EFFECT OF INTERFERON AND RIBAVIRIN ON HEPATITIS C VIRUS

A Project Report Submitted in Partial Fulfilment of the Requirements

For The Degree of

Bachelor of Technology in Biomedical Engineering

By

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(Roll No.- 107BM007)

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NATIONAL INSTITUTE OF TECHNOLOGY ROURKELA

ROURKELA-769008, ODISHA

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Under the Guidance
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CERTIFICATE

This is to certify that project entitled ”EFFECT OF INTERFERON AND RIBAVIRIN ON HEPATITIS C VIRUS” submitted by SARASWATI TUDU (Roll No. – 107BM007) in partial fulfilment of the requirements for the award of Bachelor of Technology in Biomedical Engineering at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the project report has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Prof. Amitesh Kumar
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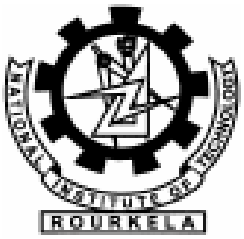
Date:



ACKNOWLEDGEMENTS

I would like to record my gratitude and sincere thanks to my honourable supervisor **Prof. Amitesh Kumar,** Faculty of Department of Biotechnology and Medical Engineering. I sincerely thank for his exemplary guidance and encouragement. It would have been impossible to carry out the present work without him who supported me and believed in my calibres. His trust and support inspired me in the most important moments of making right decisions and I am glad to work under his supervision.

I would like to extend my gratitude to the Head of the Department, Dr. S. Paul for providing us valuable facilities. I would also like to thank all my senior students of the department who have always encouraged and supported me in the progress of project report.

Last, but not the least I cannot forget to thank my friends at NIT Rourkela who were a great moral and practical support during my work.

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ABSTRACT:

Hepatitis C Virus (HCV) is a blood-borne infection that can lead to many liver diseases and hence leads to its failure [1]. The current treatment for HCV infection involves combination therapy of pegylated Interferon and Ribavirin [2]. Combination therapy induces long term response in almost 50% of the patients [2-3]. No alternative therapy exists to those patients who do not respond to the combination therapy [3]. Therefore, it is necessary to identify the markers of the disease that leads to its progression and also the factors which can result in the long term response of the drugs. Beside this the exposure to the drug should be minimised; it should not cause side effects and should be inexpensive. With the onset of therapy, the viral load declines in a biphasic or triphasic manner. A biphasic decay pattern is observed in those patients who respond to the therapy. In biphasic decay, there is a sharp decline initially and then decreases gradually until the infection is not detected. In certain patients there could be relapse of the infection after the therapy and in some cases there might not be any second phase decline, i.e. in non-responders. So these changing patterns of the viral load under therapy, holds clues for the HCV pathogenesis and outcomes of therapy.

So a mathematical model for the suppression of HCV in the presence of the potential drugs was developed. From experimental and theoretical kinetic data, we can work on how we can decrease the level of viral RNA (ribonucleic acid) in hepatocyte cells. Using the model, we can verify the estimates for the efficiency of the effect of potential drugs on replication of viral RNA and viral protein processing. We have written the code in FORTRAN; the code has been tested against the published result and a good match is found. The developed mathematical model may serve as a tool for the evaluation of the efficiency of potential drug on the HCV genome.

CHAPTER 1

INTRODUCTION

1.1 Background:

Chronic hepatitis C virus (HCV) infection is the main cause of cirrhosis, i.e. the end stage of liver disease, where the liver tissue is replaced by fibrosis, leading to loss of liver function. So there becomes a need of liver transplantation. The reason behind HCV infection is not properly understood, but upto 25% of chronically infected individuals may develop this complications. So there is a need for therapy in patients who are affected with advance disease. The purpose of therapy is to eradicate viremia, it is the medical condition when virus enter the bloodstream and have access to rest of the body, and as a consequence no hepatic fibrosis occurs which improve the survival of hepatocytes. The patient can respond to therapy in three categories, sustained virological response (SVR), end-of-treatment response (ETR) and non-responders. When the concentration of viral load falls below the detection level during therapy and remains undetected for 6 months after cessation of the therapy, this is defined as SVR. Patients showing SVR are cured of infection. When viral load becomes undetected during therapy and relapses after the cessation of therapy then the condition is defined as ETR. SVR can be achieved in such patients with the onset of therapy once again. In those patients where the viral load remains detectable through-out the treatment are defined as non-responders. It has been observed that viral clearance from serum have increased from 10% with interferon monotherapy to 56% with pegylated interferon and ribavirin [3,4].

Interferons are proteins made and released by lymphocytes in response to the presence of pathogens such as viruses. They allow communication between cells to trigger the protective defences of the immune system that eradicate pathogens or tumours. Polyethylene glycols (PEG) are amphophilic polymers with varying average molecular weights that can be chemically linked to proteins. So pegylated interferon is produced by chemical conjugation of a PEG molecule with an average molecular weight interferon. All patients treated with interferon show a rapid decline in the viral particles in the hepatocyte cells within a short period of time.

Ribavirin is synthetic purine nucleoside. Ribavirin is phosphorylated into ribavirin monophosphate (RMP), ribavirin diphosphate (RDP), ribavirin triphosphate (RTP) inside the cell. RTP is analogous to GTP (Guanosine triphosphate, it is a purine molecule). When RTP is incorporated into replication instead of GTP it leads to chain termination. So it inhibits viral polymerase activity. T-cell response has also been observed in the patients undergoing combination therapy. Ribavirin monotherapy does not lead to SVR. Interferon in addition with the Ribavirin improves SVR than interferon monotherapy.

If sustained virological eradication is not achieved then alternative way can be achieved by supressing the hepatic fibrosis by using anti-fibrogenic drug. For this an extensive study of molecular basis of hepatic fibrosis is required. So there is a requirement of new therapy for relapser and non-responder in case where the combination of interferon and ribavirin does not work.

1.2 Literature Review:

HCV dynamics model help us to analyse HCV pathogenesis in vivo and the mechanisms of action of interferon and ribavirin against HCV [5]. First, therapy gives us an accurate description of drug pharmacokinetics. The models of HCV dynamics that incorporate the pharmacokinetics of interferon have been suggested [6]. The pharmacokinetics of ribavirin, which exhibits a three phase plasma concentration-time profile, remains poorly described [7]. Secondly, ribavirin acts against the HCV by mutagenesis [8-9]. Ribavirin does not induce long-term response alone but it helps in enhancing the antiviral activity of the interferon when treated combined [10]. Finally, link between long–term response to therapy and markers of disease state such as viral load and alanine amino-transferase is yet to be proved [11-12]. Viral and drug characteristics are important for disease progression, in the meantime genetics of patient is also main cause for characterising, which is difficult to determine.

Nevens et al. [13] have proposed a method of therapeutic viral E1 envelope vaccine for treating HCV. They suggest that the vaccination with an envelope protein (mainly glycoprotein E1 or E2) might alter the course of infection and hence it is beneficial for chronically infected patients. Originally it was designed to study the tolerability and safety of truncated E1 proteins in chronic hepatitis C patients. After 24 weeks, it was analysed that there was an immune and bio-chemical response in vaccinated patient. So, it was demonstrated that there was an increase in the humoral response. After second phase of vaccination it was observed that there was an increase in T-cell response. Even though it did not decrease the HCV-RNA (Ribonucleic acid) but there was a change in the alanine amino-transferase level. Serum alanine amino-transferase level, which is the best surrogate marker for hepatocellular necrosis, usually decreases instead of increasing and hence giving an evidence of viral decay in the second phase. It was well tolerated in the biological system. But study by Nevens et al. [13] showed that no patient had viral clearance. Basing upon the decrease of alanine amino-transferase level it cannot be said that the hepatocyte cells have achieved bio-chemical response. So the study failed.

Herrmann et al. [14] analysed HCV-RNA in different patients. Patients were either given pegylated interferon alfa alone, pegylated interferon alfa with ribavirin or standard interferon and ribavirin. It showed that ribavirin did not affect ε (effectiveness with which it lower the viral production from infected cells) but affected δ (death of infected cells) kinetics. Secondly, it was found that the viral decay was seen in triphasic decay in a large population. It has a typical first phase, a flattened second phase and a third phase which ultimately related to viral clearance. This was not unique to pegylated interferon treatment but assuming that the third phase is due to immune clearance of HCV infected hepatocytes. Ribavirin takes days or weeks to achieve its activity which is hence not detected in first 24 hours of its treatment. But clinical treatment has

shown that addition of Ribavirin with the Interferon increases the rate of end- of- treatment and sustained virological responses. The third phase decay is hypothetical and not all patients respond to such treatment, it is found only in few patients.

Layden et al. [15] analysed the effect of standard interferon alone or interferon and ribavirin on African-American and white patients separately. The investigation resulted that ribavirin had no effect on either ε and δ, and then comparing with the white patients, African-American patients showed lower values for both ε (0.89 vs. 0.98) and δ (0.13 vs. 0.20). These results supported the previous studies [6]. These were different from Herrmann et al. [14]; it showed no effect of ribavirin on δ and no triphasic response. These differences might be due to difference in interferon preparation, dose and dosing schedules and problems in statistical analysis. Study of Layden et al. [15] depended upon high dose of interferon and Herrmann et al. [14] depended on the standard dose of interferon on the patients. Due to this, there might be a flattened second phase and a third phase as there is an intermittence of full interferon action. The second problem was that the study was conducted on small number of patients which might have led to statistical error. So, a sizeable portion of patient showed a little or no second phase response and some showed no response at all. When the results were calculated and there average were taken it showed that the patients who responded to second phase showed little or no difference in between African-American and white patients. From this, it was concluded that difference between the races was categorical; the frequency of null response was high in African-American patients than white patients on the onset of interferon treatment. But the quality of response in both the races was similar.

The interferon affect the virus by two different mechanisms: blocking the de novo infection of cells with effectiveness η and lowering the viral production from infected cells with effectiveness ε [16]. Therefore, the aim of the present investigation is to study the effect of these parameters on the HCV RNA and the infected hepatocytes numerically.

CHAPTER 2

MATHEMATICAL MODELLING

2.1 Governing differential equations

After the successful study of models of the dynamics of human immunodeficiency virus (HIV) [17-18] and hepatitis B virus (HBV) infections [19], Neumann et al. [20] adapted the basic models of viral dynamics to HCV. A steady state level of serum HCV-RNA is observed in untreated patients due to balance between the production and clearance of viruses. During antiviral treatment, we get the kinematic information of the viral RNA replication. A simplified view of HCV infection is assumed which describes the response to interferon therapy through the coupled evolution of three populations with the following equations.

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

$$\frac{dV}{dt} = (1-\varepsilon)pI - cV \quad (2)$$

$$\frac{dI}{dt} = (1-\eta)\beta VT - \delta I \quad (3)$$

Where T represents the uninfected hepatocytes, s represents the rate of production, the death of uninfected hepatocytes with first order rate constant is represented by d. V represents the free virions, which infect the uninfected hepatocytes at rate βVT. This in return produce productively infected hepatocytes I. The loss of infected hepatocytes due to immune mediated killing is at rate δ.

Free virions are produced at rate p per infected hepatocytes and are cleared from circulation at the rate c. The interferon can act on virus in two possible ways, i.e. by blocking of infection in cells from the beginning with effectiveness η or by lowering the viral production from infected cells with effectiveness ε.

At steady state, i.e. before treatment or in untreated patients, we assume η=0 and ε =0. Here the viral production is balanced by the rate of production of infected cells and by the rate of clearance of infected cells. Uninfected hepatocytes are also in steady state which is determined by its death, production and loss due to infection. Here, we have a slight change in the equation of free virions and productively infected hepatocytes

$$\frac{dV}{dt} = pI - cV \quad (4)$$

$$\frac{dI}{dt} = \beta VT - \delta I \quad (5)$$

Steady state solutions of the above equations give the initial value of infected cell density I_o and the uninfected cell density T_o. Once the therapy was started, there was a decline in the virions. HCV-RNA serum under Interferon therapy is studied by either completely blocking the infection from the beginning (η=1) or completely blocking virion production (ε=1). From equation (4) and (5) we get the values of T_o which is equal to $c\delta/p\beta$, the infected cell density is given by $I_0=cV_0/p$.

2.2 Solution approach

The set of governing differential equations (1)-(3) is highly nonlinear, the solution of which require computational resources. Therefore, the stiff equations (1)-(3) are solved using an implicit three time level scheme which ensures us the second order accuracy in time for unsteady problems, as the explicit scheme demands use of very small time step for numerical stability. Implicit scheme requires evaluation of right hand side of these equations at the current time level; therefore, at each time step iterations are performed to obtain the converged solution for that particular time step. Once the converged solution is obtained the numerical solution is advanced for the next time step and so on till the end of the treatment period.

2.3 Code validation

To check the accuracy of the developed numerical model, the model is validated against the published result of Dixit [16]. The result has been validated for the case when the viral production from infected cell is zero, i.e. ε=0, and effectiveness with which de novo infection of cells is blocked is 80%, i.e. η=0.8. The other parameters are: initial viral load is assumed to be $V_0=10^7$ copies per ml, $\beta=2.25\times10^{-7}$ ml per day per virions, δ is 1 per day, p is 2.9 virions per cell per day and c is 6 per day. The governing differential equations are solved for a period of 14 days and the effect of drug on HCV RNA is assessed. Figure 1 shows the variation of HCV RNA with time and compares the present numerical result with the result of Dixit [16]. A good match between the present numerical result and the result of Dixit [16] can be observed thereby validating our numerical model.

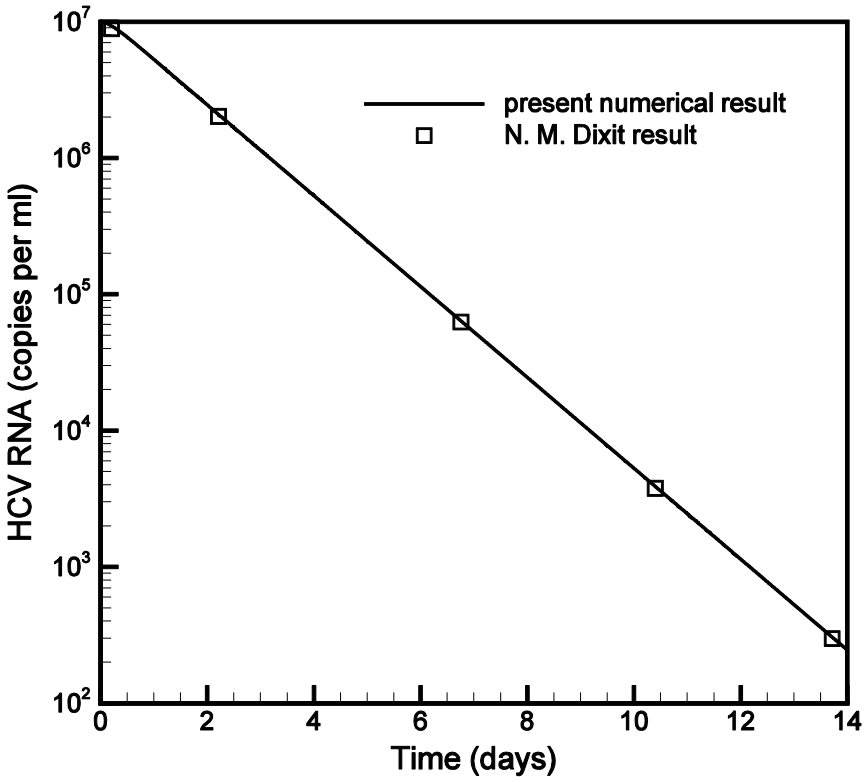

Figure 1.Validation of the present numerical model

CHAPTER 3

RESULTS AND DISCUSSION

It is assumed that interferon can suppress virus by two possible mechanisms; either by blocking the de novo infection of cells with effectiveness η or lower the viral production from infected cells with effectiveness ε. Therefore, in the present chapter, the effect of different combinations of η and ε are considered and the results are presented for the effect of these parameters on HCV RNA and the infected hepatocytes, I. The value of η is taken equal to 0.2, 0.4, and 0.6 and that of ε is taken equal to 0.2, 0.4, and 0.6.

3.1 Effect of Interferon on HCV RNA

Figure 2 depicts the variation of HCV RNA with time for ε=0.2, η=0.2; ε=0.4, η=0.4; and ε=0.6, η=0.6. From the figure it can be observed that, the more is the efficiency the more is the decline in viral load. When ε=0.2 and η=0.2 we get almost a straight line and it takes a lot of time for the infection to recover. When ε=0.4 and η=0.4 we get a biphasic decay of viral load, we can see that there is a sharp decline initially and then there is a gradual decline in the viral load. When the values of ε and η are increased to 0.6, we get more declines in the first phase decay and there is a gradual second phase decay which ceases earlier than the other cases studied.

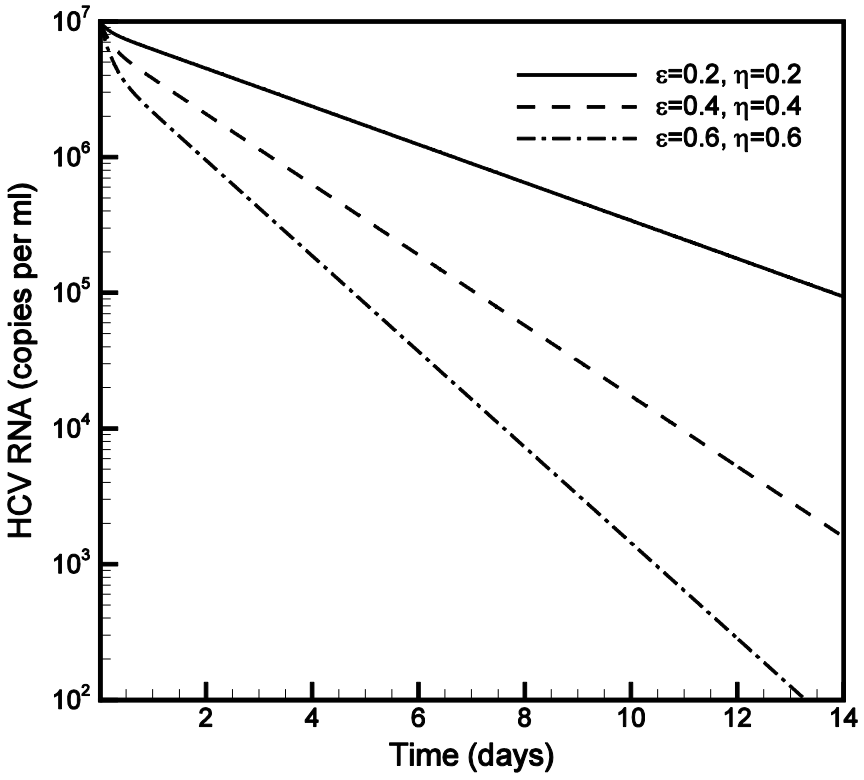

Figure 2.Variation of HCV RNA with time

From here we can conclude that with the increasing effectiveness of interferon, the decline of the HCV-RNA is more and the SVR attained for this is earlier.

3.2 Effect of Interferon on infected hepatocytes

Figure 3 shows the variation of infected hepatocytes with time for ε=0.2, η=0.2; ε=0.4, η=0.4; and ε=0.6, η=0.6. A single phase decay of infected hepatocytes with respect to time is observed in the figure, with the onset of interferon treatment, irrespective of the values of ε and η. Here we can see that as we increase the efficiency of interferon there is quick attainment of first phase representing the quick recovery of the patient. This shows that SVR achieved takes time with low ε and η. As we increase the values of ε and η we can see that there is an increase in slope of the straight line. This shows that the SVR achieved by patient becomes fast hence, more clearance of the infected hepatocyte cells.

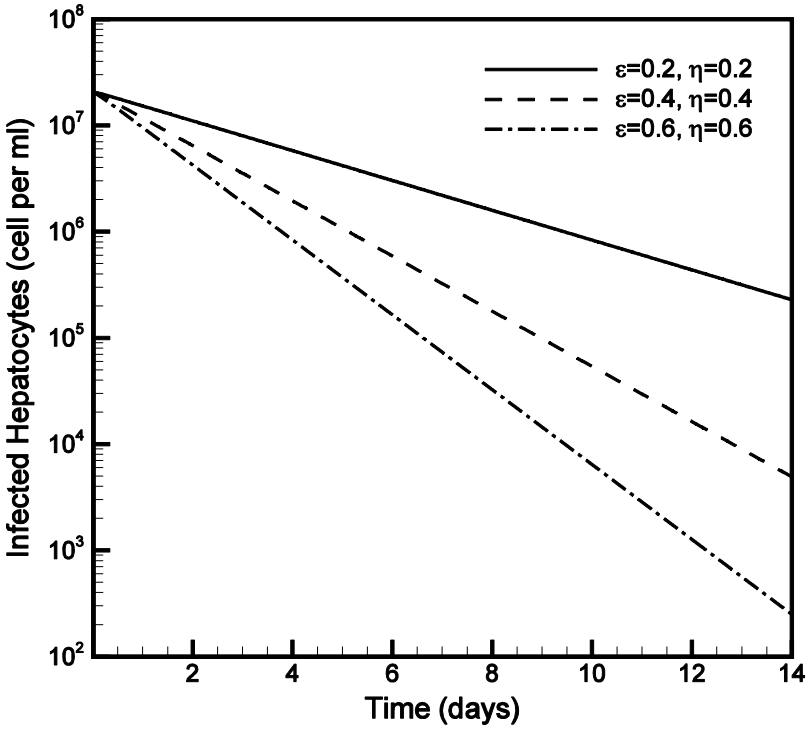

Figure 3.Variation of infected hepatocytes with time

CONCLUSION:

A numerical model is developed to study the effect of Interferon on the Hepatitis C Virus (HCV). The set of differential equations have been solved using implicit time marching scheme to avoid the stability criterion posed by explicit method. The validity of developed numerical model is checked with the published results. It has been observed that for lower value of ε and η the HCV RNA decays in a single phase. But with the increase in these values, the response changes from single phase decay to biphasic decay. The first phase of which is strongly dependent on ε and η; with increase in these values the first phase is achieved much quickly. Also, it has been observed that the infected hepatocytes decreased almost linearly with respect to time for all the studied cases. The recovery of the patient is fast with higher values of ε and η.

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