Doxorubicin encapsulated in sterically stabilized liposomes for the treatment of a brain tumor model: biodistribution and therapeutic efficacy

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 Anthracyclines entrapped in small-sized, sterically stabilized liposomes have the advantage of long circulation time, reduced systemic toxicity, increased uptake into systemic tumors, and gradual release of their payload. To date, there is no information on the behavior of these liposomes in brain tumors. The objective of this study was to compare the biodistribution and clinical efficacy of free doxorubicin (F-DOX) and stealth liposome--encapsulated DOX (SL-DOX) in a secondary brain tumor model. Nine days after tumor inoculation Fischer rats with a right parietal malignant sarcoma received an intravenous dose of 6 mg/kg of either F-DOX or SL-DOX for evaluation of drug biodistribution. For therapeutic trials a single dose of 8 mg/kg was given 6 or 11 days after tumor induction, or alternatively, weekly doses (5 mg/kg) were given on Days 6, 13, and 20. Liposome--encapsulated DOX was slowly cleared from plasma with a $t_{1/2}$ of 35 hours. Free-DOX maximum tumor drug levels reached a mean value of 0.8 $\mu$g/g and were identical in the adjacent brain and contralateral hemisphere. In contrast, SL-DOX tumor levels were 14-fold higher at their peak levels at 48 hours, declining to ninefold increased levels at 120 hours. A gradual increase in drug levels in the brain adjacent to tumor was noted between 72 and 120 hours (up to 4 $\mu$g/g). High-performance liquid chromatography analysis identified a small amount of aglycone metabolites within the tumor mass from 96 hours and beyond, after SL-DOX injection. Cerebrospinal fluid levels were barely detectable in tumor-bearing rats treated with F-DOX up to 120 hours after drug injection (≤ 0.05 $\mu$g/ml), whereas the levels found after SL-DOX were 10- to 30-fold higher. An F-DOX single-dose treatment given 6 days after tumor inoculation increased the rats’ life span (ILS) by 135% over controls ($p < 0.05$) but was not effective if given on Day 11. In contrast, SL-DOX treatment resulted in an ILS of 168% ($p < 0.0003$) with no difference when given after 6 or 11 days. Treatment with three weekly doses of SL-DOX produced an ILS of 189% compared to 126% by F-DOX ($p < 0.0002$). The authors conclude that the use of long-circulating liposomes as cytotoxic drug carriers in brain tumor results in enhanced drug exposure and improved therapeutic activity, with equal effectiveness against early small- and large-sized brain tumors.

**KEY WORDS** • brain neoplasm • cerebrospinal fluid • doxorubicin • liposome • pharmacokinetics

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**MALIGNANCIES** within the central nervous system pose unique chemotherapeutic problems because the blood-brain barrier (BBB) may limit penetration of antineoplastic drugs into brain tumors. Major elements governing the passage of chemotherapeutic agents across the intact BBB (namely, molecular weight, polarity, and lipid solubility) differ from the factors that influence the amount of drug that diffuses passively from capillaries into brain tumors. The latter factors include the permeability of the capillaries with respect to the specific drug, the luminal surface area of the capillaries available for exchange, blood flow to the tumor, the concentration of unbound drug in the plasma, and the length of time the drug circulates through the capillaries.

Despite the concern that the BBB impairs chemotherapy entry, this barrier is selectively disrupted at the site of the malignant lesion, and therefore, the tumor will receive higher doses of systemically administered chemotherapy than the adjacent normal brain. This concept is supported by the fact that brain metastases sometimes respond to systemic chemotherapy. However, in both animal and human studies, most agents are more effective against systemic tumors than against the metastases to the brain. This is probably related to an inadequate and slow influx of compounds into brain tumors, an outcome of reduced blood flow, and increased interstitial pressure resulting from edema formation and increased intracranial pressure. Thus, the difference in drug uptake between brain metastases and their original systemic tumor is a quantitative rather than qualitative phenomenon. This would also imply that many brain tumors are exposed to subtherapeutic levels of chemotherapy because most agents are given as a bolus and have short distribution half-lives, resulting in a slim chance to circulate through the tumor vascular bed.

Liposome-based anticancer chemotherapy may offer the advantage of reduced systemic toxicity combined with selective drug delivery into tumor. Recent studies revealed
that new formulations of small-sized (< 100 nm), long-circulating liposomes (also referred to as Stealth or sterically stabilized liposomes) appear to offer selective tumor localization. This localization is probably related to liposome longevity in circulation and to liposome extravasation through the abnormally permeable microvasculature of systemic tumors. It has also been demonstrated that the selective tumor localization of doxorubicin (DOX) encapsulated in Stealth liposomes (SLs) is associated with superior therapeutic activity over free drug activity in various systemic tumor models.

The above characteristics make SLs a potentially advantageous delivery system for brain tumor chemotherapy. To date, there is no information on the behavior of SLs in experimental brain tumor models. This study evaluates the biodistribution and therapeutic efficacy of doxorubicin encapsulated in SLs (SL-DOX) in an experimental brain tumor model.

Materials and Methods

Tumor Cell Line

The tumor used for the in vivo experiments is an unselected rat tumor line of a methylcholantrene-induced malignant fibrous histiocytoma. For the in vitro chemosensitivity testing we used the T-749 cell line derived from the above tumor. The tumor used for in vivo experiments is maintained in our laboratory in syngeneic Fischer rats by serial subcutaneous transplantations. Tumor cell suspension was prepared as previously described and assayed for viability by the Trypan blue exclusion test, and resuspended at the appropriate inoculum concentration in RPMI medium.

Stereotactic Brain Inoculation

The tumor cell suspension or RPMI medium (the vehicle used in control animals) was injected into the right cerebral hemisphere of adult female Fischer rats (weighing 180–200 g) anesthetized by intraperitoneal injection of 30 mg/kg pentobarbital, using a small-animal stereotactic apparatus. With bregma as zero reference point, intraperitoneal injection of 30 mg/kg pentobarbital, using a small-

Quantitative Measurements of Vascular Permeability in Tumor-Bearing Rats and Matched Controls

The evaluation of vascular permeability was performed 9 days after stereotactic injection of tumor cell suspension or vehicle. One hour before the rats were sacrificed, Evans blue dye (2 ml/kg of 2% dye in 0.9% NaCl) was injected intravenously. Immediately after sacrificing the animals by an overdose of pentobarbital, the blood was cleared from the circulation by transcardiac perfusion (150 ml 0.9% NaCl at 100 mm Hg pressure). The brain was rapidly removed, placed in a cutting chamber, and cut into 3 mm-thick coronal sections. With the aid of a dissection microscope the following brain regions were separated from appropriate brain sections: the visible tumor mass, the 1- to 2-mm surrounding brain area (brain adjacent to tumor), the contralateral matching anatomical brain area, and the ipsilateral frontal lobe. In vehicle-injected control rats identical brain areas were sampled except for the tumor mass, which was replaced by the injection site of vehicle as its control. Tumor cell line of a methylcholantrene-induced malignant fibrous histiocytoma. For the T-749 cell line derived from the above tumor. The tumor used for in vivo experiments is maintained in our laboratory in syngeneic Fischer rats by serial subcutaneous transplantations. Tumor cell suspension was prepared as previously described and assayed for viability by the Trypan blue exclusion test, and resuspended at the appropriate inoculum concentration in RPMI medium.

Biodistribution Studies

Studies were performed 9 or 16 days after tumor inoculation in two stages of tumor progression, representing either mild or marked increase in intracranial pressure that was signified by the degree of microshift. The animals received an intravenous bolus injection of DOX (6 mg/kg of body weight) in the form of either F-DOX or SL-DOX either on Day 9 or Day 16 after the stereotactic procedure. Following drug injection, blood, cerebrospinal fluid (CSF), and tissue samples were obtained after 4, 24, 48, 72, 96, and 120 hours. Blood samples were drawn from the hearts of anesthetized rats immediately prior to initiation of transcardiac perfusion. Transcardiac perfusion was required to clear the intravascular compartment of any residual circulating drug. Afterward, the brain and the liver were removed for drug tissue quantitation. The brain was cut as described above and the following brain regions were dissected: the visible tumor mass, the 1- to 2-mm surrounding brain area adjacent to tumor, and the contralateral matching anatomical brain region. In vehicle-injected control rats identical brain areas were sampled except for the tumor mass, which was replaced by its contralateral matching anatomical brain region. All tissues were weighed and stored at −20°C. Cerebrospinal fluid samples (0.08–0.1 ml) were obtained from anesthetized rats by cisternal puncture and only waterlike, clear fluids were used.

In the plasma, separation of liposome-associated DOX from protein-bound and F-DOX was performed on fresh samples, using Dowex resin columns. To determine DOX and DOX equivalents by a fluorescence assay, the samples were treated with 0.075 N HCl in 30% isopropanol (1 ml per mg w/v) as previously described. Measurement was determined by the intensity of fluorescence emission at 590 nm with an excitation wavelength of 470 nm using a spectrofluorometer. The fluorometric reading was converted to micrograms per milliliter by interpolation with the readings of a standard curve of DOX in the linear range.

Doxorubicin metabolites in the brain tumor tissue were detected by high-performance liquid chromatography (HPLC) analysis. The extraction procedure and the HPLC system have been described elsewhere.

Therapeutic Efficacy Studies

The lethal toxicity of F-DOX and SL-DOX was examined in 10-week-old female Fischer rats using either a single intravenous injection or three weekly intravenous injections of escalating doses of...
drug. Survival time was recorded for a total of 90 days posttreatment to cover the acute and delayed toxic deaths caused by DOX. Drug doses used for the therapeutic studies were selected according to the results of these toxicity evaluations.

To test therapeutic efficacy, stereotactic inoculation of tumor cells was performed as described above. Animals were then randomized for treatment with one of the following schedules: 1) a single intravenous treatment with 8 mg/kg of F-DOX or SL-DOX given 6 days or 11 days after tumor inoculation; 2) three intravenous treatments, each with 5 mg/kg of F-DOX or SL-DOX given weekly on Days 6, 13, and 20 after tumor implantation; or 3) matching intravenous injections of saline (control, no treatment groups). Survival times were recorded, and the median survival (95% confidence intervals of the median) and percentage increase in median survival of treated (T) over control (C) animals (T/C) were calculated. The statistical significance of differences in survival time was analyzed by means of the Wilcoxon ranking test.

Sources of Supplies

Evans blue dye and dimethylformamide were obtained from Sigma Chemical Co. (St. Louis, MO). Free DOX Adriamycin is manufactured by Famitalia-Carlo Erba (Milan, Italy). Biomakor (Rehovot, Israel) produces the monoclonal antibodies OX42 and ED8. Stealth was obtained from Sequus Pharmaceutical (Menlo Park, CA).

Results

Description of the Tumor Model

The stereotactic intracerebral inoculation of tumor cells resulted in the development of a tumor mass that produced the clinical signs of weight loss, apathy, and scissoring of hindlimbs in response to tilting. Macroscopically, a point-like tumor mass could be detected on the cut surface of the brain 6 days after tumor induction. After 9 days, the tumor mass, easily recognized by the naked eye, had a mean diameter of 3 ± 0.5 mm. On microscopic evaluation, the tumor behaved like a metastatic lesion that is clearly outlined from the adjacent brain. Some inflammatory reaction was identified by immunohistochemical staining using OX42 and ED8 monoclonal antibodies. It was characterized as activated microglia and macrophages that infiltrated the interface of the tumor and the adjacent brain. Discrete necrotic foci were sometimes detected within the tumor mass.

Measurements of Vascular Permeability in Tumor-Bearing Rats and Matched Controls

Measurements of vascular permeability were performed 9 days after the stereotactic procedure. Figure 1 presents the results of measurements obtained in tumor-bearing animals and in a matched group of vehicle-injected controls. After injection of the vehicle, significant (p < 0.05) but mild increase in dye extravasation was present at the injection site. This suggests that the stereotactic procedure induced mild disruption of the BBB at the injury site, which was still present 9 days after the injection. The brain adjacent to the injury site had normal permeability indicating that the surrounding brain was not edematous.

A sevenfold increase in dye extravasation was measured within the tumor mass as compared to the injection site of vehicle in controls (Fig. 1). When related to permeability of normal brain regions (frontal lobe, contralateral hemisphere) the increase in tumor vascular permeability reached 20-fold. Dye extravasation in the brain adjacent to tumor was augmented twofold compared to controls (p < 0.02), suggesting that an edematous area was surrounding the tumor mass. The permeability in the contralateral brain did not differ between tumor-bearing animals and controls.

Biodistribution of F-DOX and SL-DOX

Plasma Clearance. Figure 2 presents the plasma clearance curves of F-DOX and SL-DOX obtained in tumor-bearing animals after an intravenous bolus drug was injected 9 days after tumor inoculation. A striking difference in plasma DOX clearance pattern was apparent between the F-DOX and SL-DOX formulation, with a rapid distribution phase for the former and a prolonged, monoeponential distribution phase for the latter. The t1/2 of SL-DOX was 35 hours, and the calculated area under the
The plasma clearance curve was 3821 mg/hr/L. In animals treated with SL-DOX at least 90% of the drug was present in the liposome encapsulated form at each of the evaluation time points. Similar results were obtained when plasma clearance was examined 16 days after tumor inoculation (data not shown).

Tissue Distribution of F-DOX and SL-DOX. Figure 3 shows the drug levels of F-DOX and SL-DOX in the tumor and the various brain regions of tumor-bearing animals (Fig. 3A and B) and in the corresponding brain areas of vehicle injected controls (Fig. 3C and D). The drugs were given 9 days after either tumor inoculation or control vehicle injection. Drug levels measured in normal brain areas (contralateral hemisphere) of tumor-bearing rats were similarly low for both F-DOX and SL-DOX. Levels of F-DOX within the brain tumor mass and in the adjacent brain tissue were significantly higher for SL-DOX than for F-DOX.

![Graphs showing drug levels in brain tissue measured in tumor-bearing rats (A and B) and in vehicle-injected controls (C and D) after an intravenous injection of 6 mg/kg of either the liposomal doxorubicin (DOX) formulation or the free DOX. Experiments started 9 days after the intracerebral stereotactic inoculation of tumor cells or vehicle. In tumor-bearing rats evaluated regions included the brain tumor mass, the brain adjacent to tumor (BAT), and the contralateral hemisphere (contralat.). The corresponding brain regions in vehicle-injected controls included the injection site of vehicle, the brain adjacent to injection site (BAI), and the contralateral hemisphere. Four rats were used to evaluate each time point. Bars = standard deviation.](image1)

![Graphs indicating brain tumor and liver drug levels measured in tumor-bearing rats after intravenous administration of 6 mg/kg of either the liposomal doxorubicin (DOX) (A) formulation or the free DOX (B). Experiments started 9 days after intracerebral stereotactic inoculation of tumor cells. Four rats were used to evaluate each time point. Bars = standard deviation.](image2)
brain were also low, showing no difference from drug levels in the normal brain. Drug levels measured in the tumor mass after treatment with SL-DOX were notably different. The peak levels were obtained 48 hours after drug injection and were greater than 10 mg DOX per gram of tumor, which is a 15-fold increase over the F-DOX levels. Stealth liposome-encapsulated DOX was cleared very slowly from the tumor with less than 50% decrease from peak levels by 120 hours after drug injection. In the brain adjacent to tumor, a sluggish and modest increase in drug levels was noted with highest levels found after 120 hours.

In vehicle-injected controls, SL-DOX drug levels measured after 48 hours at the injection site were twofold higher than those obtained after F-DOX administration. Otherwise, levels were similar in all the evaluated brain regions. This probably represents tissue penetration of SL-DOX through the partially disrupted BBB as demonstrated by the dye-extravasation studies presented in Fig. 1. Still, the highest levels obtained in the injection site were sixfold lower than those measured within the tumor at the same time point.

Tissue drug levels were also evaluated in tumor-bearing animals injected with the drug after 16 days. The tumor masses were much larger after 16 days when compared with findings after 9 days, and most of the animals in the latter stage had apparent right to left shift of midline structures implying the presence of marked increase in intracranial pressure. Nevertheless, between these two phases of tumor progression drug levels did not differ significantly in any of the evaluated brain areas, including the tumor masses (data not shown).

Figure 4 compares the drug uptake by the brain tumor and the liver after treatment with either F-DOX or SL-DOX. Following SL-DOX injection, the highest drug levels were obtained in the liver after 24 hours and in the brain tumor after 48 hours. The peak dose level did not differ between the liver and the tumor, and the tumor/liver drug ratio remained close to one (ranging between 1.17–0.97) after 48 hours. In contrast, after F-DOX injection, tumor/liver drug ratios were substantially lower, ranging between 0.07 and 0.06 at all time points.

**Analysis by HPLC of Tumor Drug Levels and Drug Metabolites**

Figure 5 shows HPLC chromatograms of the tumor mass extract obtained from untreated control animals and from rats treated with F-DOX or SL-DOX. High-performance liquid chromatography analysis validated the spectrophotometric measurements revealing poor penetration of F-DOX into the tumor mass, whereas high levels of DOX were detected after SL-DOX injection. It also confirmed that throughout the study period, most of the drug was retained in the form of DOX. However, at 96 and 120 hours after injection of SL-DOX, the 7-deoxyaglycone metabolites of DOX were clearly identified within the tumor mass, suggesting that DOX is released from liposomes and metabolized. The appearance of metabolites at the same time that drug levels begin to decrease is consistent with a slow process of drug efflux from liposomes, enabling the interposition of bioavailability and clearance mechanisms.

**Drug Levels in the CSF.** Following injection of either F-DOX or SL-DOX (Fig. 6), the CSF of control animals

![Graph](image-url)
Fig. 6B) contained low levels of the drug that did not increase over time despite the long distribution phase of SL-DOX. This suggests that the blood–CSF barrier was not disrupted by the stereotactic procedure and that the intact barrier precluded drug filtration into the CSF. In contrast, the results obtained in tumor-bearing rats (Fig. 6A) injected with SL-DOX and F-DOX are dissimilar. Cerebrospinal fluid drug levels measured in tumor-bearing animals between 4 and 72 hours were 10- to 30-fold higher after SL-DOX injection as compared with CSF drug levels found after F-DOX administration. This suggests that the permeability of the blood–CSF barrier is abnormally increased in the presence of a brain tumor, allowing some penetration of SL-DOX into the CSF during the period of highest plasma drug concentrations.

In Vitro Chemosensitivity Testing

For the in vitro chemosensitivity testing we used the T-749 cell line derived from the original tumor. The in vitro cytotoxic effect of F-DOX or SL-DOX was assayed colorimetrically as previously described. The IC₅₀ values were 3 × 10⁻⁷ M for F-DOX and 2.3 × 10⁻⁶ M for SL-DOX. These values concur with our previous findings that DOX entrapped in similar liposomal formulations has reduced in vitro cytotoxicity when compared to F-DOX, secondary to slow release of the entrapped drug. Although the in vitro cytotoxicity tests are not predictive of the in vivo antitumor activity of liposome-entrapped drugs, they are useful in understanding the interaction of liposome-entrapped drugs with tumor cells. Our in vitro findings are consistent with the suggestion that there is no uptake of these types of liposomes into tumor cells; their main effect is to enable a slow drug release in the extracellular compartment.

Therapeutic Efficacy Studies

A pilot toxicity study was performed in tumor-free rats to determine the maximum tolerated dose for therapeutic studies. A single intravenous injection of 9 mg/kg of F-DOX or SL-DOX was not associated with early death. A high rate of delayed death (60% to 80%) occurred in only F-DOX treated animals after 60 to 80 days. A dose of 6 mg/kg given weekly for 3 consecutive weeks was not associated with acute death for either F-DOX or SL-DOX. Based on these results, we chose an 8-mg/kg dose for single-dose therapeutic trials and a 5-mg/kg dose for multiple-dose therapeutic trials.

Figure 7 shows the survival curves of brain tumor–bearing rats treated with a single dose of drug, administered either 6 days (Fig. 7A) or 11 days (Fig. 7B) after tumor inoculation. Early treatment with F-DOX was marginally effective yielding an increased life span (ILS = median survival Treated × 100/Control) of 35% (p < 0.05). A delayed treatment with F-DOX given after 11 days proved ineffective. In contrast, early or delayed treatment with SL-DOX were both equally effective with respective ILS values of 165% (p < 0.0003) and 168% (p < 0.0002). These results indicate that SL-DOX treatment is effective against both a small- or large-sized brain tumor, whereas F-DOX is mildly effective only when a small-sized tumor is present.

The survival curves of brain tumor–bearing animals treated with three weekly doses (Fig. 8) strengthen the trend seen with a single-dose therapy. It is evident that the results for F-DOX are similar to those obtained with a single-dose treatment (ILS = 123%; p < 0.05) given 6 days after tumor inoculation, with no further gain obtained by the addition of two more treatments. Remarkably different are the results observed with three SL-DOX treatments yielding an ILS of 189% (p < 0.00001), which differed significantly from the effect obtained by a similar schedule of F-DOX treatment (p < 0.0002).

Discussion

Our findings point at selective localization of SL-DOX in the brain tumor with mounting tumor–drug concentrations that equilibrate with liver–drug levels after 48 hours. The selective tumor localization was associated with an improved therapeutic index similar to previous studies in which various systemic tumor models were used. It is therefore suggested that SLs overcome the unique obstacles posed by brain tumors for efficient drug delivery.
Liposomal doxorubicin in brain tumor

**Drug Penetration Into the Brain Tumor**

The basic features of brain tumors that determine penetration of systemically injected drugs are the selective disruption of the BBB at the site of the tumor and the fact that intravascular injected materials accumulate slowly within the tumor, thus producing a very slowly equilibrating second compartment. Consequently, water-soluble and amphipathic drugs with short plasma distribution half-life (similar to F-DOX) penetrate these slowly equilibrating tumor centers at subtherapeutic concentrations. Our tumor model clearly manifests all these recognized features of brain tumors. The selective disruption of the BBB at the tumor site was unequivocally demonstrated by the measurements of Evans blue dye penetration (Fig. 1). In addition, the assay of tumor drug concentrations proved that drug penetration into the brain tumor is a slow process. Peak drug levels within the brain tumor mass were obtained after 48 hours, 24 hours later than in the liver (Fig. 4), a result consistent with the concept that brain tumors represent an exceptionally slow equilibrating second compartment.

The ability of SLs to deliver a substantial amount of their drug payload into brain tumor is undoubtedly related to their longevity in the circulation, because the distribution half-life was 35 hours in our experimental model. With such a long circulation time, access to the tumor microcirculation is granted for a significant fraction of the injected dose. Therefore, liposome extravasation into the brain tumor area becomes the rate-limiting factor for tumor targeting of this drug formulation, much as in the case of systemic tumors. Liposomes probably permeate through the leaky endothelium by passive convective transport, which is the dominant mode of transvascular transport for macromolecules and nanoparticles. Therefore, chances for extravasation improve with a prolonged circulation half life and a greater number of circulation passages through the tumor bed; yet factors such as increased tumoral interstitial pressure, lack of lymphatic drainage, and increased intracranial pressure may adversely affect the transport of macromolecules and liposomes into the tumor compartment. However, the results of the present study suggest that the long circulation time of SL-DOX may counterbalance these factors. We found that SL-DOX treatment resulted in similar tumor–drug levels in the smaller-sized brain tumors treated after 9 days and in the larger tumors treated after 16 days, when morphological signs of markedly increased intracranial pressure were evident. Likewise, when SL-DOX antitumor effect was evaluated, we detected no decline in the therapeutic gain with delayed onset of treatment, unlike the results obtained with F-DOX therapy.

We infer from the current data that tumor targeting of SLs depends on extravasation through endothelial fenestrations in permeable vessels with consequent liposome accumulation in the extracellular space. Because labeled liposomes are found in the interstitial fluid, outside tumor cells, it appears that drug delivery to tumor cells depends on the rate of release of drug from these extravasated liposomes. In fact, it has been recently reported in a human tumor xenograft model that after extravasation, liposomes form perivascular clusters and do not move significantly in the interstitial space. Thus, the slow clearance of the liposomal drug from the tumor area in our model is consistent with an effect of local depot delivery within the brain tumor.

Several previous studies tried to achieve a similar effect in brain tumors, either by direct instillation of liposomal chemotherapy into the tumor bed or by osmotic opening of the BBB, which requires intracarotid administration of mannitol or etoposide prior to the injection of liposomal chemotherapy. When compared to these approaches, SL-DOX offers the advantage of a selective and noninvasive delivery system of chemotherapy that produces favorable tumor-to-normal tissue distribution by extravasating into the tumoral extracellular space, thereby acting as a local depot preparation. One interesting observation derived from the biodistribution studies is that the peak concentration of SL-DOX in the brain tumor is nearly 15-fold greater than that of F-DOX. This is considerably higher than the three- to fourfold enhancement factor obtained in the subcutaneously implanted solid tumors and suggests that SL-DOX therapy may translate into a relatively greater gain for brain tumors than for systemic tumors. Similar to the results obtained with SLs in systemic animal tumor models, a superior antitumor effect for the liposomal drug was found in this brain tumor model. It should be noted that the therapeutic gain was achieved at subtoxic doses, and therefore, it does not rely on liposomal buffering of the drug’s systemic toxicity.

A common observation with SLs is the lack of liposomal uptake by tumor cells. As a result, drug uptake by tumor cells depends entirely on its release from those liposomes present in the extracellular space. These observations appear to hold true for the cell line used in this study, as suggested by the reduced in vivo cytotoxicity of SL-DOX when compared to F-DOX. Clearly, the potent in vitro antitumor activity of SL-DOX indicates that drug release from liposomes in the interstitial fluid is a more efficient process than drug release in the in vitro cytotoxic assay.

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Clearance of SL-DOX From the Brain Tumor Extracellular Space

The mechanisms and pathways responsible for clearance of edema fluids, extravasated serum protein, and particulate matter from the extracellular space of the brain are not completely understood. The concept of clearance of edema fluid through the CSF is generally accepted. A recent study concluded that the primary route for clearance of extracellular edema proteins is the subarachnoid CSF pathway, accessed via the extracellular space of the cortical neuropile by a process that involves diffusion.23 Therefore, we assumed that this pattern may be applicable for clearance of liposomes from brain tumor extracellular space. If this is valid, liposomes will first diffuse into the edematous brain adjacent to the tumor and will eventually get into the CSF pathways. The results of measurements of drug levels in the brain adjacent to tumor may imply that this concept is correct. We observed that a delayed and slow increase in DOX levels in the edematous brain surrounding the tumor (Fig. 3) was concomitant with the decrease of DOX within the tumor mass; yet the evaluation of CSF drug levels seems to rule out the possibility that DOX-containing liposomes are clear by CSF pathways from the brain tumor mass. Our findings indicate that SL-DOX penetrates into the CSF when plasma drug levels are the highest. This suggests that in tumor-bearing rats, some disruption of the blood–CSF barrier is probably present, because this barrier would normally restrict the filtration of macromolecules from the blood into the CSF. However, it is unlikely that this reflects a selective localization of SL-DOX within a leptomeningeal tumor that would probably be characterized by slow drug permeation and delayed peak drug concentrations, as in the case of ascitic tumors.13

Conclusions

The present findings indicate that with the use of SL-DOX a brain tumor should not be regarded as located within a privileged compartment, shielded from exposure to cytotoxic concentrations of intravenously administered antineoplastic agents. The prolonged circulation time of this carrier guarantees efficient drug delivery and improved therapeutic efficacy even when treatment is given to relatively large-sized tumors producing a marked increase in intracranial pressure. These results also have important implications with regard to the use of liposomes as carriers of a variety of other agents with recognized potential in the treatment of brain tumors (other cytotoxic agents, radiosensitizers, or even growth factors inhibitors) once the appropriate technology for the stable entrapment of each agent within the liposomes is fully defined.

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