Modeling Hepatitis C Virus Kinetics under Therapy using Pharmacokinetic and Pharmacodynamic Information

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Abstract

Background—Mathematical models have proven helpful in analyzing the virological response to antiviral therapy in hepatitis C virus (HCV) infected subjects.

Objective—To summarize the uses and limitations of different models for analyzing HCV kinetic data under pegylated interferon therapy.

Methods—We formulate mathematical models and fit them by nonlinear least square regression to patient data in order to estimate model parameters. We compare the goodness of fit and parameter values estimated by different models statistically.

Results/Conclusion—The best model for parameter estimation depends on the availability and the quality of data as well as the therapy used. We also discuss the mathematical models that will be needed to analyze HCV kinetic data from clinical trials with new antiviral drugs.

Keywords

Direct antivirals; interferon; hepatitis C virus; mathematical modeling; pegylated interferon; NS5B polymerase inhibitor; NS3/4A protease inhibitor

1. Introduction

1.1 Hepatitis C virus infection

Hepatitis C virus (HCV) is a positive sense RNA virus in the *Flaviviridae* family. It infects and replicates in hepatocytes (liver cells), but extrahepatic infection in peripheral blood mononuclear cells may also occur [1-3]. HCV is genetically variable and genotypes 1 to 7 have been assigned [4]. Globally, approximately 170 million people are chronically infected with HCV and 3 to 4 million persons are newly infected each year [5]. Genotype 4 is the most prevalent in Africa while genotype 1 is the most prevalent in the other continents [6]. HCV is a major risk factor for developing cirrhosis and hepatocellular carcinoma (HCC, liver cancer), which are the major causes of liver transplantation. In the United States, 16,000 individuals are waiting for livers to become available for transplantation [7]. Reducing the prevalence of HCV infection and progression to HCC are important health care issues.

1.2 Therapy for chronic HCV infection

Due to the lack of an HCV vaccine, antiviral therapy is currently the only available treatment. However, antiviral therapy is not effective in all patients. Sustained virological response

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(SVR), which is defined as virus being undetectable at the end of therapy and 6 month later, is achieved only in 50% of treated patients when pegylated interferon α-2a or 2b (PEG-IFN) [8, 9] plus ribavirin, the current standard of care, is used.

Recombinant forms of naturally occurring interferon (IFN), IFN α-2a [10] and IFN α-2b [11], were commonly used to treat chronic HCV infected patients in the 1990s. Due to the fact that IFN is a protein that would be digested if taken orally, IFN is administered by injection. Both forms of IFN have a short in vivo half-life (5.1 hours for IFN α-2a, and 2-3 hours for IFN α-2b), and thus the recommended dosing interval for IFN was three times a week. In order to increase the dosing interval peginterferon α-2a (PEG-IFNα-2a) [9] and peginterferon α-2b (PEG-IFN α-2b) [8] were introduced and approved by the FDA. Both PEG-IFN α-2a and PEG-IFN α-2b are recombinant IFN-α with a polyethylene glycol (PEG) chain covalently attached [12-14]. The two forms of PEG-IFN not only differ in the type of IFN used but also in the size of the PEG molecule attached to IFN. The molecular weight of the PEG molecule is 40 kD in PEG-IFN α-2a and 12 kD in PEG-IFN α-2b. The large PEG molecule reduces IFN's rate of elimination from the body [14] (Table 1, Fig. 1) [15]. Therefore, PEG-IFN is administered only once weekly, compared with the three times a week administration for standard IFN, which increases compliance and is easier for patients to tolerate. In addition, clinical trials have shown that there is an improvement in the SVR rate when PEG-IFN instead of standard IFN is used in combination with daily ribavirin for 12 months (~45 % vs. ~55 %) [16-18].

To improve treatment outcome, there are many HCV drugs under development: both new IFN-based drugs [19, 20] and direct antivirals that inhibit HCV enzymes such as the HCV protease and HCV polymerase [21-26]. These new drugs alone or in combination with PEG-IFN are expected to be the standard care for the treatment of chronic HCV infected patients in the next decade [21, 23].

2. Mathematical modeling: Basic to state-of-the-art

2.1 Why do we need mathematical modeling?

Mathematical modeling of viral infections under therapy has been successful in unraveling the dynamic aspect of chronic infection by providing estimates of key parameters. These estimates were obtained by analyzing the response of individual patients to antiviral therapy and then using nonlinear least squares regression to fit mathematical models to patient data. For example, using this approach to analyze human immunodeficiency virus (HIV) infection [27, 28], it was discovered that the dynamics of HIV infection were not slow even though HIV takes ~10 years, on average, from infection to cause full blown AIDS. Analysis of patient response to therapy showed that virus was both being produced and cleared at extremely rapid rates with close to the entire viral population being renewed every day of infection [29].

Analyses of HCV infection and treatment also showed that the viral half-life is short (~2-3 h) and the half-life of infected hepatocytes ranged from 2 to 70 days [30]. Moreover, by modeling HCV RNA declines under treatment, it was proposed that the primary mode of action of interferon involved blocking viral production from infected cells rather than preventing cells from being infected [30]. This approach was also instrumental in providing new insights into the mode of action of ribavirin [31] as well as shedding light on the differences in response of diverse patient populations[32].

Another important clinical benefit of viral kinetics was the prediction of long term therapy outcome based on early decreases in viral load. For example, SVR is much more likely to be achieved in patients that have a rapid virological response (absence of detectable virus at 4 weeks after the initiation of therapy) or an early virological response (absence of detectable virus at week 12 or >2 $\log_{10}$ decline from baseline) than in patients with slower responses

[33-38]. Researchers also have found a correlation between achieving SVR and HCV RNA decline for shorter times, such as 1 day [39, 40], 1 week [41] or 2 weeks[18, 42] after the initiation of therapy. As shown below, some viral kinetic parameters and pharmacokinetic/pharmacodynamic (PK/PD) parameters have been shown to be associated with SVR achievement.

2.2 Basic mathematical model of HCV dynamics under therapy

Under high-dose (5, 10, 15 million international unit) daily administration of IFN α-2b, the serum level of HCV RNA typically declines in a biphasic manner with a rapid first phase followed by a slower second phase [30]. To describe the biphasic kinetics of decline, a basic mathematical model was designed (Fig. 2A). In this model, cells susceptible to infection, i.e., target cells, T, are infected by HCV, V, with rate constant β, resulting in infected cells, I. which produce new virions. The equations describing this system are [30, 41, 43-47].

$$
\begin{aligned}
dT/dt &= s - \beta V\, T - dT \\
dI/dt &= \beta VT - \delta I \\
dV/dt &= p\,(1 - \varepsilon(t))\,I - cV.
\end{aligned}
\tag{1}
$$

where T and I are the number of target cells and infected cells, respectively, and V is the viral load.. The target cells are assumed to be generated at a constant rate s, and die at rate d per cell. Infected cells are lost at rate δ per cell, and free virions are produced at rate p per infected cell and are assumed to be cleared by a first order process with rate constant c. In principle, treatment with IFN α-2b could block de novo infection (reducing β) or interfere with virion production reducing p. Analyses of these possibilities showed that the primary mode of IFN action was to partially block virion production [30]. This mode of action is incorporated into the model given by Eq. (1) by introducing the parameter $\varepsilon(t)$, the effectiveness of IFN. The drug effectiveness, $\varepsilon(t)$, is bounded between 0 and 1, where virion production is not suppressed if $\varepsilon(t)=0$ or is fully suppressed if $\varepsilon(t)=1$. To fit the kinetics of viral load decline under high dose daily IFN treatment, it was assumed that (*i*) the number of target cells, the number of infected cells and the HCV concentration in serum are in steady state before the initiation of therapy, (*ii*) the number of target cells remains constant for a short period after the initiation of therapy, and (*iii*) the drug effectiveness is initially zero but after some delay, t_0, to account for both the pharmacokinetics of the drug and its need to bind cellular IFN receptors and stimulate expression of interferon regulated genes, the effectiveness increases to a constant level that is sustained until the end of therapy (Fig. 2B). That is, the drug effectiveness is essentially constant during treatment, $\varepsilon(t)=\varepsilon$ once it becomes active at the site of infection (Fig. 2B). Because of this assumption we call this model a constant effectiveness (CE) model. With these assumptions, the CE model above can be solved explicitly and the solution is

$$
V(t) =
\begin{cases}
V_0 & (t \leq t_0) \\
V_0 \{A \exp(-\lambda_1(t - t_0)) + (1 - A)\exp(-\lambda_2(t - t_0))\} & (t > t_0)
\end{cases}
\tag{2}
$$

where $\lambda_{1,2} = (1/2)\left[c + \delta \pm \sqrt{(c-\delta)^2 + 4(1-\varepsilon)c\delta}\right]$ and $A = (\varepsilon c - \lambda_2)/(\lambda_1 - \lambda_2)$.

The slope of the first phase of HCV kinetic decline is dependent on the virion clearance rate, c; while the absolute level of decline is related to the effectiveness ε, such that $\varepsilon=0.9$ corresponds to ~1 $\log_{10}$ decline, $\varepsilon=0.99$ to ~2 $\log_{10}$ decline, etc. The second phase slope is approximated by the maximal drug effectiveness, ε, times the infected cell loss rate, δ [30] (Fig. 3A). In patients who have larger $\varepsilon\delta$, the virus become undetectable by the end of treatment.

Thus, we can expect that the patients who have larger $\varepsilon\delta$ may achieve an end of treatment response (ETR), defined as virus being undetectable at the end of therapy, and perhaps also an SVR.

The solution of the CE model, Eq. (2), has four parameters: t_0, ε, c and δ, as well as V_0, the initial viral load, which can be directly measured. Thus this equation, when it is fit to HCV kinetic data, allows one to estimate the viral kinetic and drug effectiveness parameters. This model has been successfully used to analyze a variety of HCV kinetic data [30, 43-45], and help make predictions or understand the response in different patient groups.

Despite its simplicity, the CE model may not always be the appropriate model to use to estimate HCV kinetic parameters or drug effectiveness. We have shown that this is the case under PEG-IFN α-2b therapy [42, 46-48]. The concentration of PEG-IFN α-2b gradually increases and decreases over the week when it is administered once weekly (Fig. 1D, and Table 1). Thus, it is likely that the effectiveness of PEG-IFN α-2b also changes substantially over the week, violating the assumption of constant effectiveness. In fact, HCV kinetic data obtained during the first two weeks of PEG-IFN α-2b therapy shows that the HCV viral load oscillates [42, 49, 50], i.e., the viral load initially decreases but then partially rebounds before the next weekly drug dose. This fluctuation of HCV RNA in serum is synchronized with a fluctuation in the PEG-IFN α-2b concentration in serum [49, 50]. These types of significant viral load fluctuations (>1 $\log_{10}$) are not observed under daily IFN α-2a [51] or IFN α-2b therapy [30]. Importantly, the CE model under assumptions (*i*)-(*iii*) given above cannot describe a viral load rebound – its solution, Eq. (2), predicts that under therapy the viral load, *V(t)*, will monotonically decrease from its initial value either reaching zero or a new constant value. If we fit the CE model to HCV kinetic data that contains a rebound and if blood is sampled frequently, the best-fit curve converges to a flat 2nd phase response in which the infected cell loss rate, δ, is estimated as zero[46]. We have analyzed this issue exhaustively in computer simulation studies [47][46] and have shown that the parameter estimates obtained with the CE model under PEG IFNα–2b therapy are not reliable.

2.3 Pharmacokinetics of IFN and PEG-IFN

PEG IFN is given subcutaneously and is absorbed into the blood from the injection site. Assuming first order processes for absorption and elimination, the time evolution of the drug concentration in plasma is determined by the following equations [42, 48, 52, 53][31].

$$\frac{dM_a}{dt} = -k_a M_a \qquad (3)$$

$$\frac{dM}{dt} = k_a M_a - k_e M \qquad (4)$$

Here, M_a is the mass of the drug at the absorption site, M is the mass of drug in blood, and k_a and k_e are first order rate constants of absorption and elimination, respectively. Solving these equations with the initial conditions $M_a(0) = FD$ and $M(0) = 0$, where D is the mass of the drug administered in a dose, with only a fraction, F, of the drug being bioavailable ($0 \le F \le 1$), gives the time evolution of the concentration of the drug in blood, $C(t)$, following a single dose at *t=0*:

$$C(t) = \frac{FD}{V_d} \frac{k_a}{k_e - k_a} \left(\exp(-k_a t) - \exp(-k_e t) \right)$$

(5)

where we have assumed that PEG-IFN is distributed through a volume V_d. Equation (5) predicts that the maximum concentration, C_{max}, is attained at $t=t_{max}$ where t_{max} is given by

$$t_{max} = \frac{\log_e (k_a/k_e)}{k_a - k_e}.$$

(6)

Both the absorption and elimination of PEG-IFN are slower than those of IFN, and the t_{max} of PEG-IFN is much larger than that of IFN (Table 1). Thus, the PEG-IFN concentration in serum changes gradually over the week when administered once weekly (Fig. 1). The drug elimination half-life and the time to peak drug concentration in serum are available [8-11]. From this information, one can calculate the pharmacokinetic (PK) parameters (Table 1) that determine $C(t)$.

2.4 Analyzing HCV kinetic data as well as PK data

Talal et al. [42] measured the HCV RNA levels as well as PEG-IFN α-2b concentration in serum frequently for the first two weeks of therapy of 24 patients coinfected with HCV and HIV. Patients received once weekly PEG-IFN α-2b therapy plus daily ribavirin for 48 weeks. We analyzed the HCV viral load data and pharmacokinetic (PK) data consisting of the drug concentration in serum using pharmacokinetic/pharmacodynamic (PK/PD) modeling. The PK/PD model assumed that the drug concentration in serum, $C(t)$, is described by Eq. (5). For the pharmacodynamic model, we set ε(t) to [52, 54]

$$\varepsilon(t) = \frac{C(t-\tau)^n}{EC_{50}{}^n + C(t-\tau)^n}.$$

(7)

Here τ is a delay between the time drug levels in serum are sensed by IFN receptors and the time it has an effect on reducing viral production, EC_{50} is the drug concentration at which PEG-IFN α-2b suppresses virion production by 50%, and the parameter n is the Hill coefficient ($n \geq 1$), which controls how abruptly the effectiveness increases with higher drug concentrations. The drug effectiveness, ε(t) gradually increases and then decreases during the first week of therapy, as $C(t)$ does the same for each patient individually (Fig. 2C).

Talal et al. [42] fitted Eq. (5) to each patient's PK data to estimate k_a, k_e and FD/V_d. Then with these parameters fixed at their best-fit values, they fitted each patient's HCV RNA kinetic data to estimate c, δ, V_0, τ, EC_{50} and n. This model generated good fits to the viral load data, and demonstrated that taking the PK/PD aspects into consideration was crucial both for explaining the viral load rebound towards the end of the dosing interval and for obtaining sensible estimates for the viral kinetic parameters. Talal et al. [42] found that EC_{50} was significantly lower in sustained virological responders (patients who achieved SVR) than in non-responders (0.04 vs. 0.15 μg/L, p=.014). Additionally, the median therapeutic quotient (the ratio between the weekly average PEG-IFN concentration and EC_{50}), and the PEG-IFN α-2b concentration at day 7 divided by EC_{50} were significantly higher in patients who achieved SVR than in patients who didn't achieve SVR.

This PK/PD model can be extended to two weeks of therapy as shown in [42], or longer if frequent HCV RNA and PEG-IFN concentration data can be obtained over longer periods. To analyze

PK data over multiple doses (e.g. two weeks of therapy), suitable modifications of the solution for $C(t)$ were determined [55]. One interesting aspect is that in some patients the values for drug absorption and elimination varied considerably from the first week to the second, indicating an effect of IFN exposure or other factors on its pharmacokinetics [42].

2.5 Simplified models of PK/PD for analyses of HCV viral load data

In many clinical studies, drug concentrations in serum are not measured. Thus, the modeling approach based on detailed PK/PD profiles is not useful. Shudo et al. [41] developed an alternative mathematical model where the drug's effectiveness changes with time in a manner similar to that observed for PEG-IFN α-2b but which is analyzable in the absence of PK data. This model was called the decreasing effectiveness (DE) model. In the DE model, the viral kinetics for the first week of PEG-IFN α-2b therapy is governed by Eq. (1) but with $\varepsilon(t)$ given by

$$\varepsilon(t) = \begin{cases} 0 & (0 \le t < t_0) \\ \varepsilon & (t_0 \le t < t_m) \\ \varepsilon \exp(-k(t - t_m)) & (t_m \le t \le 7) \end{cases}$$

(8)

where $t_m = t_0 + t_1$ (Fig. 2D). In this model, after some delay, t_0, the drug effectiveness increases instantaneously to a high level, ε, which is sustained for t_1 days and then declines exponentially. This model ignores the PK details of PEG-IFN α-2b, and describes an approximation to the PD of the drug given by Eq. (8) (Fig. 2D). Thus, the DE model has fewer parameters than the full PK/PD model. With this simplification, we can estimate parameters without knowledge of drug concentration data. Shudo et al. [41] applied this model to 19 of the patient's analyzed in Talal et al. [42]. The fits were done for fixed delays t_0 of 4 hours, 8 hours, and 12 hours, and the best delay was chosen based on the sum of squared residuals. The average drug effectiveness multiplied by the infected cell loss rate, which is the relevant parameter affecting the steepness of the second phase slope of viral load decline, was significantly larger in sustained virological responders than in non-responders (p=.03) [42].

2.6 Similarity and difference between PK/PD model and DE model

Both the PK/PD model and the DE model can describe the viral load rebound caused by fluctuations in drug effectiveness. However, the patterns of viral dynamics described in the two models are slightly different. For some patients (*i.e.* choices of parameters), the viral load rebound as described by the DE model occurred more abruptly than that described by the PK/PD model (Fig. 3B). The infected cell loss rate estimated by the DE model also tended to be higher than the one estimated by the PK/PD model (in 13/19 patients). By contrast, the average drug effectiveness over the first week of therapy estimated by the DE model and the PK/PD model were similar to each other. This suggests that even if PK data is unavailable, one can obtain an estimate of the average drug effectiveness that is close to the one estimated by HCV kinetic data plus PK data. Larger studies will be needed to establish this more definitively.

3. Selection of a model to analyze HCV RNA decline kinetics under therapy

3.1 HCV kinetic models under therapy

In addition to PEG-IFN α-2b treatment, there are other circumstances in which the CE model seems inappropriate. In clinical studies, the drug dose may be changed during therapy. For example, Bekkering et al.[56] modeled a case in which 15 patients received 10 MU of IFN once daily for the first 3 days of therapy, followed by 3 MU IFN daily for 52 weeks. On average, viral load rebounded by 1.5 $\log_{10}$ HCV RNA copies/ml between days 3 and 4. Bekkering et al. [56] modified the CE model, and described the viral load rebound that was observed right

after the dose change. Moreover, recently, a variety of other viral kinetic models have been developed, some including additional biological details, such as the proliferation of uninfected and infected cells [57, 58], possible enhancement of the infected cell loss rate during therapy due to an immune response [59], and extrahepatic infection [60]. These detailed models can sometimes describe more complex viral load declines, such as a triphasic decline (i.e., a decline consisting of three phases, with a viral load plateau between two decays) [57-59]. However, these models also introduce additional parameters, which in general, cannot be uniquely estimated from viral load data alone. Even if we find the "best parameter set" by nonlinear least square regression using the Levenberg–Marquardt algorithm [61], which is one of the most commonly used algorithms, the result we get might critically depend on the initial parameter guesses from which we started the algorithm, i.e., the algorithm may find a local optima not the global one. To avoid this problem, one can perform a global search, e.g. a grid search, in which the residuals are evaluated at each point on a grid spanning a biologically realistic range for each parameter. However, this method is computationally intensive. If one wants to evaluate the residuals at 100 values of each parameter using a mathematical model that has 4 parameters, 10^8 determinations are required. In addition, if the data is limited, a complex model with a larger number of parameters, such as the model of Dahari et al [58, 62] that incorporates liver-cell proliferation, which in full generality has 10 parameters, will never lead to reliable estimates for all of the parameters.

3.2 Usefulness of the CE model

Before the DE model was described, the CE model was fit to HCV kinetic data obtained under PEG-IFN α-2a therapy because PK data was unavailable [43-45] and in general PEG-IFN α-2a concentrations do not decline as rapidly between dosing interval as do PEG-IFN α-2b concentrations (Table 1). We recently fit both the CE model and the DE model to HCV kinetic data obtained during the first week of therapy of HIV/HCV coinfected patients who received PEG-IFN α-2a therapy from the study in [44]. We compared the suitability of the two models using an F-test since the models are nested. We found the use of the CE model rather than the DE model was preferred for the analysis of this data (Shudo, unpublished). Because PEG-IFN α-2a is eliminated more slowly than PEG-IFNα-2b (Table 1 and Fig. 1C-D), the assumption of decreasing drug effectiveness in the DE model (Fig. 2C) adds additional parameters that are not justified statistically.

HCV kinetics under PEG-IFN α-2a therapy looks biphasic more often than under PEG-IFN α-2b therapy. We found that when using PEG-IFN α-2a, only 2 of 5 patients in one study [44] and 3 of 15 patients in another study (unpublished data) showed a rebound of HCV viral load during the first week of therapy. By contrast, in a study using PEG-IFN α-2b, a viral load rebound was observed in 13 of 19 patients [42].

As another example of the usefulness of the CE model, we fit a model that incorporates hepatocyte proliferation[57, 58] as well as the CE model [30] to hepatitis B virus kinetic data that looks like it has a triphasic decline [57, 58]. Still, an F-test supported the use of the CE model, which only generates a biphasic decline, rather than the extended model with liver proliferation [63], which can generate a triphasic decline.

4. Expert Opinion

We have reviewed a number of different models that describe the kinetics of HCV RNA decline under therapy: the CE model [30], a PK/PD model [42, 48] and the DE model [41]. Deciding which model to use to analyze HCV RNA kinetic data is not a simple matter. First, if the drug being used is PEG-IFN α-2b given once weekly, drug concentration and presumably the drug's effectiveness, ε, will vary between doses. In such cases, the best approach is to measure the drug concentration at each blood draw when HCV RNA is measured. If blood is drawn

frequently, as in the study by Talal et al. [42], then a PK/PD model can be used to fit the data and estimate viral dynamic, pharmacokinetic and pharmacodynamic parameters. If drug concentration data is not available, then either the CE or DE model can be used. The solution to the CE model, given by, Eq. (2), will describe a biphasic HCV RNA decline but it is not able to describe a dataset in which HCV RNA rebounds. Thus, the data would need to be examined and if rebounds were prominent (e.g. $>1 \log_{10}$), Eq. (2) would not be an appropriate model. For such cases, if the viral load rebound is thought to be due to a decrease in drug concentration, as has been seen for PEG-IFN α-2b, then the DE model might be more suitable. The DE model has more parameters than the CE model and thus requires more HCV RNA measurements. Equation (1) with $\varepsilon(t)=\varepsilon$ in which target cells are allowed to vary, can also describe a viral load rebound, but in this case the rebound is due to an increase in target cells in the presence of insufficient drug effectiveness [57, 58]. Thus, if the rebound is taking many weeks and is not occurring towards the end of each weekly dosing interval, this model may be appropriate, but numerical solution of the differential equations (1) will be required rather than the use of Eq. (2).

One practical difficulty in using any of the three kinetic models is that frequent HCV RNA measurements need to be made if all the model parameters are to be estimated. For example, the rate of virion clearance, c, and the initial delay before HCV RNA begins to decline, t_0, affect only the early viral dynamics. In order to estimate these parameters, frequent data needs to be collected during the first 1-2 days of therapy. Thus, in many studies these parameters are fixed to values found in studies with intense early sampling such as that of Neumann et al.[30] The viral load at baseline before the first dose of drug is given, which we have called V_0, can be directly measured or estimated from the data. We have found that in some patients in which frequent viral loads have been obtained early there are fluctuations in the HCV RNA level and it is better to fit V_0 than to use a single viral load measurement.

At the moment, analysis of viral load data is still somewhat of an art and the choice of model and which parameters to fix and which to fit depend on the drug being used and the objective of the clinical study. HCV models are still evolving and some studies are aimed at improving the models and our understanding of HCV pathogenesis, while other studies are of a more clinical nature and simply want to compare the effects of drug A vs drug B.

A large number of new drugs are under development or are becoming available for the treatment of HCV infection [24, 26]. As in the case of IFN and PEG-IFN, mathematical models should be useful in evaluating these new agents. Using mathematical models one can estimate *in vivo* parameters that are not directly measurable such as the drug's effectiveness, ε, in blocking viral production. In addition, the modeling approaches described above also provide information about the effects of drug dose and dosing interval in inducing viral declines and allow one via extrapolation to estimate how long therapy will be needed to totally eliminate the virus and all infected cells.

4.1 Mathematical models to describe the HCV RNA declines observed with new agents

Albuferon® (Albinterferon) [19, 20], which is a new IFN based product, is a recombinant IFN α-2b molecule with albumin attached. Albumin weighs ~60 kD, which is larger than either of the two forms of polyethylene glycol used in PEG-IFN (40 kD: PEG-IFN α-2a; 12 kD: PEG-IFN α-2b). Because of this large albumin molecule, albinterferon is eliminated much more slowly (elimination half life ~ 144 hours; k_e=0.12 day^{-1}) [19] than PEG-IFN α-2a [9] (elimination half life ~80 hours; k_e=0.21 day^{-1}) or PEG-IFN α-2b (elimination half life ~40 hours; k_e=0.42 day^{-1})[8] (Table 1). Albinterferon, thus, has the advantage of being administered subcutaneously only once every 2 weeks [19]. As in the case of once weekly administered PEG-IFN, modeling the effects of albinterferon on HCV RNA decline may require the use of PK/PD models or the DE model. Frequent viral kinetic data over the two week dosing interval will have to be

analyzed to decide whether viral load rebounds are observed and whether drug pharmacokinetics needs to be taken into account. It will also be informative to study whether the early therapeutic quotient of this new formulation is predictive of SVR.

Another class of drugs in an advanced stage of development is the so called direct antivirals. These compounds interfere with specific steps of the HCV replication cycle. Inhibitors have been developed for both the NS3/4A serine protease and the NS5B RNA-dependent RNA polymerase of HCV. Remarkably potent in vivo antiviral activity has been demonstrated in early clinical trials for the protease inhibitors telaprevir (VX-950) [64] and boceprevir (SCH503034)25. Both nucleoside analog polymerase inhibitors, such as R1626 [23, 65], and non-nucleoside NS5B polymerase inhibitors, such as GS919026, have also been developed. Although the actions of these drugs in reducing viral production can be modeled, as in standard models, through the appropriate estimation of a constant value for the drug effectiveness, ε, more specific models might be desired. For example, because these drugs are enzyme inhibitors, models based on competitive and non-competitive enzyme inhibitors could be explored in addition to the simple Hill function type pharmacodynamic relationship given by Eq. (7). In addition, the NS3/4A protease is not only involved in viral polyprotein processing, an essential step in viral replication, but it also cleaves two cellular proteins involved with IFN-signaling[66]. Therefore it is speculated that protease inhibitors might have a double effect and play a role in both inhibiting viral replication and restoring endogeneous synthesis of IFN [67]. Lastly, because it is envisaged that some of these new drugs will ultimately be used in the context of combination therapy, PD models that incorporate the possibility of synergy between these inhibitors may lead to important clinical understanding of their interactions.

As we have studied for IFN based regimens, it will be interesting to determine from clinical trial data with these new direct antivirals, if viral kinetic and PK/PD parameters estimated early in therapy can distinguish between sustained virological responders and non-responders. To study this, we need to be careful in choosing appropriate mathematical models or, if necessary, to develop new models that take into account the drug dose, the dosing schedule, and PK parameters estimated *in vitro* or in early clinical studies.

The viral load declines more steeply under the effect of direct antivirals in combination with PEG-IFN than with direct antivirals alone [21-23, 25, 26]. However, modeling combination therapy will be more difficult than modeling monotherapy. The drug effectiveness caused by direct antivirals administered every 8 hours or every 12 hours [22, 23, 25] may fluctuate more rapidly than the drug effectiveness caused by PEG-IFN administered every week. Further, synergistic interactions may occur that will need to be incorporated into pharmacodynamic relationships.

In addition, the emergence of drug resistant mutants is an important issue when patients are treated with direct antivirals [21, 22], because they impose a strong selective pressure. Serrazin et al. [24] observed that the viral load rebounded (breakthrough) a median of 2 $\log_{10}$ during 14 days of telaprevir monotherapy in 13/28 patients. In these 13 patients, the rebounding HCV virus contained drug resistant mutations. By contrast, viral load rebound was not observed in any of 12 patients during 28 days of therapy when a combination of telaprevir, PEG-IFN and ribavirin was used [22]. Thus, mathematical modeling that takes into account the difference of direct antiviral effectiveness againstwild-type HCV and against drug-resistant HCV will also be required to analyze HCV kinetic data from patients treated with direct antivirals.

4.2 Mathematical model of intracellular dynamics

Is the antiviral effect enhanced additively or synergistically when direct antivirals and IFN are used at the same time? How much drug should be administered to get the best outcome? These questions are being addressed by clinical studies [19], however we may ask if modeling can make

a contribution. To answer this question, one can introduce models of the intracellular dynamics of HCV RNA replication, as has been done for the replicon system [22], and then incorporate into these models the proposed effects of IFN and/or direct antivirals. The effect of IFN has been studied experimentally in HCV replicon systems [68-70], but its detailed mechanism of action is still not fully understood. Further, we do not know how direct antivirals, IFN and ribavirin interact with each other in hepatocytes when these drugs are used at the same time. For example, there are some suggestions that ribavirin might upregulate IFN stimulated genes in the presence of IFN thus leading to a synergy between these two drugs [71]. In the future, detailed computer simulation models could be developed that include both viral replication and host cell signaling pathways so that the effects of IFN, ribavirin and of direct antivirals could be included. With such models one might be able to predict how much HCV RNA would be reduced by therapy with direct antivirals, IFN-based drugs, ribavirin and combinations of these drugs [72].

Acknowledgments

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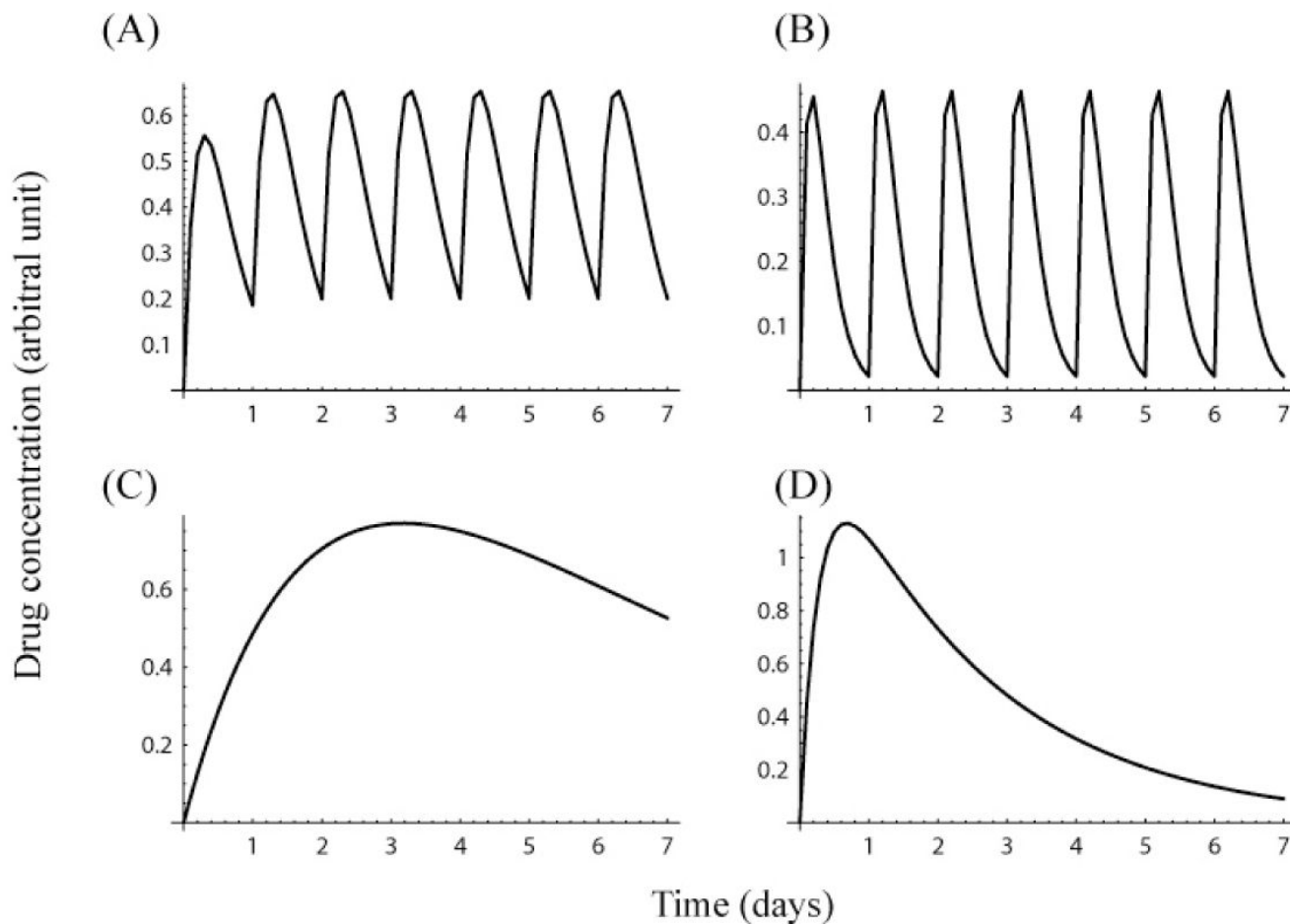
Figure 1.
Drug concentration in patient serum: IFN α-2a (A), IFN α-2b (B), PEG-IFN α-2a (C), and PEG-IFN α-2b (D). Simulation of drug concentrations in serum when IFN (A,B) is administered once daily, on days 0 through 6; and PEG-IFN (C,D) is administered once weekly at day 0 only. The curves represent the solution of the PK model, Eq. (5), using representative PK parameters for each drug. Parameters are: k_e=3.26 day^{-1} and k_a=3.31 day^{-1} (A), k_e=7.0 day^{-1} and k_a=5.0 day^{-1} (B), k_e=0.21 day^{-1} and k_a=0.45 day^{-1} (C), and k_e=0.42 day^{-1} and k_a=3.61 day^{-1} (D). For all drugs, we used an arbitrary value, 1.5 units, for FD/V_d.
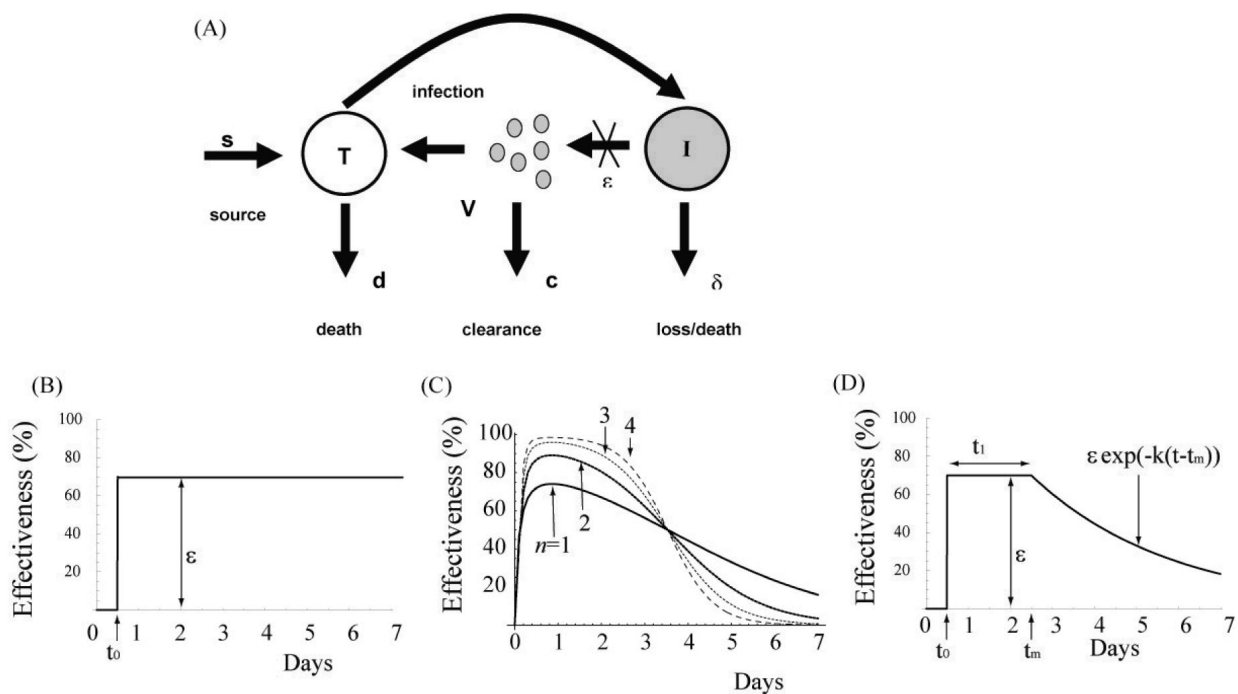
Figure 2.
(A) Schematic illustration of the model used to analyze HCV RNA kinetic data under therapy. Here T represents targets cells, I infected cells and V virus. Therapy is assumed to reduce the amount of virus produced per day by an infected cell from p to $(1-\varepsilon(t))p$. The effectiveness of therapy in blocking viral production, $\varepsilon(t)$, is chosen differently in different models. (B) $\varepsilon(t)$ for the CE model, (C) $\varepsilon(t)$ for the PK/PD model, and (D) $\varepsilon(t)$ for the DE model.
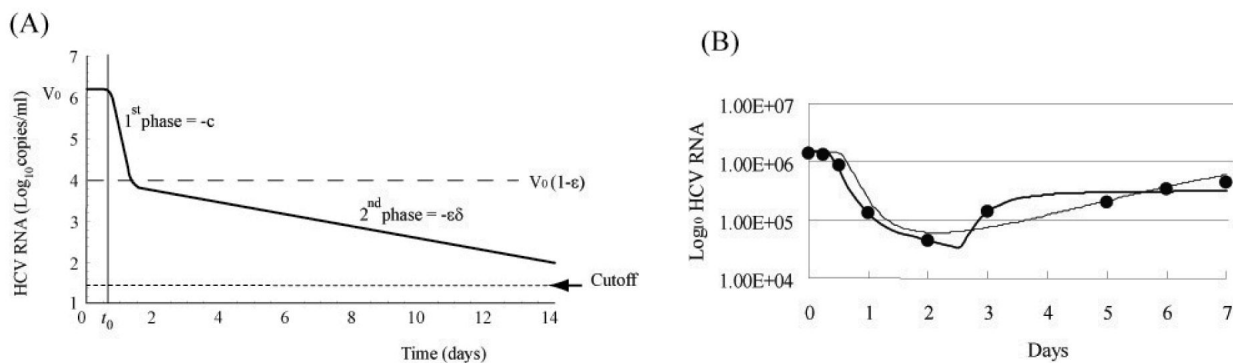
Figure 3.
HCV RNA kinetics described by (A) the CE model, (B, thin line) the PK/PD model and (B, heavy line) the DE model. The CE model can not describe viral rebound. (B) also shows the best-fit of the PK/PD (thin line) and DE models (thick line) to HCV RNA data (filled circles) from a representative patient from [42] during the first week of PEG-IFN α-2b therapy.

Table 1

Pharmacokinetic parameters of IFN-based drugs.

Product	Elimination half life (hours)	Absorption half life (hours)	t_{max} (hours)	Elimination rate (day^{-1})	Absorption rate (day^{-1})
Roferon A (IFN α-2a)	5.1 (3.7-8.5)	N/S	7.3	3.26 (4.50-1.96)[*]	3.31 (2.32-5.12)[+]
Intron A (IFN α-2b)	(2-3)	N/S	(3-12)	(5.55-8.32)[*]	(0.14 -11.1)[+]
PEGASYS® (peginterferon α-2a)	80 (50 -140)	N/S	(72 - 96)	0.21 (0.33-0.12)[*]	(0.18-0.72)[+]
PEG-Intron™ (Peginterferon α-2b)	40 (22-60)	4.6	(15 -44)	0.42 (0.76- 0.28)[*]	3.61[+] (0.38 - 4.86)[*]

Half life (absorption and elimination) and t_{max} as reported in 8-11 for subcutaneous infusion with the exception of the elimination rate of IFN α-2a, which was given for intravenous infusion. Averages (and ranges between parentheses) are shown. N/S - not shown in 8-11.

[*] Estimated parameters using a non-compartmental model [73].

[+] Estimated from t_{max}, the time to the peak of concentration, given by Eq. (6).