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Modeling HCV cure after an ultra-short duration of therapy with direct acting agents

Ashish Goyal^{1,2}, Yoav Lurie³, Eric G. Meissner^{4,5}, Marian Major⁶, Natasha Sansone^{1,7}, Susan L. Uprichard^{1,8}, Scott J. Cotler¹, and Harel Dahari^{1,*}

¹The Program for Experimental and Theoretical Modeling, Division of Hepatology, Department of Medicine, Loyola University Medical Center, Maywood, IL, United States

²Theoretical Biology & Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, United States

³Liver Unit, Digestive Disease Institute, Sha'are Zedek Medical Center, Jerusalem, Israel

⁴Division of Infectious Diseases, Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, United States

⁵Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

⁶Division of Viral Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland, United States

⁷Department of Microbiology and Immunology, University of Illinois Chicago, Chicago, IL, United States

⁸Department of Microbiology and Immunology, University of Illinois Chicago, Chicago, IL, United States

Abstract

Background—Cases of sustained-virological response (SVR or cure) after an ultra-short duration (< 27 days) of direct-acting antiviral (DAA)-based therapy, despite HCV being detected at end of treatment (EOT), have been reported. Established HCV mathematical models that predict the treatment duration required to achieve cure do not take into account the possibility that the infectivity of virus produced during treatment might be reduced. The aim of this study was to develop a new mathematical model that considers the fundamental and critical concept that HCV RNA in serum represents both infectious virus (V_i) and non-infectious virus (V_{ni}) in order to explain the observation of cure with ultrashort DAA therapy.

Methods—Established HCV models were compared to the new mathematical model to retrospectively explain cure in 2 patients who achieved cure after 24 or 27 days of paritaprevir, ombitasvir, dasabuvir, ritonavir and ribavirin or sofosbuvir plus ribavirin, respectively.

*Corresponding Author: The Program for Experimental & Theoretical Modeling, Division of Hepatology, Department of Medicine, Loyola University Medical Center, 2160 S. First Ave., Maywood, IL 60153; (p)708-216-4682, (f)708-216-6299; hdahari@luc.edu.

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Results—Fitting established models with measured longitudinal HCV viral loads indicated that in both cases, cure would not have been expected without an additional 3 to 6 weeks of therapy after the actual EOT. In contrast, the new model fits the observed outcome by considering that in addition to blocking V_i and V_{ni} production ($\epsilon \sim 0.998$), these DAA + ribavirin treatments further enhanced the ratio of V_{ni} to V_i , thus increasing the $\log(V_{ni}/V_i)$ from 1 at pretreatment to 6 by EOT, which led to <1 infectious-virus particle in the extracellular body fluid (i.e., cure) prior to EOT.

Conclusions—This new model can explain cure after short duration of DAA+ribavirin therapy by suggesting that a minimum 6-fold increase of $\log(V_{ni}/V_i)$ results from drug-induced enhancement of the V_{ni}/V_i .

Introduction

Treatment of chronic hepatitis C virus infection has dramatically improved with $>90\%$ cure (or sustained virological response, SVR) rates using all-oral direct-acting-antivirals (DAAs), with shorter treatment and minimal side-effects, relative to interferon-based treatment (WHO, 2015). Theoretically, cure is achieved when there is less than one virus particle and HCV-infected cell in the body (termed cure boundary) (Dixit et al., 2004; Snoeck et al., 2010). The typical HCV decline under DAA treatment is biphasic, consisting of a 1st phase rapid virus decline (12-48 hours), followed by a slower 2nd phase virus decay (Dahari et al., 2016). Mathematical modeling of HCV kinetics can reproduce this biphasic viral decline and can estimate the duration of treatment needed to clear the virus (and infected cells) to reach the cure boundary. For example, real-time modeling successfully predicted the duration of interferon-free therapy with silibinin plus ribavirin required to achieve cure (Dahari et al., 2015). Recently, the cure boundary concept was retrospectively examined using data from 58 HCV genotype-1 infected patients treated for 12 weeks with three different approved sofosbuvir (SOF)-based regimens (Dahari et al., 2016). Modeling predicted that the majority of patients would reach cure with 8 weeks of therapy, which is in agreement with clinical studies showing high cure rates with 8 weeks of treatment in non-cirrhotic HCV genotype-1 patients (Buggisch et al., 2016; Kowdley et al., 2014; O'Brien et al., 2016). Moreover, 43% of patients treated with SOF-based regimens were predicted to reach cure within 6 weeks.

We previously observed cure in two patients treated with DAAs for less than 4 weeks, despite having detectable HCV RNA close to end of treatment (EOT) (Hasin et al., 2016; Meissner et al., 2014). HCV RNA detected at EOT in patients who subsequently develop SVR might be due in part to the presence of non-infectious RNA or virus (V_{ni}), which has been identified in-vivo in HCV-infected chimpanzees and in-vitro (Feinstone et al., 1981; Major et al., 2002; Sansone et al., 2014). Further, in vitro inhibition experiments utilizing HCV DAAs directed against NS5A (such as ombitasvir prescribed to patient 1 in this study) have documented a reduction in the infectivity of the virus produced during treatment resulting in an increase in the relative amount of noninfectious particles (V_{ni}) to infectious particles (V_i) (Sansone et al., 2014). In the current study we show that established mathematical models cannot explain cure in these two patients and suggest a new model in which the cure boundary is related not to the total viral load in blood, but to the number of infectious viral particles.

Methods

Patients

Viral kinetics were measured from two patients treated in clinical practice who achieved cure after 24 days of paritaprevir, ombitasvir, dasabuvir, ritonavir (PODr) and ribavirin (RBV) (Hasin et al., 2016) (patient 1) or 27 days of SOF+RBV (Meissner et al., 2014) (patient 2).

Mathematical model

Since the standard biphasic (Dahari et al., 2016) and the multiscale (Guedj et al., 2013) models (termed established models) would have predicted treatment failure in these cases (Eq. S1 and S2, Supplementary text), we developed a new extended biphasic model (Figure 1), reminiscent of an extended multiscale model presented by Nguyen et al (Nguyen et al., 2015), that incorporated assumptions about infectious and non-infectious virus, as shown here:

$$\begin{aligned}\frac{dI}{dt} &= \beta T_0 V_i - \delta I \\ \frac{dV_i}{dt} &= (1 - \epsilon) p_i \exp(-gt) I - c V_i \\ \frac{dV_{ni}}{dt} &= (1 - \epsilon) p_i (1 - \exp(-gt)) I + (1 - \epsilon) p_{ni} I - c V_{ni}\end{aligned}\quad (\text{Eq. 1})$$

where V_i and V_{ni} represent infectious and non-infectious HCV, respectively. V_i infects target cells, T_0 , with constant rate β , generating infected cells, I , which produce new infectious and non-infectious virions at rate p_i and p_{ni} per infected cell, respectively. Both V_i and V_{ni} are assumed to be cleared at rate c per virion. DAA-containing regimens such as PODr inhibit HCV replication which effects both V_i and V_{ni} production with a constant effectiveness, ϵ . However, the ability of DAAs (such as NS5A inhibitors (Sansone et al., 2014)) and RBV (Dahari et al., 2007; Dixit et al., 2004) to alter the infectivity of secreted virus would result in a gradual increase in the ratio of non-infectious to infectious virus, which is modeled by $\exp(-gt)$, where g is a constant and t is time post treatment initiation.

Parameter estimation

We assume the target cell (i.e., hepatocyte) level per ml is constant at 1×10^7 cells (Dahari et al., 2007). Thus, target cell number was kept constant and initial infected cell number is given by $I_0 = \beta V_{i0} T_0 / \delta$, where V_{i0} represents pre-treatment level of infectious virus. Reverse titration studies in chimpanzees have provided quantification of HCV RNA copy number and infectious titers within HCV-positive samples (Engle et al., 2008; Major et al., 2002), suggesting a pretreatment ratio of V_i and V_{ni} of 1:11 (i.e., $p_i/p_{ni} = 1/11$). Thus, we have $V_{i0} = V_{tot0}/12$ where V_{tot0} is the measured pre-treatment HCV level. Employing initial steady state conditions also yields $p_i = c V_{i0} / I_0$ and $p_{ni} = 11 p_i$. Parameter β was set to 7×10^{-8} mL/virion/day to target a low fraction of HCV-infected hepatocytes (~4%), consistent with a

previously estimated range (Liang et al., 2009). Due to lack of frequent sampling in patient 1, especially during the first 2 days of therapy, the effectiveness of PODr+RBV in blocking HCV production was set to $\epsilon=0.998$ as recently estimated (Gambato et al., 2016) and HCV clearance rate was set to $c=6/\text{day}$ (Dahari et al., 2016). In the SOF+RBV treated patient (patient 2), the availability of frequent HCV measurements allowed for the estimation of both ϵ and c parameters.

Cure boundary—The time to eradication of the last infected cell is more speculative due to the lack of experimental data on the infected cell number (Dahari et al., 2016). Therefore we focused on the viral cure boundary, i.e., <1 virus particle in the extracellular body fluid (approximately 13.5 L) which corresponds to one virion/13,500 mL $=7 \times 10^{-5}$ (Dahari et al., 2016).

Fitting and sensitivity analysis—The available 2 patient datasets were used to estimate unknown parameters of the three models (Eq. S1 and S2, and Eq. 1) using fitting procedure lsqnonlin in MATLAB R2016a. The estimation procedure also yields Jacobian matrix and covariates which were then used to determine 95% CI of the estimated parameters using 'nlparci' in MATLAB R2016a (Bates and Watts, 1988). It should also be noted that the estimated value of parameter g represents a minimum estimate due to the inability to measure V_i . A robustness analysis of the estimated model parameters is further described in the Supplementary text.

Results

Established HCV mathematical models fail to explain cure

The standard model (Eq. S1) predicts a time-to-cure for the two cases of 8 to 10 weeks with an estimated loss of infected cells (δ) of 0.278/day [95% confidence intervals, 95%CI: 0.229-0.337] for patient 1 (Fig.S1; Table S1). For patient 2, δ , c and ϵ were estimated at 0.042/day [95%CI:0.001, 2.177], 4.87/day [95%CI:3.797-6.254] and 0.999 [95%CI: 0.997-0.999], respectively, with time-to-cure being greater than 52 weeks (Fig S1; Table S1). The multiscale model (Eq.S2) predicts time-to-cure to be greater than 8 and 18 weeks for patients 1 ($\delta=0.246/\text{day}$ [95%CI:0.218-0.278]) and patient 2 ($\delta=0.080/\text{day}$ [95%CI: 0.016-0.388] and $\epsilon=0.995$ [95%CI:0.962-0.999]) respectively (Fig.S2; Table S2). Thus, both established models suggested a time-to-cure of at least 4 additional weeks of therapy than what both patients actually received.

A model that accounts for infectious and non-infectious virus can explain cure

The new extended biphasic model (Fig.1; Eq.1) fits well with measured viral load and predicts cure with ultra-short therapy (Fig.2). The new model predicts that DAAs plus RBV not only block HCV production but in addition reduce the infectivity of the virus produced, which is modelled through parameter g . For patient 1, the estimated infected cell loss, δ , and g were 0.281/day [95%CI:0.229-0.343] and 0.366/day [95%CI:0.305-0.440], respectively. For patient 2, $\delta=0.042$ [95%CI:0.005-0.374], $g=0.404$ [95%CI:0.330-0.496], $c=4.875/\text{day}$ [95%CI:3.801-6.251], and $\epsilon=0.999$ [95%CI:0.997-0.999](Table 1). Despite the reduced production of infectious virions, the mean loss of infected cells was estimated to be similar

as in the standard model (0.278/day vs 0.281/day for patient 1; 0.042/day vs 0.042/day for patient 2)(Table 1; S1). The new model can explain a time-to-cure within 24 days of treatment for patient 1 and 27 days of treatment for patient 2.

Discussion

Established HCV mathematical models were used to predict on treatment (real time) treatment duration needed to achieve cure with silibinin+RBV (Dahari et al., 2015), and retrospectively under approved DAAs (Dahari et al., 2016; Gambato et al., 2016). However, in the current study we showed that these models cannot explain two cases of cure after ultrashort (<4 weeks) DAA therapy. A rapid viral decline was observed in the two cases presented here (HCV RNA levels were <500 IU/mL by day 2 and 7, in patient 2 and 1, respectively). Similarly, a phase 2a study documented cure after 3 weeks of DAA (without ribavirin) therapy in all patients with HCV RNA levels<500 IU/mL by day 2 and suggested that established models failed to predict time-to-cure (Lau et al., 2016). We developed a new model to explain two ultra-short successful therapy cases that includes infectious (V_i) and non-infectious (V_{ni}) virions. The new model assumes that in addition to blocking V_i and V_{ni} production ($e \sim 0.998$), treatment further reduced the infectivity of secreted virus (parameter g 0.372/d) thus increasing the $\log(V_{ni}/V_i)$ ratio from 1 at baseline to 6 by EOT. A conceptual implication of the new model is that while HCV RNA (i.e., $V_i + V_{ni}$) was detected close to EOT (termed EOT+), V_i reached the cure boundary before EOT.

The main limitation to validating the hypothesized relative change in V_i and V_{ni} during DAA therapy (via parameter g in the model) is the inability to measure HCV infectivity (i.e. V_i) in patient samples. However, a reduction in secreted HCV infectivity under NS5A inhibitor treatment has been shown in vitro (Sansone et al., 2014) and proposed for RBV (Dahari et al., 2007; Dixit et al., 2004; Hofmann et al., 2007; Lutchman et al., 2007). In fact, parameter g in the model would be relevant not only to ribavirin and NS5A inhibitors, but to other drugs or endogenous immune responses that might directly or indirectly alter virion infectivity. For example, restoration of the host immune response (e.g. improved neutralizing antibody response), due to a significant decline in viral burden within 2 days after initiation of DAA treatment, might contribute to the predicted increase in V_{ni}/V_i ratio. As suggested for HIV (Conway and Perelson, 2015), prevention or reversal of T cell exhaustion might result from HCV viral decline under DAA therapy, contributing to viral suppression. Moreover, the host immune response could potentially also increase death/loss of HCV-infected cells (parameter δ in the model), although an increase in death/loss of HCV-infected cells would not increase V_{ni}/V_i ratio and thus could not explain cure in the 2 cases presented here. This scenario of enhanced death might lead to complete HCV clearance after treatment was stopped (Meissner et al., 2014; Veerapu et al., 2011) but this is difficult to model due to a lack of supporting data.

In conclusion, we developed a new mathematical model that addresses the fundamental relationship between infectious and non-infectious virions during DAA therapy and explains two cases of HCV cure after an ultra-short treatment (Hasin et al., 2016; Meissner et al., 2014). The new model has the potential to explain cases of DAA therapy in which established models have failed to predict cure (Lau et al., 2016; Nguyen et al., 2015).

Further studies are needed to confirm whether the new model with a cure boundary based on the eradication of infectious virus (rather than total virus) will be accurate in predicting the duration of DAA therapy needed to achieve cure and the outcomes of ultra-short treatment regimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Schematic representation of the extended model (Eq. 1)

In the figure, infected cells (I) produce both infectious virions (V_i) and non-infectious virions (V_{ni}). Only V_i infects target cells, T_0 , with constant rate β , generating infected cells, I , which produce new infectious and non-infectious virions at rate p_i and p_{ni} per infected cell, respectively. Both V_i and V_{ni} are assumed to be cleared at rate c per virion. DAA-containing regimens such as PODr inhibit HCV replication which effects both V_i and V_{ni} production with a constant effectiveness ϵ but under the effect of some DAAs +/- RBV, which alter progeny virion infectivity, there is a time-dependent reduction in secretion of infectious virions relative to non-infectious virions which is modelled through the term $\exp(-g^*t)$.

Figure 2. Extended biphasic model curve fits with measured viral kinetic data and predicted time-to-cure

Total viral load (Dashed line) was fit to the measured HCV RNA (Squares) with mean estimated infected cell loss/death rate of $\delta=0.281/\text{d}$ and $\delta=0.042/\text{d}$ for patients 1 and 2, who were treated with PODr+RBV and SOF+RBV, respectively. To bring the predicted infectious viral load (Solid line) to cross the cure boundary (7×10^{-5} IU/mL represented by horizontal dotted line) before PODr+RBV was stopped at day 24 and 27 for P1 and P2 respectively (vertical dotted line), minimum values of parameter g were estimated as $0.366/\text{d}$ and $0.404/\text{d}$ for patient 1 and patient 2 respectively. Pretreatment total viral load, V_{tot0} was at 2,000,000 IU/ml and 147,910 IU/mL for patient 1 and 2 respectively, as measured before either PODr+RBV and SOF+RBV therapy was initiated. Pretreatment infectious virus level $V_{tot0}/12$ was assumed in both patients based on empirically measured HCV specific infectivity in chronically infected chimpanzees as explained in Methods. Parameters $c=6/\text{d}$ and $\epsilon=0.998$ were kept fixed in patient 1 as explained in Methods while they were estimated at $4.875/\text{d}$ and 0.999 in patient 2.

Table 1

Estimated parameters of the extended model.

Parameter	Patient 1 (PODr+RBV) estimate [95% CI]	Patient 2 (SOF+RBV) estimate [95% CI]
δ	0.281 [0.229 0.343]	0.042 [0.005 0.374]
$\#_g$	0.366 [0.305 0.440]	0.404 [0.330 0.496]
c	6.0 *	4.875 [3.801 6.251]
ϵ	0.998 *	0.999 [0.997 0.999]

δ , HCV-infected cell death/loss rate [1/d]; c , HCV clearance rate in blood [1/d]; ϵ , drug efficacy in blocking HCV production; g , drug-induced increase rate in the ratio of non-infectious to infectious virus [1/d]; PODr, paritaprevir, ombitasvir, dasabuvir, ritonavir; RBV, ribavirin; SOF, sofosbuvir; CI, confidence interval

$\#$ minimal estimate

* due to lack of measured viral kinetic data sampling c and ϵ were fixed as noted in Methods.