Triphasic Decline of Hepatitis C Virus RNA During Antiviral Therapy

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When patients chronically infected with hepatitis C virus (HCV) are placed on antiviral therapy with pegylated interferon (IFN)-α or IFN-α plus ribavirin (RBV), HCV RNA generally declines in a biphasic manner. However, a triphasic decline has been reported in a subset of patients. A triphasic decline consists of a first phase (1-2 days) with rapid virus load decline, followed by a "shoulder phase" (4-28 days) in which virus load decays slowly or remains constant, and a third phase of renewed viral decay. We show that by including the proliferation of both uninfected and infected cells, a viral kinetic model can account for a triphasic HCV RNA decay. The model predicts that a triphasic decline occurs only in patients in which a majority of hepatocytes are infected before therapy. The shoulder phase does not represent the intrinsic death rate of infected cells, but rather the third phase slope is close to the intrinsic death rate of infected cells when overall drug efficacy is close to 1. ***Conclusion:*** **Triphasic responses can be predicted from a generalization of existent viral kinetic models through the inclusion of homeostatic proliferation of hepatocytes. This generalized model can also explain the viral kinetics seen in flat partial responders. Finally, the enhanced third phase in patients treated with IFN-α in combination with RBV versus patients treated with IFN-α alone can be explained by a mutagenic effect of RBV against HCV.** (HEPATOLOGY 2007;46:16-21.)

Approximately 200 million individuals worldwide are currently infected with hepatitis C virus (HCV).[1] Current therapy involves the use of pegylated interferon (IFN)-α plus ribavirin (RBV).[2-8] The typical HCV RNA decay during therapy with IFN-α alone or in combination with RBV is characterized by an initial rapid viral decline (first phase) followed by a slower decay (second phase).[9-12] In some patients, a triphasic decline has been observed in which there is a rapid initial decline in viral load, followed by a "shoulder phase" lasting 4-28 days in which HCV RNA decays slowly or remains constant, and a third phase of renewed viral decay.[12-15] For example, Herrmann et al.[12] observed triphasic declines in 8 of 10 patients treated with pegylated IFN-α plus RBV, in 9 of 17 patients treated with pegylated IFN-α alone, and in 4 of 7 patients treated with IFN-α plus RBV. Why some patients respond to therapy with a biphasic decline whereas others respond in a triphasic manner has not been addressed previously and is the subject of our report.

In patients treated with IFN-α plus RBV, compared with patients treated with IFN-α alone, RBV tends to increase the last-phase slope (i.e., the second phase slope in biphasic viral decays and the third phase slope in triphasic viral decays).[10,12] Modeling HCV RNA kinetics during therapy suggested that the increase in last-phase slope could be due to a delayed immunomodulatory effect of RBV, which increases the death rate of HCV productively infected cells after the shoulder phase.[12] In particular, in the model of Herrmann et al.,[12] the slope of the "shoulder phase" in patients with triphasic viral decay represents the pretreatment death rate of infected cells and the third-phase slope represents the treatment-enhanced death rate of infected cells due to the immunomodulatory effect of RBV. Thus, to obtain a flat second phase in this model requires the assumption that the pretreatment death rate of infected cells is close to 0 and then increases due to the effect of RBV. However, we contend that a flat second phase can be obtained without assuming

Abbreviation: HCV, hepatitis C virus; IFN, interferon; RBV, ribavirin.

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that the pretreatment death rate of infected cells is close to 0, if the capacity of hepatocytes to replicate is included in the model.[16,17] Furthermore, we show that a triphasic viral decay can occur assuming constant death rate for infected cells throughout therapy. Thus, the presence of a triphasic viral decay is not necessarily evidence that treatment enhances the immune response against HCV-infected cells.

If one assumes that RBV does not act as an immune modulator, how can we explain the more rapid third phase seen in patients treated with IFN-α plus RBV compared with patients treated with IFN-α alone? Hermann et al.[12] suggested—and Dixit et al.[10] showed explicitly—that if RBV has a mutagenic effect that leads to the generation of less-infectious or noninfectious virus particles, then de novo infection would be slowed and the last-phase slope would be increased.[18]

We present an extended model of HCV dynamics during IFN-α plus RBV therapy[10] that accounts for proliferation of uninfected and infected cells. The model explains the "shoulder phase" and provides conditions under which a three-phase HCV RNA decline is expected to occur. Finally, the model shows that the last phase of viral decay (i.e., the second phase in biphasic models and the third phase in triphasic models) is determined by the loss rate of infected cells and the effectiveness of therapy.

Model Description and Results

The extended model for the dynamics of HCV infection that includes proliferation of uninfected hepatocytes (T) and infected hepatocytes (I) as well as the effects of RBV as a mutagen, is given by:

$$\frac{dT}{dt} = s + r_T T\left(1 - \frac{T+I}{T_{\max}}\right) - d_T T - \beta V_I T$$

$$\frac{dI}{dt} = \beta V_I T + r_I I\left(1 - \frac{T+I}{T_{\max}}\right) - \delta I$$

$$\frac{dV_{\mathrm{I}}}{dt} = (1-\rho(t))(1-\varepsilon)pI - cV_I$$

$$\frac{dV_{\mathrm{NI}}}{dt} = \rho(t)(1-\varepsilon)pI - cV_{NI} \qquad (1)$$

where V_{I} represents infectious virions and V_{NI} represents noninfectious virions. The model assumes uninfected hepatocytes (T) are produced at a constant rate s, die at rate d_{T} per cell, and are infected by infectious virions (V_I) at constant rate β. Uninfected and infected hepatocytes can proliferate with maximum proliferation rates r_{T} and r_{I}, respectively, according to a blind homeostasis process in which there is no distinction between infected and uninfected cells.[19] Due to the burdens of supporting HCV replication, we assume infected cells may proliferate slower than uninfected cells (i.e., $r_{\mathrm{I}} \leq r_{\mathrm{T}}$). If the total hepatocyte population ($T + I$) reaches a maximum level ($T_{\max}$), hepatocyte proliferation stops. Infected hepatocytes are lost at a constant rate δ per cell. In the absence of treatment, each productively infected cell releases new infectious virions at rate p. Interferon therapy lowers p by a factor $(1 - \epsilon)$, where ϵ is the effectiveness of IFN-α in blocking virion production.[9] Of the virions released, we assume that RBV renders a fraction $\rho(t)$ noninfectious, giving rise to the population V_{NI}. To mimic the slow accumulation of RBV in plasma, we let the RBV effectiveness increase with time on therapy [i.e., $\rho(t) = \rho_{\max}(1 - exp(-t/t_a))$, $t_a = 5.6$ days] as described previously.[10] Free virions are cleared from plasma at rate c per virion. The measured viral load $V = V_{\mathrm{I}} + V_{\mathrm{NI}}$.

The model has two steady states. One is the uninfected steady state corresponding to a sustained virological response in which there is no virus and no infected cells. In this state, the total number of uninfected hepatocytes is equal to:

$$\bar{T}_0 = \frac{T_{\max}}{2r_T}\left[r_T - d_T + \sqrt{(r_T - d_T)^2 + \frac{4r_T s}{T_{\max}}}\right] \qquad (2)$$

The other steady state is an infected steady state, corresponding to chronic infection, with

$$\bar{V} = \frac{(1-\varepsilon)p\bar{I}}{c},$$

$$\bar{\mathrm{I}} = \bar{T}\left(\frac{A}{r_I} - 1\right) + T_{\max} - B,$$

$$\bar{T} = \frac{1}{2}\left[-D/H + \sqrt{(D/H)^2 + \frac{4sT_{\max}}{r_T H}}\right] \qquad (3)$$

where

$$A = \frac{(1-\varepsilon_{tot})p\beta T_{\max}}{c},\ B = \frac{\delta T_{\max}}{r_I},\ H = \frac{A^2}{r_I r_T} + \frac{A}{r_I} - \frac{A}{r_T}$$

$$\text{and } \mathrm{D} = \mathrm{A}\left[\frac{T_{\max}}{r_T}\left(1 + \frac{d_T}{A}\right) - B\left(\frac{1}{r_T} + \frac{1}{A}\right)\right]$$

For simplicity, we have combined the IFN-α and RBV efficacies into a single term for overall drug effectiveness, ϵ_{tot}, where $1 - \epsilon_{\mathrm{tot}} = (1 - \rho_{\max})(1 - \epsilon)$. Before treatment, a patient with chronic HCV infection is assumed to be in this infected steady state with $\epsilon = \epsilon_{\mathrm{tot}} = 0$.

For HIV, the notion of a critical efficacy, ϵ_{c}, has been introduced, such that if $\epsilon_{\mathrm{tot}} > \epsilon_{\mathrm{c}}$, viral levels will continually decline on therapy, while if $\epsilon_{\mathrm{tot}} < \epsilon_{\mathrm{c}}$ viral loads will initially decline but ultimately stabilize at a steady state

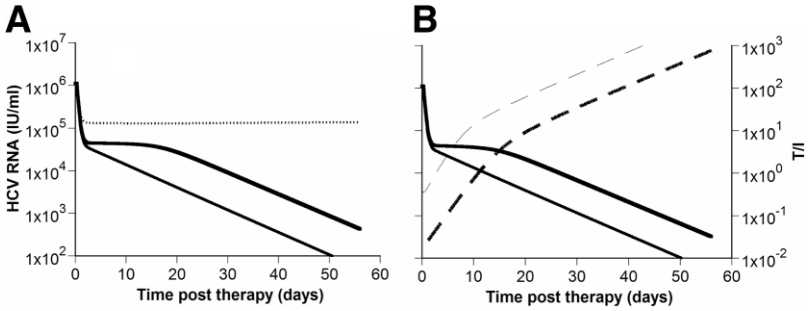

Fig. 1. Biphasic or triphasic HCV decline during drug therapy. We solved our model (Equation 1) numerically using Berkeley-Madonna software, version 7.0.2. (A) The predicted HCV RNA kinetics can account for partial responders ($\epsilon_{tot} = 0.88$) with large δ (dotted line) or sustained responders ($\epsilon_{tot} = 0.96$) (solid lines) depending on whether the total efficacy ϵ_{tot} is smaller or larger than the critical efficacy ($\epsilon_c = 0.902$). Moreover, a biphasic decline (thin solid line) or triphasic decline (thick solid line) is predicted depending on the time under therapy that it takes for the ratio of infected cells to uninfected cells to reach ≈ 1 (see panel B). For example, if we increase the ratio of target cell to infected cell replication rates from $r_T/r_I = 2$ to $r_T/r_I = 5$, the "shoulder phase" of the triphasic decline (thick solid line) disappears and we obtain a biphasic viral decline (thin solid line). (B) The "shoulder phase" occurs (thick solid line) when the majority of hepatocytes are infected at baseline (i.e., T/I is <1) before therapy (thick dashed line). For $r_T/r_I = 2$, the "shoulder phase" ends ($\approx$21 days of therapy) when T/I ≈ 1. For $r_T/r_I = 5$, the "shoulder phase" shortens so the viral decline curve is biphasic (thin solid line). Here T/I is approximately ≈ 1 at day 2 of therapy (thin dashed line). Except as noted, model parameters used were: $T_{max} = 9.13 \times 10^6$ cells, $d_T = 0.013$ day^{-1}, $p = 4.3$ virions cell^{-1} day^{-1}, $\beta = 3.5 \times 10^{-7}$ ml day^{-1} virions^{-1}, $c = 3.5$ day^{-1}, $\delta = 0.22$ day^{-1}, $r_T = 0.5$ day^{-1}, $r_T/r_I = 2$, $\epsilon_{tot} = 0.96$, $\rho_{max} = 0$, and $s = 4$ cells ml^{-1} day^{-1}. For these parameters and based on Equation 4, the critical efficacy for successful treatment is $\epsilon_c = 0.902$. For $r_T/r_I = 5$, the critical efficacy for successful treatment is $\epsilon_c = 0.899$.

level lower than baseline.[20-22] Our model predicts similar behavior where:

$$\varepsilon_c = 1 - \frac{c(\delta T_{max} + r_I(\bar{T}_0 - T_{max}))}{p\beta T_{max}\bar{T}_0} \qquad (4)$$

In the case of successful drug therapy (i.e., sustained virological response, $\epsilon_{tot} > \epsilon_c$), HCV RNA is predicted to decline in a biphasic or triphasic manner, while for $\epsilon_{tot} < \epsilon_c$, the system will converge to a new infected steady state with lower levels of virus ($\bar{V}$) than at baseline (Fig. 1A). Thus, in this model if drug effectiveness is not high enough a patient will not clear virus.

Upon successful drug therapy ($\epsilon_{tot} > \epsilon_c$), the viral load will decline until the uninfected steady state is reached. The model predicts that this decline may be biphasic or triphasic (Fig. 1A, dotted line) depending on whether a shoulder phase exists. During the shoulder phase, the viral load does not decline, which implies in our model that during this phase the number of productively infected cells must be approximately constant. If we allow for loss of infected cells (e.g., $\delta = 0.22$ day^{-1} in Fig. 1) the shoulder phase will only occur if the rate of proliferation of infected cells plus the rate of de novo infection of target cells equals the rate of infected cell loss. Although new infection of uninfected cells may occur, proliferation of infected cells is the main source of infected cells in the presence of potent antiviral therapy. Thus, models without proliferation do not account for the shoulder phase. In our model, proliferation of hepatocytes is under homeostatic control (i.e., proliferation compensates for cell loss). Thus, the proliferation rate of infected cells is sensitive to the total number of hepatocytes in the liver (i.e., to both the number of infected and uninfected cells). During treatment, uninfected cells increase faster than infected cells due to a possibly higher proliferation rate and by generation of new uninfected cells through differentiation of precursors. Because of homeostatic processes, as the number of uninfected cells increase, the proliferation of infected cells slows. When this proliferation slows to the point at which it no longer keeps up with the rate of infected cell loss, the number of infected cells start to decline. This demarks the transition from the shoulder phase to the third phase of viral decline.

We find that a shoulder phase, and hence a triphasic viral decay, occurs if the number of uninfected cells is much lower than the number of infected cells before therapy and uninfected cells proliferate faster than infected cells. Under these conditions, the generation of new uninfected cells to a level at which they slow infected cell proliferation takes a substantial length of time. This delay causes the shoulder phase. The shoulder phase persists until the ratio between uninfected cells and infected cells is approximately 1. Conversely, when the ratio of uninfected cells to infected cells at baseline is greater than 1, the shoulder phase does not exist (Fig. 1B). Thus, this model can account for the fact that not all patients show a triphasic viral decline.

Lastly, we find for drug efficacies close to 1 ($\epsilon_{tot} \approx 1$) that the slope of the final phase of viral decline is approximately the death rate of infected cells (δ). However, for efficacies less than 1, there is continuing infection of new cells; hence the final phase decline slope, which reflects the

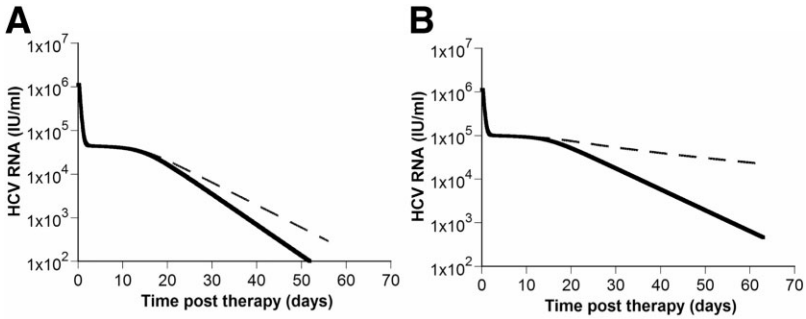

Fig. 2. Third phase viral decline is enhanced by RBV. We compared model predictions for the rate of viral decline when using IFN-α plus RBV ($\rho_{max} = 0.5$) (solid line) with IFN-α alone ($\rho_{max} = 0.0$) (dashed line). The model predicts that RBV enhances the third phase slope without affecting the death rate of infected cells. For high IFN-α effectiveness ($\epsilon = 0.96$), the RBV effect on viral decay ($\rho_{max} = 0.5$) is less significant (A) than with low IFN-α effectiveness ($\epsilon = 0.91$) (B). Parameter values are the same as in Fig. 1.

net loss of infected cells, is less than δ (Fig. 2) and depends on additional model parameters, such the rate of infection β and the total drug efficacy.

Because the final phase decline slope reflects the net loss of infected cells, by decreasing HCV infectivity through the use of RBV in combination with IFN-α we find the final HCV decline slope is more pronounced with RBV ($\rho_{max} > 0$) than without ($\rho_{max} = 0$). This is particularly true when the effectiveness of therapy is low, as previously observed[10] (Fig. 2).

To show that our model is consistent with experimental data, we fit the model to HCV RNA data that exhibit triphasic declines obtained from patients treated with pegylated IFN-α2a alone[12] (Fig. 3A) and in combination with RBV[12] (Fig. 3B) and with daily IFN-α2b alone[13] (Fig. 3C).

Discussion

The paradigm of HCV RNA changes during antiviral therapy has been a biphasic decline. However, triphasic viral declines have also been observed in many patients in whom the frequency of measurement allows it. Hermann et al.[12] noted that 8 of 10, 9 of 17, and 4 of 7 patients treated with pegylated IFN-α plus RBV, pegylated IFN-α alone, and IFN-α plus RBV, respectively, had triphasic viral decays. In this study, all patients were evaluated at day 1 (6 and 12 hours after the first dose) and days 2-5, 8, 11, 15, 22, 29, 43, and 57. Thus, the frequency of measurements was adequate to observe the shoulder phase. In addition, note that triphasic viral decay was observed in patients treated with pegylated IFN-α alone or with RBV. Thus, RBV is not necessary for a triphasic decline. Other studies[13-15] have also observed triphasic viral decays. In particular, Bergmann et al.[15] observed triphasic viral declines in a large fraction (30%-40%) of IFN-α–treated patients (n = 200).

Whereas the final phase of a triphasic viral decline could be due to an enhancement of an immune response, as suggested by Hermann et al.,[12] the shoulder phase is more difficult to explain in conventional models, because it requires the assumption that infected cell loss is close to 0 before treatment and before therapy-induced enhance-

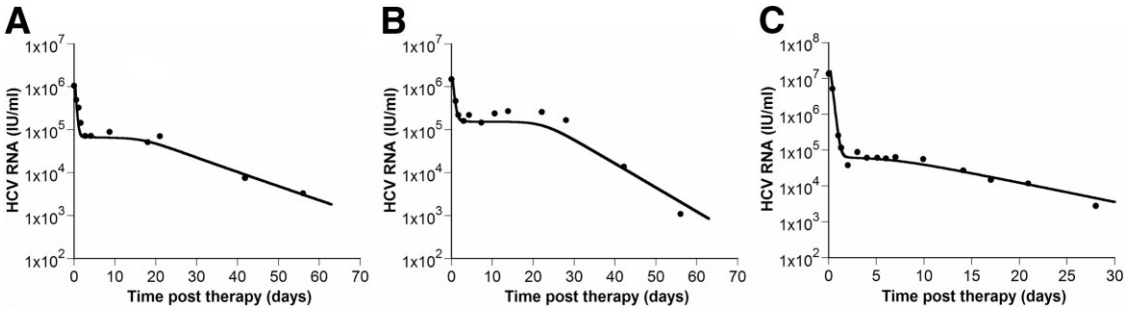

Fig. 3. Agreement of the model with experimental data. To show that the extended model agrees with experimental data (●) exhibiting triphasic viral decays, we digitized HCV RNA levels of 2 patients (figures 2A and 2B from Hermann et al.[12]) treated with (A) pegylated IFN-α2a alone and (B) pegylated IFN-α2a plus RBV and (C) 1 patient (figure 2A from Bekkering et al.[13]) treated with IFN-α2b. The analytical solution for $V(t)$ (i.e., equation 4 in Neumann et al.[9]) was first fitted to the HCV RNA using Berkeley-Madonna software, version 7.0.2, to estimate the delay time before viral decay begins (t_0), the IFN-α effectiveness (ϵ), and the viral clearance rate constant (c). We then fitted our model (solid line) to the HCV RNA data (●) with t_0, ϵ, and c held fixed at their estimated values, and found values for the parameters s, d, δ, p, r_I, r_T, T_{max}, β and ρ_{max} for each patient that generated viral load decays consistent with the data. For patients treated with IFN-α monotherapy, ρ_{max} was set to 0. Parameter values found in panels A, B, and C, respectively, are: $T_{max} = 0.51 \times 10^7$, 0.6×10^7, and 1.5×10^7 ml^{-1}; $s = 7.3$, 3.9, and 5.1 cells day^{-1} ml^{-1}; $d_T = 12.9 \times 10^{-3}$, 8.7×10^{-3}, and 2.4×10^{-3} day^{-1}; $\delta = 0.22$, 0.19, and 0.13 day^{-1}; $\beta = 3.5 \times 10^{-7}$, 2.6×10^{-7}, and 0.3×10^{-7} virions^{-1} day^{-1}; $r_T = 0.5$, 0.5, and 1.1 day^{-1}; r_T: $r_I = 2.0$, 2.2, 4.2; $c = 3.5$, 2.4, 5.4 day^{-1}; $t_0 = 0.30$, 0.19, 0.22 days; $p = 4.4$, 4.3, and 13.2 virions cell^{-1} day^{-1}; $\epsilon = 0.939$, 0.899, and 0.995; $\rho_{max} = 0.0$, 0.6, and 0.0.

ment. Here we have shown that this assumption is not needed and both the flat second phase and the subsequent third phase may be a simple consequence of liver homeostasis in which proliferation of hepatocytes compensates for the loss of infected cells. We have also shown that the final phase slope, which appears to be enhanced when RBV is included in treatment regimes[12] may be due solely to RBV's mutagenic effect. We note that in our model, if we postulate that RBV has a gradual effect in enhancing either the loss rate of infected cells (δ) or the effectiveness of therapy (ϵ), then we do not observe a shoulder phase nor a biphasic decline (data not shown); rather, the second phase slope slowly increases.

In the field of HIV therapy, the notation of a critical drug efficacy has been introduced.[20-22] If efficacy is not high enough (i.e., below its critical value), then theory predicts that HIV rather than declining monotonically during therapy, will decline initially but ultimately stabilize at a therapy-induced set point. We show that the same concept applies to HCV treatment, and estimate ϵ_c, the critical drug efficacy needed for ultimate clearance of HCV (i.e., sustained virological response). Below the critical efficacy HCV RNA levels converge to a new steady state lower than that before therapy (Fig. 1). The critical efficacy is patient-specific and depends on parameters that characterize both the virus and the host response. Thus, the same therapy may be above the critical efficacy for some patients and below the critical efficacy for others. For patients in whom the efficacy is below the critical value, our model suggests a response to therapy that corresponds to what previously has been called a flat partial response (i.e., when viral loads initially fall but then stabilize at a lower value). Notably, the new viral plateau under drug therapy can exist even if the death rate of infected cells (δ) is large (Fig. 1). Indeed, the slope of the "shoulder phase" does not represent δ. Rather, the final phase slope, second phase in biphasic viral decay, or third phase in triphasic viral decay will reflect δ. Moreover, it is only for drug efficacies close to 1 that the last-phase slope is approximately the death rate of infected hepatocytes δ, and for lower efficacies this slope is only a minimal estimate of δ.[23]

In the model presented here (Eq. 1), viral clearance has been assumed to occur at a constant rate c per virion. In models that assume constant levels of target cells, the rate constant c includes any virion loss due to virions entering cells at a rate proportional to βT per virion, because βT is a constant. Here we allow target cell numbers to vary, and with treatment their numbers increase. Thus, in principle, virion clearance due to cell entry could increase with time on therapy and thus should be treated separately from the loss at constant rate c. In a preliminary examination of this effect, we find that such additional clearance does not affect our findings about triphasic decays; however, it can change the shoulder length and, when ϵ is not close to 1, the rate of HCV RNA decay observed during the third phase. A fuller examination of these effects will be published elsewhere.

The Neumann et al.[9] model of HCV RNA kinetics during therapy, which analyzed data over the first 2 days or first 14 days of therapy, assumed that over these time periods the uninfected cell population has only a minor effect on viral decay profiles and held this population constant when fitting the model to patient data. Some[10,11] but not all[12,24] later models also made this simplifying assumption. This simplified model, which has fewer parameters than models that incorporate target cell kinetics, could be fit to the observed biphasic viral decay in treated patients to estimate the effectiveness of drug therapy (ϵ), the clearance of free virions (c), and the approximate death rate of infected cells (δ). However, the simplified model does not explain viral decay in flat partial responders and triphasic responders, and the rapid (1-2 weeks) viral resurgence to pretreatment levels after cessation of therapy.[23] We show that including hepatocyte regeneration in the Dixit et al.[10] model allows one to predict triphasic viral decay and the enhancement of the third phase by RBV without assuming that after a delay therapy enhances immune responses and causes an increase of the infected cell death rate. Unfortunately, the model with proliferation has additional parameters that characterize uninfected and infected cells kinetics, which cannot be estimated from HCV RNA decline data. Thus, a future challenge will be to collect additional data about hepatocyte populations. For example, estimates of the fraction of infected cells in pretreatment biopsies could be used to test the prediction that triphasic responses occur only when the ratio of uninfected to infected cells is less than 1.

In conclusion, we have shown that triphasic responses can be predicted from a generalization of existing viral kinetic models by the inclusion of homeostatic proliferation of infected and uninfected hepatocytes. Furthermore, we have shown that the model can explain the viral kinetics seen in flat partial responders. Lastly, we have shown that the enhanced third phase seen in patients treated with interferon in combination with RBV versus patients treated with IFN-α alone[12] can be explained by a mutagenic effect of RBV without invoking an effect of RBV in enhancing the anti-HCV immune response. If RBV does not act as an immune modulator, future therapies with combinations of direct antivirals may be able to replace both IFN-α and RBV.

Disclaimer: This study is the sole responsibility of the authors and does not necessarily represent the official

views of the NIH or the National Center for Research Resources.

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