D in which patients were given setrobuvir for 3 days and then sampled at days 1, 1.5, 2, 2.5, 3, 4 and 10 were used for data fitting.

HCV RNA concentrations were determined by the COBAS AmpliPrep/COBAS TaqMan HCV (CAP/CTM HCV) Test at Cenetron Diagnostics, Austin, Texas. HCV RNA levels were above the limit of quantification of CAP/CTM HCV for all samples. The lower limit of quantification is $1.6 \log_{10}$ IU/mL. All measured HCV RNA levels were included in the present analysis.

Mathematical modeling of setrobuvir PK/VK during monotherapy

PK model—To fit setrobuvir plasma concentrations, a one- and a two-compartment PK model using first-order absorption, and either a lag-time or not were tested (Fig. 1A). The models were compared using the Bayesian information criterion (BIC). The model was

parametrized with T_{lag} for the lag-time, k_a for the first-order -absorption rate; k_e for the elimination rate; V_d for the volume of distribution; k_{12} and k_{21} for the transition rates between the central and peripheral compartments, if necessary.

VK model—The kinetics of viral decline under setrobuvir therapy was assumed to follow the standard model of HCV kinetics (10) (Fig. 1B).

$$\begin{aligned}\frac{dI}{dt} &= \beta TV - \delta I \\ \frac{dV}{dt} &= (1 - \varepsilon(t))pI - cV\end{aligned}$$

where T represents target cells, I , infected cells and V , free virus. Target cells, T , are infected by virus, V , with rate constant β , generating infected cells, I . These infected cells, in turn, produce new virus at rate p per infected cell. Infected cells are removed at a rate δ per infected cell and virus is assumed to be cleared at rate c per virion (Fig. 1B). Due to the short treatment duration, we assumed as done previously (10), that the target cell level remained constant at its pre-treatment steady state level of $T_0 = c\delta/\beta p$. Thus the model does not purport to account for the possible proliferation of uninfected or infected cells during this short treatment period.

The effect of setrobuvir, which inhibits viral replication, is modeled in the same way as previously done for the nucleoside polymerase inhibitor mericitabine (11) and the nucleotide polymerase inhibitors danoprevir (17), sofosbuvir and GS-0938 (18), by adding the factor $(1 - \varepsilon(t))$, where $\varepsilon(t)$ is the effectiveness of drug in preventing viral production.

The standard model for HCV viral kinetics (10), was combined with the PK model to allow the effectiveness of setrobuvir, $\varepsilon(t)$, to vary as a function of the setrobuvir concentration.

Setrobuvir pharmacodynamics—We assume that the drug effectiveness, $\varepsilon(t)$, varies as a function of central compartment concentration, C_c , according to the $E_{\max}$ model given below, where the maximum effectiveness is $E_{\max}$. EC_{50} represents the concentration necessary to reach 50% of the maximum effectiveness. The Hill coefficient, h , is a shape parameter determining how steeply the effectiveness varies with drug concentration.

$$\varepsilon(t) = E_{\max} \frac{C_c(t)^h}{EC_{50}^h + C_c(t)^h}$$

Data fitting and statistical methods—For the current analysis we assume that patients did not miss any doses as they were confined to a clinical research unit for the duration of the study. The population parameter estimates and inter-individual variability (IIV) estimates were obtained using a maximum-likelihood method using the stochastic approximation expectation-approximation (SAEM) algorithm (19) implemented in MONOLIX version

2016R1 (<http://www.lixoft.com/>). Further details about mixed effect models and the population approach used here are given in the Supplementary Information.

To fit the data, we tested for each response (i.e. the setrobuvir concentration and the viral load) an additive, a proportional or a combined error model (Supplementary information).

First the PK parameters were estimated from the PK data alone. Then the VK parameters were estimated based on the VK data, using individual PK parameters as regressors. For the PK model, the parameters estimated were $Tlag$, k_a , k_e , V_d , k_{12} and k_{21} . For the VK model, the parameters estimated were E_{max} , EC_{50} , V_d , c and δ , and the Hill coefficient, h . For each parameter, we report the population estimates and their relative standard errors.

Inter-individual variability (IIV) was represented by an exponential model for all parameters. If IIV was below 10% for a parameter, the model was tested without IIV for this parameter and IIV was removed if it improved the BIC. Individual parameters were estimated using the empirical Bayes method (20). In the following we present only the significant results. The VK model was fitted to $\log_{10}$ viral load. Please note that the number of uninfected and infected cells were not measured. The association between HCV genotype and the model parameters was assessed with a Wald test. However, due to the relatively small number of patients per group, this approach can lead to an inflated type I error, we followed the recommendation of Laouénan et al. (21) to compute a corrected p-value, P_c (Supplementary Information). Goodness of fit plots for both the PK and VK data are presented in Fig. S1. A visual predictive check is given Fig. S2 and results on the corrected p-values are given in Fig. S3.

Results

HCV-infected patients

We analyzed data from a phase 1 multiple ascending dose study of setrobuvir in chronically HCV-infected patients, randomized to receive oral setrobuvir or placebo for a period of 3 days. All patients were treatment naïve. Treated patients were divided into 3 arms (Table 2). In arm 1, 11 patients received 200 mg bid. In arms 2 and 3, 8 patients received 400 mg bid and 800 mg bid, respectively. In a fourth arm, 8 patients received placebo treatment and were not included in the analysis.

Among the 27 treated patients, 12 (44%) were infected with genotype 1a and 15 (56%) with genotype 1b virus (Table 2). The HCV genotype distribution was not significantly different between the cohorts ($P=0.56$, Chi-square test). The dose received was not significantly associated with the initial viral load ($P=0.35$, Spearman's rank correlation coefficient: 0.18) (Table 2).

Setrobuvir pharmacokinetics

According to BIC (Supplementary Table S1) (22), a two-compartment model with a lag-time and a combined error model provided the best fit to the PK data.

As observed from the PK data, the plasma concentrations of setrobuvir were predicted to accumulate between doses (Fig. 2A). As summarized in Table 3 for HCV-infected individuals, after a lag-time of 1.68 hr, setrobuvir was absorbed at rate $k_a=0.26 \text{ hr}^{-1}$. The estimated volume of distribution V_d of setrobuvir was 5.11 L and the estimated drug elimination rate k_e was 0.063 hr^{-1} . The transition rates between the central and peripheral compartments were estimated as 0.12 hr^{-1} and 0.071 hr^{-1} for k_{12} and k_{21} , respectively. The PK parameter estimates for individual patients is given in Supplemental Table S2.

Setrobuvir's half-life, computed as $t_{1/2} = \ln(2)/k_e$, was estimated as 11.4 hr for HCV-infected patients and 10.2 hr for healthy volunteers. It is commonly accepted that steady-state is reached after 3 to 5 half-lives (23), leading in the case of setrobuvir to a prediction of steady-state being reached after 1.4 to 2.4 days in HCV-infected patients.

HCV viral kinetics

The observed viral load decline induced by 3-day therapy of setrobuvir was biphasic with median declines of $2.08 \pm 0.87 \log_{10}(\text{IU/mL})$, $2.46 \pm 0.62 \log_{10}(\text{IU/mL})$ and $2.88 \pm 0.49 \log_{10}(\text{IU/mL})$ in the 200 mg bid, 400 mg bid and 800 mg bid dosing cohorts, respectively (Table 2).

The estimated parameters for the VK model are presented in Table 2. Fits of the models to the individual patient viral load data are given in Fig. 2B and the parameter estimates for individual patients are given in Supplemental Table S3. An additive error model and a model with IIV on all parameters except E_{max} was found to best describe the data. The residual error for the VK data is $a=0.20 \log_{10} \text{ IU/mL}$. The estimated initial viral load is $6.35 \log_{10}(\text{IU/mL})$, the maximal treatment effectiveness E_{max} is 0.997 and the estimated loss rate of infected cells, δ is 0.093 d^{-1} . δ was not estimated as accurately as the other parameters (r.s.e.=52%) and showed a larger inter-individual variability (167%) than the other parameters. This results from the short therapy and sparse samples collected during the second decline phase. For patients infected with genotype 1a virus, the virus clearance rate, c (3.86 d^{-1} vs. 6.98 d^{-1}), and the Hill coefficient, h (3.46 vs. 15.6), were significantly lower ($P_c=0.004$ and <0.001 respectively) and the EC_{50} (17.8 ng/mL vs. 8.45 ng/mL) was significantly higher ($P_c=0.014$) than in patients infected with genotype 1b (Fig. S3).

Many DAA trials have been conducted using 2-week treatments (11,17,24). To assess how setrobuvir might perform over a similar period, we simulated setrobuvir average PK/VK for a treatment duration of 14 days, with the population parameters. We predicted that the viral load decline at the end of therapy depends on the HCV-genotype and regimen (Fig. 3. A–C, Table 4) and varies at day 14 between $1.83 \log_{10} \text{ IU/mL}$ ($\epsilon=0.960$) for patients infected with genotype 1a virus receiving 200 mg bid of setrobuvir and $3.11 \log_{10} \text{ IU/mL}$ ($\epsilon=0.997$) for patients infected with genotype 1b virus and receiving 200 to 800 mg. This reflects that for a concentration $<100 \text{ ng/mL}$ the setrobuvir effectiveness is lower for patients infected with genotype 1a HCV than with genotype 1b HCV (Fig. 3D).

Discussion

In the past, mathematical modeling has provided significant insights into the viral kinetics observed during PEG-IFN/RBV based therapy for HCV (9,10,26). More recently, viral kinetic models have been used for characterizing treatment with direct acting antivirals (DAAs) including the protease inhibitors telaprevir (13,27), and danoprevir (17,28), the NS5A inhibitor daclatasvir (29), the nucleoside polymerase inhibitor mericitabine (11) and the nucleotide polymerase inhibitor sofosbuvir (18) or a treatment combinations of mericitabine and danoprevir (24) or sofosbuvir with daclatasvir, simeprevir or ledipasvir (30). Combined PK/VK models have been developed for the combination of PEG-IFN and RBV (31–33), telaprevir with PEG-IFN and RBV (27) and for monotherapy with the NS3/4A protease inhibitor danoprevir (17). The viral kinetics observed with a non-nucleoside polymerase inhibitor, such as setrobuvir, have never been modeled. Even though further clinical development of setrobuvir has been stopped, the accumulated data on setrobuvir provide a means of assessing how well viral kinetic models can characterize the effects of therapy with a NNI.

This work describes the first PK/VK model to estimate treatment parameters for monotherapy with an NNI, setrobuvir. Patient PK and VK data was fitted using a two-compartment with zero-order absorption and lag time PK model and a standard VK model, respectively.

Importantly, the model estimated a significantly ($P_c=0.014$) lower EC_{50} and therefore a higher effectiveness, for patients infected with genotype 1b virus (8.45 ng/mL) than for those infected with genotype 1a virus (17.8 ng/mL). The Hill coefficient, h , which determines how steeply the effectiveness varies with setrobuvir concentration is significantly higher ($P_c<0.001$) in patients infected with genotype 1b virus (15.6) than in patients infected with genotype 1a virus (3.46). This is consistent with replicon assay data (34). The difference in activity has been previously associated with the variation in position 415 in the genetic sequence of the NS5B protein, encoding either for a tyrosine or a phenylalanine (35). Interestingly, the estimated viral clearance rate is also higher ($P_c=0.004$) in patients infected with genotype 1b virus (6.98 d^{-1}) than in patients infected with genotype 1a virus (3.86 d^{-1}).

Similar to other DAAs, patients treated with setrobuvir monotherapy exhibited a biphasic viral load decline. After 14 days of treatment, the predicted effectiveness in patients infected with genotype 1a HCV and treated with 200 mg bid of setrobuvir ($\epsilon=0.960$) was in a similar range as the predicted effectiveness for 100 mg bid of the nucleotide polymerase inhibitor danoprevir ($\epsilon=0.976$) (17). For patients infected with genotype 1b, setrobuvir monotherapy effectiveness ($\epsilon=0.997$) at day 14 was higher than that predicted for danoprevir monotherapy ($\epsilon=0.992$ for 200 mg tid and 300 mg bid) and similar to that predicted for 1500 mg bid of the nucleotide polymerase inhibitor mericitabine ($\epsilon=0.997$) (11), 200 mg tid of the protease inhibitor daclatasvir ($\epsilon=0.996$) (28) and 100 mg tid danoprevir + 500 mg bid mericitabine ($\epsilon=0.998$)(23) (23). However these results must be interpreted with caution as the infected cell loss rate, δ , which characterizes the second phase slope was estimated with a limited number of samples. Trials with longer setrobuvir therapy would be necessary to validate this predictions.

The short duration of this therapy (i.e. 3 days) necessarily lead to a short second phase of decline and both the lack of very frequent blood samples and the longer time needed for patients infected with genotype 1a virus to reach high effectiveness led to transitions from first to second phase decline that were difficult to characterize (Fig. 2B), which is the reason why the estimate of δ is not as accurate (relative standard error: 40%) and the inter-individual variability is larger (IIV: 163% than for other parameters (Table 3)). Longer studies would be necessary to precisely estimate δ .

We showed that sotravir, a non-nucleoside polymerase inhibitor, can be characterized using the same type of PK/VK model that has been used previously to characterize other DAA drug classes. Further, we showed that sotravir can demonstrate high antiviral effectiveness and that its effectiveness depends on the virus subtype, with the drug being more effective against genotype 1b virus than against genotype 1a virus. Whether such differences apply to other NNIs needs to further explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Combined PK/VK model used to describe viral kinetics during setrobuvir monotherapy

(A) Schematic of two-compartment PK model following zero-order absorption of setrobuvir. The model was parametrized with T_{lag} for the lag-time, k_a for the first-order -absorption rate; k_e for the elimination rate; V_d for the volume of distribution; k_{12} and k_{21} for the transition rates between the central (Q_1) and peripheral (Q_2) compartments (B) Target cells T are infected by virus, V , with rate constant β to produce infected cells I . Infected cells, I , are lost with rate constant δ and virus, V , is cleared from the circulation with rate constant c .

Figure 2. Pharmacokinetics (A) and viral kinetics (B) best fit curves of individual patients (Eqs. 1–4) under setrabenavir monotherapy. Each box represents a patient. **For the PK**, best fit curves (solid lines) of the model and kinetic data (black dots) during 200 mg bid (blue), 400 mg bid (orange) and 800 mg bid (pink) of setrabenavir. **For the VK**, best fit curves (solid lines) of the model and kinetic data (black dots) in patients infected by genotype-1a virus (red) and -1b virus (blue).

Figure 3. Comparison of viral kinetics and pharmacokinetics population predictions of the PK/VK model between cohorts during 2 weeks of treatment with senvobuvir monotherapy

(A) Model predicted time-varying plasma concentration of senvobuvir for 200 mg bid (green line), 400 mg bid (orange line) and 800 mg bid (green line). The black line and the grey line represent EC_{90} i.e. the concentration leading to 90% of the maximal effectiveness in patients infected with genotype-1b and -1a virus, respectively. **(B)** Predicted senvobuvir $\log_{10}$ transformed effectiveness in patients infected with subtype 1a (bold colors) and 1b (light colors) HCV under 200 mg bid (green lines), 400 mg bid (orange lines) and 800 mg bid (pink lines). **(C)** Predicted HCV viral load decay in patients infected with subtype 1a (bold color) and 1b (light color) HCV under 200 mg bid (green lines), 400 mg bid (orange lines) and 800 mg bid (pink lines). **(D)** Predicted effectiveness depending on senvobuvir (STV) concentration for HCV genotype 1a infection (grey line) and HCV genotype 1b infection (black line).

Table 1

Pharmacokinetic studies designs

Study	Design	Participants	Doses	Design	Blood sampling times
A	Single dose	Healthy volunteers N=12	800 mg, 2000 mg	Single dose at D0	0 (before dose), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 12.5, 13, 14, 15, 16, 17, 18, 20, 22, 24, 30, 36, 48, 96, 144 hr
B	Multiple ascending dose	Healthy volunteers N=23	400 mg, 600 mg 800 mg	Single dose at D0, From D3 10 QD	0 (before dose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72 hr
C	Danoprevir interaction	Healthy volunteers N=15	200 mg	BID during 6 days	0 (before dose), 24, 48, 144, 192, 216, 216.5, 217, 217.5, 218, 218.5, 219, 219.5, 220, 221, 222, 224, 226, 228 hr
D	Ascending dose	HCV infected patients N=27	200 mg, 400 mg, 800 mg	BID during 3 days	0 (before dose), ~12 hr after dose on days 1, 2, 3

Baseline characteristics of the patients and observed total viral decline after 3 days of setrobuvir treatment

Table 2

Arm	Dose (regimen)	Genotype (n)		Initial viral load Log ₁₀ IU/mL Median (range)	Total viral decline Log ₁₀ IU/mL Median (range)
		1a	1b		
1	200mg (bid)	5	6	6.46 (5.24–7.32)	2.37 (0.49–3.32)
2	400mg (bid)	3	5	5.71 (4.28–6.75)	2.48 (1.73–2.85)
3	800mg (bid)	4	4	6.89 (5.87–7.34)	2.99 (2.17–3.39)

Table 3

Population parameter estimates obtained using the PK/VK model

For the PK, the additive error is $a_1=5.38$ ng/mL (RSE=5%) and the proportional error $b=0.15$ (RSE=4%). For the VK, additive error is $a_2=0.20$ log₁₀ UI/mL (RSE=7%).

Parameter (unit)	$Tlag$ (hr)	k_a (hr ⁻¹)	V_c (L)	ke (/hr)	$k12$ (/hr)	$k21$ (/hr)	Emax	EC ₅₀ (µg/mL)		h		V_o (log ₁₀ IU/mL)	c (d ⁻¹)		δ (d ⁻¹)
								1a	1b	Ia	Ib		Ia	Ib	
Estimates (R.S.E. %)	1.68 (8)	0.26 (19)	5.11 (17)	0.063 (17)	0.12 (28)	0.07 (11)	0.997 (~0)	17.8 (19)	8.45 (16)	3.46 (12)	15.6 (19)	6.35 (2)	3.86 (12)	6.98 (6)	0.0933 (52)
IIV % (R.S.E. %)	52 (12)	42 (16)	21 (18)	21 (21)	60 (14)	42 (25)	-	45 (19)		10 (158)		9 (14)	14 (42)		167 (24)

Table 4

Maximal viral load decline depends on virus genotype and dosing regimen.

Predicted viral load decline ($\log_{10}$ IU/mL) after 14 days of treatment depends on virus genotype and setrobuvir dosing regimen.

	Virus genotype	
	1a	1b
200 mg bid	2.18	3.21
400 mg bid	3.02	3.22
800 mg bid	3.16	3.22