Modelling clinical data shows active tissue concentration of daclatasvir is 10-fold lower than its plasma concentration

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Objectives: Daclatasvir is a highly potent inhibitor of hepatitis C virus. We estimated the active tissue concentration of daclatasvir in vivo.

Methods: We developed a mathematical model incorporating pharmacokinetic/pharmacodynamic and viral dynamics. By fitting the model to clinical data reported previously, we estimated the ratio between plasma drug concentration and active tissue concentration in vivo.

Results: The modelling results show that the active tissue concentration of daclatasvir is \( \sim 9\% \) of the concentration measured in plasma (95% CI 1%–29%).

Conclusions: Using plasma concentrations as surrogates for clinical recommendations may lead to substantial underestimation of the risk of resistance.

Keywords: hepatitis C virus, resistance, pharmacokinetics/pharmacodynamics, mathematical modeling

Introduction

Daclatasvir is a direct-acting antiviral agent that targets the non-structural protein encoded by the NS5A protein of hepatitis C virus (HCV).1 It reduces the viral load by 3–4 log in patients after 1–2 days of monotherapy.2 Combination therapies involving daclatasvir have achieved high cure rates in clinical trials.3–5 Despite these promising characteristics, resistance can be detected after as few as 3 days of monotherapy.2 Because direct measurement of drug efficacy in the liver is not feasible, the risk of resistance is often assessed based on the mutant resistance profile measured in vitro and the pharmacokinetics of daclatasvir measured in the plasma.1,6 However, the active tissue concentration, defined as the effective drug concentration acting at the site of infection,7 is affected by multiple factors in vivo and therefore is likely to differ from the plasma concentration. Ignoring this difference may lead to biased conclusions and harmful clinical recommendations.5 We present a modelling approach to assess this difference based on clinical viral load data and show that the active tissue concentration of daclatasvir is substantially lower than the plasma concentration.

Methods

HCV model and parameter values

We first estimate the efficacy of the drug in vivo. Before treatment, the viral population is at a high level at equilibrium. Mathematically, the viral load at the equilibrium, \( V_0 \), can be expressed as:\[ V_0 = \frac{p \cdot I_0}{c} \] (1)

where \( p \) is the production rate of virions from infected cells, \( I_0 \) is the equilibrium level of infected cells before treatment and \( c \) is the clearance rate of the virus.

After daclatasvir treatment begins, the HCV viral load declines in several phases.6,10 After 3–4 days of rapid decline (due to the clearance of free viruses), the viral population enters a quasi-equilibrium where the reduction in viral load is set by the clearance rate of infected cells (assuming the initial baseline strain remains dominant).10 The viral load during this phase, \( V^* \), can be expressed as \( V^*(t) = (1 - \bar{\epsilon}_{ave}) \cdot \frac{p \cdot I(t)}{c} \), where \( \bar{\epsilon}_{ave} \) is the
average drug efficacy in vivo and \( I(t) \) is the abundance of infected cells at time \( t \). In patients treated with a potent inhibitor, such as daclatasvir, the number of newly infected cells during the initial period of treatment is negligible. Hence, the population of infected cells declines exponentially: \( I(t) = I_0 \cdot \exp(-\delta \cdot t) \), where \( \delta \) is the natural death rate of infected cells.\(^9\) Thus:

\[
V^*(t) = (1 - \epsilon_{\text{ave}}) \cdot V_0 \cdot \exp(-\delta \cdot t)
\]

Taking the ratio of Equations (1) and (2), we obtain:

\[
\epsilon_{\text{ave}} = 1 - \Gamma(t) \cdot \exp(\delta \cdot t)
\]

where \( \Gamma(t) = V^*(t)/V_0 \). Using this equation, we can estimate the value of \( \epsilon_{\text{ave}} \) from viral load kinetics measured in clinical trials.

Next, we relate the estimated average drug efficacy to the measured pharmacokinetic parameters in the plasma. Here, we assume the active tissue concentration is proportional to the plasma concentration and we define \( \eta \) as the ratio of these two concentrations. The active tissue concentration between doses can be described as:\(^{11}\)

\[
C(t) = \begin{cases} 
\eta \cdot \left( C_{\text{min}} + \frac{(C_{\text{max}} - C_{\text{min}})}{\tau} \right) \cdot t & 0 < t < \tau \\
\eta \cdot C_{\text{max}} \cdot \exp(-w \cdot (t - \tau)) & \tau < t < T
\end{cases}
\]

where \( \tau \) is the time to reach peak concentration after taking a dose, \( C_{\text{max}} \) and \( C_{\text{min}} \) are the peak and trough concentrations measured in plasma, respectively, and \( T \) is the interval between doses. We calculate the decay rate of the drug, \( w \), as:

\[
w = \frac{1}{\tau} \cdot \log\left( \frac{C_{\text{max}}}{C_{\text{min}}} \right)
\]

The drug efficacy over time, \( \epsilon(t) \), is a function of the active tissue concentration, \( C(t) \), the cooperativity of the drug, \( h \) (\( h = 1 \) for daclatasvir; H. Qi, C. A. Olson, N. C. Wu, R. Ke, C. Loverdo, J. O. Lloyd-Smith and R. Sun unpublished results), and the half maximal effective concentration of the baseline virus as measured in vitro, \( EC_{50} \): \( \epsilon(t) = 1/(1 + EC_{50}/C(t))^{h} \). Adapting earlier results,\(^{11}\) we calculate the average drug efficacy, \( \epsilon_{\text{ave}} \), over a single dosing interval:

\[
\epsilon_{\text{ave}} = \frac{1}{T} \int_{0}^{T} \frac{1}{1 + EC_{50}/C(t)} \, dt = \frac{\tau}{T} \cdot \frac{1}{\frac{\tau}{T} + \frac{\tau \cdot EC_{50}}{w - \eta \cdot (C_{\text{max}} - C_{\text{min}})}} \cdot \log\left( \frac{EC_{50} + \eta \cdot C_{\text{max}}}{EC_{50} + \eta \cdot C_{\text{min}}} \right)
\]

Combining the two expressions of \( \epsilon_{\text{ave}} \) in Equations (3) and (5), we derive the relationship between the value of \( EC_{50} \), drug pharmacokinetics measured in plasma, and the viral load:

\[
1 - \Gamma(t) \cdot \exp(\delta \cdot t) = \frac{1 + \frac{1}{T} \cdot \left( \frac{\tau}{w - \eta \cdot (C_{\text{max}} - C_{\text{min}})} \right) \cdot \log\left( \frac{EC_{50} + \eta \cdot C_{\text{max}}}{EC_{50} + \eta \cdot C_{\text{min}}} \right)}{\eta \cdot \exp(-\delta \cdot t)}
\]

This equation enables us to estimate the ratio, \( \eta \), numerically using pharmacokinetic parameters (including \( C_{\text{max}}, C_{\text{min}}, T \) and \( \eta \) reported by Nettles et al.,\(^6\)) and the viral load at day 4 of treatment (\( t = 4 \)) from a subset of patients (Patients E, G, J and N) in Fridell et al.,\(^{2}\) (see Table 1 for the values used). We used the viral load at day 4 of treatment in these four patients to ensure that the viral load has reached quasi-equilibrium (>3 days) and that the baseline virus (which is wild-type in these four patients) is still dominant.\(^{10}\) At timepoints before day 4, the viral dynamics are still dominated by transients and in other patients reported by Fridell et al.,\(^{2}\) resistant mutants have risen to high frequency by day 4; these factors are not considered in the model, so we restrict the data accordingly. Other parameter values used in the estimation are \( \delta = 0.14 \text{ day}^{-1} \), \( T = 1 \text{ day} \) and \( \tau = 1.5 \text{ h} \).\(^{6,9}\) The overall estimate of \( \eta \) is calculated by averaging the values estimated based on data from individual patients.

**Uncertainty analysis**

To derive the 95% CI for \( \eta \) for each patient, we re-estimated \( \eta \) from 10 000 parameter sets sampled randomly from plausible ranges of values and report the appropriate percentile values. Assumed ranges for the values of \( EC_{50} \) and the pharmacokinetic parameters are shown in Table 1; \( \delta \) is sampled from a triangular distribution from 0.01 to 0.27 with mode at 0.14 day\(^{-1}\)\(^{9,12}\) and \( \tau \) is sampled from a triangular distribution from 1 to 2 with mode at 1.5 h.\(^6\)

**Predicting resistance**

We define a mutant as resistant if its reproductive number under drug treatment, \( R_{0,\text{drug}} \), is \( >1 \), where \( R_{0,\text{drug}} \) can be expressed as:

\[
R_{0,\text{drug}} = (1 - \epsilon_{\text{ave}}) \cdot W_{\text{mut}} \cdot R_{0,\text{WT}}
\]

where \( \epsilon_{\text{ave}} \) is the average efficacy of the drug against that mutant, which can be calculated by substituting the \( EC_{50} \) of the

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genotype</th>
<th>( EC_{50} ), nM (SE)</th>
<th>Treatment</th>
<th>( C_{\text{max}}/C_{\text{min}} ), nM (SD)</th>
<th>Log(_{10} ) ( \eta )</th>
<th>Estimated ( \eta ) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>1a</td>
<td>0.0059 (0.0038)</td>
<td>10 mg once daily</td>
<td>216/20.4 (88.6/10.0)</td>
<td>-3.2</td>
<td>0.098 (0.02, 0.27)</td>
</tr>
<tr>
<td>G</td>
<td>1b</td>
<td>0.0026 (0.0009)</td>
<td>10 mg once daily</td>
<td>216/20.4 (88.6/10.0)</td>
<td>-3.6</td>
<td>0.109 (0.06, 0.25)</td>
</tr>
<tr>
<td>J</td>
<td>1a</td>
<td>0.0059 (0.0038)</td>
<td>30 mg once daily</td>
<td>653/55.5 (163/18.9)</td>
<td>-3.1</td>
<td>0.028 (0.01, 0.07)</td>
</tr>
<tr>
<td>N</td>
<td>1a</td>
<td>0.0059 (0.0038)</td>
<td>60 mg once daily</td>
<td>1902/176 (247/44.0)</td>
<td>-4.3</td>
<td>0.142 (0.02, 0.31)</td>
</tr>
</tbody>
</table>

Mean of estimated \( \eta \) (95% CI): 0.094 (0.01, 0.29).

\(^a\)Data collected from Nettles et al.,\(^6\)

\(^b\)Data collected from Fridell et al.,\(^2\)
Table 2. Comparison of clinical data on resistant mutants and the probabilities of resistance predicted by two models assuming $\eta = 0.094$ or $\eta = 1$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dominant resistant mutants observed in the clinical trial reported by Fridell et al.$^2$</th>
<th>Genotype</th>
<th>Patient(s)</th>
<th>Probability of resistance $\eta = 0.094$</th>
<th>Probability of resistance $\eta = 1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg once daily</td>
<td>Y93H$^a$</td>
<td>1a</td>
<td>E</td>
<td>0.684</td>
<td>0.236</td>
</tr>
<tr>
<td></td>
<td>L31V</td>
<td>1a</td>
<td>F</td>
<td>0.999</td>
<td>0.947</td>
</tr>
<tr>
<td></td>
<td>L31M + Y93H</td>
<td>1b</td>
<td>G</td>
<td>0.669</td>
<td>0.499</td>
</tr>
<tr>
<td></td>
<td>L31V + Y93H</td>
<td>1b</td>
<td>G</td>
<td>0.720</td>
<td>0.572</td>
</tr>
<tr>
<td>30 mg once daily</td>
<td>Q30E</td>
<td>1a</td>
<td>I, J, K</td>
<td>0.933</td>
<td>0.893</td>
</tr>
<tr>
<td></td>
<td>Y93H$^a$</td>
<td>1a</td>
<td>J</td>
<td>0.556</td>
<td>0.021$^b$</td>
</tr>
<tr>
<td>60 mg once daily</td>
<td>Q30H + Y93H$^a$</td>
<td>1a</td>
<td>M</td>
<td>0.869</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>M28T</td>
<td>1a</td>
<td>N</td>
<td>0.006$^b$</td>
<td>0.000$^b$</td>
</tr>
<tr>
<td></td>
<td>Q30E</td>
<td>1a</td>
<td>N, O</td>
<td>0.927</td>
<td>0.782</td>
</tr>
<tr>
<td></td>
<td>Q30R</td>
<td>1a</td>
<td>P</td>
<td>0.102</td>
<td>0.000$^b$</td>
</tr>
<tr>
<td>100 mg once daily</td>
<td>M28T</td>
<td>1a</td>
<td>R</td>
<td>0.0005$^b$</td>
<td>0.000$^b$</td>
</tr>
<tr>
<td></td>
<td>Q30R + H58D</td>
<td>1a</td>
<td>S</td>
<td>1.00</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>L31V + Q54H + Y93H$^b$</td>
<td>1b</td>
<td>T</td>
<td>0.981</td>
<td>0.113</td>
</tr>
</tbody>
</table>

$^a$Bold mutant names denote mutants for which the predicted probabilities of resistance are substantially different between the two models ($>0.4$ in probability).

$^b$Predicted probabilities of resistance $<5\%$.

Results and discussion

Based on viral load data from clinical trials and pharmacokinetic parameters from plasma,$^{2,6}$ we estimated the ratio of the active tissue concentration to the plasma concentration of daclatasvir, $\eta$, to be $0.094$ (95% CI 0.01 – 0.29; Table 1). This estimation is consistent across different dosing regimens (Table 1). Thus, the active tissue concentration of daclatasvir in the liver is much lower than the concentration measured in plasma, contrary to common assumptions.$^{1,5}$ To test our method, we used the model to predict resistant mutants in vivo based on resistance profiles measured in replicon systems for HCV genotypes 1a and 1b.$^{2,6}$ In general, the model predictions agree well with clinical data (Table 2). Almost all mutants that appeared as dominant resistant mutants in clinical trials were correctly predicted by the model, with the exception of M28T (probability of resistance $<5\%$). M28T has a low value of $EC_{50}$ in vitro,$^2$ but it appeared as resistant in two patients treated with 60 mg and 100 mg once daily regimens. This discrepancy may arise from additional differences in the viral genomes or the difference between the replicon system and in vivo conditions. Several other mutants were predicted to be resistant, but did not rise to high frequencies in clinical trials. Again, this could arise from artefacts of replicon systems or the mutants could be resistant in vivo but remain at low frequencies due to competition from other resistant mutants with higher replicative fitness, such as Q30E in genotype 1a.

If we assumed no difference between the active tissue concentration and the plasma concentration (i.e. assumed $\eta = 1$), the model underestimated the resistance potential for numerous mutants (Table 2). Two resistant mutants identified in clinical trials, Y93H and Q30R, were predicted to have very low probabilities of resistance. Also, the model substantially underestimated the probability of resistance for two mutants, Q30H + Y93H and L31V + Q54H + Y93H, compared with predictions assuming $\eta = 0.094$.

Altogether, these results suggest that the active tissue concentration for daclatasvir is ~10-fold lower than its plasma concentration. There are several possible reasons for this low active concentration: (i) the drug may not penetrate well into liver tissue or hepatocytes; (ii) the drug may be bound by proteins or other chemicals in the liver; (iii) the conformation or local environment of NSSA is different, which may reduce the accessibility or affinity to the drug; and (iv) heterogeneities in the distribution of drug and/or virus in the liver may cause infected cells to be exposed to lower drug concentrations.$^7$ Neglecting this difference can lead to substantial underestimation of the resistance potential of mutant viruses. Another possibility is that the $EC_{50}$ measured in vitro differs from the $EC_{50}$ in vivo, though this has not been found for other drugs.$^{13–15}$ If this was the case, then $\eta$ can be interpreted as a composite parameter incorporating both the difference in $EC_{50}$ and tissue concentration of daclatasvir in the liver is much lower than

...
and the difference in tissue versus plasma concentrations; this does not alter its importance when assessing resistance risk using EC50 measured in vitro and drug concentration measured in plasma.

Our work highlights the importance of estimating the active therapeutic concentration to make accurate predictions about the resistance profile of a drug. We have used a modelling approach, including uncertainty analyses to account for the challenge that pharmacokinetic data are not available from the same patients as virological data. Future studies would be strengthened by datasets that collect all pertinent information for the same individuals. We believe this method is also applicable to other highly potent antiviral drugs for which the quasi-equilibrium state is reached before resistant strains are selected to significant frequency. However, for certain drug classes, such as drugs that act by blocking viral entry, the approach to quasi-equilibrium may be too slow and alternative methods will be needed to assess active tissue concentrations.

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**Transparency declarations**

None to declare.

**References**