Cellular Membrane Permeability of Anthracyclines Does Not Correlate with Their Delivery in a Tissue-isolated Tumor

Marc Heijn, Sylvie Roberge, and Rakesh K. Jain

Department of Radiation Oncology, Edwin L. Steele Laboratory, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114

ABSTRACT

The clearance of anthracyclines from the vasculature was studied in perfused tissue-isolated tumors. Human tumor lines MCF-7, U87, and LS174T were implanted in the ovarian fat pad of immune-deficient mice and grown isolated from the surrounding tissue. The initial and continuous tissue uptakes of doxorubicin, daunorubicin, and idarubicin were measured. The clearance of these anthracyclines from the perfused vasculature of the tissue-isolated tumor was calculated using BSA as an intravascular marker. The measured clearances ranged from 50–200 μl/min/g tumor tissue, and the fractional clearances were between 0.30 and 0.70. On the basis of the in vitro cellular uptake rates of the anthracyclines, we expected a higher clearance of idarubicin than of doxorubicin. No significant differences were found among the clearances of the anthracyclines or among the tumor lines. The observed similarities in clearance of the anthracyclines was largely explained by differences in their protein binding and tissue diffusion gradients.

INTRODUCTION

The anthracycline antibiotics, such as DOX, DNR, and IDA, constitute one of the most important groups of drugs used for the treatment of a wide variety of tumors. Despite years of research in developing new and better drugs, DOX remains the most effective anthracycline for the treatment of solid tumors (1).

In vivo and in vitro data do not clarify why DOX is active against solid tumors, whereas DNR and IDA are much less effective. The most important difference between these drugs is their cellular uptake rate. Compared with DOX, the plasma membrane is 14 and 40 times more permeable to DNR and IDA, respectively (2, 3). The in vitro toxicity of DOX and DNR are comparable (IC₅₀, 10–20 nM). The toxicity for IDA is somewhat higher (IC₅₀, 0.15–6 nM; Refs. 2–5).

The initial in vivo clearance from the blood of these three anthracyclines is similar (t₁/₂, ~2 min; Refs. 6–8), indicating that the initial uptake by the tissue of the drugs is similar. The maximal tolerated dose in vivo of each anthracycline correlates with the cellular toxicity measured in vitro (9). The similar initial clearances and maximal tolerated doses correlate with the cellular toxicity, suggesting that the normal tissue toxicity can be predicted by in vitro data. However, the effectiveness of the different anthracyclines in vivo cannot be predicted by these in vitro data.

The uptake of drugs by solid tumors may be different from that of normal tissue. Both blood supply and tissue structure of solid tumors are significantly different from that of most normal tissues (10, 11). This may lead to a significant difference in extravasation and tissue penetration of drugs between tumor and normal tissue, as well as between drugs. To measure the uptake of drugs into solid tumors separately from systemic influences, we used the tissue-isolated tumor model developed by Gullino and Grantham (12). This model is especially suited to study tumor-specific pharmacokinetics (13, 14).

To investigate a wide variety of human tumor xenografts, we adapted the tissue-isolated tumor model to immune-deficient athymic nude and SCID mice (15).

We studied the uptake of the three anthracyclines in tumor cell lines derived from a human glioblastoma (U87), a colon adenocarcinoma (LS174T), and a mammary carcinoma (MCF-7), which were grown as tissue-isolated tumors. On the basis of cellular uptake rates, we expected the clearance by the tumor to be highest for IDA and lowest for DOX. On the basis of clinical effectiveness of anthracycline treatment of the different tumors (glioblastomas < colon adenocarcinomas < mammary carcinomas), we anticipated lowest clearance by U87 and highest clearance by MCF-7.

MATERIALS AND METHODS

Chemicals. ρ-BSA was purchased from Molecular Probes (Eugene, OR). The following anthracyclines were used (see Fig. 1): DOX hydrochloride from Pharmachemie B.V. (Haarlem, the Netherlands), DNR hydrochloride from Ben Venue Labs Inc. (Bedford, OH), and IDA hydrochloride from Pharmacia S.P.A. (Milan, Italy). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals. Female SCID and NCr/Sed-nu/nu athymic mice, bred in the animal facility of the Edwin L. Steele Laboratory and maintained under specific pathogen-free conditions, were used. The animals were fed sterilized standard laboratory rodent chow and sterilized water ad libitum. The body weight of the animals was 20–25 g. Before surgical procedures, animals were anesthetized with a s.c. injection of ketamine (10 mg/kg) and xylazine (1 mg/kg) in 0.9% NaCl solution. Animal care was provided in accordance with institutional guidelines.

Tumors and Transplantation. The human adenocarcinoma LS174T, glioblastoma U87, and mammary carcinoma MCF-7 were obtained from American Type Culture Collection and maintained by serial transplantation in immune-deficient mice. The transplantation and growth of tissue-isolated tumors is described in detail elsewhere (15). Briefly, for transplantation, the tumor was removed from the host animal on euthanasia and washed in HBSS (Life Technologies, Inc., Grand Island, NY). After removal of macroscopically nonviable tissue, the tumor tissue was minced into a slurry with a few drops of HBSS. The final slurry was forced several times through an 18- and 20-gauge needle in series. Nude athymic mice were used for the LS174T tumors. U87 and MCF-7 tumors were grown in SCID mice, where they grew better than in nude athymic mice. In brief, a connective tissue pedicle containing the left ovarian vessels was prepared surgically in a female mouse and positioned subsequently in a s.c. pouch wrapped in a sterile paraffin bag. Before the bag was sealed, 0.02 ml of tumor slurry was injected in fat at the distal end of the pedicle. Tumors reached the desired size in 2–4 weeks.

Perfusion experiment. The tumor was perfused, as shown in Fig. 2. The perfusion medium [118 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 2.55 mM CaCl₂, 11.1 mM Glucose, 700 units/l Na-heparin, and 2.5% (w/v) BSA] was stored in an upstream container. Gas (95% O₂ and 5% CO₂) was introduced into the perfusion media container through a semipermeable...
tube (Silastic tubing; Dow-Corning, Midland, MI). The medium was pumped (ACCU FM-40 with RH00 head; Scilog, Madison, WI) through a 0.2-μm filter (25 mm, 0.2 μ cellulose acetate membrane; Corning Corp., Corning, NY). The pressure was measured online with a pressure transducer (Gould, Inc., Valley View, OH). A six-channel injection valve (Upchurch, Oak Harbor, WA) was placed immediately upstream of the tumor entrance.

To achieve isolated perfusion of the tumor, all blood vessels supplying nontumor tissue were ligated, as described in detail elsewhere (15, 16). The inferior vena cava and aorta were cannulated with polyethylene tubing and perfused at a constant rate between 50–120 μl/min. The tumor was cleared of blood within about 10 min and could be perfused for 90–120 min. The mouse was kept on a heating pad at 37°C throughout the experiment. At the beginning of each experiment, the geometric resistance of the mouse was measured (16).

The viscosity of the perfusion medium was measured to be 1.5 centipoise (12.5 × 10⁻⁴ mm Hg/min). Oxygen was measured in the afferent and efferent perfusion medium using an ABL 330 gas analyzer (Radiometer, Copenhagen, Denmark) at the beginning and the end of the experiment. Oxygen consumption was calculated as the difference between the oxygen tensions in the afferent and efferent media relative to the oxygen tension in the afferent medium. The perfusion flow was measured by weighing the collected samples, assuming a perfusate density of 1.0. The inflow rate and the resistance of the tubing were measured separately in each experiment. The difference between inflow and outflow rates was attributed to leakage from the vessels and fluid loss from the tumor surface (Fig. 3).

The anthracycline and p-BSA were added either simultaneously to the perfusate as a pulse injection or were administered continuously to the tumor in the perfusion medium. For the applied pulse, the volume of injection was 24 μl and the concentrations of the anthracycline and p-BSA were 100 μM and 0.1 mg/ml, respectively. At the moment of the injection, the time was set to zero. The outflow pattern was measured with and without a tumor. For continuous infusion, the container with the perfusate was changed at time zero to one that contained 5 μM anthracycline and 50 μg/ml p-BSA. The concentration in the outflow was followed as a function of time. A single tumor could be used for one pulse and one continuous perfusion or for three pulse measurements. No difference in clearance of a drug was seen between the first and third pulse.

**Analysis of Outflow Pattern.** The concentrations of p-BSA and anthracyclines or calcein were measured fluorometrically in the same sample [DOX, DNR (480/585 nm), IDA, DOCh (541/585 nm), p-BSA (494/517 nm)]. The value at the wavelength pair of one fluorochrome was corrected by subtracting the value of cross contamination of the other fluorochrome at that wavelength pair.

The outflow concentrations were all normalized to the input concentration of the injected medium or perfusate. The outflow profiles after bolus injection were analyzed by calculating the area under the curve (auc) and the mean transit time (mtt). The mtt was corrected for the mean transit time of the compounds in the tubing before the tumor. Depending on the flow rate, it ranged from 0.55–0.75 min (mean, 0.66 ± 0.05). Secondary pharmacokinetic parameters, such as recovery ratio ($F_r = \frac{auc_{r,BSA}}{auc_{l,BSA}}$), mean elimination time ($l_{el} = mtt(1-F_r)$), initial distribution volume ($V_i = Q_{out}mtt/F_r$), and intrinsic clearance ($Cl = V_i/l_{el}$), were derived as described by Ohkouchi et al. (13). The clearance during continuous infusion was calculated according to:

$$Cl = \frac{Q_{out}}{C - C_{out}}$$

where $C$ is the normalized output concentration of the anthracycline or BSA.

**RESULTS**

To verify the quality of the perfused tumors, we measured the basic parameters, as summarized in Table 1. The fluid loss during the perfusion was about 30%. Part of the 30% oozed out from the tumor surface (~8%). The rest leaked out of the vessels before the perfusate reached the tumor. This unavoidable leakage was due to damage of delicate structures during preparation of mice for tumor perfusion and to fluid loss at the connection between the aorta and the cannula. The geometric resistance was first calculated with the measured flow rate out of the tumor, $Q_{out}$, and the pressure drop across the tumor. On the basis of measurements with BSA indicating that about 10% of the perfusate leaves from the tumor surface (see below), we corrected the flow rate by $Q_{out}/0.9$. This corrected flow rate may provide a more accurate estimation of the geometric resistance.

After 2 h of perfusing the tumor, the oxygen consumption dropped from 80% to 76%. This small decrease in oxygen consumption (5%) shows that the tumors were still in a good condition after 2 h. The initial oxygen tension of the afferent perfusate was 700 mm Hg.

**Continuous Infusion.** The concentration profiles of p-BSA and anthracyclines in the effluent, following a continuous perfusion, are shown in Figs. 4 and 5 for MCF-7 and U87, respectively. After ~30 min, the concentration in the effluent reached a steady state. The first 30 min represent the time necessary for perfusate from the container with the anthracycline and p-BSA to fill the tubing between the container and the tumor. The concentration profile measured before the tumor had a similar profile in time (data not shown). After the
steady-state level was reached, the inflow rate was reduced. The normalized concentrations of the anthracyclines and ρ-BSA in the effluent before and after the reduction in flow are given in Table 2. For ρ-BSA no change in concentration was found. In most cases, the concentration of the anthracyclines decreased after reduction of the flow. The calculated clearance at steady state before the flow reduction is shown in Table 2. No change in clearance was observed after reduction in the flow rate (Figs. 4 and 5).

BSA was more concentrated in the effluent than in the infused perfusate. The relative steady-state concentration of ρ-BSA was 1.1 in all three tumor lines, indicating that about 9% of the perfusate was filtered in the tumor.

**Anthracycline Pulse.** Fig. 6 shows the mean effluent profiles of ρ-BSA, DOX, DNR, and IDA from MCF-7, U87, and LS174T tumors. The pharmacokinetic parameters calculated according to Ohkouchi et al. (13) are summarized in Table 3. The mtt for the anthracyclines was longer than for ρ-BSA. IDA had the longest transit time. These differences are reflected in the differences in distribution volume. No significant difference was found in the intrinsic clearance between the different anthracyclines in the three tumor lines or between the tumor lines. The recovery ratio and fractional clearance of the anthracyclines showed even less variation.

To separate the kinetics of cellular uptake from interstitial uptake, we studied the clearance of calcein (molecular weight, $M_r$ 623). Calcein has approximately the same weight as the anthracyclines (DOX, $M_r$ 580), but is not taken up by the cells. Table 3 shows the pharmacokinetic parameters of calcein given as a pulse to MCF-7 tumors. The fractional clearance of calcein approximated the fluid loss from the tumor.

**DISCUSSION**

In this study, we showed that three anthracyclines that differ in their lipophilicity have the same clearance by perfused tissue-isolated tumors. Oxygen consumption and geometric resistance of the perfused tumors are comparable with data published previously (16). The largest resistance found in the smallest tumor (MCF-7) was observed earlier (17), but the calculated geometric resistance may be underestimated when the outflow rate from the tumors is used in the calculation. In this study, we corrected for intratumor fluid loss to obtain a more accurate estimation.

The parameters calculated from the pulse experiments assume that BSA does not cross the vascular barrier. Although the vessel wall is permeable to BSA, the loss from the vascular compartment is negligible. Outflow profiles of BSA and other intravascular tracers are similar to those found by Eskey et al. (18) and by us for fluorescent microspheres with a diameter of 0.5 μm in LS174T tumors (data not shown). The distribution volume of ρ-BSA is in agreement with studies using a similar technique [7% (18); 80–100 μl/g (13)], as well as different techniques (11).

The BSA in the effluent was 1.1 times more concentrated than in...
the tissue-isolated tumor (19) or leakage is similar to values obtained with different techniques in behind in the vessel, causes this increase. This extent of fluid entering perfusate. Net extravasation of fluid, leaving BSA present as mean ± SE of at least four experiments.

In the present study, DOX, DNR, and IDA showed similar clearance rates in the three tumor lines. Differences in lipophilicity account for the different cellular uptake rates of these drugs in vitro (2, 3). However, higher lipophilicity allows for greater binding of albumin, which may conversely reduce the uptake rate. Using the data of Demant and Friche (20), we calculated the percentage-free drug at a BSA concentration of 2.5% as 60%, 50%, and 20% for DOX, DNR, and IDA, respectively. Under these conditions (2.5% BSA), the cellular uptake rates of DNR and IDA in vitro are, respectively, 12 and 13 times, instead of 14 and 40 times, that of DOX, assuming that the anthracyclines were free in solution during the uptake experiments of Mülër et al. (2) and Mankhetkorn et al. (3).

One reason for the observed similarities in clearance rates might be that part of the flow does not reach the capillary bed of the tumor because of arterial-venous shunts. When even the slowest clearing compound (DOX) is cleared completely from the nonshunted perfusate, the observed clearance rates for all anthracyclines will be similar and equal to the nonshunted flow.

Several facts argue against the presence of a shunt. First, the vascular architecture of tumors revealed little anatomical evidence for shunts (21). Second, the residence time distribution of D₂O measured in tissue-isolated tumors in rats could not be satisfactorily explained by intravascular shunting (18). Third, in this study, we find a fractional clearance of O₂ of 80%, whereas the fractional clearance for anthracyclines is 40–70%. This is not what we would expect in the case of shunting. The clearance of oxygen cannot be greater than that of the anthracyclines because the anthracyclines should be cleared almost completely from the nonshunted perfusate. Finally, when we reduced the flow during continuous infusion, we saw a reduction in the anthracycline concentration in the effluent. This effect was most clear in the case of IDA in the MCF-7 tumor line. If a shunt were, in fact, present, we would not expect to see a change in drug concentration in the effluent after reduction of the flow, unless the drug with the slowest cellular uptake rate (DOX) is incompletely cleared at a high flow rate. In that case, however, one would see a reduction in the concentration most clearly for DOX instead of for IDA.

A more likely explanation for the similar clearances of the different drugs is that other factors besides the cellular uptake rate may affect the clearance. The endothelial barrier consists not only of the endothelial plasma membrane, but also of histological structures, including fenestrae and endothelial channels (22). Drugs diffuse through these channels in a plasma-like environment instead of the lipid environment of the plasma membrane. After passing the endothelial barrier, drugs enter the interstitial space from where they can enter tumor

<table>
<thead>
<tr>
<th>Relative concentrationa</th>
<th>Inflow 100%</th>
<th>Inflow 50%</th>
<th>Ratio (100%-50%)</th>
<th>Clearance (μl/min/g)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOX</td>
<td>0.94 ± 0.15</td>
<td>0.84 ± 0.13</td>
<td>1.12 ± 0.05f</td>
<td>41 ± 15</td>
<td>(4)</td>
</tr>
<tr>
<td>DNR</td>
<td>0.97 ± 0.12</td>
<td>0.86 ± 0.10</td>
<td>1.12 ± 0.09</td>
<td>40 ± 10</td>
<td>(4)</td>
</tr>
<tr>
<td>IDA</td>
<td>0.89 ± 0.10</td>
<td>0.69 ± 0.08</td>
<td>1.28 ± 0.16f</td>
<td>58 ± 18</td>
<td>(4)</td>
</tr>
<tr>
<td>BSA</td>
<td>1.11 ± 0.10</td>
<td>1.11 ± 0.09</td>
<td>1.01 ± 0.03</td>
<td></td>
<td>(12)</td>
</tr>
<tr>
<td>U87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOX</td>
<td>1.04 ± 0.13</td>
<td>0.81 ± 0.06</td>
<td>1.29 ± 0.08f</td>
<td>26 ± 23</td>
<td>(4)</td>
</tr>
<tr>
<td>DNR</td>
<td>0.82 ± 0.37</td>
<td>0.79 ± 0.29</td>
<td>1.02 ± 0.11</td>
<td>41 ± 46</td>
<td>(4)</td>
</tr>
<tr>
<td>IDA</td>
<td>0.85 ± 0.10</td>
<td>0.92 ± 0.06</td>
<td>0.92 ± 0.04</td>
<td>28 ± 16</td>
<td>(4)</td>
</tr>
<tr>
<td>BSA</td>
<td>1.09 ± 0.10</td>
<td>1.12 ± 0.06</td>
<td>0.97 ± 0.07</td>
<td></td>
<td>(12)</td>
</tr>
<tr>
<td>LS174T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA</td>
<td>1.11 ± 0.03</td>
<td></td>
<td></td>
<td></td>
<td>(4)</td>
</tr>
</tbody>
</table>

- The relative concentration in the effluent normalized to the input concentration and the clearance of anthracyclines and BSA are presented as mean ± SD (n).
- The ratio of the relative concentration at normal and reduced flow rates was calculated for each experiment.
- There were significant decreases in the concentration after flow reduction (P < 0.05, two-tailed paired t test). The clearance was calculated according to equation 1.

Fig. 6. Outflow profiles of BSA, DOX, DNR, and IDA after bolus injection. Data are presented as mean ± SE of at least four experiments.
and is set to $f_\text{free}$; 

**Concentration and volume of distribution in the vascular, interstitial, and surface compartment**;

**Fig. 7. Compartmental model of the tumor.**

**Table 3** Pharmacokinetic parameters from injection

<table>
<thead>
<tr>
<th>Drug</th>
<th>$m_{tt}$ (min)</th>
<th>$auc$ (C/min)</th>
<th>$V_i$ (µl)</th>
<th>$V_v$ (µl/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOX</td>
<td>0.53 ± 0.16</td>
<td>0.28 ± 0.05</td>
<td>48 ± 19</td>
<td>198 ± 50</td>
</tr>
<tr>
<td>DNR</td>
<td>0.64 ± 0.18</td>
<td>0.30 ± 0.11</td>
<td>71 ± 19</td>
<td>235 ± 88</td>
</tr>
<tr>
<td>IDA</td>
<td>1.14 ± 0.78</td>
<td>0.27 ± 0.03</td>
<td>120 ± 93</td>
<td>833 ± 67</td>
</tr>
<tr>
<td>BSA</td>
<td>0.23 ± 0.08</td>
<td>0.20 ± 0.06</td>
<td>18 ± 7</td>
<td>65 ± 38</td>
</tr>
<tr>
<td>Calcein</td>
<td>0.45 ± 0.24</td>
<td>0.30 ± 0.14</td>
<td>36 ± 12</td>
<td>114 ± 48</td>
</tr>
<tr>
<td>U87</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOX</td>
<td>0.66 ± 0.33</td>
<td>0.25 ± 0.08</td>
<td>61 ± 32</td>
<td>177 ± 95</td>
</tr>
<tr>
<td>DNR</td>
<td>0.61 ± 0.21</td>
<td>0.17 ± 0.04</td>
<td>72 ± 31</td>
<td>211 ± 88</td>
</tr>
<tr>
<td>IDA</td>
<td>1.35 ± 0.47</td>
<td>0.22 ± 0.09</td>
<td>138 ± 71</td>
<td>376 ± 203</td>
</tr>
<tr>
<td>BSA</td>
<td>0.24 ± 0.17</td>
<td>0.26 ± 0.06</td>
<td>19 ± 16</td>
<td>50 ± 41</td>
</tr>
<tr>
<td>LS174T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOX</td>
<td>1.18 ± 0.27</td>
<td>0.33 ± 0.04</td>
<td>118 ± 58</td>
<td>185 ± 109</td>
</tr>
<tr>
<td>DNR</td>
<td>0.81 ± 0.26</td>
<td>0.22 ± 0.09</td>
<td>113 ± 50</td>
<td>167 ± 97</td>
</tr>
<tr>
<td>IDA</td>
<td>4.41 ± 1.08</td>
<td>0.27 ± 0.12</td>
<td>450 ± 142</td>
<td>645 ± 166</td>
</tr>
<tr>
<td>BSA</td>
<td>0.33 ± 0.31</td>
<td>0.37 ± 0.10</td>
<td>68 ± 29</td>
<td>100 ± 43</td>
</tr>
</tbody>
</table>

The values are presented as mean ± SD. The fractional clearance is calculated as $(1 - F_{\text{free}})k_r$. The clearances ($\mu l/min$) are calculated as described in Fig. 7. The basic parameters are given in the text.

**Table 4** Estimated clearance rates of the anthracyclines

<table>
<thead>
<tr>
<th>Drug</th>
<th>DOX</th>
<th>DNR</th>
<th>IDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic parameters</td>
<td>921</td>
<td>3099</td>
<td>2306</td>
</tr>
<tr>
<td>10% perfused</td>
<td>120</td>
<td>818</td>
<td>887</td>
</tr>
<tr>
<td>75-µm tissue depth</td>
<td>64</td>
<td>157</td>
<td>118</td>
</tr>
<tr>
<td>150-µm tissue depth</td>
<td>49</td>
<td>96</td>
<td>75</td>
</tr>
</tbody>
</table>
quasi-steady state will be reached in the first minutes during continuous infusion. In this case, the total clearance can be estimated as shown in Fig. 7.

Using the parameter values described above, we calculated clearances for DOX, DNR, and IDA as shown in Table 4. The calculated values are more than 20 times higher than the observed values given in Table 2. This difference is difficult to explain with this simple model. One explanation is that only part of the tumor is perfused due to the existence of necrotic areas and spatial and temporal changes in perfusion rate (27, 28). In this case, part of the tumor is not reached by perfusate, unlike shunting, in which some of the perfusate does not reach the tissue. A 10-fold reduction of both the endothelial surface area and the number of cells reached by perfusate yields clearances that predict the observed values more accurately. Second, we assumed that all compartments are well-mixed. In practice, this condition is not met (29), as evidenced by findings such as interstitial gradients of anthracyclines (30). These gradients will affect drug uptake in the tumor, favoring the uptake of less lipophilic drugs. An approximate model for this effect can be obtained by considering one-dimensional diffusion of drug under quasi-steady state conditions. The interstitial concentration \( C(x) \) then satisfies D \( d^2C/dx^2 = CIC \) where \( x \) is the distance from the vessel, \( D \) is the effective diffusion coefficient in the tissue, and \( C \) is the cellular clearance per volume tissue. The solution of this equation is \( C(x) = C_0 \exp(-k_{eq}x) \), where \( k_{eq} = \sqrt{CD} \). The average extracellular concentration over a tissue depth \( L \) is \( C_0(1 - \exp(-k_{eq}L))/(Lk_{eq}) \). Assuming a tissue depth of 75 \( \mu \)m or 150 \( \mu \)m, we used this factor to correct the cellular uptake for the presence of concentration gradients in the interstitium. The resulting estimates of the clearance rates show an improved fit to the observed data. From this model, we expect that DOX is more uniformly taken up by all of the cells, whereas IDA is mostly taken up by the cells nearest to the blood vessel. This difference would give a steeper gradient across the vessel wall for IDA. As a consequence, reduction of the flow rate would affect the clearance of IDA more than that of DOX, as suggested by Fig. 4.

These differences in drug exposure may lead to different toxicities in vivo for drugs that have similar toxicities in vitro. In addition, the in vivo toxicity may be lower due to a higher cell-cell interaction for cells in tumor tissue compared with cells in a monolayer culture (31). However, this would probably affect the toxicity of different anthracyclines to a similar extent.

The influence of the gradient on the uptake rate will be less in most nontumor tissues where the mean distance between cells and the blood vessel are normally smaller compared with that in solid tumors. This will give a ratio of the uptake in tumor and nontumor tissue for DOX that is higher than that of DNR and IDA. This is a possible explanation for the greater clinical efficiency of DOX.

Conclusion. In summary, we find similar clearance rates for different anthracyclines with a tissue-isolated tumor model. We argue that a combination of anthracycline-protein binding and a diffusion gradient in the interstitium could cause this similarity despite differences in cellular uptake rates, as determined in vitro.

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