Selecting ideal drugs for encapsulation in thermosensitive liposomes and other triggered nanoparticles

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ABSTRACT

Objective: Thermosensitive liposomes (TSL) and other triggered drug delivery systems (DDS) are promising therapeutic strategies for targeted drug delivery. However, successful designs with candidate drugs depend on many variables, including nanoparticle formulation, drug properties, and cancer cell properties. We developed a computational model based on experimental data to predict the potential efficacies of drugs when used with triggered DDS, such as TSL.

Methods: A computer model based on the Krogh cylinder was developed to predict uptake and cell survival with four anthracyclines when delivered by intravascular triggered DDS (e.g., TSL): doxorubicin (DOX), idarubicin (IDA), pirarubicin (PIR), and aclacinomicin (ACLA). We simulated three tumor types derived from SVR angiosarcoma, LLC lung cancer, or SCC-1 oral carcinoma cells. In vitro cellular drug uptake and cytotoxicity data were obtained experimentally and incorporated into the model.

Results: For all three cell lines, ACLA and IDA had the fastest cell uptake, with slower uptake for DOX and PIR. Cytotoxicity was highest for IDA and lowest for ACLA. The computer model predicted the highest tumor drug uptake for ACLA and IDA, resulting from their rapid cell uptake. Overall, IDA was most effective and produced the lowest tumor survival fraction, with DOX being the second best. Perivascular drug penetration was reduced for drugs with rapid cell uptake, potentially limiting delivery to cancer cells distant from the vasculature.

Conclusion: Combining simple in vitro experiments with a computer model could provide a powerful screening tool to evaluate the potential efficacy of candidate investigative drugs preceding TSL encapsulation and in vivo studies.

INTRODUCTION

Thermosensitive liposomes (TSL) are triggered drug delivery systems (DDS) where the encapsulated drug is released in response to hyperthermia (typically >40°C). Combined with local hyperthermia, TSL enable the targeted drug delivery to a specific tissue region. TSL have potential clinical applications in the delivery of cancer therapeutics, as well as for other diseases, such as the treatment of infections and inflammation. Multitudes of TSL formulations have been proposed since the first TSL were described more than 40 years ago [1–6]. In addition, TSL have been employed to deliver a variety of different drugs, with varying delivery efficacy.

Most nanoparticle DDS have been based on the enhanced permeability and retention (EPR) effect that enables the preferential accumulation of nanoparticles in tumors [7,8]. However, there has been consensus in recent years on the limitations of the EPR effect, including high variability between and within tumors, more pronounced accumulation in animal tumors compared to human tumors, and an apparent upper limit on how much drug can be delivered to tumors [7,8]. Recent reviews highlight the need for alternate delivery approaches not dependent on the EPR effect [7–9]. Many of the more recent TSL formulations are based on the intravascular triggered release paradigm that is independent of EPR. For DDS based on intravascular triggered release (IV-DDS), drug release occurs while the IV-DDS (e.g., TSL) pass through the vasculature where the release trigger (e.g., heat) is present [10–14]. TSL based on intravascular triggered release enable the delivery of tumor doses up to 25 times higher than after the administration of unencapsulated drug [15].

Few past studies investigated how drug properties affect the delivery efficacy when a drug is encapsulated within TSL. One recent study suggested that drugs with rapid cellular uptake, such as idarubicin are preferable [16], and another study indicated that drugs with rapid tissue uptake (i.e., high extraction fraction) are ideal candidates for encapsulation by TSL and other IV-DDS [14]. Direct experimental comparisons between drugs are difficult since TSL release kinetics depend on the encapsulated compound [17], and each drug typically requires a different TSL formulation for optimal release kinetics [16].
Computer models have been used in prior TSL studies to compare liposomal formulations \cite{12,18,19}, to study the impact of tissue properties \cite{14}, and to compare hyperthermia strategies \cite{20–24}. One disadvantage of computational models is that they typically require a large number of parameters, which are often estimated or obtained from prior publications \cite{14}. Here we employ computational models based on the commonly used Krogh-cylinder model, where a representative capillary with surrounding tissue is stimulated \cite{25,26}. We developed mathematical models predicting cell uptake and viability based on \textit{in vitro} studies with four anthracycline drugs, in three cancer cell lines. \textit{In vitro}-based cell uptake and cytotoxicity were integrated with the Krogh cylinder drug delivery model. Drug release from IV-DDS (e.g., TSL) was simulated, followed by uptake from surrounding tissue and cells. Based on this drug delivery model we directly compared the efficacy of the four drugs in terms of tumor uptake and tumor viability.

**Materials and methods**

**Cell culture**

Three established cancer cell lines were used in these experimental models: murine Lewis lung carcinoma cells (LLC) \cite{27} with a luciferase-reporter gene were obtained from ATCC (cat. no. CRL-1642-LUC2; Rockville, MD, USA); murine angiosarcoma SVR cells \cite{28} were obtained from Dr. Nancy Klauber-DeMore (Hollings Cancer Center, MUSC); human UM-SCC-1 squamous carcinoma cells (SCC herein) \cite{29} were obtained from Sigma–Aldrich (cat. no. SCC070; St. Louis, MO, USA). All cell lines were cultured at 37 °C in a humidified 5% CO\textsubscript{2} incubator in a complete medium: Dulbecco’s modified Eagle’s medium (cat. no. 11995-065; Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum (cat. no. FB-11; Omega Scientific, Tarzana, CA, USA).

**Drugs**

Four anthracycline chemotherapeutic drugs were used in these experimental models (Table 1): doxorubicin (DOX) hydrochloride was obtained from Sigma-Aldrich (cat. no. PHR1789), idarubicin (IDA) hydrochloride from APEXBio (cat. no. A2476; Houston, TX, USA), aclarubicin (ACLA) hydrochloride from MedChemExpress (cat. no. HY-N2306A; Monmouth Junction, NJ, USA), and pirarubicin (PIR) from AdooQ Bioscience (cat. no. A10735; Irvine, CA, USA). To prepare stock concentrates, all drugs were reconstituted in water or dimethyl sulfoxide according to supplier instructions and stored as aliquots protected from light at −20 °C.

**Cellular drug uptake measurements**

For individual drug concentrations and exposure times, cell lines were seeded in separate T-150 flasks in a complete medium and grown to sub-confluency. For drug exposure, the medium was removed from the flask and replaced with a fresh complete medium containing a particular drug diluted to the indicated concentration. Cells were then

| Drug Structure | MW | pKa | logP |
|----------------|-----|-----|------|
| Doxorubicin HCl (DOX) | 580.0 | 8.00, 9.93 | 1.41 |
| Idarubicin HCl (IDA) | 534.0 | 8.04, 10.04 | 1.69 |
| Aclarubicin HCl (ACLA) | 848.3 | 7.47, 8.64 | 2.79 |
| Pirarubicin (PIR) | 627.6 | 7.99, 9.09 | 2.06 |

\textsuperscript{a}From \url{https://pubchem.ncbi.nlm.nih.gov}.

\textsuperscript{b}Molecular weight.

\textsuperscript{c}Strongest acidic, strongest basic (predicted).

\textsuperscript{d}Predicted log\textsubscript{10} partition coefficient.
returned to the 37 °C incubators for the indicated exposure time before removing the medium and washing the cells three times with phosphate-buffered saline (PBS, pH 7.4). Cells were then detached by trypsinization and transferred to centrifuge tubes for two additional PBS washes. Before the final wash, cells were counted by an automated cell counter (Bio-Rad, Hercules, CA, USA) to correlate total cell numbers from each flask to corresponding drug quantification. After the final wash, drugs were extracted from cell pellets by overnight incubation at 4 °C in a lysis-extraction solution of 85% 2-propanol/0.75 N HCl, a modification of a previously published protocol [30]. The total drug released into this solution was quantified by fluorescence detection in a spectrophotometer (Exc 485 nm, Em 590 nm) and compared to a standard curve of serial dilutions of free drug in the same lysis-extraction solution. Values were then normalized by the total drug extracted per 10^6 cells.

**Cell viability assays**

For each cell line, drug, and exposure time, cells were seeded in separate 96-well plates at 5 x 10^4 cells/well to assay seven drug concentrations with 4–6 replicates each. The following day, the medium was removed and replaced with a fresh complete medium containing indicated drug at 1:6 serial dilutions plus control (100, 10, 1, 0.1, 0.01, 0.001, and 0 µg/mL [control]). Plates were then returned to the 37°C incubators for the indicated drug exposure time. The medium was then removed and wells were carefully washed 3 x with 200 µL Hanks’ buffered saline solution (HBSS) before adding 100 µL complete medium and returning plates to the incubator for 24 h. Cell viability was then determined by directly adding 15 µL MTS reagent from the CellTiter 96 AQueous One Solution Cell Proliferation Assay kit (cat. no. G3582; Promega, Madison, WI, USA) to the medium and returning plates to the incubator. Every hour (2–4 total measurements), color development was measured by absorbance at 490 nm. Background absorbance (medium + MTS from wells with no cells) was subtracted from cell-well absorbance. For each drug concentration, % cell viability was calculated as (avg. absorbance of drug conc. replicates/avg. absorbance of 0 µg/mL control replicates) x 100. The half maximal inhibitory concentrations (IC50) for each drug were determined by four parameters logistic (4PL) calculations.

**Mathematical models**

**Cellular drug uptake model**

Cellular uptake was mathematically modeled considering two rate constants representing drug influx (k12) and efflux (k13) (Equation S9). The rate constants were determined based on optimization using the Nelder-Mead Simplex Method [31] in the software MATLAB. The optimization was based on the experimental results from the in vitro cell uptake studies described above. Results with extracellular drug concentrations (c_e) of 1, 5, and 10 µg/mL were considered, for exposure durations of 1, 15, and 60 min. The three extracellular concentrations were selected to cover the range between minimum detectable level, and maximum anticipated level in the model. The lowest exposure durations were selected to cover the initial rapid uptake dynamics. Maximum exposure duration was chosen in agreement with typically used hyperthermia durations of 60 min in prior studies [15,32,33]. We optimized specific durations and concentrations we used in preliminary experiments (data not shown). Individual cell uptake rate constants were determined for all four drugs, and for each drug in all three cell lines.

**Cell cytotoxicity model**

A cell survival model was developed to predict drug cytotoxicity. The model was based on the extracellular AUC model described earlier [34,35], where the tumor cell survival fraction S was modeled as:

\[
S = \frac{1}{1 + \frac{A}{A^{\text{AUC}}} + \frac{1}{A^{\text{AUC}}} - \text{AUC}} 
\]

where A and n were survival model parameters, and AUC is the area under the curve calculated for the extracellular drug concentration c_e as shown in Equation (1). The two model parameters were determined by optimization as described for the cell uptake model. The optimization was based on the experimental results of the in vitro cytotoxicity studies described above, using data for 5, 15, and 60 min drug exposure for drugs with fast uptake (IDA, ACLA). Cytotoxicity data for 15, 60, and 180 min drug exposure was used for drugs with slow uptake (DOX, PIR).

**Krogh cylinder model**

We developed a computational model based on the commonly used Krogh-cylinder model (Figure 1), where a representative capillary with surrounding tissue is stimulated [24,25]. Two computer models were coupled: (1) a flow model to simulate blood flow along the tumor capillary and diffusion across the capillary wall, and (2) a drug delivery model to simulate drug diffusion within the interstitium (extracellular- extravascular space (EES)) and cellular drug uptake. The diffusion across the capillary wall composed of endothelial cells was represented by an effective vascular permeability parameter. This parameter can also model plasma protein binding of drugs (i.e., in cases of substantial protein binding, the effective vascular permeability would be lower than the true permeability for that drug). The second model (light pink outer cylinder in Figure 1) included two compartments: an interstitial (EES) compartment, and a second compartment representing cells. Drug transport between EES and cells was modeled based on the cell uptake model derived from our in vitro studies. We assumed that the whole tumor was exposed to hyperthermia (41 °C). We further assumed that blood enters the tumor at body temperature via a supplying artery, and immediately heats to 41 °C once entering the capillary. Blood equilibrates with surrounding tissue very rapidly within capillaries as a result of the small vessel diameter and slow blood flow velocity [36]. We further assumed ideal IV-DDS (e.g., TSL), where complete drug release occurs once blood enters the capillary and...
heats to 41 °C. For one drug (DOX), we performed an additional simulation based on the actual TSL release kinetics of a specific TSL-DOX formulation based on prior data [24], for comparison to ideal TSL (Figure S1). The models were simulated assuming 2-D axisymmetry and the finite element method was used to solve the model equations (COMSOL Multiphysics 5.3). Inside the capillary, we assumed laminar flow with a no-slip boundary condition at the vessel wall. A no flux boundary condition was assumed at the outer tissue cylinder. The most relevant model parameters are shown in Table 2, and a complete list of equations, boundary conditions, and model parameters is available as Supplementary Materials (Table S1). As result, we present tumor drug concentration maps, where we calculated tissue drug concentration considering interstitial and cellular concentrations and their volume fractions (Equation S10).

Results

Cellular drug uptake model

We present cell uptake results for SVR cells for all four drugs in Figure 2. Additional results for other cell lines are available in the Supplementary Materials (Figures S2–S5). For all cell lines, uptake was very rapid for IDA and ACLA with substantial uptake within a few minutes, whereas DOX and PIR had much slower uptake. Uptake kinetics could be adequately represented by our mathematical model (Figures 2, S2–S5).
The uptake parameters for all drugs and cell lines are summarized in Table 3.

### Cytotoxicity model

Cell survival following drug exposure for all four drugs is shown in the SVR cell line together with mathematical model predictions in Figures 3(a–d). Results for other cell lines are available in Supplementary Materials (Figure S6). IDA was the most cytotoxic, followed by PIR, DOX, and ACLA. To show variability between cell lines, cytotoxicity data for all three cell lines exposed to either DOX or IDA for 60 min are plotted in Figure 3(e). These results indicated that IDA was more cytotoxic than DOX in all 3 cell lines and that SCC cells were most resistant to either drug. Parameters of the cytotoxicity model and IC50 values for each drug and cell line are shown in Table 4. Overall, ACLA was the least cytotoxic with IDA and PIRA showing the highest cytotoxicity.

### Plasma drug concentration along capillary is governed by vascular permeability

We assume that blood with encapsulated drug enters the tumor via an artery and that 100% of the drug is released from IV-DDS (e.g., TSL) upon entering the capillary. The free drug concentration at the capillary inlet was 51.4, 41.1, 48.0, and 54.8 μg/ml for DOX, IDA, PIR, and ACLA, respectively. These values differ as all drugs were administered at different doses, i.e., at their maximum tolerated dose (MTD) [41–43,40] (Table 2).

Once entering the capillary, the drug diffuses across the capillary wall as blood flows along the capillary. Initially, tissue (i.e., interstitium and cells) are free of drugs and the total amount of drug extracted from plasma in the capillary corresponds to the initial extraction fraction ($EF$) (Figure 4). As more drug is taken up by cells and interstitium, the amount extracted from plasma will decrease. Among the four drugs, DOX is extracted less rapidly compared to the other three drugs due to its lower vascular permeability. Due to the relatively small capillary diameter, no radial plasma concentration gradient is apparent (i.e., any radial concentration differences are rapidly equilibrated by diffusion within plasma).

### Spatial tissue drug concentration gradients

We determined drug concentration in the tissue surrounding the capillary (incl. interstitium (EES) and cells) 10 min...
following a 60 min hyperthermia duration. The 10 min delay was to allow for the backflow of drugs not taken up by cells from the interstitium into plasma [12,21]. After 10 min there is negligible interstitial drug remaining and the tissue concentration is primarily dictated by intracellular drug concentration. The average concentration in the tissue cylinder for

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**Figure 3.** *In vitro* cytotoxicity of four drugs in the SVR angiosarcoma cell line, shown for (a) DOX, (b) IDA, (c) PIR, and (d) ACLA. (e) *In vitro* cytotoxicity for DOX and IDA (60 min exposure), for all three cell lines (LLC, SCC-1, SVR). In all graphs, data points indicate experimental results, and lines indicate mathematical model predictions.

**Table 4.** Survival model parameters (A and N) and IC50 values.

| Cell line | DOX     | IDA     | PIRA    | ACLA    |
|-----------|---------|---------|---------|---------|
|           | A (μg/s/mL) | N       | IC50 (μg/mL) | IC50 (μg/mL) | IC50 (μg/mL) | IC50 (μg/mL) |
| LLC       | 1.62 x 10^{-3} | 0.68    | 3.0 ± 0.6 | 0.5 ± 0.1 | 5.2 ± 0.6 | 0.54  | 9.1 ± 1.3 | 0.6 ± 0.1 | 6.0 ± 0.6 | 0.48   | 0.54   | 0.43   |
| SVR       | 1.73 x 10^{-3} | 0.57    | 1.55 ± 0.3 | 0.3 ± 0.05 | 5.95 ± 0.3 | 0.0534 | 5.5 ± 0.6 | 0.5 ± 0.05 | 2.5 ± 0.7 | > 50   | 0.25   |
| SCC       | 3.13 x 10^{-3} | 0.99    | 3.13 ± 10^{-3} | 3.13 ± 10^{-3} | 7.83 ± 10^{-3} | 0.54  | 5.1 ± 0.1 | > 50 | 16.5 ± 3.2 | 1.62 ± 0.6 | 0.48   | 0.54   | 0.43   |

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the four drugs based on SVR cells was 242.1 µg/g (DOX), 412.8 µg/g (IDA), 422.5 µg/g (PIR), and 559.9 µg/g (ACLA). The tissue concentration was largely determined by cell uptake rate, with drugs with rapid uptake (IDA, ACLA) showing higher tissue concentrations than those with slow uptake (DOX, PIR) (Figure 5). Purely based on the amount of drug delivered, ACLA and IDA would be the preferred drug candidates for encapsulation in TSL or other IV-DDS.

**Cancer cell survival prediction**

Based on the drug concentration predicted in our computer model, we used the cell survival model to predict cancer cell viability. The area under the curve (AUC) of the interstitial (extracellular) drug concentration was calculated to estimate cell survival based on Equation (1) [34,35]. We report cell survival 10 min post the cessation of 60 min heating for SVR tumors with 4 different drugs (Figure 6; Table 5). Beyond 10 min, the interstitial drug concentration is negligible, i.e., the AUC of interstitial concentration and predicted cytotoxicity would not further increase after this time. Cytotoxicity was highest for IDA, followed by DOX, PIR, and ACLA. IDA killed 73–99% of cells within 60 min of heating (Figure 6(b)). DOX had a killing efficacy of 64–72% (Figure 6(a)), PIR had an efficacy of 58–76% (Figure 6(c)), and ACLA had an efficacy of 26–47% (Figure 6(d)). While ACLA had the most rapid cell uptake (Figure 2) and highest tissue uptake (Figure 5), cytotoxicity was substantially lower than for the other drugs (Figure 3), resulting in overall poor kill efficacy (Figure 6(d)). This suggests that ACLA is a poor choice for TSL encapsulation. Overall, the computer model predicts that among the four drugs, IDA is the best choice for TSL encapsulation, followed by PIR and DOX (Figure 6, S8).

**Figure 4.** Plasma drug concentration inside capillary for (a) DOX, (b) IDA, (c) PIR, and (d) ACLA after 1 min of delivery. Plasma concentration decreases along the capillary due to drug diffusion across the capillary wall. Results are shown for SVR cells, but since uptake is dependent primarily on vascular drug permeability, results for other cell lines are almost identical.

**Figure 5.** Spatial variation in tissue drug concentration for (a) DOX, (b) IDA, (c) PIR, and (d) ACLA. Results are shown based on SVR (angiosarcoma) cells 10 min post cessation of 60 min delivery with IV-DDS (i.e., 60 min heating for TSL). Since concentration between different drugs spans several orders of magnitude, results are plotted on a logarithmic scale to enable comparison between drugs. Results for other cell lines are available in Supplementary Materials (Figure S7).

**Figure 6.** Tumor cell survival for (a) DOX, (b) IDA, (c) PIR, and (d) ACLA encapsulated in ideal TSL. Results are for SVR (angiosarcoma) cells, and cell survival was calculated for 60 min delivery duration. Results for other cell lines are available in Supplementary Materials (Figure S8).

| Table 5. Tissue drug concentration ($c_t$) and survival ($S$) for all drugs and cell lines. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | DOX             | IDA             | PIR             | ACLA            |
| LLC $c_t$ (µg/g) $S$ (%)    | 206.1 (171.2–354.9) 31.9 (24.2–34.6) | 279.7 (137.4–927.3) 21.4 (8.4–29.3) | 316.3 (185.4–1066.1) 21.9 (13.7–25.7) | 417.2 (32.5–4219.5) 67.5 (30.5–83.2) |
| SVR $c_t$ (µg/g) $S$ (%)    | 242.1 (214.1–346.6) 33.8 (28.4–35.6) | 412.8 (148.3–1522.6) 12.8 (1.1–26.9) | 422.5 (271.8–1167.8) 36.4 (23.6–41.8) | 559.9 (165.3–2533.5) 68.1 (53.1–74.4) |
| SCC $c_t$ (µg/g) $S$ (%)    | 175.9 (83.0–561.4) 67.7 (52.2–72.2) | 216.8 (27.2–3267.6) 65.5 (27.2–79.9) | 214.5 (152.1–480.1) 55.9 (43.2–60.6) | 283.3 (57.5–1634.1) 82.3 (55.1–90.5) |

Table lists tissue mean, with the range indicated in brackets.
Comparison of ideal TSL and realistic TSL formulation

In all results above we assumed ideal IV-DDS/TSL that instantaneously release all drug upon entering the tumor capillary. Here we compare the results of ideal TSL to a fast-releasing lysolipid-based formulation encapsulating DOX (LTSL-DOX). We considered LTSL-DOX release data at 41°C based on published data [24] (Figure S1). Upon entering the capillary, LTSL continuously release DOX while LTSL move with blood flow along the capillary. We compare capillary concentration, tissue drug concentration, and cytotoxicity following 60 min heating and a 10 min cool-down period (Figure 7). As anticipated, ideal TSL-DOX resulted in higher drug uptake and greater cytotoxicity compared to LTSL-DOX. The average tissue drug concentration was 242.1 μg/g for ideal TSL-DOX and 130.9 μg/g for LTSL-DOX. In contrast to ideal TSL-DOX where the highest plasma concentration is at the capillary entrance, for LTSL-DOX the drug concentration increases along the capillary length due to continuous release and reaches a maximum inside the capillary (Figure 7(a)).

Discussion

Thermosensitive liposomes (TSL) belong to the category of triggered drug delivery systems (DDS), where drug release is initiated by a specific signal (e.g., heat for TSL) [3,4,14,51]. The more recent TSL formulations are based on the concept of intravascular triggered release, where drug release is
Drug delivery based on IV-DDS depends on the complex interplay between DDS, drugs, and the in situ tumor micro-environment. Computer models can aid in the optimization of DDS and have been used in past studies to compare different TSL formulations and the impact of hyperthermia methods [12,14,18,23,24]. Here, we developed a model that simulates drug delivery based on intravascular triggered release from IV-DDS, such as TSL. To predict the effects of various drugs, we first developed mathematical models that describe the cellular uptake kinetics and cytotoxicity of four anthracycline drugs (doxorubicin (DOX), idarubicin (IDA), pirarubicin (PIR), aclorubicin (ACLA)). Furthermore, each drug was characterized in three cancer cell lines: angiosarcoma (SVR), Lewis lung carcinoma (LLC), and oral cavity squamous cell carcinoma (SCC-1). We used the Krogh cylinder model, where a representative tumor capillary with surrounding tissue and cancer cells is represented (Figure 1) [25,26]. Importantly, for our computer model, we used in vitro experimentally-determined parameters rather than parameter estimates as is often the case (see Tables 3 and 4).

The cellular uptake kinetics in the computer model were based on in vitro studies where we measured uptake for the four drugs in the three cell lines. The uptake rate varied widely between drugs, with the two drugs ACLA and IDA showing substantially faster uptake compared to DOX and PIR (Figure 2). Previous studies comparing different anthracycline agents have shown that in general, more lipophilic drugs, such as IDA are taken up by cells more rapidly, as they can more easily cross membranes [53,54]. A mathematical model based on two rate constants for influx and efflux ($k_{12}$, $k_{21}$) was able to adequately describe uptake kinetics for each drug in each specific cell line (Figure 2; Table 3). Uptake also varied for each particular drug between cell lines (Figures S2–S5).

For predicting cancer cell survival in our model, we performed in vitro cytotoxicity studies which indicated that IDA was the most cytotoxic agent, with ACLA being the least cytotoxic (Figures 3(a–d)). For all drugs, the SCC cell line was the most sensitive (Table 4). We developed mathematical models that predict viability based on the area under the curve (AUC) of extracellular concentration (Equation 1) [34,35], and list the two parameters of the cytotoxicity model for each drug-cell line combination in Table 4.

We integrated the cell uptake and the cytotoxicity model in our 3-D model where we simulate a tumor capillary with surrounding tissue (Figure 1). We assumed ideal IV-DDS (e.g., TSL), where the drug is completely and instantaneously released from DDS upon entering the capillary. Specifically for TSL, we assume that the tumor is heated to 41°C. TSL is carried to the capillary by plasma within a tumor-feeding artery. For larger vessels than capillaries, the blood may be at lower temperatures compared to surrounding tissue [55]. Due to the small diameter and low blood flow velocity within the capillary, we can assume that blood temperature immediately equilibrates with surrounding tissue temperature [36] and heats up to 41°C, resulting in drug release from TSL at the capillary entrance. As drug and plasma move along the capillary, the drug is continuously extracted by the surrounding tissue, resulting in a longitudinal drug

Figure 8. (a) Radial concentration gradient for DOX and IDA in comparison to a prior in vivo study, where intravital fluorescence microscopy was employed to estimate concentration [16]. DOX results in significantly more uniform drug uptake compared to DOX and PIR (Figure 2).

(b) Schematics indicate transport kinetics for a drug with slow cell uptake (e.g., DOX). Diffusion is dominating over cell uptake, allowing drug penetration distant from the capillary. Blue arrows indicate drug transport, red circles represent cancer cells. (c) For a drug with rapid cell uptake (e.g., IDA), cell uptake by cells close to the capillary depletes the drug available for more distant cells.
concentration gradient within the capillary (Figure 4). The amount extracted depends on the permeability of the vascular wall, which varies by drug (Table 2; Table S1). Therefore, the drugs with high permeability, such as IDA are extracted better (Figure 4).

Following drug transport across the capillary wall, the drug diffuses radially through the tissue surrounding the capillary and is simultaneously taken up by cells. Figure 5 shows the predicted drug concentration after 60 min of triggered release for the four drugs. Notably, the two drugs ACLA and IDA that have the most rapid cellular uptake (Figure 2) also resulted in the highest tissue uptake, indicating that cellular uptake rate is a dominating factor for tissue drug uptake when delivered by IV-DDS (e.g., TSL). As a result of the longitudinal gradient within the capillary, a similar longitudinal gradient was observed within the tissue cylinder surrounding the capillary. In addition, a radial concentration gradient is apparent that considerably varies between drugs. This radial gradient was more pronounced for drugs with rapid cell uptake, since drug uptake by cells close to the capillary depletes drug available for uptake further distant from the vessel (e.g., ACLA, IDA in Figure 4). This radial gradient has been experimentally measured in vivo for DOX and IDA in a prior study based on intravital fluorescence microscopy [16]. In Figure 8(a), we show a direct comparison of the radial concentration gradient (averaged along the capillary) between our model and this prior in vivo study for DOX and IDA, where a steeper gradient for IDA was observed both experimentally and in our models. For drugs with slower cell uptake, such as DOX, diffusion dominates, resulting in a more uniform radial concentration gradient (Figure 8(b)). For drugs with rapid cell uptake, such as IDA, cell uptake dominates, resulting in a steeper gradient (Figure 8(c)). That is, there is a competition between cellular uptake and radial drug diffusion. This reduced penetration due to rapid cellular drug uptake is similar to the concept of binding site barriers, where macromolecular ligands are prevented from deep tumor penetration by excessive binding to targeted receptors close to the delivery site (e.g., vessel) [56].

In the results above, we assumed ideal IV-DDS (e.g., TSL) with complete and instantaneous drug release once entering the capillary. In reality, release occurs more slowly, for example in the range of seconds to minutes for common TSL formulations [2,3,17]. For comparison to ideal TSL, we simulated delivery based on the delivery kinetics of a liposom-based TSL formulation (LTSL-DOX) [2,24]. We assumed a temperature of 41°C, where this LTSL releases most of the encapsulated DOX within ~2s (Figure S1). As a result, plasma concentration increases upon entrance of LTSL into the capillary, and maximum plasma DOX concentration occurs distant from the entrance—in contrast to ideal TSL where maximum plasma concentration is at the entrance (Figures 7(a,b)). As anticipated, ideal TSL-DOX resulted in higher drug uptake and greater cytotoxicity compared to LTSL-DOX (Figures 7(c–f)). These results suggest that the model may also be employed to compare specific TSL formulations or types of IV-DDS in terms of delivery efficacy and resulting cytotoxicity.

The proposed model has limitations: while we measured several parameters experimentally, some parameters, such as vascular permeability were estimated based on published literature. Further, in vitro response of cancer cells (e.g., cytotoxicity) may not be identical to the in vivo response of these cells as the tumor microenvironment is not considered. Also, we did not consider active uptake mechanisms or drug metabolism, which may be relevant for certain drugs or cell lines. We did not include any hyperthermia-induced changes in vascular permeability, cell uptake, or cytotoxicity. Plasma stability of TSL impacts drug uptake [33], and here we only consider perfectly stable TSL. Finally, the model would require in vivo validation to establish the accuracy of the predicted results.

Conclusions
We developed a computer model approach integrated with in vitro experiments to establish a platform that may serve for screening of drugs and to select the compounds most likely to have optimal efficacy when delivered by IV-DDS, such as TSL. In addition, the model enables the comparison of different IV-DDS based on their release kinetics. Our results suggest that among the four tested drugs, IDA is the most effective. Overall, drugs with rapid cell uptake are preferred as these will accumulate at a high concentration within the targeted tumors.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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