Chronic hepatitis B virus and liver fibrosis: A mathematical model

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Abstract

Hepatitis B virus (HBV) infection is a liver disorder that can result in cirrhosis, liver failure and hepatocellular carcinoma. HBV infection remains a major global health problem, as it affects more than 350 million people chronically and kills roughly 600,000 people annually. Drugs currently used against HBV include IFN-α that decreases viremia, inflammation and the growth of liver fibrosis, and adefovir that decreases the viral load. Each of these drugs can have severe side-effects. In the present paper, we consider the treatment of chronic HBV by a combination of IFN-α and adefovir, and raise the following question: What should be the optimal ratio between IFN-α and adefovir in order to achieve the best 'efficacy' under constraints on the total amount of the drugs; here the efficacy is measured by the reduction of the levels of inflammation and of fibrosis? We develop a mathematical model of HBV pathogenesis by a system of partial differential equations (PDEs) and use the model to simulate a 'synergy map' which addresses the above question.

Introduction

Hepatitis B virus (HBV) infection is a liver disorder that can result in cirrhosis, liver failure, and hepatocellular carcinoma; it is one of the most prevalent infectious diseases associated with human liver diseases, including acute, fulminant and chronic hepatitis [1]. Despite the availability of HBV vaccine and the development of antiviral therapies, HBV infection remains a major global health problem. Chronic HBV affects more than 350 million people of whom roughly 600,000 die annually from HBV-related liver diseases [1–3].

To understand the virus biology and pathogenesis in HBV-infected patients, several animal models have been developed to mimic hepatic HBV infection and the immune response against HBV [1, 4, 5]. However, as observed in [1], the narrow host range of HBV infection and lack of a full immune response spectrum in animal models remain significant limitations. For this reason, it may be useful to develop a mathematical model of the progression of HBV, which includes the host response to treatment. In the present paper we introduce such a model and use it to study the effect of a combination therapy with two currently used drugs, interferon alpha (IFN-α) [5–7] and adefovir [8–11]. The model shows that it may be possible
to reduce HBV infection and viral load while, at the same time, decrease inflammation and control liver fibrosis.

Monotherapy of HBV infection with IFN-α decreases viremia, inflammation and the progression of liver fibrosis. However, only a small fraction of patients respond positively to treatment [5, 6]. Monotherapy with adefovir decreases the viral load by blocking reverse transcriptase, an enzyme that is crucial for the reproduction of HBV in the body [8, 12, 13].

Both IFN-α and adefovir can have severe side-effects when administered alone, including brain disorder that may lead to a stroke, a heart attack or lung fibrosis for IFN-α [14], and kidney damage, a low amount of phosphate in the blood, a stroke or pancreatitis for adefovir [15]. A combination therapy with smaller dose may be as effective, or even more effective, than monotherapy with a larger dose if there is a ‘positive synergy’ between the two drugs. We shall use our mathematical model to determine the synergy between IFN-α and adefovir.

In their study of HBV infection in a humanized mouse model where the mice are inoculated with clinical isolates HBV in vivo, the authors in [16] showed that a persistence of liver disease due to HBV infection is associated with infiltration of M2 macrophages. They found that chronic HBV infection in the liver of humanized mice is associated with human hepatic stellate cells (HSCs) activation/infection and human liver fibrosis. It was also observed in [16] that infection of M1 and M2 macrophages with HBV results in increasing level of IL-10 and decreasing level of IL-12. Although the authors in [16] do not demonstrate that HBV infects macrophages, they show that inoculation of macrophage cultures with HBV results in macrophage activation, however the mechanism of activation is unknown. Since the possibility that HBV could infect macrophages remains to be conclusively proven, we do not assume that macrophage infection is a major source of virus as compared to HSCs.

Methods

In the course of chronic HBV infection, M1 macrophages produce pro-inflammatory cytokines IL-6 [17–19], IL-12 [17, 20, 21] and TNF-α [22, 23], while M2 macrophages produce anti-inflammatory cytokines IL-1β [17, 18, 20, 24], IL-4 [25, 26], IL-10 [17–19, 27, 28], IL-13 [17–19, 27–29] and TGF-β [30], and chemokine PDGF [31, 32]. The HBV environment includes T cells: Th1 and Th2. Th1 is activated by IL-12 [33] and it produces IL-2 [17], IFN-α [34] and IFN-γ [17, 20, 35]. Th2 is activated by IL-4 [36]. Th2 is activated by IL-4 [37] and it produces IFN-α [38], IL-4 [25, 26], IL-10 [27, 28] and IL-13 [27, 28, 39]. Th1 and Th2 are mutually antagonistic [36, 40, 41]. In addition to macrophages and T cells, the mathematical model will include other types of cells closely associated with chronic HBV: hepatic stellate cells (HSCs), fibroblasts and myofibroblasts. HSCs enhance the proliferation of fibroblasts by producing hyaluronic acid [42–44]. Fibroblasts are transformed into myofibroblasts by PGDF and TGF-β [30, 45–47]. Fibroblast and, more effectively, myofibloblast, produce collagen [47–50]. MMP produced by M2 macrophages disrupts collagen cross-linking and increases scarring, especially under excessive collagen concentrations [31, 32, 51]. Since M2 macrophages produce TGF-β and PDGF which enhance the presence of fibroblasts, M2 macrophages contribute to the progression of fibrosis. In chronic HBV, the virus infects both M1 and M2 macrophages, as well as hepatic stellate cells. The infected cells secrete C-C Motif Chemokine Ligand 3 (CCL3) [45, 52, 53] which attracts macrophages into the liver [31, 54], resulting in increased inflammation. The replication of the intracellular virus is blocked by several cytokines, including IFN-α, TNF-α, TGF-β and IFN-γ [20, 35, 55].

A schematic diagram of our model is shown in Fig 1, where the nodes are defined in Table 1. We represent the cells by square nodes, but the fibrosis formation pathway by diamond nodes, and the virus and cytokines by circular nodes. The nodes with reddish colors...
(red and orange) indicate the variables that include and/or induce infection or fibrosis formation. The nodes with greenish colors (blue, green and silver) indicate the variables that counteract the infection. We shall use our model to compare the efficacy of treatment by a single agent (IFN-α or adefovir) with a combination, whereby the amount of each agent is less than in the monotherapy. We shall accordingly develop a ‘synergy map’ which addresses the question of achieving a desired target of efficacy in a combination therapy while using the smallest dose of these two drugs.

Mathematical models for population dynamics of HBV transmission have been developed [56–63]. In [62], the authors used a SEIR-type mathematical model by a system of ordinary differential equations (ODEs) to study the dynamics of HBV infection under administration of vaccination and treatment. A compartmental mathematical model by a system of partial differential equations (PDEs) was also developed.
differential equations (PDEs) to predict the dynamics of HBV transmission and evaluate the long-term effectiveness of the vaccination program was developed in [63]. Another class of models that consider uninfected hepatocytes, infected hepatocytes and free virus have been developed to analyze changes in HBV levels during drug therapy [56–61]. Techniques in [58, 59] where used in [56, 60, 61] to develop mathematical models by a system of ordinary differential equations to understand the factors that govern HBV infection dynamics, and to analyze the immune mechanisms responsible for viral clearance in the case of acute HBV infection. They found that the viral load initially increases exponentially, then decreases and seems to approach a plateau after some time. Interestingly, they obtained that the number of infected cells, the number of uninfected cells and the number of CD8\(^+\) T cells increase in acute HBV infection, leading to clearance of the viral load. A more recent model by Friedman and Hao [64] uses reaction-diffusion equations to study the evolution of fibrosis in the liver, without specifically targeting the cause of the disease.

Mathematical model

In this section, we develop a mathematical model of liver fibrosis due to infection by HBV. In our model that builds on the model in [64], infection takes place in some region \( \Omega \) of the liver. The variables used in the model are given in Table 1. These variables satisfy a system of PDEs in \( \Omega \).

### Table 1. Variables of the model. All densities and concentrations are in units of g/cm\(^3\).

| Variables | Descriptions | Variables | Descriptions |
|-----------|--------------|-----------|--------------|
| \( H \)  | density of healthy HSCs | \( H_i \) | density of infected HSCs |
| \( M_1 \)  | density of healthy M1 macrophages | \( M_{1i} \) | density of infected M1 macrophages |
| \( M_2 \)  | density of healthy M2 macrophages | \( M_{2i} \) | density of infected M2 macrophages |
| \( T_1 \)  | Th1 cell density | \( T_2 \) | Th2 cell density |
| \( f \)  | density of fibroblast | \( m \) | density of myofibroblast |
| \( \rho \)  | density of ECM | \( S \) | scar density |
| \( V_e \)  | density of external virus | \( V_{i2} \) | density of virus in \( M_{2i} \) |
| \( V_{i1} \)  | density of virus in \( M_{1i} \) | \( V_{iH} \) | density of virus in \( H_i \) |
| \( G \)  | concentration of PDGF | \( H_A \) | concentration of hyaluronic acid |
| \( I_{1\beta} \)  | concentration of IL-1\( \beta \) | \( I_2 \) | concentration of IL-2 |
| \( I_4 \)  | concentration of IL-4 | \( I_6 \) | concentration of IL-6 |
| \( I_{10} \)  | concentration of IL-10 | \( I_{12} \) | concentration of IL-12 |
| \( I_{13} \)  | concentration of IL-13 | \( I_{w} \) | concentration of IFN-\( \alpha \)/\( \beta \) |
| \( I_{\gamma} \)  | concentration of IFN-\( \gamma \) | \( T_{\alpha} \) | concentration of TNF-\( \alpha \) |
| \( T_{\beta} \)  | concentration of TGF-\( \beta \) | \( M_F \) | concentration of MMP |
| \( T_P \)  | concentration of TIMP | \( C_{3} \) | concentration of CCL3 |

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where

\[ \varepsilon_1 = \left( \lambda_{Mi} \frac{I_2}{I_2 + K_2} + \lambda_{Mi1} \frac{T_2}{T_2 + K_2} \right) \frac{1}{1 + I_10/K_{10}}, \]

\[ \varepsilon_2 = \lambda_{Mi} \frac{I_3}{I_3 + K_4} + \lambda_{Mi1} \frac{I_{13}}{I_{13} + I_{13}}. \]

The term \( \nabla \cdot (M_1 \chi_C \nabla C_3) \) is the chemotactic effect of \( C_3 \) on M1 macrophages; \( \chi_C \) is the chemotactic coefficient. M2 macrophages can become M1 macrophages under the influence of IFN-\( \gamma \) and TNF-\( \alpha \), a process resisted by IL-10 \([65–67]\), and M1 macrophages can become M2 macrophages under the influence of IL-4 and IL-13 \([29, 68]\). The fourth term on the right-hand side is the infection of M1 macrophages by external virus, \( V_e \).

Infected cells produced chemokine CCL3 \([45, 52, 53]\) in order to attract immune response. Monocytes circulate in the blood. They are attracted to the liver tissue by CCL3 \([31, 54]\), and then they differentiate into either M1 or M2 macrophages \([69–71]\). We denote \( M_{10} \) and \( M_{20} \) the densities of the monocytes from the vascular system which differentiate into M1 and M2 macrophages, respectively. Accordingly, on the boundary of each blood capillary, there is an influx of M1 macrophages into the tissue,

\[ D_M \frac{\partial M_1}{\partial n} + \tilde{b}(C_3)(M_{10} - M_1) = 0, \]

where \( \tilde{b}(C_3) \) is an increasing function of \( C_3 \). Using a homogenization method as in \([52]\), we can replace the boundary conditions on the blood capillaries by a source term in the tissue, which we take to be \( \tilde{b}(C_3) = \frac{\rho M_1}{K_C + \rho} \), where \( K_C \) is a constant. Hence the final equation for the density of M1 macrophages is the following:

\[ \frac{\partial M_1}{\partial t} - D_M \Delta M_1 = \tilde{b}(C_3)(M_{10} - M_1) - \nabla \cdot (M_1 \chi_C \nabla C_3) + \lambda_{Mi} \varepsilon_1 + \lambda_{Mi1} \varepsilon_2 M_2 - \lambda_{Mi} \varepsilon_1 M_1 - \lambda_{Mi1} \varepsilon_2 M_2 - \mu_{Mi} M_1. \]

(1)

The density \( M_{1i} \) of infected M1 macrophage satisfies the following equation:

\[ \frac{\partial M_{1i}}{\partial t} - D_M \Delta M_{1i} = \lambda_{Mi1} V_e M_1 - \mu_{Mi1} M_{1i}. \]

(2)

where we assume that the death rate of \( M_{1i} \) is increased due to the viral load represented by \( V_{1i} \).

The density of healthy M2 macrophages satisfies an equation similar to Eq (1):

\[ \frac{\partial M_2}{\partial t} - D_M \Delta M_2 = \tilde{b}(C_3)(M_{20} - M_2) - \nabla \cdot (M_2 \chi_C \nabla C_3) + \lambda_{M1} \varepsilon_1 + \lambda_{M12} \varepsilon_2 M_1 - \lambda_{M1} \varepsilon_1 M_2 - \lambda_{M12} \varepsilon_2 M_2 - \mu_{M2} M_2. \]

(3)
The third, fourth and fifth terms in the right-hand side of Eq (3) are complementary to the corresponding terms in Eq (1).

The density $M_2$, of infected M2 macrophages satisfies an equation similar to Eq (2):

$$
\frac{\partial M_{2i}}{\partial t} - D_2 \Delta M_{2i} = \frac{\lambda_{M_2 M_2} V_e M_2}{M_2 - M_{2i}} - \mu_{V_e} (1 + \mu_{V_e} V_{i2}) M_{2i},
$$

where we assume that the death rate of $M_{2i}$ is increased due to the viral load $\mu_{V_e} V_{i2}$.

**Equations for hepatic stellate cells (HSCs) density.** In homeostasis, HSCs have a source $A_H$ and death rate $\mu_H$. In HBV, the proliferation of the healthy population of HSCs is enhanced by PDGF and TGF-β [45, 53, 72] and is reduced when they become infected by the extracellular virus. Hence the equation for $H$ is the following:

$$
\frac{\partial H}{\partial t} - D_H \Delta H = A_H \text{source} + \left( \frac{\lambda_{H_G} G}{G + K_G} + \frac{\lambda_{H_T} T}{T + K_T} \right) H - \frac{\lambda_{H_V} V_e H}{H + H_e} - \mu_H H \text{death}
$$

The infected HSCs satisfy the equation

$$
\frac{\partial H}{\partial t} - D_H \Delta H = \frac{\lambda_{H_H} V_e H}{H + H_e} - \mu_H (1 + \mu_{H_H} V_{iH}) H, 
$$

where the death rate of infected HSCs is increased due to the viral load $\mu_{H_H} V_{iH}$.

**Equations for T cells density.** Th1 cells are activated from naive T cells ($T_0$) by contact with M1 macrophages, under IL-12 environment [33]; this process is resisted by IL-10 and IL-13 [17, 73]. IL-2 enhances the proliferation of Th1 cells [17, 65]. Hence the equations for the densities of Th1 and Th2 cells are given as follows:

$$
\frac{\partial T_1}{\partial t} - D_T \Delta T_1 = \left( \frac{\lambda_{T_1 M_1} T_0 M_1}{M_1 + K_{M_1} I_{12} + K_{12} 1 + I_{10} / K_{10}} + \lambda_{T_1 T_2} I_3 + K_2 T_1 \right) \frac{1}{1 + I_{13} / K_{13}} - \mu_{T_1} T_1 \text{death}
$$

T0 cells in the liver may also be activated into Th2 cells ($T_2$) by contact with $M_2$ in an $I_4$ environment [36]. The activation of $T_2$ is antagonized by $T_1$ [36, 40, 41].

$$
\frac{\partial T_2}{\partial t} - D_T \Delta T_2 = \frac{\lambda_{T_2 M_2} T_0 M_2}{M_2 + K_{M_2} I_4 + K_4 1 + T_1 / K_{T_1}} \frac{1}{1 + I_{13} / K_{13}} - \mu_{T_2} T_2 \text{death}
$$

**Equations for fibroblast ($f$) and myofibroblast ($m$) densities.** Hyaluronic acid ($H_A$) enhances the proliferation of fibroblasts [44]. Fibroblasts are transformed into myofibroblasts
by G and $T_\beta$ [30, 46, 47, 74, 75]. Hence equations of $f$ and $m$ are:

$$\frac{\partial f}{\partial t} - D_f \Delta f = \frac{H_A}{H_A + K_{H_A}} f - \left( \frac{\lambda_{mfT_\beta}}{T_\beta + K_{T_\beta}} + \frac{\lambda_{mG}}{G + K_G} \right) f - \frac{\mu_f f}{m}$$

$$\frac{\partial m}{\partial t} - D_m \Delta m = \left( \frac{\lambda_{mfT_\beta}}{T_\beta + K_{T_\beta}} + \frac{\lambda_{mG}}{G + K_G} \right) f - \frac{\mu_m m}{m}$$

Equations for ECM ($\rho$) and scar ($S$) densities. The ECM consists primarily of fibrillar collagens and elastins, but it includes also fibronectins, lamina and nitrogen that support the matrix network by connecting or linking collagens [49]. For simplicity we represent the ECM by the density of collagens. ECM is produced by $f$, $m$ and by HSCs [47, 48]. The production of ECM by $m$ is enhanced by $T_\beta$ [47, 50]. We assume that MMP degrades the ECM at a rate proportional to $M_p \rho$. Hence the equation of $\rho$ is as follows:

$$\frac{d\rho}{dt} = \frac{\lambda_{mf} \left( 1 - \frac{\rho}{\rho_0} \right)^+ + \lambda_{pm} \left( 1 + \frac{\lambda_{pm}}{f} \right)}{M_p + K_{M_p}} + \lambda_{pH} H + \lambda_{psH} S$$

$$-d_{\rho M_p} \left[ M_p \rho \right] \frac{\rho}{m} - \frac{\mu_r \rho}{m}$$

where $\left( 1 - \frac{\rho}{\rho_0} \right)^+ = 1 - \frac{\rho}{\rho_0}$ if $\rho < \rho_0$, $\left( 1 - \frac{\rho}{\rho_0} \right)^+ = 0$ if $\rho \geq \rho_0$.

Fibrotic diseases are characterized by excessive scarring due to excessive production and deposition of ECM and disruption of normal healthy protein cross-linking. MMP disrupt collagen cross-linking and increases scarring in cases of excessive collagen concentrations [51]. Accordingly we model the growth of a scar as, in [51], by the formula

$$S = \lambda_s \left( \rho - \rho^* \right)^+ \left( 1 + \frac{M_p}{M_p + K_{M_p}} \right)$$

where $\rho^*$ is the concentration of collagen in normal healthy tissue.

Equations for cytokines and other proteins.

PDGF ($G$) is produced by $M_2$ [31, 32]. Hence,

$$\frac{\partial G}{\partial t} - D_G \Delta G = \frac{\lambda_{GM_2} M_2 + \lambda_{GM_3} M_3}{M_p + K_{M_p}} - \frac{\mu_s G}{m}$$

Hyaluronic acid ($H_A$) is produced by HSCs and is degraded by sinusoidal epithelial cells [42, 43]. Hence $H_A$ satisfies the following equation

$$\frac{\partial H_A}{\partial t} - D_{H_A} \Delta H_A = \frac{\lambda_{H_A} H_A + \lambda_{H_A, H} H}{M_p + K_{M_p}} - \frac{\mu_{H_A} H_A}{m}$$

where, for simplicity, the degradation of $H_A$ is taken at a constant rate.
Hence, given as follows:

IL-1β is secreted by healthy and infected $M_2$, and this process is inhibited by $I_\alpha$ and $I_\alpha$ [17, 18, 20, 24]. Hence,

$$\frac{\partial I_{1\beta}}{\partial t} - D_{i\beta} \Delta I_{1\beta} = \frac{\lambda_{\beta, M_2} M_2 + \lambda_{i, M_2} M_i}{1 + I_\beta/K_\beta} \frac{1}{1 + I_\alpha/K_\alpha} - \mu_{i\beta} I_{1\beta}$$  \hspace{1cm} (15)$$

IL-2 is secreted by $T_1$ [17]; hence,

$$\frac{\partial I_2}{\partial t} - D_{i2} \Delta I_2 = \frac{\lambda_{i, T_1} T_1}{\text{production}} - \mu_i I_2$$  \hspace{1cm} (16)$$

IL-4 is secreted by $T_1$, $M_2$ and $M_{2i}$ [25, 26], so that the equation of $I_4$ is given by

$$\frac{\partial I_4}{\partial t} - D_{i4} \Delta I_4 = \frac{\lambda_{i, M_2} M_2 + \lambda_{i, M_{2i}} M_{2i} + \lambda_{i, T_2} T_2}{\text{production}} - \mu_i I_4$$  \hspace{1cm} (17)$$

IL-6 is produced by healthy and $M_1$ macrophages, a process enhanced by TNF-α [17–19]. Hence,

$$\frac{\partial I_6}{\partial t} - D_{i6} \Delta I_6 = \frac{\lambda_{i, M_1} M_1 + \lambda_{i, M_{1i}} M_{1i}}{1} \left(1 + \frac{T_i}{T_i + K_{1i}}\right) - \mu_{i6} I_6$$  \hspace{1cm} (18)$$

IL-10 is produced by $M_2$ and $M_{2i}$ [17–20], and by $T_2$ [27, 28]; so that

$$\frac{\partial I_{10}}{\partial t} - D_{i10} \Delta I_{10} = \frac{\lambda_{i, M_2} M_2 + \lambda_{i, M_{2i}} M_{2i} + \lambda_{i, T_2} T_2}{\text{production}} - \mu_{i10} I_{10}$$  \hspace{1cm} (19)$$

IL-12 is produced by $M_1$ and $M_{1i}$, a process inhibited by IL-10 and IL-13 [17, 20, 21]. Hence

$$\frac{\partial I_{12}}{\partial t} - D_{i12} \Delta I_{12} = \frac{\lambda_{i, M_1} M_1 + \lambda_{i, M_{1i}} M_{1i}}{1} \left(1 + \frac{T_i}{T_i + K_{1i}}\right) - \mu_{i12} I_{12}$$  \hspace{1cm} (20)$$

IL-13 is produced by $M_2$ and $M_{2i}$ [17–20], and by $T_2$ [27–29]. Hence the equation of $I_{13}$ is given as follows:

$$\frac{\partial I_{13}}{\partial t} - D_{i13} \Delta I_{13} = \frac{\lambda_{i, M_2} M_2 + \lambda_{i, M_{2i}} M_{2i} + \lambda_{i, T_2} T_2}{\text{production}} - \mu_{i13} I_{13}$$  \hspace{1cm} (21)$$

IFN-α is produced by $T_1$ and $T_2$, a process resisted by IL-1β [34], so that

$$\frac{\partial I_\alpha}{\partial t} - D_{i\alpha} \Delta I_\alpha = \frac{\lambda_{i, T_1} T_1 + \lambda_{i, T_2} T_2}{\text{production}} \left(1 + \frac{I_\beta}{K_{i\beta}}\right) - \mu_i I_\alpha$$  \hspace{1cm} (22)$$
IFN-γ is secreted by $T_I$ [17, 20, 35], a process resisted by IFN-α [23]. Hence IFN-γ satisfies the equation

$$\frac{\partial I}{\partial t} - D_I \Delta I_I = \gamma \frac{1}{1 + I_1/K_1} I_I - \mu_f I_I$$  \hspace{1cm} (23)$$

TNF-α is secreted by $M_1$, $M_{1i}$ and $M_{2i}$, a process resisted by IL-10 [22] and IL-13 [23]. Hence the equation of $T_\alpha$ is given by

$$\frac{\partial T_\alpha}{\partial t} - D_{T_\alpha} \Delta T_\alpha = \left( \gamma_T S, M_1 + \gamma_{T,i} M_{1i} + \gamma_{T,a} M_{2i} \right) \frac{1}{1 + I_{10}/K_{10}} \frac{1}{1 + I_{13}/K_{13}} - \mu_{T_\alpha} T_\alpha$$  \hspace{1cm} (24)$$

TGF-β is produced by $M_2$ and $M_{2i}$, a process enhanced by IL-13 [30, 75]. Hence,

$$\frac{\partial T_{\beta}}{\partial t} - D_{T_{\beta}} \Delta T_{\beta} = \left( \gamma_{T_2}, M_2 + \gamma_{T,2i} M_{2i} \right) \left( \frac{1}{1 + I_{13}/K_{13}} \right) - \mu_{T_\beta} T_{\beta}$$  \hspace{1cm} (25)$$

MMP and TIMP are produced by $M_2$ and $M_{2i}$ macrophages [31, 32], and depleted by binding to each other [73]. Hence the equations for $M_p$ and $T_p$ are given, respectively, as follows:

$$\frac{\partial M_p}{\partial t} - D_{M_p} \Delta M_p = \gamma_{M_p}, M_2 + \gamma_{M_p,i} M_{2i} - d_{M_p} T_p M_p - \mu_{M_p} M_p \hspace{1cm} (26)$$

$$\frac{\partial T_p}{\partial t} - D_{T_p} \Delta T_p = \gamma_{T_p M_2} M_2 + \gamma_{T_p M_{2i}} M_{2i} - d_{T_p} M_p T_p - \mu_{T_p} T_p \hspace{1cm} (27)$$

CCL3 is produced by infected $M_1$ and $M_2$ macrophages, as well as infected HSCs [45, 52, 53]. CCL3 is degraded naturally, but is also lost when internalized by $M_1$ [64]. Hence $C_3$ satisfies the equation:

$$\frac{\partial C_3}{\partial t} - D_{C_3} \Delta C_3 = \gamma_{C_3}, H + \gamma_{C_3, M_1} M_1 + \gamma_{C_3, M_{2i}} M_{2i} - d_{C_3} C_3 M_1 - \mu_{C_3} C_3 \hspace{1cm} (28)$$

**Equations for intracellular** ($V_{1i}$, $V_{1e}$, $V_{1f}$) **and extracellular** ($V_2$) **viruses.** In order to explain the dynamics of the viral loads, we introduce the following notations: $C$ will denote any of the cells $M_1$, $M_2$ or $H$, and $C_i$ will denote their infected states. $V_i$ will denote any of the intracellular viral loads $V_{1i}$, $V_{1e}$ or $V_{1f}$. We assume that the growth rate of $V_i$ is proportional to $C_i$, and is resisted by $I_{10}$, $T_\alpha$, $T_\beta$ and $I_p$, with the effect of $I_p$ being modulated by $I_6$ [20, 35, 55], thus reducing the growth rate coefficient $\lambda$ by a factor $(1 + I_6/K_{16})(1 + T_\alpha K_{16})(1 + T_\beta K_{16})(1 + I_p K_{16})$. There is also an increase of $V_i$ at a rate proportional to $C_V$, when external viruses are internalized by C cells [20]. We assume that the rate of this increase is $\lambda_{CC} N_V C_V$, where $N_V$ is a dimensionality coefficient which is of order of magnitude of (mass of 1 virus)/(mass of 1 cell). We assume that when one infected
cell, $C_i$ dies at rate $\mu_C = \mu_C(1 + \mu_V V_i)$, $N$ viruses are released. We conclude that the density of intracellular viruses in $C_i$ satisfies the equation

$$\frac{\partial V_i}{\partial t} - D_C \nabla^2 V_i = \frac{\lambda C_i}{(1 + I_i/K_i)(1 + T_i/K_{T_i})(1 + T_j/K_{T_j})(1 + I_j/(K_j + I_j))} + \lambda_{C_i} N_i CV_i - \mu_C (1 + \mu_V V_i) NV_i.$$  

Hence, we have the following equations for $V_{i1}$, $V_{i2}$, $V_{iH}$ and $V_c$:

$$\frac{\partial V_{i1}}{\partial t} - D_{M_i} \nabla^2 V_{i1} = \frac{\lambda_{Y_{i1}} M_{i1}}{(1 + I_i/K_i)(1 + T_i/K_{T_i})(1 + T_j/K_{T_j})(1 + I_j/(K_j + I_j))} + \lambda_{M_i} N_i M_{i1} V_i - \mu_{M_i} (1 + \mu_{V_{i1}} V_{i1}) NV_{i1}$$  

$$\frac{\partial V_{i2}}{\partial t} - D_{M_i} \nabla^2 V_{i2} = \frac{\lambda_{Y_{i2}} M_{i2}}{(1 + I_i/K_i)(1 + T_i/K_{T_i})(1 + T_j/K_{T_j})(1 + I_j/(K_j + I_j))} + \lambda_{M_i} N_i M_{i2} V_i - \mu_{M_i} (1 + \mu_{V_{i2}} V_{i2}) NV_{i2}$$  

$$\frac{\partial V_{iH}}{\partial t} - D_{H} \nabla^2 V_{iH} = \frac{\lambda_{Y_{iH}} H_i}{(1 + I_i/K_i)(1 + T_i/K_{T_i})(1 + T_j/K_{T_j})(1 + I_j/(K_j + I_j))} + \lambda_{H_{i1}} N_i H_i V_i - \mu_{H_{i1}} (1 + \mu_{V_{iH}} V_{iH}) NV_{iH}$$

Note that the diffusion coefficients of intracellular viruses were taken the same as the diffusion coefficients of their hosts. Note also that we have included the factor $\frac{1}{1 + A/K_A}$ in the first terms on the right-hand sides of Eqs (29)–(31). This factor accounts for the control of the viral load within infected cells by a drug, such as adefovir, that inhibits the growth of the virus. If the drug is not given, then $A = 0$ and the factor $\frac{1}{1 + A/K_A}$ may be dropped.

$V_c$ is increased when infected cells die and release the virus that they contained. There is a reduction in $V_c$ when cells internalize extracellular viruses and become infected. Hence,

$$\frac{\partial V_c}{\partial t} - D_{V_c} \nabla^2 V_c = N [\mu_{H_{i1}} (1 + \mu_{V_{i1}} V_{i1}) V_{i1} + \mu_{M_i} (1 + \mu_{V_{i2}} V_{i2}) V_{i2} + \mu_H (1 + \mu_{V_{iH}} V_{iH}) V_{iH}]$$

released by infected cells

$$-\frac{\lambda_{M_i} M_{i1} + \lambda_{M_i} M_{i2} + \lambda_{H_{iH}} H_i}{V_c} N_i V_e - \mu_{V_c} V_c,$$

$$\text{Boundary Conditions.} \text{ We simulate Model (1)–(32) in a rectangular domain } \Omega. \text{ We assume that the fibrosis occurs in } \Omega \text{ only, and hence we consider no-flux boundary conditions for all the variables.}$$
Initial Conditions. We prescribe the following initial conditions in a unit of g/cm³:

Cells: $M_1(0) = 3.48 \times 10^{-2}$, $M_1(0) = 4.07 \times 10^{-3}$, $M_2(0) = 4.02 \times 10^{-2}$, $M_3(0) = 4.86 \times 10^{-2}$, $H(0) = 4.97 \times 10^{-2}$, $H_1(0) = 2.67 \times 10^{-4}$, $T_1(0) = 9.44 \times 10^{-3}$, $T_2(0) = 3.01 \times 10^{-2}$, $f(0) = 6.2 \times 10^{-3}$, $m(0) = 3.31 \times 10^{-4}$;

ECM: $\rho(0) = 2.7 \times 10^{-5}$;

Cytokines and other proteins: $G(0) = 1.81 \times 10^{-11}$, $H_A(0) = 1.44 \times 10^{-6}$, $I_{1A}(0) = 5.5 \times 10^{-10}$, $I_2(0) = 1.47 \times 10^{-17}$, $I_4(0) = 2.3 \times 10^{-10}$, $I_6(0) = 1.29 \times 10^{-10}$, $I_{10}(0) = 2.2 \times 10^{-9}$, $I_{12}(0) = 4.19 \times 10^{-9}$, $I_{13}(0) = 3.53 \times 10^{-10}$, $I_{14}(0) = 7.42 \times 10^{-10}$, $I_{16}(0) = 1.77 \times 10^{-10}$, $T_{A}(0) = 1.82 \times 10^{-10}$, $T_{B}(0) = 6.22 \times 10^{-7}$, $M_P(0) = 1.32 \times 10^{-5}$, $T_{P}(0) = 3.86 \times 10^{-7}$, $C_3(0) = 1.22 \times 10^{-14}$;

HBV: $V_A(0) = 3.9 \times 10^{-12}$, $V_{i1}(0) = V_{i2}(0) = V_{i3}(0) = 1.6 \times 10^{-9}$.

However, we note that the simulation results with different initial conditions do not appreciably change after a few days.

Results

All the computations are done using Python 2.7.6. The parameter values of the model equations are estimated in Section A in S1 File and are listed in Tables C–F in S1 File. The techniques used for the simulations are also described in Section B in S1 File.

Fig 2 is a simulation of the averages of the model variables for 180 days. We see that most of the variables are not in steady state, in agreement with the progressive state of HBV in chronic stage. The densities of uninfected phagocytic cells ($M_1$, $M_2$ and $H$) decrease while the densities of the infected phagocytic cells ($M_1i$, $M_2i$ and $H_i$) increase. The increase in the densities of infected phagocytic cells results in an increase of the inflammation as seen in the profiles of IFN-γ and TNF-α, in agreement with chronic HBV progression. While $M_2i$ decreases and $M_2i$ increases, the production term $\lambda_{M_1, M_2} M_2 + \lambda_{M_1, M_2} M_2$, of $M_P$ increases, as seen in the increasing profile of $M_P$. The simulations show that the densities of both Th1 cells and Th2 cells increase, while the Th2 cells are dominant. The increase in IL-10 and decrease in IL-12 suggest that the immune system is not strong enough to confront the infection. Cytokines produced by Th1 cells tend to reduce the load of intracellular viruses, while cytokines produced by Th2 cells work in the opposite direction. It is therefore interesting to note that, since $T_2$ dominates $T_1$, the viral loads (mostly represented by the intracellular HBV) are continuously increasing. Our model also shows that the density of fibroblasts decreases monotonically while the density of myofibroblasts increases, which enhances the production of ECM; the transition from fibroblast to myofibroblast is induced by PDGF and TGF-β, and it increases in time. The concentrations of MMP and TIMP also increase in time. The scar continues to grow, as a result of the increase in MMP and ECM.

We can also simulate the spatial variations of the variables, however we are interested, in this paper, only in the average profiles of the variables. One is tempted to use the simpler ODE model to compute averages. We note however that the diffusion coefficients of cytokines and extracellular viruses are several orders of magnitude larger than the diffusion coefficients of cells. For this reason the ODE system cannot adequately represent the spread of the infection, and hence, the averages of the variables. Indeed, in the simulation of the ODE system (not shown here) the infected macrophages decrease, rather than increase; healthy HSCs are increasing, rather than decreasing, and the intracellular and extracellular viruses decrease and stabilize, rather than monotonically increase, as in Fig 2.
Treatment

In this section, we consider two drugs that are commonly used for the treatment of HBV, namely IFN-α and adefovir [5, 6, 12, 13]. Treatment with IFN-α in chronic HBV adult patient ranges between 16–52 weeks, while treatment with adefovir is continuous for 2–3 years [39, 76]. In oncology, it is found that in case of intermittent therapies in which the drug amount administered per unit time is constant, the response is significantly influenced by the amount of drug given and the period of time within which the drug is given [77–79]. Moreover, if negative side effects are ignored, then by doubling the amount of drug and correspondingly shortening the period of treatment (keeping the total amount of drug fixed) we may get better results in reducing the infection [65]. Assuming that the total amount of each drug is fixed, we use the mathematical model to evaluate the efficacy of combination therapy with IFN-α and adefovir. We start the treatment after 90 days of control and administer the drugs for 90 days.

**Treatment with IFN-α**. In our model, injection of IFN-α is represented as a source term, $c_α(t)$, in Eq (22), which then takes the form

$$ \frac{\partial I_a}{\partial t} - D_a \Delta I_a = c_α(t) + \lambda_{T_1} T_1 + \lambda_{I_2} I_2 M_{I_2} \left( \frac{1}{1 + I_{I_2}/K_{I_2}} \right) - \mu_{T_1} I_a . \tag{33} $$

**Treatment with adefovir (A)**. We denote the source of the drug by $c_a(t)$. The drug is diminished by absorption by infected cells, and by natural degradation. Hence, the equation of
adefovir, $A$, is given by

$$\frac{\partial A}{\partial t} - D_A \Delta A = \underbrace{c_A(t)}_{\text{source}} - \underbrace{(\lambda_{M_{1i}A}M_{1i} + \lambda_{M_{2i}A}M_{2i} + \lambda_{H_{i}A}H_{i})}_{\text{absorption}} \frac{A}{A + K} - \underbrace{\mu_A A}_{\text{degradation}}. \quad (34)$$

**Combination therapy with IFN-α and adefovir.** Combination therapy with IFN-α and adefovir is modeled by using Eqs (33) and (34) together with System (1)–(32). Adefovir is given daily in one or two tablets [80], and its effective time is about 10 hours [81]. For simplicity, we assume a daily average constant level $c_A$ taking

$$c_A = \begin{cases} 10^{-4} \text{ g/cm}^3 \text{ d}^{-1}, & 90 < t < 180; \\ 0, & \text{elsewhere}. \end{cases} \quad (35)$$

IFN-α is given in shots three times a week, or in slow injection, as pegylated IFN-α, once a week [82]. We assume that it is given once a week at level $c_\alpha$, so that after $t$ days its level reduces to

$$c_\alpha e^{-\alpha t}, \quad \text{for some } \alpha > 0,$$

and at the end of the week ($t = 7$ days) its level is negligible, taking

$$c_\alpha e^{-7\sigma} = \frac{1}{X} c_\alpha, \quad X = e^{\sigma},$$

so that $\sigma = 1$. The average level during the week is

$$\frac{1}{7} \int_0^7 c_\alpha e^{-\alpha t} dt = \frac{c_\alpha}{7} (1 - e^{-7}) \sim \frac{c_\alpha}{7}.$$

We wish to simplify the simulations by replacing the intermittent treatment of injections at level $7c_\alpha$ by a constant level of the drug at the level $c_\alpha$, that is, by

$$c_\alpha(t) = \begin{cases} 10^{-7} \text{ g/cm}^3 \text{ d}^{-1}, & 90 < t < 180; \\ 0, & \text{elsewhere}. \end{cases} \quad (36)$$

In order to justify this simplification, we simulated in Figs 3 and 4 the average densities of all the variables with treatment (IFN-α–orange, adefovir–green and combination–red) and without treatment when the drugs are given intermittently (Fig 3) and continuously (Fig 4).

We see that the profiles of the virus and scar are almost the same under Figs 3 and 4. Hence, for simplicity, we shall use the scheme for Fig 4, that is, Eqs (35) and (36), to compute the efficacy and synergy maps.

**Therapy with continuous drug delivery.** From Fig 4, we see that IFN-α reduces the viral load by 30.36%, which agrees with reported clinical results by Paul and Han [83]; adefovir reduces by 42.28% and the combination reduces by 57.66%. The reduction in the scar is 20.41% by IFN-α, 28.24% by adefovir, and 37.8% by the combination, although the scar still keeps increasing. We also see that the drugs (in monotherapy and in combination therapy) effectively reduce the inflammation by considerably reducing the densities of infected phagocytic cells ($M_{1i}$, $M_{2i}$, and $H_{i}$), and the pro-inflammatory cytokine TNF-α. The pro-inflammatory cytokines IL-6 and IL-12 already have decreasing profiles in the control case, making the effect of drugs on them less significant. We observe in Fig 4 that the drugs have little (or no)
effect on the profiles of Th1 and Th2 cells, and hence also on IFN-γ and IL-2 which are secreted by T cells.

We proceed to consider the effect of therapy for a range of doses of adefovir and IFN-α. We denote by $S_X(180)$ and $V_iX(180)$ the densities of $S$ and $V_i$, respectively, at day 180 under administration of drug $X$, where $X$ is either adefovir alone, or IFN-α alone, or their combination. We denote by $S(180)$ and $V_i(180)$ the densities of $S$ and $V_i$, respectively, at day 180 in the control case (no drugs), and define two efficacy functions:

$$E_S(X) = \frac{S(180)}{S_X(180)} \times (100\%)$$

$$E_{V_i}(X) = \frac{V_i(180)}{V_iX(180)} \times (100\%).$$

(37)

Fig 5A is an efficacy map for the scar density under the combined therapy with $X = (c_A, c_α)$, and Fig 5B is the corresponding efficacy map for the viral load $V_i$, the color columns colors show the efficacy values.

Both IFN-α and adefovir can have severe side-effects when administered alone. These side-effects could be aggravated if both drugs are given simultaneously with the same doses as in monotherapy. Thus, there is the need to find a strategy for combination therapy that maximizes the efficacy while reducing the sides effects. The dose-response relationship of the
amount of drugs to the severity of negative side-effects has been extensively studied [84–86]: at higher doses, undesired side-effects appear and grow stronger with increases in the dose.

We compare treatment of combination therapy ($c_\alpha, c_A$) with monotherapy $I_\alpha$ and monotherapy $A$. For monotherapy $I_\alpha$, we take $(1 + \theta_\alpha) c_\alpha$, and for monotherapy $A$, we take $(1 + \theta_A) c_A$, with $\theta_\alpha > 0, \theta_A > 0$, to reflect the highest toxicity expected when combining the two drugs. If $E_S(c_\alpha, c_A)$ is larger than both $E_S((1 + \theta_\alpha) c_\alpha, 0)$ and $E_S(0, (1 + \theta_A) c_A)$, then we say that the ‘synergy’ in scar density reduction for the combination ($c_\alpha, c_A$) is positive, and otherwise, we say that the synergy is negative [87]. Similarly, if $E_V(c_\alpha, c_A)$ is larger than both $E_V((1 + \theta_\alpha) c_\alpha, 0)$ and $E_V(0, (1 + \theta_A) c_A)$, then we say that the synergy in viral load reduction for the combination ($c_\alpha, c_A$) is positive, and otherwise, we say that the synergy is negative [87]. More generally, we define the synergy in scar density reduction $\sigma_S = \sigma_S(c_\alpha, c_A)$ by the formula (as in [87]):

$$\sigma_S(c_\alpha, c_A) = \frac{E_S(c_\alpha, c_A)}{\max \{E_S((1 + \theta_\alpha) c_\alpha, 0), E_S(0, (1 + \theta_A) c_A)\}} - 1.$$  

Then $\sigma_S(c_\alpha, c_A) > 0$ (positive synergy) if the combination ($c_\alpha, c_A$) reduces the level of scar density more than either one of the single agents $(1 + \theta_\alpha) c_\alpha$ or $(1 + \theta_A) c_A$. Negative synergy occurs in the reverse case where instead of a combination therapy with ($c_\alpha, c_A$), we achieve better reduction of the level of scar density if we apply only one drug, $(1 + \theta_\alpha) c_\alpha$ or $(1 + \theta_A) c_A$. Similarly we...
define the synergy in reducing the viral load by the formula:

\[
\sigma_V(c_a, c_A) = \frac{E_V(c_z, c_A)}{\max \{E_V((1 + \theta_I) c_z, 0), E_V(0, (1 + \theta_A)c_A)\}} - 1. \tag{39}
\]

There are negative side-effects such as lactic acidosis in the case of treatment with adefovir, and serious depression in the case of IFN-\(\alpha\)\cite{88}. Since the mechanisms of these side-effects are not well understood, and since it is difficult to evaluate and compare the severity of these side-effects, we take for definiteness \(\theta_I = \theta_A = 2\); this choice is arbitrary and could be made more precise as more clinical data become available that will inform on the side-effects associated with IFN-\(\alpha\) and adefovir, separately or in combination.

Fig 6 shows the ‘synergy map’ associated with Model (1)–(32) where we compute the values for the synergy as in Eq (38) related with the drugs IFN-\(\alpha\) (\(I_\alpha\)) and adefovir (\(A\)). The color columns assign a “synergy number” (SN) to any level of adefovir and IFN-\(\alpha\); SN varies from \(-0.16\) to \(0.64\) for the level of scar density, and from \(-0.15\) to \(0.69\) for the viral load. Given any level of \(c_a\) as \(c_A\) initially increases so does the SN, until \(c_A\) reaches a level \(c_A^*\) which depends on \(c_a\); i.e., \(c_A^* = c_a(c_a)\). Thereafter, the SN begins to decrease as \(c_A\) increases. The biological explanation of the color patterns in the synergy maps can be traced to the fact that the virus proliferation is inhibited by the product \((1 + I_\alpha/\bar{K}_\alpha)(1 + A/\bar{K}_A)\), as seen in Eqs (29)–(31). This product is highly correlated, after scaling, to the product \((1 + c_a)(1 + c_A)\). Viewing this product
as representing the efficacy of the combined therapy, the resulting expression for the synergy is then a function of the following type:

\[ f(c_\alpha, c_A) = \frac{(1 + c_\alpha)(1 + c_A)}{\max(1 + 3c_\alpha, 1 + 3c_A)} - \text{constant}. \]

We now observe that \( f(c_\alpha, c_A) \) is an increasing function in \( c_A \) if \( c_A < c_\alpha \) and a decreasing function in \( c_\alpha \) if \( c_A > c_\alpha \), and similarly, \( f(c_\alpha, c_A) \) is increasing in \( c_\alpha \) if \( c_A < c_\alpha \) and is decreasing in \( c_\alpha \) if \( c_A > c_\alpha \), and this behavior is reflected in the synergy maps. In both synergy maps Fig 6A and 6B, SN is negative when \( c_A < 1.25 \times 10^{-5} \text{ g/cm}^3 \text{ per day} \), or \( c_\alpha < 0.15 \times 10^{-8} \text{ g/cm}^3 \text{ per day} \). Negative synergy indicates that it is more beneficial to use monotherapy with either \( 3c_\alpha \) or \( 3c_A \) instead of combination therapy with \( (c_\alpha, c_A) \). However this conclusion does not take into account negative side-effects.

**Discussion**

Hepatitis B virus (HBV) infection is one of the most prevalent infectious diseases associated with human liver. Despite the availability of a vaccine, HBV remains a global health problem, which affects more than 350 million people annually, with 600,000 deaths resulting from HBV–related liver diseases. One of the hallmarks of the disease is the development of liver fibrosis. Anti–HBV drugs currently in use include adefovir, which is anti–viral therapy, and IFN–α, which is decreases viremia, inflammation and liver fibrosis. The treatment with each of
these drugs typically extends over a period of many months, and incurs severe negative side-effects; kidney damage, low amount of phosphate in the blood, stroke and pancreatitis for adefovir, brain disorder, heart attack and lung fibrosis for IFN-α.

In the present paper, we considered the effect of these two drugs on slowing the growth of liver fibrosis associated with HBV. With the aim of using the least amount of drugs, we asked the following question: Is a combination of adefovir and IFN-α at a certain level more effective in reducing the fibrotic scar than a monotherapy with either adefovir or IFN-α at an ‘appropriate’ increased level? If so, we say that the combination therapy is synergistic. We developed a mathematical model by a system of PDEs and used the model to address this question. The model includes cells and cytokines that significantly affect the fibrosis of the liver in HBV. Some of the parameters were taken from the literature, while others were estimated under some assumptions; simulations of the model validated these assumptions.

The main result of the paper is the “synergy maps” in Fig 6. The color columns assign a “synergy numbers” (SN) to any level of adefovir (c_A) and IFN-α (c_α). Given any level of c_α, as c_A initially increases so does each SN, until c_A reaches a level c_A* which depends on c_α; i.e., c_A* = c_α(c_A). Thereafter, the SN begins to decrease as A increases. We suggest that the dosage of IFN-α should be related to adefovir so that the proportion should lie as closed as possible to both solid curves in Fig 6.

In Fig 6, negative synergy means that it is more beneficial to treat the patient with either IFN-α or adefovir as a single agent, rather than with combination of the two drugs. In the definition of efficacy we compared treatment of combination therapy (c_α, c_A) to monotherapy with either (1 + θ_1_α)c_α or (1 + θ_2_A)c_A, where we took θ_1_α = θ_2_A = 2. This choice was quite arbitrary. If it turns out that the negative side-effects in the combined therapy are much more severe than the negative side-effects of a monotherapy with either IFN-α and adefovir, then it would be more appropriate to increase the values of θ_1_α and θ_2_A in the synergy map. On the other hand, if the negative side-effects from the combination are not significantly more severe than the negative side-effects from either one of the two drugs, then it would be appropriate to decrease the chosen values of θ_1_α and θ_2_A in the synergy map. A meta-analysis for the comparison of efficacy in the combination IFN-α-adefovir versus IFN-α in monotherapy is done in [89]. In this work that does not include the effect of drug toxicity, the authors show that the efficacy of IFN-α plus adefovir combination therapy is superior to IFN-α monotherapy. However, combined clinical data on efficacy and toxicity are quite limited at this time. Hence, our model should be viewed as setting up a computational framework, to address the question of optimal efficacy in combination therapy with IFN-α and adefovir.

Supporting information

S1 File. Supporting information—Chronic hepatitis B virus and liver fibrosis: A mathematical model. Parameter estimates (Section A in S1 File), parameter values (Tables C–F in S1 File) and numerical methods used (Section B in S1 File).

(PDF)

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