Effect of Heterogeneous Microvasculature Distribution on Drug Delivery to Solid Tumour

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Abstract

Most of the computational models of drug transport in vascular tumours assume a uniform distribution of blood vessels through which anti-cancer drugs are delivered. However, it is well known that solid tumours are characterised by dilated microvasculature with non-uniform diameters and irregular branching patterns. In this study, the effect of heterogeneous vasculature on drug transport and uptake is investigated by means of mathematical modelling of the key physical and biochemical processes in drug delivery. An anatomically realistic tumour model accounting for heterogeneous distribution of blood vessels is reconstructed based on magnetic resonance images of a liver tumour. Numerical simulations are performed for different drug delivery modes, including direct continuous infusion and thermosensitive liposome-mediated delivery, and the anti-cancer effectiveness is evaluated through changes in tumour cell density based on predicted intracellular concentrations. Comparisons are made between regions of different vascular density, and between the two drug delivery modes. Our numerical results show that both extra- and intra-cellular concentrations in the liver tumour are non-uniform owing to the heterogeneous distribution of tumour vasculature. Drugs accumulate faster in well-vascularised regions, where they are also cleared out more quickly, resulting in less effective tumour cell killing in these regions. Compared with direct continuous infusion, the influence of heterogeneous vasculature on anti-cancer effectiveness is more pronounced for thermosensitive liposome-mediated delivery.

Keywords: drug delivery, heterogeneous vasculature, liver tumour, mathematical model, MR imaging, thermosensitive liposome

1. Introduction

Microvasculature has a significant influence on the growth, metastasis and drug delivery to tumours. The organisation of microvasculature depends strongly on the tumour type, its growth rate and location in the tumour [1]. As a tumour develops, the pre-existing vasculature may become tortuous, enlarged, dilated or incorporated into the tumour [2, 3]. On the other hand, as a result of angiogenesis occurring at the capillary level, neovascularization can originate from venules within the tumour and in the host tissue. The structures of newly formed microvessels are irregular, especially in fast growing tumours [2, 3].

For a solid tumour at a specific growth stage, the distribution of microvessels is spatially heterogeneous. In tumours with peripheral vascularisation, the centre is often poorly perfused and vasculatures are predominantly located near the boundaries, resulting in the formation of a necrotic core. This may also occur when a tumour is fully grown and invades into its host tissue. In tumours with central vascularisation, microvessels may proliferate to form a tree-like structure in the centre, resulting in higher vessel density [4, 5].

Previous numerical studies of drug transport have typically assumed a homogeneous distribution of microvasculature in non-necrotic regions. A numerical platform for studying drug transport in solid tumours was established by Baxter and Jain [6-8] with a focus on understanding the influences of various factors on the concentration of antibodies in the extracellular space. El-Karech [9] and Eikenberry [10] used mathematical modelling to determine doxorubicin concentration in the extra- and intra-cellular space with an idealised model and a tumour cord model, respectively. Goh *et al.* [11] investigated drug concentration in a 2-D hepatoma model which was assumed to have a uniformly distributed microvasculature in the tumour region excluding the necrotic core,. A 3-D computational model was applied to murine sarcoma by Zhao *et al.* [12] in order to study the effects of spatial variation of fluid filtration rate and vascular permeability on tumour microenvironment and drug transport, without incorporating the effect of anti-cancer drug on tumour cells.

In the present study, an improved mathematical model is applied to a realistic model of human liver tumour, with an aim to determine the effect of heterogeneous micro-vessel density on drug concentrations in the extra- and intra-cellular space as well as the effectiveness of anti-cancer treatment. The mathematical model incorporates the key physical and biological drug delivery processes, including the time-dependent plasma clearance, liposome and drug transport across the blood and lymphatic vessel walls, drug binding with proteins, lymphatic drainage, interactions with normal tissue, tumour cell uptake, tumour cell proliferation and physical degradation, as well as cell killing by drugs. The therapeutic efficacy is evaluated in terms of tumour cell density by directly solving the pharmacodynamics model based on predicted intracellular drug concentration. Two different drug delivery modes are examined and compared: continuous infusion of drug in its free form, and thermosensitive liposome-mediated delivery, where anticancer drugs are encapsulated in the interior of thermosensitive liposomes (TSL), and drug-loaded TSL are administrated via intravenous infusion. TSL are designed to release the encapsulated drugs upon heating to a designated temperature, which is usually in the range of 42 – 45°C. TSL-mediated drug delivery has been developed to overcome the limitations of conventional chemotherapy by prolonging plasma half-life and reducing systemic cytotoxicity. It also has the advantage of being a localised therapy, as drug release is triggered by heating, which can be targeted at the tumour site.

2. Methods

2.1 Model Formulation

The mathematical models consist of the mass and momentum conservation equations for interstitial fluid flow, mass transfer equations for the free and bound drug, transport equations for liposome encapsulated drug, as well as equations describing the intracellular drug concentration and pharmacodynamics. Details of the mathematical equations have been reported previously [13], and a brief summary of these is given in Appendix.

First, the interstitial fluid flow equations (Table A-1 in Appendix) are solved to provide the basic biomechanical environment for drug transport. This is followed by solution of the mass transfer equations for drug transport (Tables A-2 and A-3), which is described schematically in Figure 1(a) and (b) for direct infusion, and TSL-mediated delivery of doxorubicin, respectively. Briefly, the tumour region consists of three compartments: blood, extracellular space and tumour cells. Within each compartment, letters F, B and L represent free, bound and liposome encapsulated doxorubicin, respectively.

(a)

(b)

Figure 1. Drug transport with (a) continuous infusion of drug in its free form, and (b) thermosensitive liposome-mediated drug delivery

The dynamics process in drug delivery includes association/disassociation with protein at the rate k_{as} and k_{ds} respectively, drug exchange between blood and extracellular space, and influx/efflux of drugs from extracellular space to tumour cells. The rate of cell killing is governed by a pharmacodynamics model based on the predicted intracellular concentration of anticancer drugs (Table A-4 in Appendix). In the case of TSL-mediated drug delivery, an additional equation describing the transport of liposomes is needed (Table A-3 in Appendix). Since extra-vascular triggered release is simulated here, drug release at the rate k_{rel} is only included in the extracellular space of tumour. This is a reasonable approximation as the temperature of extracellular space is always higher than that of blood plasma during hyperthermia [14].

2.2 Model Geometry

The geometry of a liver tumour is reconstructed from post-contrast MR images acquired from a patient using a 3.0-Tesla MR scanner (DISCOVERY MR750, GE, Schenectady, New York, USA). Multislice anatomical images of the liver were acquired in three orthogonal planes with GR sequence at three time points after systemic infusion of the tracer Gd-DTPA, with each image comprising 256×256 pixels. Other imaging parameters are given in Table 1.

Table 1. MR imaging parameters

Parameter (unit)	Pixel Size (mm)	Field of View (mm)	Slice Thickness (mm)	Repetition Time (ms)	Echo Time (ms)
	1.3	420	5.0	3.808	1.808

In order to build a 2D model of the tumour and its surrounding normal tissue, a representative transverse image is chosen, as shown in Figure 2 (a). The chosen section covers the maximum dimension of the tumour. Image processing is carried out by using Mimics (Materialise HQ, Leuven, Belgium), with the tumour and its sub-regions being segmented based on pixel intensities and contrast enhancement. It can be seen that the tumour is located at the corner with a maximum dimension of 41 mm and an area of $9.0 \times 10^{-4} \text{ m}^2$. The tumour is divided into 6 sub-regions based on differences in signal intensities which are correlated to vessel densities as described later. Region_1 is further divided into two regions in order to compensate for any potential boundary effect caused by the lack of surrounding normal tissue. The resulting smoothed contours of the tumour and normal tissues are imported into ANSYS ICEM CFD to generate computational mesh. The dimensions of the reconstructed model are approximately 118.8 mm (maximum width) by 126.8 mm (maximum depth), and the area of the normal tissue is $8.7 \times 10^{-3} \text{ m}^2$. The final mesh consists of 88594 triangular elements. This is obtained based on mesh sensitivity tests which are carried out until the difference in predicted drug concentration between the adopted mesh and a 10-time finer mesh is less than 3%.

Figure 2. Model geometry: (a) MR image of the liver tumour (in red) and its surrounding tissue (in blue); (b) the reconstructed 2-D geometry.

2.3 Model Parameters

A large number of model parameters are required for the computational simulation; these include physical and transport properties for doxorubicin, liposome, as well as tumour and normal tissues. Essential model parameters adopted in the present study are the same as those used in our previous work [13]. For brevity, only differences are described here, and a complete list of model parameters [and sources](#) is given in Appendix (Tables A-6, A-7 and A-8).

Surface Area of Blood Vessels per Unit Volume of Tumour Tissue (S/V)

The concentration of Gd-DTPA tracer in the interstitial fluid is described by:

$$\frac{dC_{Gd}}{dt} = P \frac{S}{V} (C_p - C_{Gd}) + F_v (1 - \sigma) C_p \quad (1)$$

where t is time, P is the permeability of the tracer, S/V is the surface area of blood vessels per unit volume of tumour tissue, C_{Gd} and C_p represent the tracer concentration in the interstitial fluid and blood, respectively, F_v is the filtration rate from the blood vessels per unit volume of tumour tissue, and σ is the osmotic reflection coefficient of the tracer ($\sigma = 0.6$ [12]).

The average interstitial concentration of Gd-DTPA has been found to be proportional to the relative enhancement of signal intensity [15]:

$$C_{Gd} \approx \frac{S - S_0}{S_0 T_{10} r_1} \quad (2)$$

where S and S_0 represent the signal with and without the tracer, Gd-DTPA, T_{10} denotes the relaxation time and r_1 is the relaxivity, both of which are constant and can be cancelled out during the evaluation of S/V .

The spatial-mean signal intensity of each tumour region at three different time points is calculated, and the relative value of surface area of blood vessel per tumour volume, $(S/V)^*$, can be obtained by

interpolation. The ratio of $(S/V)^*$ in each tumour region to the average value for the entire tumour region is then used to calculate values for S/V , by scaling with the standard value from the literature which is 200 cm^{-1} . The scaled values are summarized in Table 2 in descending order of microvasculature density.

Table 2. The relative and scaled values of surface area of blood vessel per tumour volume (cm^{-1})

	Average	Region_1	Region_2	Region_3	Region_4	Region_5	Region_6
$(S/V)^*$	776.52	1741.73	860.84	554.73	502.23	428.69	324.48
S/V	200.00**	448.60	221.72	142.88	129.35	110.41	83.57

** baseline value from literature [11, 16, 17]

Plasma Pharmacokinetics

An exponential decay function is adopted to describe doxorubicin concentration in blood plasma. For direct continuous infusion, this follows a tri-exponential decay function [9]. For liposome encapsulated doxorubicin, its plasma pharmacokinetics is described by a 2-exponential decay function [18]. The effect of binding of free doxorubicin with proteins has been taken into account in the same way as reported previously [13]. Relevant equations are given in Table A-5 in Appendix.

2.4 Numerical Methods and Boundary Conditions

The mathematical equations are solved by means of a finite volume based computational fluid dynamics (CFD) code, ANSYS FLUENT, together with tailor-made routines using the User Defined Scalar (UDS) function. The second order UPWIND scheme is adopted for spatial discretization, while temporal discretization is achieved by employing the second order implicit backward Euler scheme. Regarding boundary conditions, the only external boundary is the outer surface of the normal tissue, where a zero relative pressure is prescribed. All variables at the internal boundaries between the tumour and normal tissue and between different tumour regions are assumed to be continuous. The governing equations for interstitial fluid flow are solved first, and the resolved pressure and velocity fields in tumour and normal tissues are then used to solve the mass transfer equations for drug transport. A fixed time-step of 10 seconds is chosen following a time-step sensitivity test. The convergence criteria for residual tolerances are 1×10^{-5} for momentum equations and 1×10^{-8} for mass transfer equations.

3. Results and Discussion

Interstitial fluid pressure (IFP) plays an important role in determining the transport of drug in tumours. IFP is obtained by solving the governing equations for interstitial flow (Table A-1 in Appendix) for the entire tumour region and its surrounding normal tissue, subject to the boundary conditions described above and a vascular pressure of 2080 Pa. As shown in Figure 3, there is no discernable difference among different tumour regions, although IFP in the tumour is much higher than that in the normal tissue. The observation that IFP is not sensitive to tumour microvasculature distribution is consistent with the results of Baxter and Jain [7] who found that even the presence of a necrotic core had virtually no effect on the IFP profile, unless the necrotic core size is greater than 90% of the tumour size. This is because in regions except for a thin layer close to the outer boundary of the tumour, IFP equilibrates with the effective vascular pressure, which is given by $p_v - \sigma_p(\pi_v - \pi_i)$; refer to Appendix for definition of symbols and

parameter values. Based on the model parameters adopted in this study, the effective vascular pressure is 1533.88 Pa; this is reached in all tumour regions except region_1 and region_2, where the mean IFP is slightly lower. This means that there is no net filtration between the microvessels and interstitium in regions 3-6 which are surrounded by regions 1 and 2. On the other hand, the rather uniform IFP in the interior of tumour implies that pressure-induced convection of interstitial fluid is weak there, and convective drug transport in the interstitium occurs mainly within a thin layer at the tumour/normal tissue interface, where there is a steep pressure gradient.

Figure 3. Interstitial fluid pressure distribution in tumour and normal tissues.

3.1 Direct Continuous Infusion

For direct infusion of doxorubicin, a total dose of 50 mg/m^2 [19] is administrated into blood within 2 hours [9]. Figure 4 shows the spatial distribution of free doxorubicin at different time points in the tumour. It is clear that extracellular concentration of doxorubicin is non-uniform, which is not surprising since the transport of drug is channelled through blood vessels, and as such, vasculature density is expected to have a strong influence on the spatial distribution of drug. Since the difference in extra- and intra-cellular concentrations between sub-regions 1_1 and 1_2 is very small these sub-regions are combined and referred to as region_1 in the following discussion.

Figure 4. Spatial distribution of free doxorubicin extracellular concentration in tumour regions.

Figure 5 shows the variation of extracellular concentration of free and bound doxorubicin in the liver tumour and each of its sub-regions of different vessel densities. In all regions, the predicted concentration increases during the infusion period, when doxorubicin is administrated continuously into the circulatory system. After the end of administration, both free and bound doxorubicin concentrations start falling

immediately. There are obvious differences in drug concentration in different tumour regions until at approximately 12 hour, when uniform doxorubicin concentrations are reached in all tumour regions (also demonstrated in Figure 4). The rate of change of doxorubicin concentration is slower in regions with low vessel density (e.g. region_6), owing to reduced drug exchange between plasma and interstitial fluid as a result of sparse vasculature.

Figure 5. Spatial mean doxorubicin extracellular concentration in tumour as a function of time. (a) Free doxorubicin, and (b) bound doxorubicin (total dose = 50 mg/m²).

Intracellular concentration also displays a non-uniform pattern among tumour regions of different vessel densities. As shown in Figure 6(a), the rate of change of intracellular concentration is slower and peak concentration is lower in poorly vascularised regions, similar to the pattern observed for extracellular concentration. The predicted anti-cancer effectiveness, assessed based on variation in the percentage of tumour cells (Figure 6(b)), suggests that cell killing is more effective in well-vascularised regions in the first few hours, but the trend is reversed thereafter. However, the difference among the various regions is small, with the maximum difference being 3% between region_1 (high vessel density) and region_6 (low vessel density). A parametric sensitivity study confirms that the qualitative trend presented here is not altered by changes in infusion duration or dose level.

Figure 6. Spatial mean (a) intracellular concentration and (b) percentage of tumour cells as a function of time (total dose = 50 mg/m²).

3.2 TSL Encapsulated Doxorubicin

For TSL-mediated delivery, it is assumed that there is no release of doxorubicin until the temperature exceeds 42°C. Heating begins at 24 hour following the administration of TSL-doxorubicin (simulating extravascular release), and the simulated heating duration is 1 hour [9]. Given that the temperature of blood plasma is always lower than that of the surrounding tissue during hyperthermia [14], no doxorubicin is assumed to be released in blood plasma.

Concentrations of encapsulated doxorubicin in blood plasma and tumour interstitial space are shown in Figure 7. Following drug administration, encapsulated doxorubicin concentration in tumour increases rapidly during the initial period and reaches equilibrium with blood plasma after 5-16 hours depending on tumour vascular density. It then follows the intravascular concentration curve until a sharp fall at 24 hour when TSL particles are heated to 42°C, causing a rapid release of doxorubicin. After heating stops at 25 hour, the encapsulated doxorubicin concentration starts to rise again and recovers to a new equilibrium level with blood plasma.

Figure 7. Concentrations of liposomal encapsulated doxorubicin in blood plasma and in tumour over the treatment duration.

It is clear from these results that the heterogeneous blood vessel distribution has a strong influence on doxorubicin concentration in tumour. The rate of change in concentration is faster in regions that are well perfused. After heating, extracellular concentration of encapsulated doxorubicin is determined by the rate of doxorubicin release and transvascular transport of liposomes. In well-vascularised regions, transvascular transport is strong, allowing more liposomes to enter; but more encapsulated doxorubicin is released at the same time. However, the rate of release is slower than that of transvascular transport, so that the overall effect is dominated by the latter. As a result, region_1 has the highest concentration of encapsulated doxorubicin after heating, and region_6 has the lowest.

Extracellular concentration profiles of free doxorubicin between 22-36 hours after administration are shown in Figure 8(a). Since no doxorubicin is released from TSL before heating, both free and bound doxorubicin concentrations remain at zero during 22-24 hours. Once the temperature reaches 42°C at the 24 hour, doxorubicin is rapidly released, leading to a sharp increase in concentration (see Figure 8(b) for a close up view). It is worth noting that the concentration of free doxorubicin is a result of the balance between the source term which is the amount of drug released from TSL, and the sink terms, which

include transvascular exchange, binding with protein and tumour cell uptake (as described in Figure 1(b)). Detailed contributions of each of these terms are displayed in Figure 9(a), with the net gain of free doxorubicin given in Figure 9(b). Within a few seconds upon heating, the rapid release of doxorubicin leads to a peak concentration. However, transvascular transport from the extracellular space also increases, resulting in a negative net gain (see inset in Figure 9(b)), hence a temporary reduction in extracellular concentration of free doxorubicin. This lasts for a few minutes only before a positive net gain is restored, so that free doxorubicin concentration continues to rise until the end of heating. Since no more doxorubicin is released from the 25 hour onwards, there is a rapid fall in concentration at this time point before a gradual reduction thereafter.

Figure 8. Extracellular concentration of free doxorubicin as a function of time (a) between 22 and 36 hours, and (b) a close up view at 24 hour.

Figure 9. Spatial mean rate of change of free doxorubicin in tumour extracellular space as a function of time. (a) Contributions of each drug transport process, and (b) net rate of change.

Similar trends are observed for extracellular concentration of bound doxorubicin, shown in Figure 10. Here the source term accounts for doxorubicin association with protein, and the sink terms account for drug disassociation with protein and transvascular exchange. Both Figures 8 and 10 show that the rate of change in extracellular drug concentration is slower in regions of lower vessel density.

Figure 10. Extracellular concentration of bound doxorubicin in the tumour as a function of time.

Intravascular concentrations of free and bound doxorubicin are shown in Figure 11(a) and (b), respectively. Because it is assumed that there is no intravascular release of encapsulated drug, all forms of doxorubicin enter blood plasma from the interstitial space by convection and diffusion. Results show that both free and bound doxorubicin concentrations increase during the heating period, and then decrease from their own peaks when heating is over. Higher peak concentrations are observed in regions of denser vasculature, where more drugs are transported across the vessel wall.

Figure 11. Intravascular concentration of doxorubicin as a function of time: (a) free doxorubicin, (b) bound doxorubicin.

The time course of intracellular concentration is shown in Figure 12. It is initially zero until doxorubicin is released at the 24 hour upon heating. During the one hour heating period, there is a rapid increase in intracellular drug concentration which peaks shortly after the cessation of heating. This is followed by a reduction in intracellular concentration at varying rates depending on vascular density in the tumour region, with slower reduction in regions of lower vessel density.

Figure 12. Spatial mean intracellular concentration of doxorubicin in tumour regions as a function of time.

The anti-cancer effectiveness is evaluated by calculating changes in the percentage of tumour cells using a pharmacodynamics model described previously [13]. As can be seen in Figure 13, the percentage of tumour cells begins to decline at the 24 hour when drug is released from TSL upon heating. There is a rapid cell killing phase in the first few hours after heating in all tumour regions, but the duration of this phase depends strongly on the density of microvasculature. This is followed by a slower cell killing phase which lasts for approximately 2 hours. With further reduction in intracellular concentration, the rate of cell killing and physiological degradation is overtaken by that of cell proliferation, and as a result, the percentage of tumour cells begins to increase. Results in Figure 13 suggest that better anti-cancer effectiveness is achieved in regions of lower vascular density.

Figure 13. Percentage of tumour cells in different regions of tumour as a function of time.

Spatial distributions of tumour cell density at different time points are shown in Figure 14. There is no difference among tumour regions at the 25 hour, as the heating period is dominated by the rapid release of doxorubicin which results in a uniformly high drug concentration in all tumour regions. After the end of heating, no more doxorubicin is released, and as a result, the level of intracellular drug concentration is strongly influenced by the rate at which doxorubicin is lost from the extracellular space, which in turn depends on local vascular density. Well-vascularised regions (e.g. region_1) lose doxorubicin more quickly through diffusion back to the plasma than poorly vascularised regions (e.g. region_6), and hence less effective tumour cell killing in the former.

Figure 14. Spatial distribution of tumour cell density for liposomal delivery.

For TSL-mediated drug delivery, the predicted anti-cancer efficacy can be influenced by a number of factors; these include release mode (intra- or extra-vascular triggered release), heating schedule (e.g. the duration and timing of heating) and drug release rate. To test the sensitivity of the results to these factors, simulations have been repeated for a longer heating duration (2 hours), an early timing of heating (at the 8 hour following TSL administration), and a faster release of doxorubicin from TSL (at 0.0541 s^{-1} based on data in [20]). Since the mathematical model does not include intravascular release, it is not possible to compare the effect of different release modes. Results corresponding to 2-hour heating duration are presented in Figure 15, which shows the same qualitative trend as that obtained for 1-hour heating, i.e. more effective tumour cell killing in regions of lower vascular density, although with 2-hour heating there is ~5% improvement in anti-tumour effectiveness. Results for an early heating at the 8 hour are virtually identical to those for heating at the 24 hour, except that the timing is brought forward accordingly. With a faster release of doxorubicin from TSL, the overall anti-tumour effectiveness is improved but the qualitative trend remains the same. These results are not presented here owing to space limitation.

Compared with continuous infusion of non-encapsulated drugs, the anti-cancer effectiveness for TSL-mediated delivery is more sensitive to the heterogeneous distribution of microvasculature. This is mainly attributed to the discontinuous release of doxorubicin from TSL during a treatment period, i.e. rapid release upon heating and no release outside the heating period. Consequently, the level of intracellular drug concentration cannot be sustained after heating is over, and the rate at which intracellular concentration falls is strongly dependent on vasculature density. This dependence results in a more rapid fall in intracellular concentration in well-vascularised regions, leading to less effective cell killing compared to poorly-vascularised regions where intracellular concentration falls more slowly, allowing for more exposure time to trigger cell killing. On the other hand, continuous infusion of non-encapsulated drug ensures a constant supply of drugs during the entire treatment period. In this case, cell killing is more effective in well-vascularised regions in the first few hours, but the trend is reversed thereafter. The quantitative effect of vascular density on the resultant cell killing is not as pronounced as for TSL-mediated delivery.

Figure 15. Spatial mean concentration of anticancer drug and cell killing among tumour regions with 2-hour heating duration.

By incorporating heterogeneous microvascular density in a realistic tumour model, the present study has provided more insights into the effect of non-uniform distribution of tumour vasculature on drug delivery to solid tumours. Nevertheless, the mathematical model employed here involves a number of assumptions. First, the tumour vascular network is treated as a distributed source term in the governing equations instead of being modelled explicitly. As a result, the effects of intravascular transport and geometric features of tumour vasculature are not included. While this may be an over-simplification, it is practical given the lack of information on realistic tumour vasculature geometry and the complexity in direct modelling of blood flow in a vascular network coupled with interstitial drug transport. Second, a 2D tumour model is used instead of a full 3D model. Based on our previous comparisons between 2D and 3D models of a prostate tumour, the 3D effect on spatial-mean parameters, such as intracellular drug concentration and tumour cell density, is negligible. This is consistent with the work of Teo et al [21] who found qualitatively similar results between their 2D and 3D models of a brain tumour.

Other assumptions include: (i) signal intensities of tracer Gd-DTPA in post-contrast MR images are used to calculate vascular density, with the tracer concentration in the plasma being assumed to be proportional to the relative change in signal intensity. This could be improved by measuring the intravascular concentration of Gd-DTPA directly in MR imaging [12]. (ii) Tumour temperature is assumed to be uniform, and changes in temperature during and after heating are ignored. This assumption can be relaxed by incorporating a bioheat transfer model to predict the temperature distribution in tumour in response to

the heating schedule applied. Related to this assumption is the constant release rate, which should be temperature-dependent, allowing for varied release rates in different regions. (iii) For TSL-mediated delivery, a fixed heating period of 1 hour at 24 hours after drug infusion are chosen as an example which may not be the optimal setting for this delivery method. Based on the results presented, it appears that a longer heating period may help to sustain high levels of intracellular drug concentration, thereby improving the anti-cancer effectiveness over a given treatment period, and (iv) uniform transport properties and uniform tumour cell density are assumed in each tumour region.

4. Conclusion

The transport of doxorubicin in a liver tumour with heterogeneous vasculature distribution has been studied under direct continuous infusion and TSL-mediated delivery. Computational results show that interstitial fluid pressure is not sensitive to microvessel density in the interior of tumour, implying that drug transport by pressure-induced convection is weak except in a thin layer close to the tumour boundary. Compared with continuous infusion of non-encapsulated doxorubicin, the heterogeneous distribution of tumour vasculature has a greater influence on the effectiveness of tumour cell killing under TSL-mediated delivery. Our results show that with TSL-mediated delivery and extravascular triggered release, better anti-cancer effectiveness is achieved in regions of low vasculature density, owing to reduced loss of drugs to the plasma; and that the qualitative trend is not affected by changes in heating duration, timing of heating, or release rate, although quantitative differences exist. These findings have important implications. First, since tumour aggressiveness is manifested through enhanced vascularisation, existing chemotherapies that work well in treating less aggressive tumours might not be as effective in treating aggressive, vascularised tumours. Second, the overall effectiveness of TSL-mediated delivery of chemotherapy drugs is influenced by a number of factors, and future research should aim to design optimal treatment methods, including heating schedule and release mode, for TSL-mediated delivery of chemotherapy drugs for the treatment of different types of tumours.

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487 Table A-1. Governing equations for interstitial fluid flow

Continuity Equation

$$\nabla \cdot \mathbf{v} = F_v - F_{ly} \quad (\text{A-1})$$

$$F_v = K_v \frac{S}{V} [p_v - p_i - \sigma_p (\pi_v - \pi_i)]; \quad F_{ly} = K_{ly} \frac{S_{ly}}{V} (p_i - p_{ly}) \quad (\text{A-2})$$

Momentum Equation

$$\frac{\partial(\rho \mathbf{v})}{\partial t} + \nabla \cdot (\rho \mathbf{v} \mathbf{v}) = -\nabla p_i + \nabla \cdot \boldsymbol{\tau} + W_L \mathbf{w} \quad (\text{A-3})$$

488

489

490 Table A-2. Governing equations for drug transport under direct continuous infusion *

Free Doxorubicin Concentration in the Interstitial Fluid (C_{fe})

$$\begin{aligned} \frac{\partial C_{fe}}{\partial t} + \nabla \cdot (C_{fe} \mathbf{v}) = & D_{fe} \nabla^2 C_{fe} + F_v (1 - \sigma_d) C_{fp} + P_{fe} \frac{S}{V} (C_{fp} - C_{fe}) \frac{\text{Pe}_f}{e^{\text{Pe}_f} - 1} - F_{ly} C_{fe} \\ & + k_d C_{be} - k_a C_{fe} + D_c V_{\max} \left(\frac{C_i}{C_i + k_i} - \frac{C_{fe}}{C_{fe} + k_e \varphi} \right); \quad \text{Pe}_f = \frac{F_v (1 - \sigma_d)}{P_{fe} S/V} \end{aligned} \quad (\text{A-4})$$

Bound-Doxorubicin concentration in Interstitial Fluid (C_{be})

$$\frac{\partial C_{be}}{\partial t} + \nabla \cdot (C_{be} \mathbf{v}) = D_{be} \nabla^2 C_{be} + F_v (1 - \sigma_d) C_{bp} + P_{be} \frac{S}{V} (C_{bp} - C_{be}) \frac{\text{Pe}_b}{e^{\text{Pe}_b} - 1} - k_d C_{be} + k_a C_{fe} \quad (\text{A-5})$$

Intracellular Doxorubicin Concentration (C_i)

$$\frac{\partial C_i}{\partial t} = V_{\max} \left(\frac{C_{fe}}{C_{fe} + k_e \varphi} - \frac{C_i}{C_i + k_i} \right) \quad (\text{A-6})$$

491 * Pe_b is defined in the same way as Pe_f in (A-4).

492

493 Table A-3. Governing equations for drug transport under thermo-sensitive liposome-mediated delivery *

Liposome Encapsulated Drug Concentration in the Interstitial Fluid (C_{le})**

$$\frac{\partial C_{le}}{\partial t} + \nabla \cdot (C_{le} \mathbf{v}) = D_l \nabla^2 C_{le} + F_v (1 - \sigma_l) C_{lp} + P_l \frac{S}{V} (C_{lp} - C_{le}) \frac{\text{Pe}_l}{e^{\text{Pe}_l} - 1} - F_{ly} C_{le} - k_{rel} C_{le} \quad (\text{A-7})$$

Free Doxorubicin Concentration in Blood Plasma (C_{fp})

$$\frac{\partial C_{fp}}{\partial t} = S_r - \frac{V_T}{V_B} F_{fp} - \frac{CL_{fp} C_{fp}}{V_D} - (k_a C_{fp} - k_d C_{bp}) \quad (\text{A-8})$$

Bound Doxorubicin Concentration in Blood Plasma (C_{bp})

$$\frac{\partial C_{bp}}{\partial t} = (k_a C_{fp} - k_d C_{bp}) - \frac{V_T}{V_B} F_{be} - \frac{CL_{bp} C_{bp}}{V_D} \quad (\text{A-9})$$

Free Doxorubicin Concentration in Interstitial Fluid (C_{fe})

$$\begin{aligned} \frac{\partial C_{fe}}{\partial t} + \nabla \cdot (C_{fe} \mathbf{v}) = & D_{fe} \nabla^2 C_{fe} + F_v (1 - \sigma_d) C_{fp} + P_{fe} \frac{S}{V} (C_{fp} - C_{fe}) \frac{Pe_f}{e^{Pe_f} - 1} - F_{ly} C_{fe} \\ & + k_d C_{be} - k_a C_{fe} + D_c V_{\max} \left(\frac{C_i}{C_i + k_i} - \frac{C_{fe}}{C_{fe} + k_e \phi} \right) + k_{rel} C_{le} \end{aligned} \quad (A-10)$$

*Bound doxorubicin concentration in interstitial fluid and intracellular concentration are the same as in Table A-2

** Pe_i is defined in the same way as Pe_f in (A-4).

Table A-4. Pharmacodynamics Model

Pharmacodynamics Model

$$\frac{dD_c}{dt} = -\frac{f_{\max} C_i}{EC_{50} + C_i} D_c + k_p D_c - k_g D_c^2 \quad (A-11)$$

Table A-5. Pharmacokinetics Model

Direct continuous infusion delivery*

$$\begin{cases} C_v = \frac{Dose}{T} \left[\left(\frac{A}{\alpha} (1 - e^{-\alpha t}) + \frac{B}{\beta} (1 - e^{-\beta t}) + \frac{C}{\gamma} (1 - e^{-\gamma t}) \right) \right] & (t < T) \\ C_v = \frac{Dose}{T} \left[\frac{A}{\alpha} (e^{\alpha T} - 1) e^{-\alpha t} + \frac{B}{\beta} (e^{\beta T} - 1) e^{-\beta t} + \frac{C}{\gamma} (e^{\gamma T} - 1) e^{-\gamma t} \right] & (t \geq T) \end{cases} \quad (A-12)$$

Liposome encapsulated drug delivery

$$C_{lp} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \quad (A-13)$$

*75% of doxorubicin binds with protein

Table A-6. Parameters for tumour and normal tissues

Symbol	Parameter	Unit	Tumour Tissue	Normal Tissue	Source
S/V	surface area of vessels per unit volume of tissue	m^{-1}	20000	7000	[6, 16, 20]
K_v	hydraulic conductivity of the microvascular wall	$m/Pa \cdot s$	2.10×10^{-11}	2.70×10^{-12}	[6, 7]
ρ	density of interstitial fluid	kg/m^3	1000	1000	estimated [11]
μ	dynamic viscosity of interstitial fluid	$kg/m \cdot s$	0.00078	0.00078	estimated [11]
l/κ	permeability of the interstitial space	m^{-2}	4.56×10^{16}	2.21×10^{17}	[6, 11, 21]
p_v	intravascular pressure	Pa	2080	2080	[6, 22]
π_v	osmotic pressure of the plasma	Pa	2666	2666	[6, 22]
π_i	osmotic pressure of interstitial fluid	Pa	2000	1333	[6, 11]
σ_p	average osmotic reflection coefficient for plasma proteins		0.82	0.91	[6, 11]
$K_{ly}S_{ly}/V$	product of hydraulic conductivity of lymphatic wall and surface area of vessels per unit volume of tissue	$(Pa \cdot s)^{-1}$	0	4.17×10^{-7}	[7, 11]
p_{ly}	pressure in lymphatics	Pa	0	0	[11, 23]
D_c	cell density	$10^5 \text{ cell}/m^3$	1×10^{10}	-	estimated [24]
V_T	total tumour volume	m^3	5×10^{-5}	-	estimated [9]
V_B	total blood volume in body	m^3	5×10^{-2}	5×10^{-2}	[9, 25]

Table A-7. Parameters for liposome

Symbol	Parameter	Unit	in Tumour	in Normal Tissue	Source
P_o	permeability	m/s	3.42×10^{-9}	8.50×10^{-10}	[26, 27]
h	thermo-enhanced fold of permeability in tumour		71	-	estimated based on [9, 28]
D	diffusivity	m^2/s	9.0×10^{-12}	5.8×10^{-12}	[27, 29]
σ_l	osmotic reflection coefficient of liposome		0.95	1.0	estimated based on [6, 30]
A_1	parameter of pharmacokinetic model	kg/m^3	6.90×10^{-3}	6.90×10^{-3}	[18]
A_2	parameter of pharmacokinetic model	kg/m^3	1.22×10^{-2}	1.22×10^{-2}	[18]
k_1	compartment clearance rate	s^{-1}	8.37×10^{-5}	8.37×10^{-5}	[18]
k_2	compartment clearance rate	s^{-1}	4.17×10^{-6}	4.17×10^{-6}	[18]
$k_{rel_37^\circ C}$	release rate at 37°C	s^{-1}	0	-	assumption
$k_{rel_42^\circ C}$	release rate at 42°C	s^{-1}	0.0078	-	[31]

Table A-8. Parameters for doxorubicin

Symbol	Parameter	Unit	Free Doxorubicin	Bound Doxorubicin	Source
P_{Tumour_o}	permeability in tumour	m/s	3.00×10^{-6}	7.80×10^{-9}	[11, 32]
h	thermo-enhanced fold of permeability in tumour		2.56	-	[33]
P_{Normal}	permeability in normal tissue	m/s	3.75×10^{-7}	2.50×10^{-9}	[11, 32]
D_{Tumour}	diffusivity in tumour	m ² /s	3.40×10^{-10}	8.89×10^{-12}	derived from [10, 21, 34-36]
D_{Normal}	diffusivity in normal tissue	m ² /s	1.58×10^{-10}	4.17×10^{-12}	derived from [10, 21, 34-36]
σ_d	osmotic reflection coefficient of doxorubicin		0.15	0.82	[6, 11]
k_a	association rate with protein	s ⁻¹	0.833	-	estimated [24]
k_d	disassociation rate with protein	s ⁻¹	-	0.278	estimated [24]
V_{max}	rate of trans-membrane transport	kg/10 ⁵ cells s	4.67×10^{-15}	-	[10, 37]
k_e	michaelis constant for transmembrane transport	kg/m ³	2.19×10^{-4}	-	[10, 37]
k_i	michaelis constant for transmembrane transport	ng/10 ⁵ cells	1.37×10^{-12}	-	[10, 37]
f_{max}	cell-kill rate constant	s ⁻¹	1.67×10^{-5}	-	[38]
EC_{50}	drug concentration producing 50% of f_{max}	kg/10 ⁵ cells	5×10^{-13}	-	[38]
$Dose$	doxorubicin dose	kg	8.56×10^{-5}	-	[11, 39]
A	parameter of pharmacokinetic model	m ⁻³	74.6	74.6	[10, 40]
B	parameter of pharmacokinetic model	m ⁻³	2.49	2.49	[10, 40]
C	parameter of pharmacokinetic model	m ⁻³	0.552	0.552	[10, 40]
α	compartment clearance rate	s ⁻¹	2.43×10^{-3}	2.43×10^{-3}	[10, 40]
β	compartment clearance rate	s ⁻¹	2.83×10^{-4}	2.83×10^{-4}	[10, 40]
γ	compartment clearance rate	s ⁻¹	1.18×10^{-5}	1.18×10^{-5}	[10, 40]
k_p	cell proliferation rate constant	s ⁻¹	3.0×10^{-6}	-	estimated [24]
k_g	cell physiological degradation rate	s ⁻¹	3.0×10^{-16}	-	estimated [24]
CL_{tumour}	clearance rate of doxorubicin in tumour	s ⁻¹	2.43×10^{-3}	0	[9, 40, 41]
CL_{normal_tissue}	clearance rate of doxorubicin in normal tissue	s ⁻¹	2.43×10^{-3}	0	[9, 40, 41]