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Modeling Hepatitis C Virus Therapies Combining Drugs and Lectin Affinity Plasmapheresis

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Key Words
Hepatitis C virus · Lectin affinity · Hemodialysis · Plasmapheresis · Mathematical models · *Galanthus nivalis* agglutinin

Abstract
Hepatitis C virus (HCV) infection can be cured by standard pegylated interferon (IFN) + ribavirin drug therapy in 30–50% of treatment-naïve genotype 1 HCV patients. Cure rate is defined as a sustained viral response measured 6 months after the end of treatment. Recently, Fujiwara et al. [Hepatol Res 2007;37:701–710], using a double-filtration plasmapheresis (DFPP) technique, showed that simple physical reduction in circulating HCV using a 1-week pretreatment increased the cure rate for treatment-naïve type 1 HCV patients from 50 (controls) to 78% (treated). For previous nonresponders, the cure rate increased from 30 to 71%. This effect occurs even though the DFPP per treatment HCV viral load reduction averaged 26%. In clinical studies discussed here, a lectin affinity plasmapheresis (LAP) device caused an estimated 41% decrease in viral load as previously reported. A more detailed analysis using normalized data to correct for any variations in initial viral load gave an average 29% per treatment viral load reduction in 5 HCV-positive dialysis patients. The latter data indicate that continuous application of LAP could bring HCV viral load to undetectable levels in 4.1 days. Compared to DFPP, the LAP approach has the advantage that no plasma losses are incurred. In addition hemopurification can be carried out for extended periods of time analogous to continuous renal replacement therapy for the treatment of acute kidney failure, making the process much more effective. Calculations based on these data predict that continuous hemopurification would substantially increase the rate of viral load reduction (approx. 14-fold) and therefore increase the cure rate for HCV standard-of-care drug therapies without adding additional drugs and their associated side effects.

Introduction

Hepatitis C virus (HCV) infections currently affect more than 3.9 million people in the USA [1] and over 180 million worldwide. The virus is responsible for liver damage that drives much of the need for liver transplants in the USA and Europe.

The current standard of care for HCV infection uses pegylated interferon (IFN-α_{2b}) combined with ribavirin [2]. The therapy is curative in about 40% of patients [3]. According to the World Health Organization, only 30–50% of infected patients respond to pegylated IFN + ribavirin treatment after a 48-week course of therapy. Cure

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rates are related to both viral and human genetics [4]. There are 6 HCV genotypes and more than 50 subtypes. Genotype 1 accounts for 70–75% of all HCV infections in the USA and is associated with a 50% response rate to drug therapy. Genotypes 2 and 3 are more common in Asia and are more responsive to drug therapy [2], with genotype 2 having the best response rate at more than 80%.

The principal problem with the current standard of care is that a majority of patients suffer substantial adverse effects from IFN that can limit patient compliance or cause people to avoid therapy altogether. The side effects include influenza-like symptoms, hematological abnormalities and neuropsychiatric symptoms [2]. Present research in this area is aimed at the development of new, more powerful drugs that can inhibit the action of viral proteins such as the RNA polymerase. Other potential targets include the viral envelope (entry inhibitors) and viral proteases.

We have been developing an alternative strategy using a highly selective extracorporeal filtration therapy analogous to hemodialysis [5, 6]. This therapy combines plasmapheresis with affinity capture using lectins. Lectin affinity plasmapheresis (LAP) has been used in vitro and in clinical trials to rapidly and selectively clear viruses from blood and plasma [7].

In a recent clinical trial, hemodialysis patients infected with HCV were treated with a lectin affinity cartridge in combination with their kidney dialysis treatment [7]. Four patients received up to 3 four-hour treatments 3 times weekly. In a follow-up case study, 1 of these patients received extended treatment consisting of 12 four-hour treatment sessions on the same schedule. The LAP device caused an estimated 41% decrease in viral load in the initial studies. However, using the current data normalized to correct for variations in initial viral load gave an average 29% per treatment viral load reduction in 5 HCV-positive dialysis patients (table 1). As shown here, the predictions based on this result indicate that LAP in combination with drug therapy could reduce HCV viral load to undetectable levels in approximately 4 days, providing a substantial increase in cure rates relative to drugs alone.

Table 1. HCV clearance values in patients

	Average HCV, IU/ml		Change %
	before	after	
Fortis study 1 dialysis controls			
C1	7.90×10^7	1.49×10^7	–81
C2	8.96×10^7	4.60×10^7	–49
C3	4.00×10^7	3.70×10^7	–91
Fortis study 2 dialysis controls			
C1	1.64×10^6	5.82×10^6	256
C3	2.30×10^6	4.23×10^6	84
Mean			24
Fortis study 1 LAP treatment + dialysis			
T1	2.62×10^7	3.03×10^7	16
T2	3.72×10^7	1.86×10^7	–50
T3	4.41×10^7	1.43×10^7	–68
Fortis study 2 LAP treatment + dialysis			
T1	2.64×10^6	7.30×10^5	–72
T3	2.45×10^6	4.50×10^5	–61
T4	2.30×10^6	4.23×10^6	48
T6	2.03×10^6	1.10×10^6	–46
T7	2.48×10^6	3.64×10^6	46
T9	7.79×10^6	4.21×10^6	–46
T10	5.87×10^6	4.48×10^6	–24
T12	2.91×10^6	1.02×10^6	–65
Mean			–29

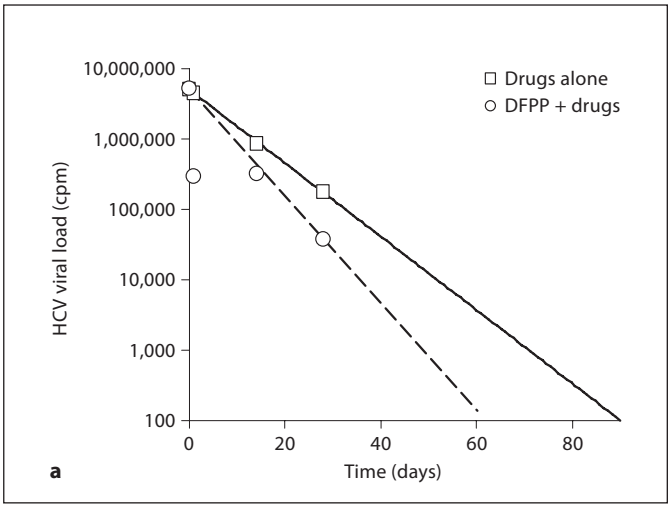
Fortis study 1, 2 = Study 1, entitled 'Controlled, Sequential, Phase I Safety Study to Evaluate the Use of the GNA Hemopurifier® during the Intermittent Dialysis of Subjects with End Stage Renal Disease', was a 3-treatment study involving 6 patients; study 2, entitled 'Single-Case Studies to Evaluate the Preliminary Effiacy of Prolonged Treatment of HCV with the Hemopurifier™ during Intermittent Dialysis', was a 12-treatment case study on 1 patient; both were ERB-approved studies conducted under the supervision of Dr. Vijay Kher at the Fortis Hospital in New Delhi; C = control; T = treatment.

Methods of Analysis

In vitro experiments have demonstrated that viral clearance using LAP is a first-order linear process in tissue culture media, plasma and human blood for a large number of different viral species [8]. Typical clearance half-times for most of these viruses range from 1 to 2 h in the absence of viral replication [6, 8]. Assuming a constant flow rate and total volume, virus clearance follows an exponential decay $C = C_o \cdot e^{-ct}$. For an apparent first-order process, the reaction half-time is related to the clearance rate by $t_{1/2} = \ln 2/c$.

Using this formulation, we can calculate a clearance rate constant (c) from the in vivo HCV clearance rate observed for HCV in our clinical trials (table 1).

In these two studies, we observed an average HCV viral load reduction of 29% per 4-hour treatment at a blood flow rate of 250 ml/min. This is equivalent to an 8.1-hour clearance half-time in the presence of in vivo viral replication. In contrast, there was a 24% increase in the average control viral loads over both studies. As is clear in the results presented, there was a large variation from sample to sample. Previous studies have shown that hemodialysis treatment can have some positive effect on HCV viral load which is quite variable from study to study [9, 10]. However, such changes tend to be transient. In one study, no significant reductions in viral load were observed when followed over the course of 13 months [11].



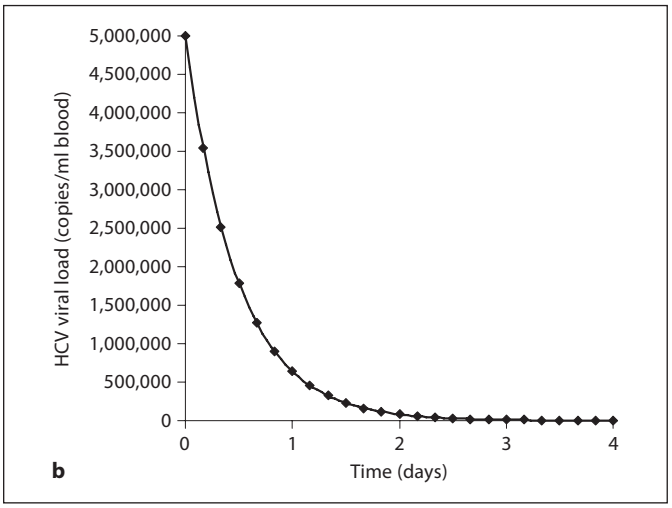


Fig. 1. a Effect of DFPP pretreatment on the response of HCV type 1 to standard-of-care drug therapy. Taken from the data of a clinical study by Fujiwara et al. [13]. The data were originally taken for 30 days and are extrapolated here for comparison with other calculations. The lines are a theoretical exponential decay. **b** Modeling HCV viral load clearance during hemopurification alone from clinical studies on HCV dialysis patients: log plot exponential theory reflecting an average clearance of 29% in 4 h that holds true during continuous affinity hemodialysis treatment and follows the same log linear process observed in vitro.

Inspection of the clearance equation makes clear that the most effective treatment regime would be one of continuous treatment (hemopurification) for as long a period of time as is safe. In continuous renal replacement therapy, commonly used to treat acute kidney failure, hemodialysis therapy is typically maintained for up to 24 h and has been used for up to 40 days on a single patient. By analogy, for continuous affinity plasmapheresis treatment, it is reasonable to suppose that a similar time frame for treatment would be safe.

In order to model the combination of the techniques, we needed an expression that combines exponential decay functions, one clearance rate for the drug treatment (k) and one for the device treatment (c). The combined expression is given by $C = C_o\, e^{-(k+c)t}$, where k = HCV virus daily clearance rate during treatment with pegylated IFN + ribavirin and c = HCV daily clearance rate during continuous hemodialysis of HCV dialysis patients and C_o = initial viral load in international units per milliliter. Using this formulation allows us to calculate the effect of combining continuous lectin affinity hemodialysis with standard-of-care HCV treatment in patients and compare it to the pattern for drug treatment alone. For the purposes of this discussion, the comparison applies primarily to a single continuous LAP treatment in combination with drug therapy where both mechanisms are operating.

Results and Discussion

The regimen we envision proposes continuous hemopurification to rapidly reduce HCV viral load for the first week of standard-of-care treatment with pegylated IFN and ribavirin [2].

A similar approach developed by Asahi has recently been reported to improve sustained viral response outcomes from 50% without double-filtration plasmapheresis (DFPP) to 78% in genotype 1 HCV patients when DFPP is used in conjunction with ribavirin and pegylated IFN [12, 13]. In DFPP, plasma from venous blood is obtained from the patient and cleared of virus particles by ultrafiltration. In this study, an average of 3 treatments were given for 3.25 h during the first week of drug therapy. Typical results showed an approximate 100-fold drop in viral load in about 1 month (fig. 1a, replotted from data of Fujiwara et al. [13]). This simple 1-week pretreatment by physical virus removal increased the rate of virus clearance by the drug by approximately 33% and allowed drug therapy to be about 50% more successful in curing the infection.

These results are not without historical precedent. Table 2 shows a list of viral infections where viral load is correlated with the severity of viral disease and disease progression. For instance, it is well known that HCV is more responsive to drug therapy when the initial viral load is less than 800,000 copies/ml.

Thus, viral load reduction prior to or early in the treatment process should be expected to improve treatment outcomes. We have therefore looked to find more efficient methods to reduce viral load without the need for fluid replacement.

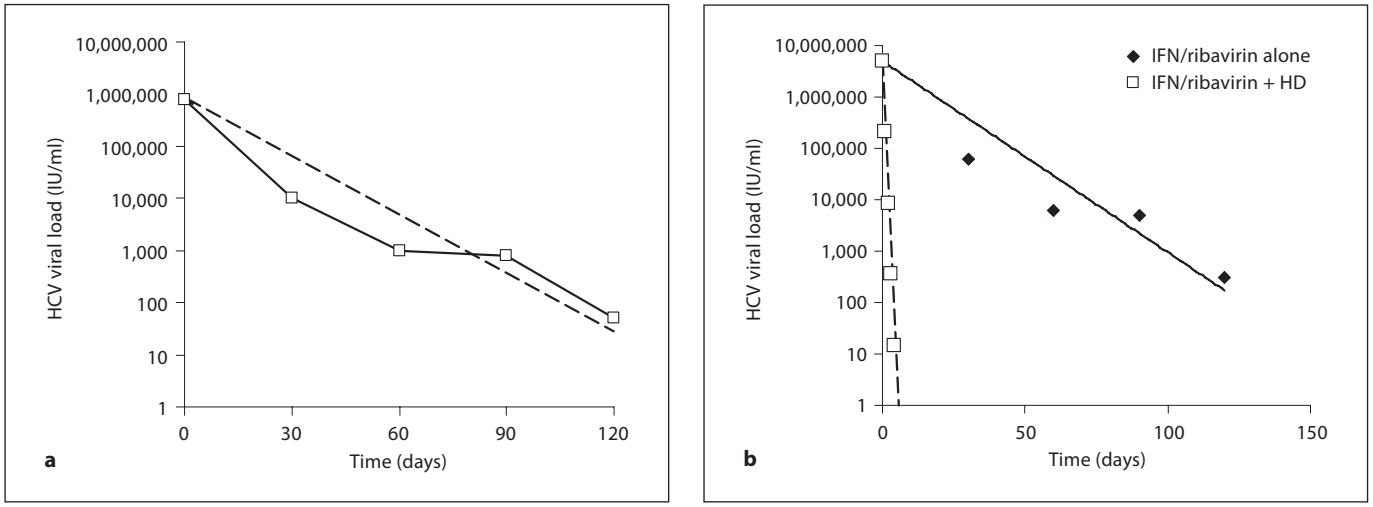


Fig. 2. a Modeling HCV viral load from patient data during IFN + ribavirin treatment (n = 34). The data for this figure were taken from Veillon et al. [33]. The log plot is fitted to a single exponential decay given by $y = 794{,}000^{-0.0857t}$ ($R^2 = 0.873$). **b** Modeling HCV viral load from patient data during IFN + ribavirin treatment in combination with affinity hemodialysis (HD). The data were recalculated and replotted against the predicted curve for the combination of continuous LAP + drug therapy. In both cases, the initial HCV viral load was set at 5×10^6 IU/ml.

Table 2. Correlation of viral load with disease severity and outcomes

Virus	Viral load correlation	Clinical benefit/ market clearance	Reference
Dengue	lethality correlates with high viral load	yes	14, 15
Ebola	lethality correlates with high viral load	yes	16, 17
Hepatitis C	pegylated IFN + ribavirin treatment	yes/yes	18
	disease severity and treatment response correlates with viral load	yes	19, 20
Herpes	antiherpetic drugs	yes/yes	21, 22
HIV	drug cocktails	yes/yes	23, 24
	long-term nonresponders (<1,000 cpm) and 'elite controllers'	yes	25–28
Influenza A	Tamiflu	yes/yes	29, 30
Marburg	plasmapheresis	yes (1 patient)	31
Sin Nombre	lethality correlates with high viral load	yes	32

LAP is a good candidate for such a process. Figure 1b shows the analysis of the HCV virological response predicted for continuous application of LAP in the absence of any other treatments. It shows a rapid reduction in HCV viral load based on a clearance rate of 29% in 4 h obtained in clinical studies. From this it may be calculated that starting from an initial viral load of 5 million IU/ml, continuous LAP should reduce HCV to undetectable levels in 2.28 days.

In order to predict the full effect of performing hemopurification in conjunction with the standard of care HCV treatment, we analyzed the kinetics of HCV drug treatment on a typical HCV patient population. Veillon et al. [33] have provided such treatment data in 34 patients using a combination of pegylated IFN-α and ribavirin. In this study, patients infected with genotype 2 and 3 HCV receiving weekly injections of pegylated IFN-α_{2a} and daily ribavirin were studied. In these patients, a more

than 100-fold decrease in viral load was predictive of a sustained virus response. Among sustained responders to combination therapy, 76 out of 96 (79.2%) had a viral load decrease of greater than 100-fold after 1 month of treatment.

A plot of the data averaged for the patients who showed a sustained virological response is shown in figure 2a. The rate of virus clearance during treatment was evaluated using a single exponential function. The clearance rate constant here was k = 0.0857 per day corresponding to an overall half-time of 8.09 days. While the data is clearly biphasic, a single exponential fit simplifies the picture and gives a reasonable correlation coefficient of 87%.

Figure 2b shows the results of combining drug treatment with 1 session of continuous LAP versus drug treatment alone. The striking feature of this comparison is that the combination treatment of continuous LAP with standard-of-care pegylated INF-α + ribavirin is predicted to proceed significantly faster than drug treatment alone. In this regard it is similar to DFPP, with the primary difference that continuous LAP-mediated virus clearance is at least 10 times faster and will also clear immunosuppressive free viral proteins and viral fragments which would be missed by DFPP.

Conclusions

Physical reduction of viral load has been demonstrated to substantially improve HCV cure rates. Based on the observed rates of virus clearance in clinical studies, we calculate that LAP applied continuously for 4.1 days would reduce HCV viral load to undetectable levels versus more than 30 days measured for DFPP in combination with drugs. This calculation suggests that 1 week of pretreatment with LAP used in combination with standard HCV drug therapy would probably lead to cure rates of more than 80%.

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