A systematic study of temperature sensitive liposomal delivery of doxorubicin using a mathematical model

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Abstract

Background: Temperature-sensitive liposomes (TSL) in combination with hyperthermia (HT) exposure have emerged as a potentially attractive option to achieve therapeutic drug concentration at targeted tumour site while reducing adverse side effects associated with systemic administration of anticancer drugs. The aim of this study is to elucidate the interplay among different kinetic steps by means of computational modelling.

Methods: A multi-compartment model for TSL-mediated delivery of doxorubicin (DOX) is developed, which incorporates descriptions of the pharmacokinetics of TSL and DOX, and their accumulation in tumour tissue following intravascular triggered release. By examining dynamic interactions among TSL properties, tumour physiological properties and treatment regimen, peak intracellular DOX concentration is predicted for continuous and pulse HT exposures.

Results: Drug release rate from TSL has a saturable effect on peak intracellular drug concentration, and no further gain could be achieved for release rates greater than 0.1018 s^{-1} . A similar effect has also been found for heating duration, such that for a given bolus injection, peak intracellular drug concentration reaches its maximum and then levels off after HT duration of 2 hours. These results suggest that both TSL release rate and HT duration can be optimised in accordance with other parameters, e.g. clearance rate of TSL and administration mode, in order to achieve a desirable level of intracellular drug concentration. However, prolonged heating is not effective for resistant tumour cells with overexpression of ABC (ATP-binding cassette) transporter proteins.

Conclusions: The results obtained in this study can be used to guide the design and optimisation of TSL parameters and treatment regimens.

Keywords: Targeted drug delivery, tumour, drug transport, hyperthermia, multi-compartment model.

1. Introduction

Liposomal encapsulation of anticancer agents, as a drug delivery system, has been developed to overcome the limitations of conventional free drug treatment, by reducing systemic cytotoxicity, prolonging plasma half-life and passively targeting tumour sites as a result of enhanced permeability and retention (EPR) effect [1]. Liposomal formulations of doxorubicin (DOX) (e.g., Doxil[®]) have been approved for clinical use in the treatment of ovarian cancer; however, their clinical efficacy is limited by insufficient local DOX concentration due to slow release kinetics. Therefore, considerable effort has been made on designing liposomes that are capable of targeting tumour actively and releasing encapsulated agents rapidly in response to specific stimulus, temperature-sensitive liposomes (TSL) being one of these [2].

As a localized therapy, TSL are expected to remain stable at body temperature but can be triggered to release the encapsulated drug rapidly (within seconds to minutes) when they are heated to a designated temperature at the tumour site [3, 4]. In addition, local hyperthermia (HT) alone has been shown to improve accumulation of TSL in solid tumour by accentuating the leakiness of tumour vasculature [5]. Among several HT applicators, such as radiofrequency, microwave, and lasers, high intensity focused ultrasound (HIFU) has a distinct advantage due to its non-invasive and controllable nature [2]. More importantly, when combined with magnetic resonance imaging (MRI), MRI guided HIFU can offer real-time temperature monitoring with a feedback control, which is critical to TSL-based delivery systems activated by HIFU [6].

The application of TSL in conjunction with local HT has shown promising prospect both in preclinical studies and in clinical trials. Current TSL research is mainly focused on formulation design, especially with regard to release kinetics in response to HT exposure.

However, among the multiple transport steps in a drug delivery process, drug release is an intermediate step that is preceded by the upstream transport (systemic circulation) and followed by the downstream transport (transvascular/interstitial and eventually intracellular transport). Furthermore, the target output, i.e. peak intracellular drug concentration, is not only influenced by interactions among these multiple transport steps, but also by the characteristics of HT applicators. It is a formidable task to measure the entire range of drug concentrations as well as a large amount of parameters experimentally. On the other hand, it is beyond our intuitive understanding to appreciate temporal variations of drug concentrations, and their dependence on the constituent parameters. For these reasons, an *in silico* model can play a key role in gaining insights into the transport processes of TSL in combination with their response to HT exposure. *In silico* experiments can be carried out as a precursor and supplement to experimental studies, in order to provide guideline for further design and optimisation of TSL drug delivery systems and their combination with various HT applicators.

Based on the concept of multi-compartment model, a number of mathematical models [7-12] have been developed to describe biological transport processes involved in stealth liposomes and TSL. These models have provided various levels of insight into the delivery of liposomal carriers, bioavailability in the tumour tissue, and its correlation to cytotoxic effect. In particular, mathematical models of TSL delivery require tempo-spatial profiles of temperature as input (stimulus) to account for temperature-dependent release kinetics. This can be achieved by assuming a homogeneous distribution of desired temperature in the entire tumour region [10], or by coupling with a bio-heat transfer model. In their recently published computational studies, Gasselhuber *et al.* [9, 13] predicted distributions of temperature and drug concentration in response to two different heating modalities, and their numerical results

showed a qualitative agreement with preliminary *in vivo* data. However, a systematic study with a thorough sensitivity analysis of the input parameters is lacking.

Employing a multi-compartment model together with the assumption of a homogeneous temperature distribution, the present study aims to provide clear-cut information on the interplays among multiple transport steps involved in the the delivery of TSL-encapsulated doxorubicin (DOX). Compared to the mathematical models employed by Gasselhuber *et al.* [9, 10, 13], an additional compartment for tumour plasma is introduced in the present study, which allows distinction of TSL in systemic plasma from those in tumour plasma. A systematic study is then performed by contrasting and comparing numerical results in response to continuous and pulse temperature stimuli. A sensitivity analysis is carried out to determine which input parameters have the greatest influence on the peak intracellular drug concentration ($c_{i,peak}^T$), and how these parameters affect $c_{i,peak}^T$ under continuous and pulse HT exposure.

2. Methods

A multi-compartment model (Figure 1) is adopted to describe the transport of liposomes and unencapsulated drug following intravenous injection of drug-loaded TSL. The model consists of three high level compartments: systemic plasma, lumped tissue and tumour. The tumour compartment is further divided into three sub-compartments, namely tumour plasma, tumour extravascular extracellular space (EES) and tumour intracellular space. There are eight variables representing drug concentrations in either liposomal or free form in different compartments and the mass conservation equation for each variable incorporates kinetic transfer and reaction processes. A general assumption underlying all compartmental models is homogeneity in each compartment, i.e. a well-mixed system with a uniform temperature

and uniform tumour cell density. In the equations described in the subsequent sections, drug concentrations are expressed in micrograms (μg) per mm^3 of the volume of their corresponding compartment.

2.1 Pharmacokinetics of TSL: systemic plasma compartment

Pharmacokinetics of TSL is described by one compartment, i.e. systemic plasma. The volume of the central compartment is set to the volume of systemic plasma (V_P^B), which implies that the latter is consistent with the volume of TSL distribution [7]. Following an intravenous injection, the concentration of TSL in systemic plasma compartment ($c_{P_Lip}^B$) is determined by:

$$V_P^B \frac{dc_{P_Lip}^B}{dt} = D/T_d * H(T_d - t) - k_{e_Lip} c_{P_Lip}^B V_P^B - kr_{37} c_{P_Lip}^B V_P^B + F_{PV}^T V_P^T c_{P_Lip}^T - F_{PV}^T V_P^T c_{P_Lip}^B \quad 1$$

where D is the dose of TSL, and T_d is the infusion duration. The Heaviside term $H(T_d - t)$ describes a constant infusion of TSL during the period of $t = 0$ to T_d only. Perfect mixing of TSL is assumed within systemic plasma. TSL are eliminated via body clearance at rate k_{e_Lip} . A first order kinetics with a rate constant of kr_{37} is used to describe the release of DOX in systemic plasma due to instability of TSL at body temperature [10].

For bolus injection, Equation 1 is reduced to:

$$V_P^B \frac{dc_{P_Lip}^B}{dt} = -k_{e_Lip} c_{P_Lip}^B V_P^B - kr_{37} c_{P_Lip}^B V_P^B + F_{PV}^T V_P^T c_{P_Lip}^T - F_{PV}^T V_P^T c_{P_Lip}^B \quad 1A$$

2.2 Pharmacokinetics of free DOX: systemic plasma & tissue compartments

Pharmacokinetics of free DOX is described by a two-compartment model, with one common systemic plasma compartment shared with TSL and the other representing lumped body tissues with significant drug uptake. Free DOX is present in systemic plasma compartment due to instability of TSLs at body temperature and is cleared from the body at rate k_e .

Exchange of free DOX between systemic plasma compartment and tissue compartment is described by a bidirectional linear kinetics at transfer rate k_p and k_t , respectively.

$$V_P^B \frac{dc_P^B}{dt} = kr_{37} c_{P_Lip}^B V_P^B - k_e c_P^B V_P^B - k_p c_P^B V_P^B + k_t c_t^B V_t^B + F_{PV}^T V_P^T c_P^T - F_{PV}^T V_P^T c_P^B \quad 2$$

The concentration of free DOX in tissue compartment (c_t^B) is described by:

$$V_t^B \frac{dc_t^B}{dt} = k_p c_P^B V_P^B - k_t c_t^B V_t^B \quad 3$$

2.3 Tumour deposition

Tumour plasma compartment

Tumour plasma compartment is linked to systemic plasma compartment via blood perfusion, which accounts for convective transport ($F_{PV}^T V_P^T c$) of TSL and free DOX between blood and tumour. Here F_{PV}^T is defined as plasma flow per tumour plasma volume. When tumour is heated to a designated temperature (e.g. 42°C), rapid release of DOX from TSL is triggered in tumour plasma, which is assumed to follow a linear kinetics at rate kr_{42} . After heating is ceased, the release rate is reduced to the rate at body temperature (kr_{37}). The concentration of TSL in tumour plasma compartment ($c_{P_Lip}^T$) is described as

$$V_P^T \frac{dc_{P_Lip}^T}{dt} = F_{PV}^T V_P^T c_{P_Lip}^B - F_{PV}^T V_P^T c_{P_Lip}^T - H(T_h - t) * kr_{42} c_{P_Lip}^T V_P^T - H(t - T_h) * kr_{37} c_{P_Lip}^T V_P^T \quad 4$$

Heaviside term $H(T_h - t)$ represents HT exposure during the period of $t = 0$ to T_h .

Unencapsulated DOX in response to HT in tumour plasma provides the driving force for transvascular drug transport into tumour EES. Diffusion is the dominant transport mechanism for transvascular drug transport as convection is negligible in tumours due to elevated interstitial fluid pressure. The diffusive flux across the interface between tumour plasma volume and tumour EES is determined by the product of vascular permeability and surface

area (PS_t) as well as the concentration difference of free DOX between tumour plasma (c_p^T) and tumour EES (c_e^T). Transvascular transport of TSL to tumour EES is assumed to be negligible for two reasons: (1) for intravascular triggered release, which is the mode of TSL release examined in the current study, release of DOX is triggered before extravasation of TSL from tumour plasma to tumour EES; (2) vascular permeability of TSL is several orders of magnitude lower than that of free DOX.

$$V_P^T \frac{dc_p^T}{dt} = F_{PV}^T V_P^T c_p^B - F_{PV}^T V_P^T c_p^T + H(T_h - t) * kr_{42} c_{P_{Lip}}^T V_P^T + H(t - T_h) * kr_{37} c_{P_{Lip}}^T V_P^T - V^T PS_t (c_p^T - c_e^T) \quad 5$$

Tumour EES

Upon entering tumour EES, free drugs are consumed by tumour cells (denoted by $d_c V^T$, the product of tumour cell density d_c and tumour volume V^T). Two different intracellular uptake mechanisms are accounted for: passive diffusive transport and active transport modeled by Michaelis-Menten kinetics. On the other hand, intracellular drugs can be pumped out of cells, most likely by membrane transporter proteins (e.g. P-glycoprotein), which could be significant in resistant tumour cells associated with overexpression of transporter proteins. In addition, binding of free drugs to interstitial proteins (e.g. albumin) should be accounted for. At binding rate k_b and reversible disassociate rate k_d , the equation for free drug concentration in tumour EES can be expressed as:

$$V_e^T \frac{dc_e^T}{dt} = V^T PS_t (c_p^T - c_e^T) - d_c V^T \left(k_{1ci} c_e^T + \frac{V_{max, in} c_e^T}{c_e^T + K_e} - \frac{V_{max, out} c_i^T}{c_i^T + K_i} \right) - k_b c_e^T V_e^T + k_d c_b^T V_e^T \quad 6$$

where the concentration of bound drug in tumour EES is described by:

$$\frac{dc_b^T}{dt} = k_b c_e^T - k_d c_b^T \quad 7$$

Tumour intracellular space

As described above, drug concentration in intracellular space (c_i^T) is governed by a bidirectional transport across the cell membrane:

$$V_i^T \frac{dc_i^T}{dt} = d_c V^T \left(k_{1ci} c_e^T + \frac{V_{max,in} c_e^T}{c_e^T + K_e v_e^T} - \frac{V_{max,out} c_i^T}{c_i^T + K_i} \right) \quad 8$$

The system of ordinary differential equations was solved by using Ode15s implemented in Matlab. In all simulations unless otherwise stated, TSL are administrated intravenously by bolus injection at a dose of 0.7 mg/kg body weight (assuming an average human weight of 70 kg). HT exposure is applied simultaneously with TSL administration and is assumed to result in a desired temperature at the target site instantly. Initial concentrations of all drugs are set to be zero except that of TSL in systemic plasma where $c_{P_Lip}^B(t = 0) = D/V_P^B$.

2.4 Parameterization

Model parameters used in the current study can be broadly classified into four categories: pharmacokinetics, drug release kinetics, tumour physiology, and cell related parameters.

Since DOX is a commonly studied agent, values of parameters describing DOX pharmacokinetics, transport and uptake are directly adopted from the literature. For TSL drug carriers, their release kinetics and pharmacokinetics vary significantly according to their formulation. TSL release kinetics reported by Hossann et al [14] was adopted as baseline values in the present study, with estimated release rates $5.09 \times 10^{-3} \text{ s}^{-1}$ and $1.0 \times 10^{-5} \text{ s}^{-1}$ at HT and physiological temperatures, respectively. Ultra-fast release kinetics has been reported in Thermodox[®] (a commercially available TSL, Celsion, Columbia, MD) with a release rate up to 0.3 s^{-1} . In addition, tumour physiological properties are highly heterogeneous even in the

same tumour, not to mention various tumour types. Therefore, simulations were carried out with baseline values first, which were extracted from the literature (Table 1). This was followed by a thorough sensitivity analysis to evaluate the relative importance of multiple kinetic transport processes. Further details about the ranges of parameters adopted in the sensitivity analysis are described later in the Results section.

3. Results

A systematic investigation of TSL delivery combined with HT was performed. Compared to conventional stealth liposomes, predicting the intracellular drug concentration with TSL-mediated delivery is more complicated as the system is controlled not only by drug signal (TSL injection) but also by hyperthermia exposure; the latter can be achieved through continuous or pulse heating. In order to focus on providing essential insight into the interplay between biological and biochemical processes involved, simulation results corresponding to continuous heating are presented first. The effects of pulse heating are then evaluated by comparing simulation results performed under different heating schemes.

3.1 Basic model dynamics: time-course of drug concentration

As shown in Figure 2(a), the concentration profiles of TSL in systemic plasma compartment $c_{P_Lip}^B$ and TSL in tumour plasma compartment $c_{P_Lip}^T$ are almost identical, following a gradual decline with time. TSL are transported to tumour plasma compartment at sufficiently high concentration via blood perfusion. Upon arrival in tumour plasma, rapid release of DOX is triggered in response to local HT, which gives rise to a sharp increase in DOX concentration in tumour plasma compartment (c_P^T). This is followed by a continuous reduction in c_P^T , since free DOX is simultaneously removed by the competing kinetic transport steps, i.e. perfusion back to systemic plasma and extravasation into tumour EES. As

the amount of DOX released from TSL at body temperature is trivial, DOX concentration in systemic plasma (c_p^B) displays a similar trend to c_p^T .

Shown in Figure 2(b) are profiles of drug concentrations in tumour compartment. It can be seen that the concentration of free DOX in tumour EES (c_e^T) reaches its peak before falling off gradually. Taking a closer look at the transport steps in tumour EES, c_e^T is regulated by different processes: (1) influx driven by unencapsulated DOX triggered by HT in tumour plasma; (2) favourable binding kinetics with nearly 3 times more DOX in bound form (c_b^T) than in free form (c_e^T); (3) effective outflow to tumour intracellular space. Of note, the extravasation flux gradually decreases due to diminished concentration difference between c_p^T and c_e^T , which cannot overcome counteracting processes. Very high intracellular DOX concentration (c_i^T) is observed which peaks at around $t=130$ min, when the profiles of c_p^T and c_e^T intersect. After the peak, c_e^T is higher than c_p^T , resulting in a reverse concentration gradient which drives free DOX in tumour EES to diffuse back to tumour plasma. As a consequence, the net flow of DOX in intracellular space becomes efflux-dominant, leading to a reduction in c_i^T .

Following the analysis of continuous HT exposure, it is more natural to understand how drug concentrations evolve over time under pulse HT exposure. Results shown in Figure 3 reveal the time-course of all drug concentrations in the baseline case, with 60 min HT exposure at 42°C. Comparisons with results in Figure 2 show that sudden changes in drug concentrations occur at cessation of heating ($t=60$ min), which causes a dramatic reduction in the release rate of DOX from TSL. Of note, c_p^T falls below c_e^T immediately after termination of heating, which drives a series of reverse transport, from tumour intracellular space to tumour EES, then back to tumour plasma. Although a similar trend is observed under continuous HT, in

which case the process is regulated by the interplay between different kinetic transport steps, the reverse transport observed under pulse HT is triggered by termination of heating.

Comparisons of results in Figure 2 and Figure 3 suggest that: (1) Peak intracellular drug concentration is reached at approximately 120 min under continuous heating; and (2) Peak intracellular drug concentration is higher under continuous heating than 60 min pulse heating.

3.2 Sensitivity analysis

Among all drug concentrations, peak intracellular drug concentration $c_{i,peak}^T$ is the variable of primary interest, as it has been found to correlate with tumour cell survival [11, 15, 16].

Using the *in silico* model described here, a local sensitivity analysis is performed to evaluate the relative change in $c_{i,peak}^T$ with respect to the baseline case when each tested parameter is increased by 10%. Results for both continuous HT and 60 min pulse HT are obtained and Figure 4 shows that: (1) Parameters governing pharmacokinetics of unencapsulated DOX (k_p , k_t , and k_e) have a minor effect on regulating $c_{i,peak}^T$; (2) Among the properties related to TSLs, clearance rate of TSLs (k_{e_Lip}) and the release rate during HT (kr_{42}) are the most sensitive parameters, with a 10% increase in k_{e_Lip} causing 3.3-6.0% reduction in $c_{i,peak}^T$ while a 10% increase in kr_{42} causing 8.0-8.3% increase in $c_{i,peak}^T$; (3) Vascular parameters also have noticeable influences on $c_{i,peak}^T$, with permeability and surface area product (PS_t) elevating $c_{i,peak}^T$ while blood perfusion rate (w_{blood}) lowering $c_{i,peak}^T$; (4) The maximum efflux transport rate (V_{max_out}) is another parameter whose effect on $c_{i,peak}^T$ cannot be ignored.

The local sensitivity analysis described above has shed new lights on the relative importance of and complex interplay among different kinetic steps. This has led to a further detailed investigation on how these parameters would influence the time-course of intracellular drug concentration and determine the peak intracellular drug concentration over a biologically reasonable parameter range under continuous and pulse HT. These are presented in Figure 5 – Figure 9 and described below.

Clearance rate of TSL (k_{eLip})

k_{eLip} is varied over a range of 0.1-10 folds of its baseline value, which is sufficient to cover the parameter values reported in the literature [17, 18]. Comparisons of temporal profiles of c_i^T obtained with the baseline value and the lower and upper bounds are shown in Figure 5(a).

As k_{eLip} is reduced, $c_{i,peak}^T$ increases, and more time is required to reach the peak value.

Lower k_{eLip} indicates longer circulation time, which helps to maintain sufficient TSL concentration in systemic plasma (and tumour plasma) for a longer period. Therefore, longer HT exposure (continuous exposure as a limiting case) is favoured for sustained release of DOX from TSL, which leads to higher intracellular drug concentration.

$c_{i,peak}^T$ is predicted over the same range of k_{eLip} under continuous and pulse HT exposure. As shown in Figure 5 (b), there are obvious differences between the two heating modes when k_{eLip} is less than 2 times of its baseline value: higher $c_{i,peak}^T$ and more rapid reduction in $c_{i,peak}^T$ are observed under continuous HT exposure. Beyond this narrow range there is hardly any difference between the two heating modes.

Release rate of DOX from TSL during HT (kr_{42})

As a characteristic parameter of TSL, kr_{42} depends on the specific composition of liposome. A range of release rates can be found in the literature, and the value chosen in the baseline calculation [14] represents a moderate release rate (complete release in ~3 min) which is comparable to release kinetics in other computational studies [e.g. 19]. Increasing kr_{42} up to 55 folds allows fast release kinetics [20] and ultrafast release kinetics for Thermodox[®] to be simulated. Figure 6 (a) shows changes in intracellular drug concentration with time at

different kr_{42} under continuous HT. As expected, $c_{i,peak}^T$ is higher with fast release kinetics, however the relationship is nonlinear and the rate of increase in $c_{i,peak}^T$ starts to drop after kr_{42} reaches a certain threshold (around 10 folds of baseline value). The burst release of DOX from TSL triggered by HT provides a powerful local source of DOX in tumour plasma to drive its downstream transport. Since varying the release rate does not affect the kinetics of downstream transport, intracellular drug concentrations reaches its peak at the same time regardless of the large variation in kr_{42} .

Figure 6(b) shows profiles of $c_{i,peak}^T$ over a wide range of kr_{42} under continuous and 1 h pulse heating exposure. It is obvious that $c_{i,peak}^T$ is higher under continuous heating than under 1 h pulse heating, and the difference widens with the increase in kr_{42} until a certain value is reached (around 15 folds of baseline). Beyond this point, the difference in $c_{i,peak}^T$ between the two heating schemes is hardly affected by any further increase in kr_{42} . These results indicate that peak intracellular drug concentration does not increase linearly with the release rate of DOX from TSL, and that there is an optimal release rate for a given heating scheme.

Blood perfusion rate (w_{blood})

The effects of blood perfusion rate, w_{blood} , on intracellular drug concentration are first presented under continuous HT in Figure 7 (a). Compared to the baseline w_{blood} , low w_{blood} generates overall high intracellular drug concentration, thus a strikingly high $c_{i,peak}^T$, but longer time is required to reach the peak intracellular drug concentration. Further results in Figure 7 (b) exhibit a distinct trend of $c_{i,peak}^T$ as a function of w_{blood} under continuous and pulse HT exposure. Within a narrow range of very low w_{blood} , there is a sharp increase in

$c_{i,peak}^T$, but beyond this range $c_{i,peak}^T$ reduces as w_{blood} increases. This is because at high perfusion rates, a large amount of bioavailable DOX released upon HT from tumour plasma is transported back to systemic plasma, where DOX is cleared rapidly by systemic clearance. Such an adverse effect has been demonstrated by a limiting case examined by Gasselhuber et al [9], where enhanced intracellular accumulation of DOX was found following vascular shutdown induced by a short intense HT exposure after a routine HT exposure. The results indicate that high $c_{i,peak}^T$ is achieved at relatively low perfusion rates.

Permeability surface area product (PS_t)

Tumour vascular network is highly irregular with dilated and tortuous vessels; as a result, vascular permeability and vascular density are heterogeneous even within the same tumour. Under the compartmental model setting, a lumped parameter (PS_t) - the product of vascular permeability of DOX and vascular surface area, is used to describe the transvascular transport of DOX. Its effect is further examined over an arbitrarily broad spectrum of values (0.1-10 folds of the baseline value), to account for differences in tumour vascular permeability and vascular density. In a recent study based on a patient-specific liver tumour reconstructed from magnetic resonance images [21], vascular density, measured in terms of surface area of blood vessels per unit volume of tumour tissue was found to vary between 0.4 and 2.2 folds of its baseline value.

The time-course of intracellular drug concentration under continuous HT is displayed at three representative values of PS_t in Figure 8 (a). PS_t is an important parameter governing the transvascular flux of drug: high PS_t allows more bioavailable DOX to be transported into tumour EES, resulting in high intracellular drug concentration in the first hours. On the other hand, high PS_t also causes more intense reverse diffusion of DOX to tumour EES when the

concentration gradient is reversed, thus lowering intracellular drug concentration after approximately 2 hours following drug administration.

Figure 8 (b) presents $c_{i,peak}^T$ over various PS_t under continuous and pulse HT exposures. The results exhibit a saturated effect of PS_t on $c_{i,peak}^T$, and follow a similar trend to that shown in Figure 6 (b). This is explained as follows: kr_{42} and PS_t exert their effects by enhancing the amount of DOX available in tumour EES, and when they reach a sufficiently large value, it is the kinetics of transmembrane transport that determines the intracellular drug concentration. It also implies that improved vascular permeability as a result of heating may enhance intracellular drug accumulation in the first 2 hours of HT exposure.

Maximum efflux transport rate (V_{max_out})

V_{max_out} is a characteristic parameter governing the kinetics of efflux trans-membrane transport. As shown in Figure 9 (a), increasing V_{max_out} reduces intracellular drug concentration in general and the time required to reach equilibrium between influx and efflux, thus bringing forward the reversal transport across cell membrane. As a result, a large fraction of free DOX released upon continuous HT is forced to exit tumour compartment and perfuse back to systemic plasma, whereas discontinuity in HT may help to reduce this reverse transport. On the other hand, when intracellular drug concentration is down-regulated by a reduction in the maximum influx rate (V_{max_in}), continued release of DOX upon constant HT can sustain the rise in intracellular drug concentration, which cannot be achieved by pulse heating.

From the physiological point of view, over-expression of membrane transporter proteins (e.g. p-glycoprotein) results in an increase in V_{max_out} , which is responsible for chemotherapeutic

drug resistance. For TSL-mediated drug delivery combined with HT, it is worth investigating how changes in V_{max_out} may affect $c_{i,peak}^T$ under continuous and pulse HT. It can be seen from Figure 9 (b) that when V_{max_out} is low (below the baseline value), a much higher $c_{i,peak}^T$ is achieved with continuous HT; when V_{max_out} is above its baseline value, the difference in $c_{i,peak}^T$ between the two heating schemes is reduced and eventually disappears when relative V_{max_out} exceeds 2. The results indicate that if the intrinsic kinetics of transmembrane transport is altered, the duration of HT can no longer compensate for this effect to achieve a desirable $c_{i,peak}^T$.

3.3 Treatment regimen

TSL infusion time (T_d)

As shown in Figure 10 (a) under continuous HT, the time-course of intracellular drug concentration is shifted to the right along the time axis with an increment in T_d . With prolonged TSL infusion, it takes more time for the transport and accumulation of DOX in the tumour intracellular space. Within this context, the time scale of HT exposure is a constraint, which may limit the level of intracellular drug concentration. Such an effect is obvious from Figure 10 (b) that peak intracellular drug concentration is almost independent of infusion time under continuous heating, while it falls continually under 1h pulse HT exposure. Results suggest that shorter infusion time is preferred under pulse HT.

Duration of pulse HT exposure

The effect of heating duration is presented in Figure 11, which shows that increasing HT exposure up to approximately 2 h can dramatically increase $c_{i,peak}^T$, but longer than 2 h heating results in little change in $c_{i,peak}^T$. This suggests that the duration of pulse HT exposure has a saturable effect on peak intracellular concentration.

4. Discussion and Conclusion

TSL in combination with HT have recently emerged as a potential solution to delivering high concentrations of chemotherapeutics to targeted tumour sites. The current study focuses on a novel release mode of TSL, by which release of encapsulated drug is triggered during transit of TSL within tumour plasma in response to HT. Intravascular triggered release is a preferred release mode for TSL delivery of DOX in combination with HT [14], as it can provide a boosted local supply of drug at the tumour site and enhance the driving force for drug penetration in tumour interstitium [22].

Although a rich body of experimental research has been centred on the design of TSL formulation for improved stability and drug release behaviour, there is a lack of understanding on the role of these properties in upstream (systemic) and downstream (transvascular and intracellular) transport steps in response to HT exposure. We aim to address this question systematically by using a multiscale computational model, which incorporates descriptions of pharmacokinetics of TSL and free DOX, and drug deposition inside the tumour combined with release triggered by HT, on the basis of mechanistic understanding of drug delivery. Different from traditional experimental research, the *in silico* experimentations presented here are performed on a well-defined TSL-tumour system with a controlled source of variability and heterogeneity to uncover the complex interplay among different kinetic steps.

Peak intracellular drug concentration ($c_{i,peak}^T$) is regarded as the target output; therefore, the current study focuses on exploring how the output value is affected by varying input parameters including TSL properties, tumour physiological properties and stimulus

characteristics (e.g. duration of HT exposure). Our local sensitivity analysis has revealed that TSL properties, especially release rate kr_{42} during HT, has the greatest influence on $c_{i,peak}^T$. Therefore, it is important that TSL should be designed to offer an ideal release rate. An interesting finding is that release rate has a saturable effect on $c_{i,peak}^T$ (Figure 6) in that no appreciable increase in $c_{i,peak}^T$ could be gained for release rates greater than 0.1018 s^{-1} (20-fold of the baseline value). The implication of this finding is that ultra-fast release may not be necessary to achieve desirable levels of $c_{i,peak}^T$.

Following a bolus injection of TSL, intracellular drug concentration is found to culminate after approximately 2 hours of HT exposure even under continuous HT. When heating duration is less than 2 hours, $c_{i,peak}^T$ continues to rise with HT exposure time. However, prolonging HT exposure beyond 2 hours does not increase $c_{i,peak}^T$ further, since equilibrium between cellular uptake and outward transport of DOX from cells has been reached. This finding is consistent with that of Gasselhuber et al [9]. Moreover, increasing heating duration may not be effective for drug-resistant tumour with overexpression of transmembrane transporters (i.e. p-glycoprotein), as heating cannot compensate for the altered kinetics of trans-membrane transport due to increased maximum efflux transport rate. Targeted TSL has been suggested as a novel strategy to overcome multidrug resistance, e.g. folate-targeted TSL developed by Gaber [23]. In addition, injection mode of TSL also affects $c_{i,peak}^T$.

Comparisons of bolus injection and continuous infusion of TSL suggest that although bolus injection offers a higher $c_{i,peak}^T$, it is also associated with high peak drug concentration in systemic plasma which is related to cardiotoxicity [24]. Consequently, continuous infusion with longer duration of HT exposure may help to maximise peak intracellular drug concentration while keeping a low level of drug concentration in systemic plasma.

In conclusion, by employing a multi-compartment model, the current study provides numerical results that help to elucidate how peak intracellular drug concentration is regulated by the complex interplay among tumour physiology, drug specific properties and treatment regimen for TSL-mediated drug delivery combined with intravascular triggered release upon local hyperthermia. The mathematical model presented here can be used as a platform to accommodate additional complexities and has the flexibility to be tailored for any specific tumour-drug systems.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Figure captions

Figure 1. A schematic diagram of the multiple compartment model, incorporating pharmacokinetics of liposome (TSLs) and DOX, and DOX transport and deposition in the targeting tumour tissue.

Figure 2. Time-course of drug concentration under continuous HT exposure: (a) in systemic and tumour plasma compartments; (b) in tumour compartment. Note that drug concentration is based on the volume of the respective compartment.

Figure 3. Time-course of drug concentration under 1h pulse HT exposure: (a) in systemic and tumour plasma compartments; (b) in tumour compartment.

Figure 4: Results of sensitivity analysis on changes in peak intracellular drug concentration (normalised with respect to the peak intracellular drug concentration corresponding to the baseline case with parameter values specified in Table 1) with 10% increase in each parameter. Positive sensitivity values indicate an increase in peak drug concentration with an increase in the tested parameter, whereas negative values predict an opposing effect. Top panel: continuous HT; bottom panel: 1h pulse HT.

Figure 5. (a) Time-course of intracellular drug concentration under continuous HT at various clearance rates of TSLs k_{eLip} ; (b) the effect of varying k_{eLip} on peak intracellular drug concentration $c_{i,peak}^T$ under continuous and 1h pulse HT exposure.

Figure 6. (a) Time-course of intracellular drug concentration under continuous HT at various release rates of TSLs upon HT kr_{42} . (b) the effect of varying kr_{42} on peak intracellular drug concentration $c_{i,peak}^T$ under continuous and 1h pulse HT exposure.

Figure 7. (a) Time-course of intracellular drug concentration under continuous HT at various blood perfusion rates w_{blood} . (b) the effect of varying w_{blood} on peak intracellular drug concentration $c_{i,peak}^T$ under continuous and 1h pulse HT exposure.

Figure 8. (a) Time-course of intracellular drug concentration under continuous HT at various permeability surface area products PS . (b) the effect of varying PS on peak intracellular drug concentration $c_{i,peak}^T$ under continuous and 1h pulse HT exposure.

Figure 9. (a) Time-course of intracellular drug concentration under continuous HT at various maximum efflux transport rates V_{max_out} . (b) the effect of varying V_{max_out} on peak intracellular drug concentration $c_{i,peak}^T$ under continuous and 1h pulse HT exposure.

Figure 10. (a) Time-course of intracellular drug concentration under continuous HT at various infusion duration T_d . (b) the effect of varying T_d on peak intracellular drug concentration $c_{i,peak}^T$ under continuous and 1h pulse HT exposure.

Figure 11. The effect of heating duration on peak intracellular drug concentration $c_{i,peak}^T$ following a bolus injection.

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Table 1. Parameters and baseline values used in the computational model

Symbol	Description	Baseline value	Reference
k_{eLip}	Rate constant of TSL clearance	2.228e-4 [1/s]	[9]
k_e	Rate constant of DOX clearance	1.1e-3 [1/s]	[9]
k_p	Transfer constant from systemic plasma to tissue	1.6e-3 [1/s]	[9]
k_t	Transfer constant from tissue to systemic plasma	4.68e-5 [1/s]	[9]
k_b	Binding rate of DOX to tissue proteins	3000 [1/h]	[15]
k_d	Dissociation rate of DOX-tissue proteins	1000 [1/h]	[15]
kr_{37}	Release rate constant from TSLs at body temperature	0.1e-4 [1/s]	[14]
kr_{42}	Release rate constant from TSL during HT (at 42°C)	50.9e-4 [1/s]	[14]
K_e	Michaelis constant for transmembrane transport	2.19e-4 [$\mu\text{g}/\text{mm}^3$]	[11]
K_i	Michaelis constant for transmembrane transport	1.37 [$\text{ng}/10^5$ cells]	[11]
$V_{\max_in}$	Maximum influx rate for transmembrane transport	0.28 [$\text{ng}/(10^5\text{cells}\cdot\text{min})$]	[11]
$V_{\max_out}$	Maximum efflux rate for transmembrane transport	0.28 [$\text{ng}/(10^5\text{cells}\cdot\text{min})$]	[11]
k_{1ci}	Rate for passive intracellular uptake	6.33e-4 [1/s]	Fit to [9]
w_{blood}	Blood perfusion rate	0.018 [1/s]	[9]
PS_t	Permeability surface area product	7e-3 [1/s]	[9]
H_{ctt}	Haematocrit for tumour microvasculature	0.19	[9]
V_t^B	Volume of body tissue	64.47 [L]	[9]
V^T	Volume of tumour tissue	8.82e-2 [L]	Estimated
v_p^T	Volume fraction of tumour	0.07452	[9]

	plasma		
v_e^T	Volume fraction of tumour EES	0.454	[9]
v_i^T	Volume fraction of intracellular space	0.454	[9]
V_c	Volume of single tumour cell	$1e-6$ [mm ³ /cell]	[11]
d_c	Tumour cell density per tumour volume	$0.454 \cdot 10^6$ [cells/mm ³]	Calculated by v_i^T/V_c
D	Total dose	49 [mg]	Calculated at a dose of 0.7 mg/kg in a 70 kg human

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

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Figure 10

Figure 11