

HCV

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After the success shown by mathematical models on HIV, in the late '90 scientists have applied the same approach to the recently discovered Hepatitis C virus. In an article appeared on Science in 1998, Perelson and collaborators, present a model of HCV kinetics, which successfully predicts the observed biphasic behavior of viral load reduction of HCV in the first two weeks since the beginning of interferon therapy.

The same approach is also adopted by Zeuzem and his group in Germany. They also describe viral kinetics by means of simple mathematical models, which don't look at the details of the specific interactions, but only at the effective presence of the various components of the system. Their results are in agreement with the ones found by Perelson.

Since these first models appeared the therapies to treat HCV have evolved. The standard therapy used now involves the presence of a second component, ribavirin, to make a combination therapy. Also, modified versions of interferon have been introduced that make it more resistant to enzymatic digestion by the host cell. These other elements, even though essential in the economy of the therapy, as shown by a greatly improved success rate of combination therapy, over regular interferon therapy, do not seem to modify the general kinetics scheme. Their effect has more to do with the specific values assumed by the various parameters. Therefore the model introduced in 1998 can still be considered applicable also to combination therapy, making the necessary adjustments in the parameters.

1 Some facts about Hepatitis C virus

Hepatitis C is a slowly progressive liver disease that can lead to cirrhosis and/or hepatocellular cancer over a period of 10 to 20 years from the first encounter with the virus.

Of the people who get in contact with HC virus, about 30% will develop major liver failure, 55% will develop a mild chronic liver inflammation, more or less asymptomatic, while the remaining 15% will win over the virus in a time period of a few months from its contraction, and become HCV-negative on their own.

Interferon therapy and interferon + ribavirin combination therapy are available, but only show at most a 50% success rate in permanently debelling the virus. The effectiveness of the therapy is strongly dependent upon the virus genotype, and is related to the virus concentration at the beginning of the therapy.

The virus has an RNA genome of about 10,000 nucleotides in length. This genome encodes for a polyprotein composed of at least 10 discrete polypeptides. Key structural features of this RNA include two large stem-loops and a pseudoknot that involves parts of the sequence near the initiator codon. In order to initiate translation a stem loop must unfold allowing the RNA to form a high affinity complex with the host ribosome. Via this process the AUG initiator codon of the virus is positioned near the ribosome decoding site and protein synthesis may begin.

HCV is found to exist in several different genotypes, and inside a single organism it can differentiate enough to escape the attack of the host immune system. Its RNA replication is error prone because of the lack of proofreading by the viral RNA polymerase. The increased viral diversity during acute phases of hepatitis, has been found to be associated with progression to chronicity, where the organisms that are able to clear the virus show a stable population (in the sense of the strains present).

HCV replicates primarily in hepatocytes, and has a production rate of about 10^{12} particles/day. Its half-life is estimated to be around 2 or 3 hours, while the half-life of infected cells is in the range of 2 - 3 days.

Given the relatively rapid turnover of hepatic cells, it is believed that the progress of the disease is due to *de novo* infection of new hepatic cells.

2 Modelling the infection

In the infection process there are three kind of cells involved: the virus cells, the active infected cells which produce new virus, and the target hepatocells which get infected by the virus.

We will indicate with V the viral load, with T the number of target cells, and with

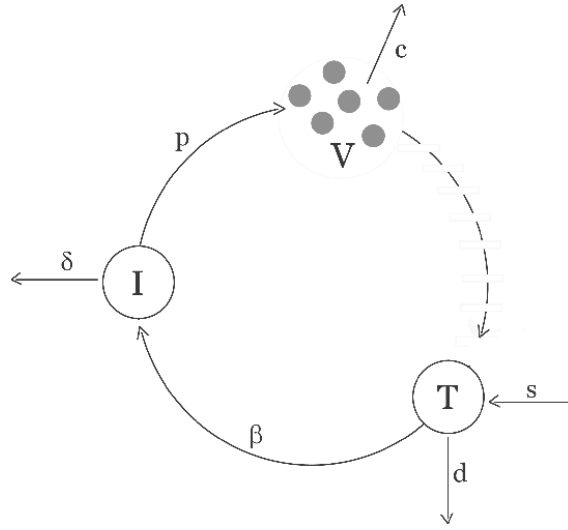


Figure 1: Scheme of HCV infection cycle

I the number of active infected cells. The following system of differential equations can be written:

$$\frac{dT}{dt} = s - dT - \beta VT \quad (1)$$

$$\frac{dI}{dt} = \beta VT - \delta I \quad (2)$$

$$\frac{dV}{dt} = pI - cV \quad (3)$$

where

- s : rate of production of target (T) cells
- d : death rate of target (T) cells
- β : rate at which cells become infected with de novo infection
- δ : death rate of infected cells
- p : rate of virions production, per cell, per day
- c : clearance rate of virions

This system allows for two stationary points at

$$(a) \quad V = 0 \quad I = 0 \quad T = \frac{s}{d}$$

$$(b) \quad V = \frac{ps}{c\delta} - \frac{d}{\beta} \quad I = \frac{s}{\delta} - \frac{cd}{p\beta} \quad T = \frac{c\delta}{p\beta}$$

The first case corresponds to the situation when the host organism wins over the virus, while the second case corresponds to a chronicization of the disease in which the number of uninfected hepatocells, settles to a value directly proportional to the death rate of both virions and infected cells, and inversely proportional to the number of virions produced per infected cells, and on their infective ability (that is how many target cells are turned into infected cells in the presence of virions).

We now analyze the stability of the two stationary solutions. We need to determine the eigenvalues of the matrix

$$\begin{pmatrix} -(d + \beta V_0) & -\beta T_0 & 0 \\ 0 & -c & p \\ \beta V_0 & \beta T_0 & -\delta \end{pmatrix}$$

the eigenvalues satisfy the equation:

$$(d + \beta V_0 + \lambda)(c + \lambda)(\delta + \lambda) = \beta p T_0 (d + \lambda) \quad (4)$$

Negative eigenvalues mean that the particular stationary point considered is stable along the direction of the corresponding eigenvector, while positive eigenvalues are characteristic of instability of the stationary point. In order to clear the virus, the ideal situation would see the three eigenvalues at (a) all negative, and the three eigenvalues at (b) all positive, so that the solution with $V = 0$ is a true stable point, while the solution at $V > 0$ is completely unstable.

Substituting V_0 and T_0 from solution (a), we find that 2 of the 3 eigenvalues are always negative, while the third one is positive if

$$\frac{p\beta s}{d} > c\delta$$

that is, if the death rate of both virions and infected cells are not large enough with respect to the rate of spreading of the infection.

From this condition one sees that good features to win the virus are small number of virions produced per cell, small infectivity of the virions, which depend on the virus, but also small production of hepatic cells and large death rate of hepatic cells, which

depend on the host organism. Obviously, the last two conditions are good in order to clear the virus, but not so good for the overall economy of the organism.

Also, a large death rate of infected cells and of the virions are needed in order to clear the virus. So one can predict that organisms which have a short half-life of hepatic cells would be more prone to clear the virus on their own. Indeed this is found also experimentally. Patients with are able to clear the virus are the one for which the death rate δ is faster.

Experimentally δ is found to be directly correlated with the transaminases ALT level, and inversely correlated with the initial viral load. ALT is an indicator of the activity of the immune system. When cytotoxic T cells are active in killing infected cells the ALT level raises. The interpretation of ALT values in the prognosis of the disease is still controversial though, since very high ALT are indicators of an acute infection but witness a high immune response, while low ALT are indicative of a low immune response that can be due both to a very mild infection or to a very poor immune response to a high infection.

For the second stationary point (b) it is less instructive to explicitly write down the three eigenvalues, since the equation to solve is a complete cubic equation. Numerically one can see that two of the three eigenvalues are negative over a very large range of the parameters, and that the third eigenvalue can change from negative to positive. In particular, increasing s , p , and β one at the time, keeping all other parameters fixed, has the effect of lowering the most negative eigenvalue, making the high-virus solution more stable (the virus wins). On the other hand, by increasing d , δ or c , the highest eigenvalue can go from negative to positive, destabilizing the high-virus solution, eventually going in favor of the zero-virus solution.

3 Therapies

The standard therapy against HCV is done through the administration of Interferon Alpha, in elevated and frequent doses for long periods of time (daily or 3 times a week injections of 10 mIU of IFN- α -2b for 48 weeks). Under this regime it is seen that the viral load decreased significantly in the first 2 days from the beginning of the treatment, and then progresses into a less rapid descent for the remaining time up to 14 days, and maybe even slower descent thereafter (this is not well documented though).

The viral load reduction is a double exponential decay with a higher slope at the beginning of the therapy. This decay is dose dependent, especially at the beginning of the treatment. After two days from the first injection the patients which are given higher doses of IFN show a larger viral load reduction than patients undergoing a less

strong regimen.

The amount by which the viral load decreases in the initial part of the treatment seems to be significant for the success of the therapy and the final elimination of the virus. Patients which show the largest drop are more likely to clear the virus after a few months of therapy.

The first phase slope does not seem to be correlated with baseline viral load or with baseline ALT, while the second phase slope is found to be inversely correlated with baseline ALT levels, and therefore with the death rate of the infected cells. Patients with high δ during the first 2 weeks of treatment are likely to be found clear from the virus after 3 months of therapy, while patients with low δ are most likely still virus positive after the same time.

Moreover, it is found that failure of IFN treatment is associated with large quasi-species diversity. The ability of HCV to easily mutate could be responsible for the failure of the therapy. An aggressive first stage of the treatment should reduce the chance of the virus to mutate, and therefore should improve the success rate of the therapy.

The specific mechanism of the interaction of interferon with the virus and with the host organism is still not clear. One possible explanation is that interferon might block the ability of HCV to infect new cells. If this were true the rates of viral release and clearance (p and c) would not be affected by IFN. Only the number of infected cells would change under IFN. To see a major impact of the drug we would then have to wait until after at least one I half-life, that is a few days. This is definitely not the case since the major changes occur in a time of just a few hours from the injection.

A more plausible explanation of IFN action is that it prevents infected cells from releasing new virus. This action could be carried both at the level of viral RNA production and at the level of viral proteins production.

At a molecular level IFN is known to activate double-stranded RNA-activated protein kinase and other pathways known to inhibit viral production.

A second generation of therapy is a combination therapy of IFN and Ribavirin. This therapy proves to be particularly effective in IFN resistant patients, bringing the success rate from 20% to about 50% for genotype 1b patients (most interferon resistant genotype).

Ribavirin is a purine nucleoside analog, introduced in the '70s for treatment of severe respiratory virus infections. It has a broad spectrum of antiviral activities against various RNA and DNA viruses. The exact mechanism of its action, both alone and in combination with interferon, is still unclear. There are currently three hypothesis on how it could work: (1) it has a direct antiviral activity targeting the viral polymerase, (2) it has beneficial effect through indirect mechanisms which could include enhancement of cytokines and cytotoxic T lymphocytes response, (3) it acts as a mutagen,

increasing the probability of mutation of the virus in a dose dependent manner, which could lead to an “error catastrophe” and diminish the fitness and infectivity of the virus.

Trial studies of therapies with both IFN and ribavirin show the same kind of general behavior than therapies with interferon alone. The major difference is in the slope of the second phase of the decline, which is greater in the presence of ribavirin, and which seems to be dose dependent. The action of ribavirin could then be related to the half-life of the infected cells, which could well be the case if ribavirin enhances the organism immune response, effectively changing the value of δ .

A third generation of therapies sees the introduction of a modified interferon which lives longer in the cell. This has the advantage of reducing the number of injections to once per week and to keep a steady level on interferon for longer periods of time. This is accomplished by combining the regular IFN- α with a pegylated protein, from which the name “peginterferon α ”. The overall qualitative action of this molecule is not different from the action of regular IFN.

In recent trial studies with peginterferon and ribavirin a new behavior of the system has appeared. After 48 hours from the first injection there is a rebound of viral load. The viral load steeply decreases for the first 2 days and then suddenly increases, or levels off, in the best case scenario. This increase seems to be related with the dose of interferon and is greater with a higher dose. Also, this rebound is not related to the clearance of the virus at the end of the therapy. The significance of this rebound is still unclear, and the models currently used to describe the kinetics of the virus are not able to predict it. It is also unknown if there is a specific effect related to ribavirin or to peginterferon, they may increase a phenomenon previously undetected.

4 Modelling IFN therapy

Mathematical models introduced in the literature so far consider the effect of interferon without entering in the specificity of its interactions. There is therefore no point in distinguishing between regular IFN or peginterferon, since the interferon molecule is not represented directly as one of the active players in the system, but it is only considered indirectly through the effect it has on the system parameters.

I have not found mathematical models including the effect of ribavirin, but the same reasoning applied to interferon could be applied to ribavirin, just by considering its effects on T , I , and/or V .

Let’s consider the model of the infection presented before. The possible effects of IFN in this model are either to reduce the production of virions from infected cells by a fraction $(1 - \epsilon)$ or to reduce the de novo rate of infection by a fraction $(1 - \eta)$.

The set of differential equation that describe the system become:

$$\frac{dT}{dt} = s - dT - (1 - \eta)\beta VT \quad (5)$$

$$\frac{dI}{dt} = (1 - \eta)\beta VT - \delta I \quad (6)$$

$$\frac{dV}{dt} = (1 - \epsilon)pI - cV \quad (7)$$

Before the therapy $\epsilon = \eta = 0$. Once the therapy is initiated $\epsilon > 0$ or $\eta > 0$ or both. As we mentioned before, the major effect of IFN is thought to be to block the production or release of the virions from the infected cells, and not to block new HCV infections. Accordingly, we will no longer consider the case $\eta \neq 0$, but we will only consider $\epsilon > 0$, $\eta = 0$.

The value of ϵ is dose dependent, higher for higher doses. Since the blocking by IFN is not perfect ϵ will always be strictly less than one.

Through equations (5), (6), and (7) we want to try to understand the biphasic behavior in viral load drop, found experimentally in patients that start IFN therapy.

We assume that before the beginning of the treatment both the viral load and the number of infected cells are in their stationary state (b) (steady values for over 6 months), at V_0 , and I_0 respectively.

Since the behavior we are interested in explaining takes place over a period of time shorter than the half-life of the infected cells, for the moment we will consider $I(t) = I_0$, and $T(t) = 0$. Having done so, the three equation decouple into two set of two equations, one for T and I, and one for V and I. We are interested in the second pair and in particular to the equation for V

$$\frac{dV}{dt} = (1 - \epsilon)pI_0 - cV \quad (8)$$

which has as solution

$$V(t) = V_0 \left[1 - \epsilon \left(1 - e^{-c(t-t_0)} \right) \right] \quad (9)$$

for $t > t_0$, where t_0 is the time at which the interferon begins to act, which is after a few hours from its injection.

The solution is one of an exponential decay. It starts from the initial value V_0 and decays with slope $c\epsilon$. Given that IFN is not 100% effective, $\epsilon < 1$, and the viral load stabilizes at $(1 - \epsilon)V_0$ for $t \gg 1/c$. If ϵ was equal to one the decay would continue and would bring V down to zero. From this solution we see how the steep part of the descent depends on the clearance rate c at which the virus subtracted from the

system.

The dependence of this first phase decline from the amount of IFN used in the therapy is reflected in its efficacy ϵ . In the medical trials ϵ was estimated to be equal to 85% for 5 mIU regimen, 95% for 10 mIU regimen, and 96% for 15 mIU regimen.

Over time periods longer than a few days, that is longer than infected cells half-lives, we can no longer assume $I(t)$ to be constant. We can still assume that the hepatocells turnover is slow, and therefore consider T to be constant at T_0 over a period of 2 weeks.

Under these assumptions the system of equations becomes

$$\frac{dI}{dt} = (1 - \eta)\beta T_0 V(t) - \delta I(t) \quad (10)$$

$$\frac{dV}{dt} = (1 - \epsilon)pI(t) - cV(t) \quad (11)$$

For $t > t_0$ the solution for the viral load is

$$V(t) = V_0 \left(A e^{-\nu_1(t-t_0)} + (1 - A) e^{-\nu_2(t-t_0)} \right) \quad (12)$$

where

$$\nu_{1,2} = \frac{1}{2} \left(c + \delta \pm [(c - \delta)^2 + 4(1 - \epsilon)c\delta]^{1/2} \right)$$

$$A = \frac{\epsilon c - \nu_2}{\nu_1 - \nu_2}$$

Indeed we have found the double exponential decay in viral load reported by medical trial.

The coefficient ν_1 dominates the behavior at small times, while ν_2 dominates at larger times. If IFN was 100% effective, we will have $\nu_1 = c$ and $\nu_2 = \delta$, from which we see the importance of the value of δ for completely clearing the virus in the long run. This finding from the mathematical model indeed agrees with the experimental evidence reported in the previous section.

In general, for values of ϵ less than one, the two slopes will depend both on c and δ . For values of ϵ close to one, we can assume $(1 - \epsilon)$ to be small and perform an expansion of the square root in the definition of $\nu_{1,2}$.

We find

$$\nu_1 \sim c \left(1 + \frac{\delta(1 - \epsilon)}{c - \delta} \right)$$

$$\nu_2 \sim \delta \left(1 - \frac{c(1 - \epsilon)}{c - \delta} \right)$$

We clearly see that one of the decay rates is essentially the virion clearance rate c . In agreement with the discussion above for $t < 2$ days, and with our knowledge that that $c > \delta$, given that the the clearance of the virus is quicker than the death of the infected cells, ν_1 dominates the first part of the decay. The slope of the second part of the decay is essentially the infected cells death rate δ .

This dependence of $V(t)$ on δ is well in accordance with the findings that the patients who win over the virus are the one for which δ assumes the larger values. Larger death rates for the infected cells will bring the viral load to small values more quickly, leaving less chances for the virus to mutate and to start to proliferate again.

In this framework the effect of ribavirin was not investigated. Given how researchers think ribavirin might behave, we could think that its effect would be to increase the value of ϵ , since, as IFN, it acts in impairing virus production. This assumption would agree with the findings of steepest slopes at low interferon doses found in clinical studies on combination therapies.

The model presented here only accounts for the virus kinetics in the first two weeks of therapy. Models that want to describe longer times, must take into account the time dependence for T as well, and solve the full set of three differential equations.

Medical studies on long periods of time have not shown any significant change of behavior after two weeks from initiation of IFN assumption. So, at this low level of detail it is probably not necessary, nor too instructive, to solve the full system.

It would be probably more interesting to try to understand the dose dependent rebound that a 20% of patients experience after 48 hours from the beginning of interferon treatment, since its understanding might bring light on more specific behavior of IFN. The fact that not all patients experience the rebound make it possibly related with the infected cells half-life, which is the parameter that mostly changes from one patient to another since it depends directly on the organism immune response. One may therefore speculate that there could be an interaction between IFN and elements of the host immune system. It would than probably be significant to include also lymphocytes in the model, especially since it is known that HCV can also infect them, even though in smaller percentages than hepatocells, and because they are the one responsible for killing of infected cells, and therefore ultimately determine the specific value of δ .

5 Possible new therapies

New strategies to win over HCV infection, focus on inhibiting viral replication.

A first kind of approach consists in targeting the virus genetic code. A technique involves hybridization of viral RNA with synthetic oligonucleotides of 15 to 40 bases

in length. Such compounds can inhibit protein expression through a variety of mechanisms, depending on what part of viral RNA the synthetic RNA inserts in. Other techniques are focused on inhibiting replication by cleavage of the HCV “internal ribosome entry site”.

A second approach involves targeting the viral protein themselves, instead than the HCV genetic code. One attractive target is NS3 protein, whose enzymatic activities are required for HCV replication in vivo. Also other proteins are considered, like HCV helicase whose movement along RNA is critical for the unwinding of the double helix during replication.

A third kind of therapy consists in treatments whose goal is not the complete elimination of the virus from the host organism, but is to minimize the damage that it causes, in particular to minimize liver fibrosis. These would be long-term therapies for those patients who do not respond to interferon therapy oriented to virus clearance. For this purpose interferon itself seems to be efficient in reducing inflammation and into slowing down the progress of the disease. Also ribavirin by itself has the positive effect of reducing necroinflammatory activity, and lowering transaminases levels.

References

- [1] A. Neumann, N. Lam, H. Dahari, D.Gretch, T. Wiley, T. Layden, A. Perelson, *Science* **282**, pp. 103-107 (1998)
- [2] A. Perelson, *The American Journal of Medicine* **107**, pp.49S-52S (1999)
- [3] F. Bekkering, C. Stalgis, J. McHutchison, J. Brower, A. Perelson, *Hepatology* **33**, pp.419-423 (2001)
- [4] M. Buti, F. Sanchez-Avila, Y. Lurie, C. Stalgis, A. Valdés, M. Martell, R. Esteban, *Hepathology* **35**, pp.930-936 (2002)
- [5] J. Layden, T. Layden, *Hepatology* **35**, pp.967-970 (2002)
- [6] A. Di Bisceglie, J. McHutchison, C. Rice, *Hepatology* **35**, pp.224-231 (2002)
- [7] B. Kronenberg, B. Rüster, J. Lee, C. Sarrazin, W. roth, G. Herrmann, S. Zeuzem, *Journal of Hepatology* **33**, pp.640-647 (2000)
- [8] S. Zeuzem, J. Schmidt, J Lee, M. von Wagner, G. Teuber, W. Roth, *Hepatology* **28** pp.245-252 (1988)