MODELING TUMOR GROWTH INCORPORATING THE EFFECTS OF NECROSIS AND THE EFFECT OF BEVACIZUMAB.

1. Abstract

A tumor growth model incorporating the necrotic part and using mixed order pharmacokinetics of the drug (bevacizumab) is presented in [8]. The model is able to show good fit to the experimentally observed results and is able to explain the important physiological phenomena. The mathematical analysis of the model is lacking. The aim of the current project is to use this model of tumor growth and do further mathematical analysis and simulations. The experimental data is available for the treatment of andenocarcinoma tumor on laboratory mice. Two types of treatments were performed: 1) a large dosage of 10 mg/Kg (0.171 mg/ml serum level) of drug at the beginning of the treatment. 2) Moderate dosage of 1/18 mg/Kg (9.5×10^{-4}mg/ml serum level) for each day for 18 days. The model is able to better fit the experimental data from continuous dosage treatment in contrast to the single dosage treatment. Further predictions are made based on the simulation results by varying the type of treatment.

2. Introduction

Angiogenesis is the formation of new blood vessels required for the growth of tumors. Inhibition of this process is ideal for fighting cancer [8]. VEGF (Vascular endothelial growth factor) is a signalling protein produced by cells that stimulates formation of blood vessels. High metabolic rates of cancer cells demands high intake of nutrients and oxygen [6]. This induces hypoxia which triggers the transcription of VEGF genes. The VEGF binds to the corresponding receptors on the surface of the epithelial cells hence inducing angiogenesis. Bevacizumab selectively binds to VEGF therefore inhibiting the binding of VEGF to cell surface receptors and in turn inhibiting the formation of blood vessels [6]. Cancers are always composed of active (proliferating cells) and inactive tumor cells. There are also specialised cells which are temporarily inactive. These quiescent cells, also called cancer stem cells, remain in reversible G0 phase until stimulated by signalling pathways and molecules like tumor protein p53, retinoblast protein (RB) and cyclin-dependent kinases inhibitors (CDKI) [8]. These quiescent cells can metastasize and remain in dormant state for a long time. The experiment for measuring tumor growth was done on C57BL/6 laboratory mice [8, 3]. C38 adenocarcinoma was subcutaneously implanted on two groups of mice (group 1 with mice C1-C5 and group 2 with mice E1-E9). Both groups were of same weight (20 g). Two kinds of therapy was given to the two groups. The first group (C1-C5) received an injection of 10 mg/Kg dosage of bevacizumab at the beginning of the treatment. The second group (E1-E9) received a continuous dosage of 1/18 mg/Kg injection of bevacizumab for 18 days. The data of the tumor volume was collected for 18 days for the two groups of mice. C38 colon adenocarcinoma typically do not metastasize [8]. Due to the subcutaneous location the limited space condition necessary for the presence of quiescent cells is not a possibility. Hence in modelling the tumor growth only the necrotic tumor cells and proliferating tumor cells are considered.

3. Mathematical Model

Let X_1 denotes the proliferating tumor volume (mm^3), X_2 denotes the necrotic tumor volume (mm^3) and X_3 denotes the inhibitor (bevacizumab) serum level (mg/ml), O denotes the compartment outside the model. The following equations describe the dynamics of tumor and the serum [8]:
(1) $X_1 \xrightarrow{a} 2X_1$ represents the growth of tumor cells with the rate a. Hence using the mass action kinetics we get $\dot{x_1} = ax_1$.
(2) $X_1 \xrightarrow{n} X_2$ represents the proliferating tumor cells becoming necrotic tumor cells at the rate n. Hence

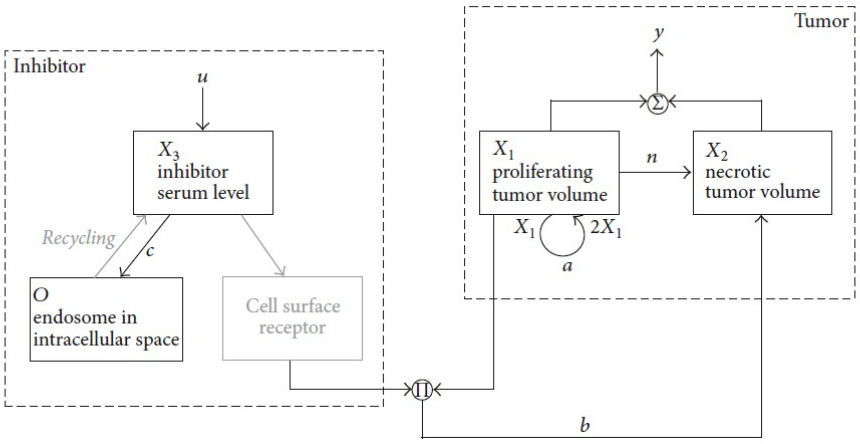

FIGURE 1. System network as shown in [8]. The items in grey represents extra details happening in the biological background which is not explicitly described in the model but is taken care of by the Michealis-Menten kinetics [8].

by the mass action kinetics we obtain $\dot{x_1} = -nx_1$ and $\dot{x_2} = nx_1$.
(3) $X_3 \xrightarrow{c} O$ represents the clearance rate of the drug or the outflow of the drug to a compartment outside the model at the rate c. By using the Michealis-Menten kinetics we get $\dot{x_3} = -c\frac{x_3}{K_B+x_3}$ where K_B is the Michealis-Menten constant for the inhibitor.
(4) $X_1 + X_3 \xrightarrow{b} X_2$ represents the inhibitor binding to the VEGF molecules whose concentration is proportional to the volume of tumor with the rate b. Hence by using the Michealis- Menten kinetics we get $\dot{x_1} = -bx_1\frac{x_3}{ED_{50}+x_3}$, $\dot{x_2} = bx_1\frac{x_3}{ED_{50}+x_3}$ and $\dot{x_3} = -b_kx_1\frac{x_3}{ED_{50}+x_3}$. Where $b_k = bk$ and k is a constant with the dimension $(mg/ml)/mm^3day$. ED_{50} is the median effective dosage which is found from [5] to be $5 \times 10^{-5}mg/ml$.

The differntial equations can be written as follows:

$$\dot{x_1} = (a-n)x_1 - bx_1\frac{x_3}{ED_{50}+x_3}. \tag{1}$$

$$\dot{x_2} = nx_1 + b\frac{x_1x_3}{ED_{50}+x_3}. \tag{2}$$

$$\dot{x_3} = -b_k\frac{x_1x_3}{ED_{50}+x_3} - c\frac{x_3}{K_B+x_3} + u. \tag{3}$$

u is a function of the input dosage rate of the inhibitor. u will vary according to the type of treatment used.

The model for the treatment of tumor using a single initial dosage is similar to the above model :

$$\dot{x_1} = (a-n)x_1 - bx_1\frac{x_3}{ED_{50}+x_3}. \tag{4}$$

$$\dot{x_2} = nx_1 + b\frac{x_1x_3}{ED_{50}+x_3}. \tag{5}$$

$$\dot{x_3} = -b_k\frac{x_1x_3}{ED_{50}+x_3} - c\frac{x_3}{K_B+x_3}. \tag{6}$$

with the condition that $x_3(0)$ is the initial serum drug level. The only single dosage treatment that will be considered throughout the project is the treatment on mice (C1-C5) with $x_3(0) = 0.171mg/ml$, which is the initial serum level of the inhibitor.

4. Analysis

Theorem 4.1. *The system (1)-(3) has a unique solution and* $\{(x, y, z) : x \geq 0, y \geq 0, z \geq 0\}$ *is a positive invariant set for the system.*

Proof. Note that $\dot{x_1}|_{x_1=0} \geq 0$, $\dot{x_2}|_{x_2=0} = nx_1 + b\frac{x_1x_3}{Ed_{50}+x_3} \geq 0$ if x_1, $x_3 \geq 0$ and $\dot{x_3}|_{x_3=0} = u \geq 0$. Hence it follows from the proposition 2.1 in [9] that $\{(x, y, z) : x \geq 0, y \geq 0, z \geq 0\}$ is a positive invariant set for the system.
The equations on the right hand side of (1)-(3) are C^1 functions in the domain $\{t : t \geq 0\} \times \mathbb{R}^3_+$. By the existence and uniqueness theorem the system (1)-(3) has a unique solution in $\{t : t \geq 0\} \times \mathbb{R}^3_+$. □

Theorem 4.2. *The proliferating tumor volume is bounded by exponential functions as follows*

$$x_1(0)e^{(a-n-b)t} \leq x_1(t) \leq x_1(0)e^{(a-n)t}. \tag{7}$$

If $(a - n) < 0$ *then eventually all the proliferating tumor will become necrotic tumors.*

Proof. From (1) it follows that

$$(a - n - b)x_1 \leq \dot{x_1} \leq (a - n)x_1.$$

Hence it follows that the solution $x_1(t)$ satisfies the inequality

$$x_1(0)e^{(a-n-b)t} \leq x_1(t) \leq x_1(0)e^{(a-n)t}.$$

□

Note that from (1)-(3) it follows that the dynamics of proliferating tumor (x_1) and the inhibitor (x_3) is independent of the dynamics of necrotic tumor (x_2). Hence we focus on the system

$$\dot{x_1} = (a - n)x_1 - bx_1\frac{x_3}{ED_{50} + x_3}. \tag{8}$$

$$\dot{x_3} = -b_k\frac{x_1x_3}{ED_{50} + x_3} - c\frac{x_3}{K_B + x_3} + u. \tag{9}$$

For all the further analysis let us assume that u is a constant function.

Theorem 4.3. *The system (8)-(9) has a locally asymptotically stable equilibrium point* $(0, u.\frac{K_B}{c-u})$ *if* $a - n < 0$.

Proof. Solving the equations

$$(a - n)x_1 - bx_1\frac{x_3}{ED_{50} + x_3} = 0.$$
$$-b_k\frac{x_1x_3}{ED_{50} + x_3} - c\frac{x_3}{K_B + x_3} + u = 0.$$

we get two equilibrium points $(\bar{x_1}, \bar{x_3}) = (0, u.\frac{K_B}{c-u})$ and $(x_1^*, x_3^*) = \left(\frac{b}{b_k.(a-n)}.(u - c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}), \frac{(a-n)ED_{50}}{b-a+n}\right)$.

Evaluating the Jacobian of the system (8)-(9) we get

$$J|_{(\bar{x_1},\bar{x_3})} = \begin{bmatrix} a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} & 0 \\ \frac{-b_k.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} & \frac{-c.K_B}{(K_B+\frac{u.K_B}{c-u})^2} \end{bmatrix} \tag{10}$$

$$J|_{(x_1^*,x_3^*)} = \begin{bmatrix} 0 & \frac{-b.x_1^*.ED_{50}}{(ED_{50}+x_3^*)^2} \\ \frac{-b_k.x_3^*}{(ED_{50}+x_3^*)} & \frac{-b_k.x_1^*.ED_{50}}{(ED_{50}+x_3^*)^2} - \frac{c.K_B}{(K_B+x_3^*)^2} \end{bmatrix} \tag{11}$$

Let us assume that the equilibrium point (x_1^*, x_3^*) is in $\mathbb{R}^2_+$. It follows that $\dot{x_2}|_{(x_1^*,x_3^*)} = x_1^*(n + b.\frac{x_3^*}{ED_{50}+x_3^*}) = a.x_1^*$, since $x_1^* \neq 0$ then the necrotic tumor x_2 increases in mass for all future time, which is absurd because the proliferating tumor is at equilibrium (with the value x_1^*) and the serum inhibitor level is a constant (with the value x_3^*). This implies that contrary to our assumption (x_1^*, x_3^*) doesn't belong to $\mathbb{R}^2_+$. We are left with the case when $x_1^* = 0$ which is possible if and only if the dosage rate is a fixed value of $u = c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$. At this dosage rate both the equilibrium points (x_1^*, x_3^*) and $(\bar{x_1}, \bar{x_3})$ are equal.

For the equilibrium point $(\bar{x_1}, \bar{x_3})$, let $c > u$ so that the equilibrium point is a positive value. From (10) it follows that the eigenvalues are $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})}$ and $\frac{-c.K_B}{(K_B+\frac{u.K_B}{c-u})^2}$. If $a - n < 0$ then the eigenvalues are negative. Hence the equilibrium point $(\bar{x_1}, \bar{x_3})$ is locally asymptotically stable. This equilibrium is a 'favourable' equilibrium since the proliferating tumor $\bar{x_1} = 0$ and from (2) it follows that necrotic tumor is stable (i.e $\dot{x_2}|_{(\bar{x_1},\bar{x_3})} = 0$). □

Theorem 4.4. *If $a - n > 0$ and $K_B.\frac{b-a+n}{a-n} + ED_{50} > 0$ then the dosage rate $u = c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$ is a bifurcation value for the system (8)-(9).*

Proof. From (10) it follows that the eigenvalues are $a-n-\frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})}$ and $\frac{-c.K_B}{(K_B+\frac{u.K_B}{c-u})^2}$. It follows that $a-n-\frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} < 0$ if and only if $u > c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$ and $a-n-\frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} > 0$ if and only if $u < c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$. Hence if $u > c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$ then the equilibrium point is locally asymptotically stable (eigenvalues are negative). If $u < c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$ then the equilibrium point is a saddle (eigenvalues are of opposite sign).

□

The biological interpretation of the previous theorem is as follows: If the tumor growth rate is greater than the necrosis rate (i.e $a > n$) and if the inhibition rate (b) is greater than the net tumor growth rate $(a - n)$ then the dosage rate u is a bifurcation parameter for the system (8)-(9). Note that the system eq(1)-eq(3) has a family of equilibrium points $\{(0, x_2, u.\frac{K_B}{(c-u)})\}$ for all x_2 such that $0 \leq x_2$. The jacobian matrix at any of these equilibrium points is given by

$$J|_{(\bar{x_1},\bar{x_3})} = \begin{bmatrix} a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} & 0 & 0 \\ n + \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})}) & 0 & 0 \\ \frac{-b_k.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} & 0 & \frac{-c.K_B}{(K_B+\frac{u.K_B}{c-u})^2} \end{bmatrix} \tag{12}$$

The eigenvalues are clearly $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})}$, 0 and $\frac{-c.K_B}{(K_B+\frac{u.K_B}{c-u})^2}$. Hence the stability of the equilibrium point is determined by the sign of the eigenvalue $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})}$.

Theorem 4.5. *There is no periodic orbit for the system (8)-(9) in the region $\{(x_1, x_3) : x_3 \geq \frac{(a-n)ED_{50}}{(b-a+n)}\}$.*

Proof. Let $f = (a - n)x_1 - bx_1\frac{x_3}{ED_{50}+x_3}$ and $g = -b_k\frac{x_1x_3}{ED_{50}+x_3} - c\frac{x_3}{K_B+x_3} + u$. Since $\frac{\partial f}{\partial x_1} + \frac{\partial g}{\partial x_3} = (a - n) - \frac{b.x_3}{(ED_{50}+x_3)} - \frac{b_k.x_1.ED_{50}}{(ED_{50}+x_3)^2} - \frac{c.K_B}{(K_B+x_3)^2} \leq 0$ when $(a - n) - \frac{b.x_3}{(ED_{50}+x_3)} \leq 0$ or equivalently when $x_3 \geq \frac{(a-n)ED_{50}}{(b-a+n)}$. Hence by the Dulac's criteria the system (8)-(9) has no periodic solution in the region $\{(x_1, x_3) : x_3 \geq \frac{(a-n)ED_{50}}{(b-a+n)}\}$.

□

Theorem 4.6. *The system (1)-(3) has no periodic orbit in the region $\{(x_1, x_2, x_3) : x_1 \geq 0, x_2 \geq 0, x_3 \geq 0\}$.*

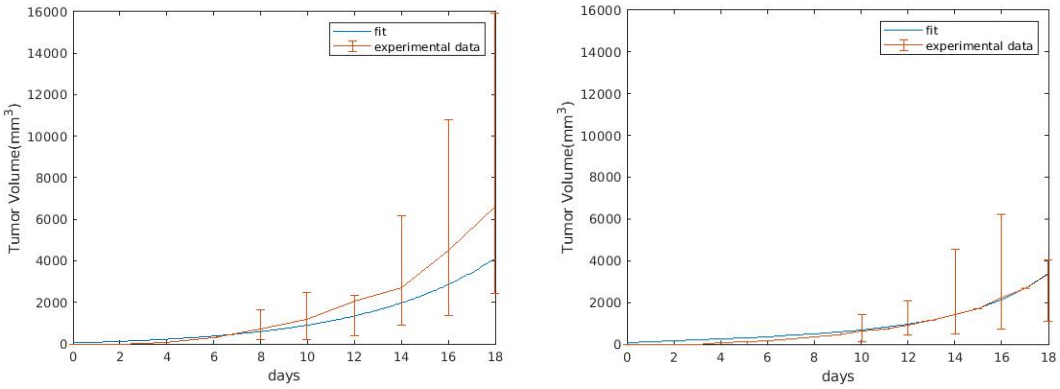

FIGURE 2. The figure shows the result of fitting the models eq(4)-(6) and eq(1)-(3) to the experimental data obtained from the single dosage and continuous dosage treatment respectively. (a) The experimentally observed average tumor volume (necrotic + proliferating tumor) in five mice (C1-C5) with treatment1 (single initial dosage of 10 mg/Kg) and the simulated total tumor volume. (b) The experimentally observed average tumor volume (necrotic + proliferating) in nine mice (E1-E9) with treatment2 (continuous dosage of 1/18 mg/Kg per day) and the simulated tumor volume.

Proof. Equation (2) implies that x_2 is an increasing function. Hence it is impossible to have a periodic orbit in the positive region. □

5. Parameter Estimation and Data Fitting

The value of ED_{50} was obtained from [5] to be 5×10^{-5} mg/ml. The value of the clearance rate c of Bevacizumab dosage of $5 - 10$ mg/Kg was obtained from [2] to be 0.18 L/day. Which is equivalent to the clearance of 0.0855-0.171 mg/ml of drug in the serum per day. The growth rate a of the tumor volume was estimated to be $0.2 - 0.7$ per day. The value of the necrosis rate n is taken to be between 0 and 1. If $n \geq 1$ then tumor volume is decreasing even without any drug action which is not possible from the experimental results. The value of b is estimated to be between 0 and 1 from [1]. The value of the Michealis-Menten constant of the inhibitor (K_B) is 0.4409 from [3].

The parameters a, n, b, b_k, $x_1(0)$ were identified by fitting to the data using the LSQCURVEFIT function in MATLAB(R2020b). This was done by minimizing the distance of the simulated total tumor volume and the experimentally observed total tumor volume. A set of parameters were identified such that the model eq(1)-eq(3) fit the experimental data for continuous dosage treatment (1/18mg/Kg per day). The list of parameters obtained are given in table(1)(a). Detailed algorithm used for parameter identification is given in supplementary data.

Similarly another set of parameters were identified such that the model eq(4)-(6) fit the experimental data for the single dosage treatment (single initial dosage of 10mg/Kg). The list of parameters obtained are given in table(1)(b). The figure(2) shows the results of the fitting both the models to the corresponding experimental data. Using the identified parameters for continuous dosage treatment for 18 days the plot of the necrotic tumor volume and the proliferating tumor volume is obtained as shown in figure(3)(a). The simulation result of the serum drug level for 18 days is shown in figure(3)(b).

Parameter	Dimension	Parameter name	1. Continuous dosage	2. Single dosage
a	$\frac{1}{day}$	Tumor growth rate	0.5696	0.6443
n	$\frac{1}{day}$	Necrosis rate	0.2564	0.2337
b	$\frac{1}{day}$	Inhibition rate	0.2556	0.2326
b_k	$\frac{mg}{ml}/mm^3/day$	Modified inhibition rate	9.498×10^{-6}	9.5×10^{-6}
c	$\frac{mg}{ml}$/day	Clearance rate	0.1502	0.1039
K_B	$\frac{mg}{ml}$	Michealis-Menten constant for inhibitor	0.4409	0.4409
$x_1(0)$	mm^3	Initial tumor volume	67.3819	47.7666
ED_{50}	mg/ml	Median effective dosage	5×10^{-5}	5×10^{-5}

TABLE 1. The parameters of the models after the identification by minimizing the distance between the total tumor volume and the simulated total tumor volume. (1) Continuous dosage: The parameter set obtained by fitting the model eq(1)-eq(3) to the experimental data from continuous dosage treatment. (2) Single dosage: The parameter set obtained by fitting the model eq(4)-eq(6) to the experimental data from single dosage treatment.

6. Simulation result

Using the parameter values from the table(1)(a), the local vector field of the system eq(1)-eq(3) and eq(8)-eq(9) are plotted in the figure(4). The value of u is taken to be a constant $\frac{9.5\times10^{-4}}{18}$mg/ml, which is the average drug dosage of the continuous treatment for 18 days. Using the identified parameters for the continuous dosage treatment (table(1)(a))

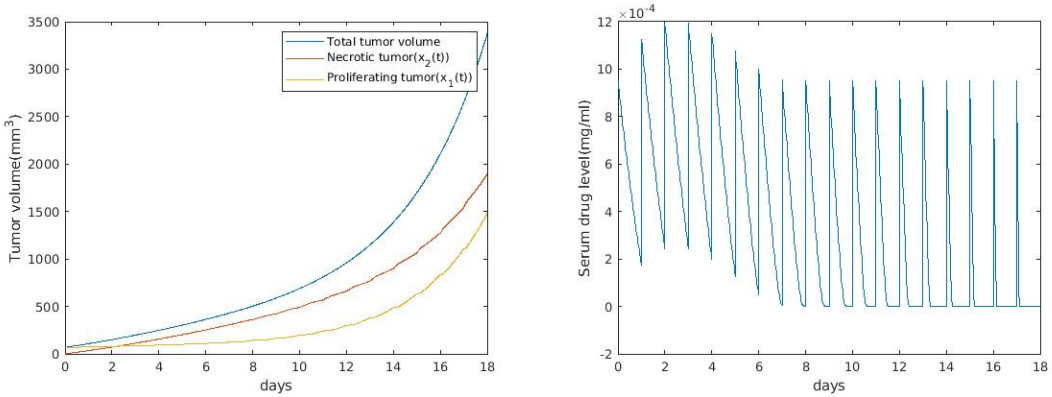

FIGURE 3. (1) The figure shows the simulation result of the necrotic tumor volume, proliferating tumor volume and the total tumor volume for the continuous treatment. (2) The figure shows the simulation result of the serum drug level in the continuous dosage treatment.

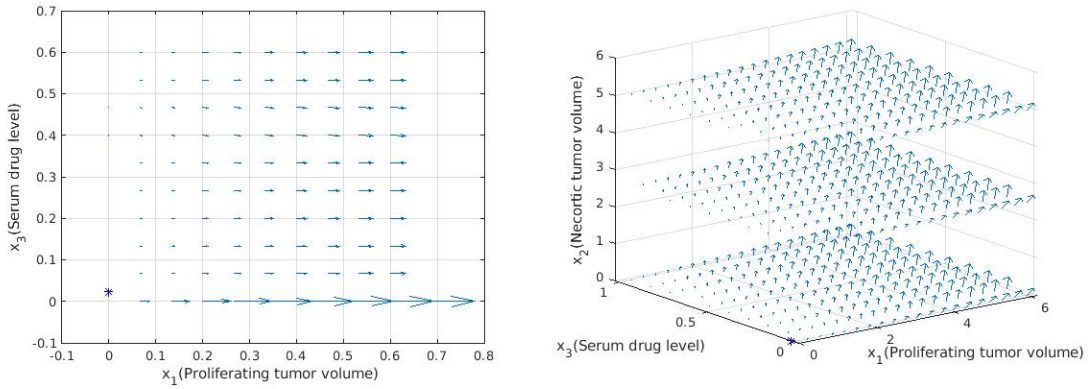

FIGURE 4. (a) The local vector field for the system eq(8)-eq(9). (b) The local vector field for the system eq(1)-eq(3). The star mark in the plots represent the equilibrium point $(0, u.\frac{K_B}{(c-u)})$ and $(0, 0, u.\frac{K_B}{(c-u)})$ respectively. The parameters used for plotting the vector fields are given in the table(1)(a). Here $u = \frac{9.5\times10^{-4}}{18}$mg/ml representing the average dosage rate for the continuous treatment.

the eigenvalue $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} = 0.1191 > 0$ and hence by eq(10) it follows that the equilibrium point $(0, \frac{u.K_B}{c-u})$ of the system eq(8)-eq(9) is a saddle node for the dosage rate $u = \frac{9.5\times10^{-4}}{18}$. From the calculations it follows that $ED_{50} + \frac{b-a+n}{a-n}K_B = -0.0825 < 0$ for the given set of parameters. It can be shown by using simple algebraic manipulations that given $a-n > 0$, then $a-n-\frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} \leq 0$ if and only if $c.ED_{50} \leq u.(K_B(\frac{b-a+n}{a-n}) + ED_{50})$. Since $c.ED_{50}$ is a positive value and $ED_{50} + \frac{b-a+n}{a-n}K_B = -0.0825$, the last inequality doesn't hold for any value of dosage rate u. Hence there is no bifurcation with the given set of parameter values as there is no change in the sign of eigenvalues of the system eq(8)-eq(9). By the theorem 4.5 there is no limit cycle in the region $\{(x_1, x_3) : x_3 \geq \frac{(a-n)ED_{50}}{(b-a+n)}\}$. Since $\frac{a-n}{b-a+n} < 0$ for the given set of parameters, there is no limit cycle in the region $\{(x_1, x_3) : x_3 \geq 0\}$ i.e there is no limit cycle in the positive region.

The figure(5) shows the simulation result of the tumor volume for 18 days with the treatment of $1.11 \times 10^{-3} mg/ml$ and $0.005 mg/ml$ of inhibitor dosage per day. The final tumor volume at the end of 18 days for the two treatments are $2239.39 mm^3$ and $1299.09 mm^3$ respectively. Hence increasing the dosage rate decreases the final tumor volume. Comparing with the final tumor volume at the end of 18 days for the single initial dosage treatment in figure(2), it follows that the continuous dosage treatment is much more effective in reducing the tumor volume. The total amount of drug used for 18 days in the case of continuous treatment with $1.11 \times 10^{-3} mg/ml/day$ is $0.02 mg/ml$, which is much lower than the single dosage treatment of $0.171 mg/ml$. The figure(6) shows the simulation results of the tumor volume for 18 days when the continuous dosage treatment of 9.5×10^{-4}mg/ml was given for the first n days, where $n = 2, 6, 12, 18$. We see that continuous dosage has to be maintained throughout the treatment period for the effectiveness of the drug.

7. Simulation prediction

By the figure(2) it is clear that the model for single dosage treatment is unable to fit the growth of the total tumor volume as experimentally observed. The final tumor volume is much higher than the simulated result. The continuous dosage model fits well to the experimental result. The only difference between

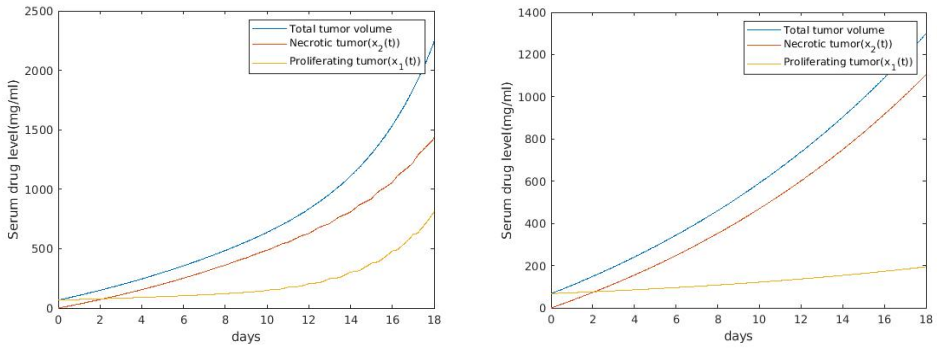

FIGURE 5. The figure shows the simulation result of the tumor volume for 18 days for the treatment of (a) $1.11 \times 10^{-3} mg/ml$ dosage of drug per day (b) $0.005 mg/ml$ dosage of drug per day.

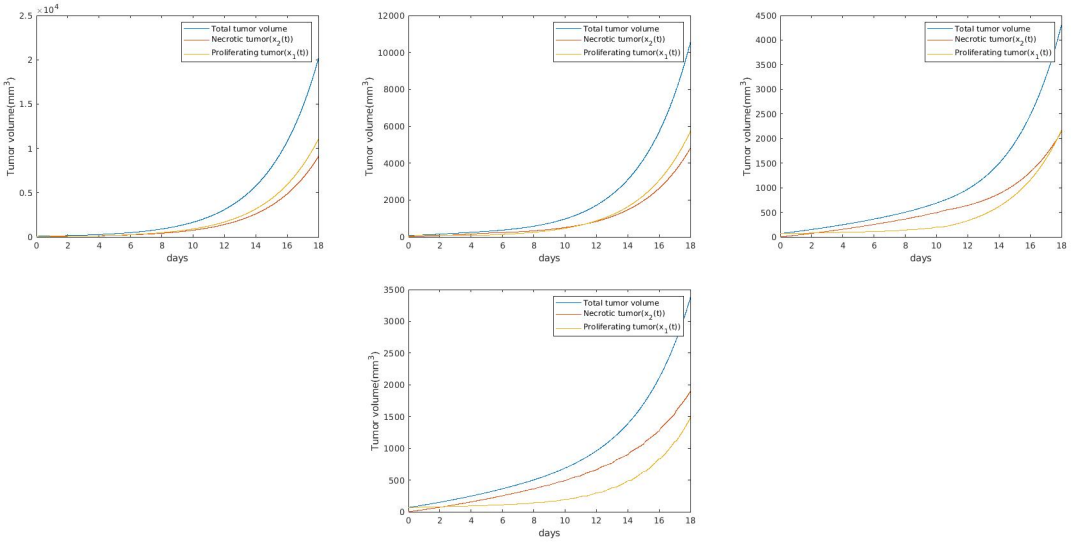

FIGURE 6. The figure shows the simulation result of the tumor volume for 18 days when the treatment of $1/18 mg/ml$ dosage of drug per day was given till the (a) second day (b) First 6 days (c) First 12 days and (d) First 18 days.

the single dosage model and the continuous dosage model is the treatment u. Moreover, according to the simulation results in figure(6), the continuous dosage has to be maintained for the entire length of the treatment for a significant result from the drug. Hence from the above observations it implies that there is some other mechanism which is effecting the bevacizumab drug which is hindering it's action, hence a continuous dosage is necessary to maintain it's action on the VEGF molecules. Hence the continuous dosage model is able to fit to the data. For this hypothesis to be verified further experiments have to be done. It is also observed that combining bevacizumab with other treatments (chemotherapy) can produce significant results compared to the monotherapy [7].

8. Conclusion and Future work

This work is a continuation or addition to the work done by Drexler *et al.* [8], where the authors tried to model the tumor growth incorporating the necrotic part and fit to the experimental results. Some errors observed in the paper (in using the dimensions of the parameters) were corrected. This

didn't change the results reported in the paper. A thorough mathematical analysis of the model was lacking, which is supplied in this work. Further simulations and predictions are made about the drug action and the proposed model. A possible drawback of this work could be that the same model as proposed in [8] is used without any modification. Detailed mechanisms were not incorporated instead approximations by Michealis-Menten kinetics were used, as discussed in section 3. This model also did not incorporate the tumor vasculature. The explanation that the authors have provided is that the tumor dynamics can be described by a first order model and that the tumor dynamics dominates the vasculature dynamics. Hence identification of the parameters corresponding to the vasculature dynamics is hard or nearly impossible [8]. The model and the identified parameters suggests that the tumor growth can't be stopped but the growth can be reduced. This result is also in accordance with the clinical practice of using other treatments with bevacizumab [7, 8, 4]. To validate the predictions made from this present work further research has to be done. A literature review of the possible mechanisms for the hindrance of the action of bevacizumab has to be conducted. The modification of the single dosage model has to be done and should be validated by fitting to the experimental result.

9. Supplementary data: Coding

The following are the codes used in the present work for the parameter estimation of the continuous dosage model and the single dosage model. The codes are written in the program $MATLAB(R2020b)$.

The file $confit.m$ is the algorithm for the parameter estimation of continuous dosage model eq(1)-eq(3) with $u = 9.4 \times 10^{-4}$ mg/ml dosage per day. The file *continuous.m* is the algorithm for solving the differential equations eq(1)-eq(3). Note that the file *continuous.m* is needed to run $confit.m$.

9.1. **Continuous treatment model:** *continuous.m*

$function$ S= $continuous(B, d)$
$tend = []$;
$Sv1 = []$;
$Sv2 = []$;
$Sv3 = []$;
$T = [B(6)]$;
$x0 = [B(6); B(6); 9.5. * 10^{-4}]$;
for $index = 2 : numel(d)$
t= $d(index - 1 : index)$;
[t, Sv]= $ode45(@DifEq, t, x0)$;
$T = cat(1, T, Sv(end, 1))$;
$tend = cat(1, tend, t)$;
$Sv1 = cat(1, Sv1, Sv(:, 1))$;
$Sv2 = cat(1, Sv2, Sv(:, 2))$;
$Sv3 = cat(1, Sv3, Sv(:, 3))$;

$x0 = Sv(end, :)$;
if $index < 20$
$x0(3) = Sv(end, 3) + 9.5. * 10^{-4}$;
end
end
$S = T$;

$function$ $dS = DifEq(-, x)$
$ED = 5. * 10^{-5}$;

$xdot = zeros(3,1);$
$xdot(1) = B(1).*x(2);$
$xdot(2) = (B(1)-B(2)).*x(2) - (B(3).*(x(2).*x(3))./(ED+x(3)));$
$xdot(3) = -(B(4).*x(2).*x(3))./(ED+x(3)) - (B(5).*x(3))./(0.449+x(3));$
$dS = xdot;$
end
end

Continuous treatment model:
$confit.m$

$Time2 = [0;1;2;3;3.98604175162936;5.11409752306497;6.01652828969137;7.04423702860133;7.99673743257489;$
$8.97430728152725;10.0269469601951;11.0295862541263;12.0070175978578;12.9843796889788;14.0368116098154;$
$14.9890350033472;15.9910510237844;17.0181364892004;17.9698058618487];$

$d = [0;1;2;3;4;5;6;7;8;9;10;11;12;13;14;15;16;17;18];$

$Sdata2 = [0;0;0;0;63.6508437276353;113.389607491590;162.020329488526;255.465185173787;348.540693603364;$
$441.739317784831;623.704399079705;717.026139013063;898.621873052264;1124.41616202032;1438.97690810178;$
$1708.84663624681;2199.95537053994;2691.18722058495;3314.64538816087];$

$options = optimset('disp','iter','LargeScale','off','TolFun',.00001,'MaxIter',1000000,'MaxFunEvals',1000000);$
$B0 = rand(6,1)*100;$
$[B] = lsqcurvefit(@continuous, B0, Time2, Sdata2, [0.2;0;0;0;0;0], [0.7;1;1;9.5.*10^{-6};0.2;100], options);$
$Mfit = continuous(B, Time2);$
$errneg = [0;0;0;0;0;0;0;0;0;0;485;0;475.1;0;968.2;0;1496.5;0;2200.3];$
$errpos = [0;0;0;0;0;0;0;0;0;0;807.496;0;1166.4;0;3087.5;0;4029.8;0;741.2];$
$plot(Time2, Mfit)$
$hold\ on$
$errorbar(Time2, Sdata2, errneg, errpos)$
$hold\ off$
$xlabel('days')$
$ylabel('TumorVolume(mm^3)')$
$legend('fit','experimentaldata','Location','Northeast')$

9.2. **Single dosage treatment:** *singledosage.m*

The file *singledosagefit.m* is the algorithm for the parameter estimation of single dosage model eq(4)-eq(6). The file *singledosage.m* is the algorithm for solving the differential equations eq(4)-eq(6). Note that the file *singledosage.m* is needed to run *singledosagefit.m*.

$function\ \ S = singledosage(B,t)$
$x0 = [B(6);B(6);0.171];$
$opt = odeset('MaxStep',0.5);$
$[T,Sv] = ode45(@DifEq,t,x0);$
$function\ \ dS = DifEq(-,x)$
$ED = 5.*10.^{-5};$
$xdot = zeros(3,1);$
$xdot(1) = B(1).*x(2);$
$xdot(2) = (B(1)-B(2)).*x(2) - (B(3).*(x(2).*x(3))./(ED+x(3)));$

$xdot(3) = -(B(4). * x(2). * x(3))./(ED + x(3)) - (B(5). * x(3))./(0.4409 + x(3));$
$dS = xdot;$
end
$S = Sv(:, 1);$
end

Single dosage treatment:
$singledosagefit.m$

$Time = [0; 2; 3.95000000000000; 5.97500000000000; 8.00000000000000; 9.97500000000000; 12.0000000000000; 1400000000000; 16.0000000000000; 17.9750000000000];$

$Sdata = [0; 0; 53.6883991429438; 323.109886746250; 724.762779308233; 1170.37037037037; 2056.87174778084; 2722.92623201714; 4490.90909090909; 6611.44781144781];$

$options = optimset('disp','iter','LargeScale','off','TolFun', .00001,'TolX', 0.0001,'MaxIter', 100000, 'MaxFunEvals', 100000);$
$B0 = rand(6, 1) * 100;$
$[B] = lsqcurvefit(@singledosage, B0, Time, Sdata, [0.2; 0; 0; 0; 0; 0], [0.7; 1; 1; 9.5.*10.^{-6}; 0.2; 100], options);$

$t = linspace(0, 18, 250);$
$errneg = [0; 0; 0; 0; 508.73; 944.29; 1644.31; 1813.24; 3130.5; 4180.3];$
$errpos = [0; 0; 0; 0; 905.64; 1309.5; 295.2; 3445.2; 6318.1; 9300.55];$
$Mfit = singledosage(B, t);$
$plot(t, Mfit)$
$hold\ \ on$
$errorbar(Time, Sdata, errneg, errpos)$
$hold\ \ off$
$xlabel('days')$
$ylabel('TumorVolume(mm^3)')$
$legend('fit','experimentaldata','Location','Northeast')$

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