ELSEVIER

Biochimica et Biophysica Acta 1609 (2003) 102–108

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Rate of biodistribution of STEALTH® liposomes to tumor and skin: influence of liposome diameter and implications for toxicity and therapeutic activity

Gregory J.R. Charrois, Theresa M. Allen*

Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Received 9 August 2002; received in revised form 28 October 2002; accepted 6 November 2002

Abstract

The influence of diameter on the pharmacokinetic and biodistribution of STEALTH® liposomes into the tumor (4T1 murine mammary carcinoma) and cutaneous tissues (skin and paws) of mice was studied to ascertain the time course of liposome accumulation and to determine if a preferential accumulation of liposomes into tumor over skin or paws could be achieved by altering liposome size. These tissues were chosen as the dose-limiting toxicity for Caelyx™/Doxil® in humans is palmar-plantar erythrodysesthesia, a cutaneous toxicity. We examined liposomes of four diameters: 82, 101, 154, or 241 nm. Liposomes with the three smallest diameters showed similar accumulation profiles that were significantly higher than the largest liposomes in all three tissues of interest. We were unable to achieve a preferential accumulation of liposomes into tumor over skin or paws based on size alone, as evidenced by the tumor to skin and tumor to paw ratios. However, there were differences in the time courses of liposome accumulation in these three tissues. Liposome levels plateaued in tumors and paws within 24 h, whereas skin levels plateaued between 24 and 48 h. The therapeutic activity of liposomal doxorubicin of three diameters (100, 157, and 255 nm) was tested in the same model. All formulations delayed tumor growth, with liposomes of 100 or 157 nm being equally efficacious and superior to liposomes of 255 nm.

Keywords: STEALTH® liposome; Biodistribution; Pharmacokinetics; Liposomal doxorubicin; Palmar-planter erythrodysesthesia

1. Introduction

Caelyx, STEALTH® liposomal doxorubicin (Doxil® in the United States), has been approved for use in AIDS-related Kaposi's sarcoma and refractory ovarian cancer, and is in clinical trials for other indications. In general, Caelyx has shown good activity against a variety of solid tumors, and its use has resulted in a reduction of doxorubicin-associated side effects, including dose-limiting cardiac toxicity and myelosuppression, as well as other side effects like alopecia, nausea, and vomiting [1–8]. The improved side effect profile is a result of alterations in the pharmacokinetics and biodistribution of doxorubicin (DXR) that arise when the drug is encapsulated within long-circulating (STEALTH®) liposomes.

Despite the large body of literature on the pharmacokinetics and biodistribution of STEALTH® liposomes, there has been little experimental effort to study their accumulation into cutaneous tissues, such as skin [9–11]. Furthermore, there have been no studies comparing the accumulation of different sizes of liposomes into tumor and cutaneous tissues, which is surprising given that the dose-limiting toxicities of Caelyx are mucocutaneous reactions such as stomatitis and palmar-plantar erythrodysesthesia [12]. Lokich and Moore [13] originally described PPE for various anticancer agents, including DXR, when given as prolonged infusions. Clinically, this syndrome starts with paraesthesias, edema, and erythema, and if left unchecked, can progress to blistering desquamation, which requires discontinuation of the therapy [12–14]. Histological evaluation of PPE lesions shows areas of necrosis in the basal layers of the skin [12,14]. Areas of skin that are under pressure, such as flexure creases on the hands, soles of the feet, or belt lines, are particularly vulnerable [12].

* Corresponding author. Tel.: +1-780-492-5710; fax: +1-780-492-8078.

E-mail address: terry.allen@ualberta.ca (T.M. Allen).

0005-2736/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0005-2736(02)00661-2

Dose reduction or delay has been used to prevent high-grade PPE once lesions start to develop, and either of these may have negative consequences on therapeutic outcome [15]. Current interventions for PPE include patient counseling, oral pyridoxine, and topical dimethylsulfoxide which partially relieve these symptoms [16–18]. A more complete understanding of the factors that lead to the development of PPE may lead to more efficacious strategies for its reduction or elimination.

It is hypothesized that the long circulation time and small size (100 nm diameter) of Caelyx allow the liposomes to extravasate in pressure areas and/or micro-traumatized areas in the skin, where they accumulate and release DXR over several days or weeks [12]. This is thought to damage cells in the basal layers of the skin, resulting in PPE. This hypothesis is supported by current clinical data; the plasma half-life ($t_{1/2}$) of Caelyx is approximately 48 h in humans, which mimics a prolonged infusion [19]. Indeed, a recent study demonstrated that the development of PPE correlated with the plasma $t_{1/2}$ of DXR in patients with metastatic breast cancer [20]. There is also a relationship between the development of PPE and the dose intensity of Caelyx therapy, with an increased incidence (up to 40% in one study of patients treated for ovarian cancer) occurring in patients receiving greater than 10–12 mg/m^2/week [2,3,12,21,22]. Further evidence to support this hypothesis is that a different liposomal formulation of DXR, Myocet™, does not produce PPE and has a different dose-limiting toxicity (leukopenia). Myocet is larger then Caelyx (160 versus 100 nm), has a substantially shorter half-life in humans (approximately 7 h), and has a much more rapid rate of drug release, as evidenced by its significantly larger volume of distribution [23].

Altering the size of liposomes can lead to changes in their biodistribution. Thus, it may be possible to reduce skin accumulation of liposomes without compromising tumor accumulation by increasing liposomal mean diameter. To test this hypothesis, we examined the accumulation of STEALTH® liposomes into tumor, skin, and paws in relation to their mean diameter and time after injection in a murine mammary carcinoma model. The goals of the study were to ascertain the time course of liposome accumulation into these tissues and to determine if it is possible to achieve a differential accumulation of liposomes into tumor relative to skin and/or paws, based solely on changes in the diameter of the liposomes. The rationale for this is that tumors have a defective endothelium with gaps between the cells in the range of 380–780 nm, rendering the tumors permeable to appropriate diameters of liposomes and macromolecules [24,25]. Because the biodistribution of liposomes is size dependent [26,27], an increase in diameter (e.g., 150–250 nm versus the 100 nm of Caelyx) may reduce liposome accumulation in normal tissues (i.e., skin) without having substantial effects on tumor uptake. In addition to biodistribution experiments, we also performed therapeutic experiments in the same tumor model, using DXR-loaded STEALTH® liposomes of various diameters, to determine the therapeutic significance of any size-dependent alternations in tissue distribution.

2. Materials and methods

2.1. Materials

Hydrogenated soy phosphatidylcholine (HSPC), methoxypolyethyeneglycol (M_r 2000)-distearoylphosphatidylethanolamine (mPEG$_{2000}$-DSPE) and doxorubicin hydrochloride (DXR) were donated by ALZA Corporation (Mountain View, CA). Cholesterol was purchased from Avanti Polar Lipids (Alabaster, AL). Sephadex-G50 and Sepharose CL-4B were from Amersham-Pharmacia Biotech (Baie d'Urfe, PQ, Canada). Minimal essential medium (MEM) was from Sigma (St. Louis, MO). Fetal bovine serum (FBS), penicillin, and streptomycin were from Life Technologies (Burlington, ON, Canada). Sterile, pyrogen-free saline was purchased from Baxter (Toronto, ON, Canada) and was supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4 (HEPES-buffered saline). Na^{125}I was purchased from Amersham (Oakville, ON, Canada) and ^{125}I-tryaminylinulin (^{125}I-TI; an aqueous space marker for liposomes) was prepared as previously described [28]. All other chemicals were of the highest grade possible.

2.2. Liposome preparation

All liposomes were composed of HSPC:CHOL:mPEG$_{2000}$-DSPE (3:1:1 w/w). For biodistribution experiments, liposomes were prepared by hydrating dried lipid films with HEPES-buffered saline (HBS) containing ^{125}I-TI. Liposomes were sized by sequential extrusion through stacked Nuclepore polycarbonate filters (0.4 μm down to 0.080 μm) using an extrusion device (Lipex Biomembranes, Vancouver, BC, Canada) at 65 °C and had low polydispersities. Free ^{125}I-TI was separated from liposome-encapsulated ^{125}I-TI by size exclusion chromatography on a Sepharose CL-4B column eluted with HBS. For therapeutic experiments, DXR was remote loaded with an ammonium sulfate gradient as previously described [29]. Briefly, lipid films were hydrated in 250 mM ammonium sulfate and preparations with low polydispersity were made by extrusion through Nuclepore filters with appropriate pore sizes. The external buffer was changed to sodium acetate (pH 5.5) by passage over a Sephadex G-50 column, and then DXR, dissolved in 10% sucrose (w/v), was incubated with the liposomes at a 0.2:1 (w) drug/lipid ratio for 15 min at 65 °C. Unencapsulated DXR was separated by passage over a Sephadex G-50 column equilibrated with HBS, pH 7.4.

Liposomes were sized by dynamic light scattering using a Brookhaven BI-90 particle sizer (Brookhaven Instruments,

Holtsville, NY). DXR concentrations were determined from a standard curve in methanol extracts at 480 nm; phospholipid concentrations were determined by the method of Bartlett [30].

2.3. Animal experiments

Female BALB/c mice (6–8 weeks) were purchased from the breeding colony at Health Sciences Laboratory Animal Services. Mice were housed under standard conditions and had access to food and water ad libitum. All protocols were in accordance with the Guide to the Care and Use of Experimental Animals set forth by the Canadian Council on Animal Care and approved by the Health Sciences Animal Policy and Welfare Committee, University of Alberta.

2.4. Tumor cell lines and tumor implantation

The 4T1 mouse mammary carcinoma cell line, a metastatic, thioguanine-resistant cell line, was donated by Dr. Fred Miller (Barbara Ann Karmanlos Cancer Institute, Detroit, MI) [31]. The cell line was maintained in MEM supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37 °C in a humidified incubator with a 5% CO_2 atmosphere. Cells were harvested for passage with the use of phosphate-buffered saline containing EDTA (PBS-EDTA; 0.54 mM EDTA, 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) followed by trypsin EDTA (0.05% trypsin in 137 mM NaCl, 5.4 mM KCl, 7 mM $NaHCO_3$, 0.34 mM EDTA).

Tumors were implanted as previously described [32]. Briefly, female BALB/c mice were anesthetized with methoxyflurane (Metafane, Janssen, Toronto, ON, Canada). A 6- to 8-mm incision was made adjacent to the mid-line in the lower abdominal region to expose the right #4 mammary fat pad where 10^5 4T1 cells were injected in 10 μl of full media. The incision was then closed with a surgical wound clip, which was removed 7 days later.

2.5. Biodistribution studies

Female BALB/c mice were implanted with the 4T1 mammary carcinoma as described above. Ten days after tumor inoculation, when tumors were well developed, mice were injected intravenously with 200 μl HBS containing 0.5 μmol of ^{125}I-TI-labeled liposomes ($1.5–2.5 \times 10^5$ cpm per mouse) with mean diameters (polydispersity) of 82 (0.111), 101 (0.122), 154 (0.073), or 241 (0.081) nm. At 24, 48, 72, or 96 h after injection, groups of 10 mice were euthanized, and organs (tumor, skin, and paws) were taken for radioactive counting (Beckman 8000 gamma counter). The skin was washed and shaved to remove hair and contaminating blood, and data from all four paws were pooled. Results are expressed as cpm per mg tissue normalized to 10^6 injected cpm. The data were corrected for the blood volume of organs as previously described [33]. Tumor to skin and tumor to paw ratios were calculated from the data. A one-way ANOVA with a Tukey–Krammer post test was used for all statistical comparisons using Graph Pad InStat version 3.01 for Windows 95/NT (GraphPad Software, San Diego CA).

2.6. Therapeutic studies

Groups of five mice were implanted with the 4T1 tumor as previously described. Four days after tumor implantation, mice were injected intravenously with liposomal DXR (6 mg/kg) of various diameters (polydispersity): 102 (0.152), 156 (0.077), or 254 (0.086) nm. The 4T1 tumor grows rapidly; therefore, therapeutic experiments were started 4 days after tumor implantation when the tumors were just palpable, even though biodistribution experiments were carried out 10 days after tumor implantation, when the tumors were large enough to excise. Tumor blood content, measured using ^{111}In-labeled murine red blood cells, showed that tumor blood content was proportional to tumor weight and volume (data not shown). Liposomes of 82 nm diameter were not used for the therapeutic studies because their pharmacokinetics and biodistribution was similar to the 101-nm liposomes. Tumor growth was monitored by measuring perpendicular diameters (a and b), and volume was calculated with the formula $v = 0.4ab^2$, where $b > a$. The experiment was repeated once with liposomes having similar mean diameters (polydispersity): 98 (0.140), 159 (0.078), or 256 (0.054) nm. The results at each liposome size were pooled as follows: 100 nm (98 and 102 nm), 157 nm (156 and 159 nm), and 255 nm (254 and 256 nm). All control mice received 200 μl of sterile saline. Results are presented as the mean ± S.D. with $n = 6–10$. There were no statistically significant differences between the end tumor volumes of the replicates as determined by a one-way ANOVA.

3. Results

3.1. Biodistribution experiments

Tumor uptake of ^{125}I-TI liposomes is shown in Fig. 1A. Tumor accumulation of liposomes was highest at 24 h for all sizes tested. The largest liposomes (241 nm) had substantially lower tumor levels than the smaller sizes at all time points ($P < 0.001$). Some statistically significant differences also occurred among the tumor uptakes of the smaller liposomes, although these may not be therapeutically significant (see results of therapeutic experiments below). For example, the 101-nm liposomes had higher accumulation in tumor than the 82-nm liposomes at 24 and 48 h after injection ($P < 0.05–0.01$) and had significantly higher accumulation than the 154-nm liposomes at all time points ($P < 0.05–0.001$). Also, the 82-nm liposomes had

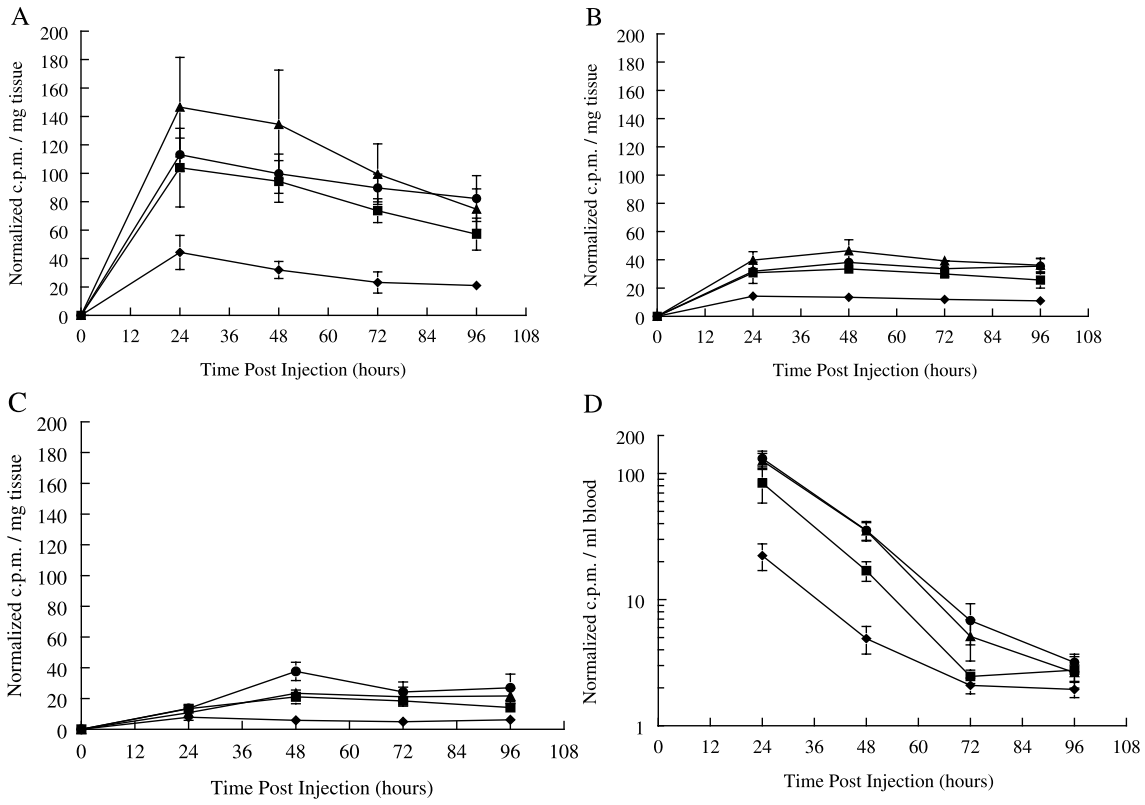

Fig. 1. Accumulation of ^{125}I-tyraminylinulin liposomes in mouse tissues as a function of time. BALB/c mice were implanted in the #4 mammary fat pad with the 4T1 tumor and injected IV 10 days later with ^{125}I-tyraminylinulin-labelled liposomes of various mean diameters: ●, 82 nm; ▲, 101 nm; ■, 154 nm; ◆, 241 nm. Data are expressed as cpm/mg tissue normalized to 10^6 cpm injected. (A) 4T1 mouse mammary carcinomas, (B) mouse paws, (C) skin, (D) blood. Data represent the mean ± S.D., $n = 10$. See text for results of statistical comparisons.

higher tumor levels than the 154-nm liposomes at 72 and 96 h after injection ($P < 0.05$ and $P < 0.001$).

Results of the uptake of liposomes into paws are shown in Fig. 1B. Liposome levels in paws were significantly lower than tumor levels. Except for the 241-nm liposomes, the level of liposomes in paws was highest at 48 h. The largest (241 nm) liposomes attained lower levels in paws than the smaller sizes of liposomes ($P < 0.01$ to $P < 0.001$). Other statistically significant differences between the paw uptake of the other liposomes were as follows: the 101-nm liposomes had higher paw accumulation than the 154-nm liposomes at all time points ($P < 0.01$ to $P < 0.001$) and had higher accumulation than the 82-nm liposomes at 24, 48, and 72 h ($P < 0.05$ to $P < 0.01$). The 82-nm liposomes had higher paw levels at 72 and 96 h than the 154-nm liposomes ($P < 0.001$).

Skin accumulation of liposomes is shown in Fig. 1C. Skin levels of liposomes were significantly lower than tumor levels, and, with a few exceptions (154 nm at 24 h, 101 nm at 48 h, and 82 nm at 96 h), were also significantly lower than paw levels ($P < 0.05$ to $P < 0.001$). Skin levels of the largest liposomes were highest at 24 h after injection, whereas the smaller sizes had peak levels at 48 h. For the 48-, 72-, and 96-h time points, the largest liposomes had significantly lower skin accumulation than the smaller liposomes ($P < 0.01$ to $P < 0.001$). Again these data show that the three smaller sizes of liposomes accumulate to a greater extent in tissue than the largest size of liposomes.

Blood levels of liposomes are presented in Fig. 1D. The blood concentrations of the two smallest liposomes (82 and 101 nm) were similar, and were significantly higher than blood concentrations for the 254-nm liposomes at all time points ($P < 0.05–0.001$) or the 154-nm liposomes, except at 96 h ($P < 0.01–0.001$). The 154-nm liposomes achieved higher blood levels that the largest liposomes for the 24-, 48- and 96-h samples ($P < 0.001$). These blood levels are consistent with other data showing that liposomal diameter influences circulation times [34].

Tumor to paw ratios are presented in Table 1A. There were no significant differences within the data columns, that is, altering liposome size did not produce a preferential accumulation of liposomes into tumor versus paws. However, for a given size of liposome, the tumor to paw ratios decreased significantly over time. At 24 h after injection, regardless of liposome size, almost four times as many liposomes accumulated in tumor than in paws and this decreased to approximately two-fold by 96 h after injection

Table 1A
Tumor to paw ratios as a function of time after injection

	24 h	48 h	72 h	96 h
82 nm	3.6 ± 0.5	2.6 ± 0.4	2.8 ± 0.7	2.3 ± 0.5
101 nm	3.7 ± 0.9	3.0 ± 1.2	2.6 ± 0.8	2.1 ± 0.6
154 nm	3.6 ± 1.4	2.8 ± 0.5	2.5 ± 0.5	2.3 ± 0.6
241 nm	3.3 ± 1.0	2.5 ± 0.7	2.1 ± 0.8	2.0 ± 0.4

Data represent the ratios of liposome levels in tumor to paw (cpm/mg tissue) for the mean ± S.D. of 10 mice. Ratios were calculated from the data in Fig. 1A and B.

($P<0.01$). The ratios at the 24-h time point for all liposome diameters were significantly higher than the other time points ($P<0.05$ to $P<0.001$).

Tumor to skin ratios are presented in Table 1B. At the 24-h time point, only the 101-nm liposomes had a significantly higher ratio than the three sizes of liposomes. ($P<0.01$ to $P<0.001$). There were no other significant differences in the tumor to skin ratios for the different sizes of liposomes at all other time points. However, for any given size of liposome, the tumor to skin ratios decreased over time. The three smaller liposome sizes had approximately 8- to 17-fold higher levels in tumor than in skin at 24 h and this decreased significantly with time to approximately 3- to 4-fold by 96 h after injection ($P<0.01$ to 0.001). The tumor to skin ratios for the largest liposomes (241 nm) also decreased over time, with the ratio at 24 h being significantly higher than the ratio at 96 h ($P<0.01$).

3.2. Therapeutic experiments

All three tested sizes of liposomal DXR delayed tumor growth (Fig. 2). The smaller liposomes (100 and 157 nm) had almost equivalent anticancer activity, which was greater than that seen for the larger liposomes (255 nm). These data are consistent with data from the biodistribution experiments, which showed greater tumor accumulation of the smaller liposomes compared with the largest ones. It is reasonable to expect that higher tumor levels of drug for the smaller liposomes would result in greater therapeutic activity. The small differences seen in the level of tumor accumulation for the smaller liposomes (101 and 154 nm), although statistically significant, resulted in no measurable differences in therapeutic activity.

Table 1B
Tumor to skin ratios as a function of time after injection

	24 h	48 h	72 h	96 h
82 nm	8.8 ± 2.3	4.5 ± 1.2	4.0 ± 1.6	3.3 ± 1.1
101 nm	17.0 ± 9.6	5.9 ± 2.2	5.1 ± 1.9	3.7 ± 1.3
154 nm	8.4 ± 3.2	4.8 ± 1.9	4.1 ± 1.0	4.1 ± 0.7
241 nm	6.3 ± 2.6	5.5 ± 1.4	5.0 ± 1.8	3.6 ± 1.1

Data represent the ratios of liposome levels in tumor and skin (cpm/mg tissue) for the mean ± S.D. of 10 mice. Ratios were calculated from the data in Fig. 1A and C.

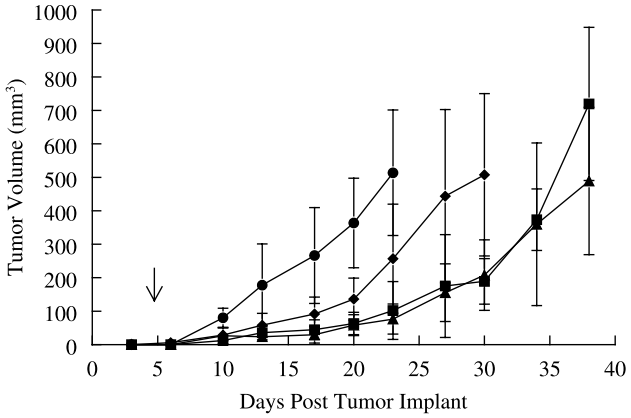

Fig. 2. Therapeutic activity of various sizes of STEALTH® liposomal doxorubicin against the 4T1 mouse mammary carcinoma. BALB/c mice were implanted with the #4 mammary fat pad with the 4T1 tumor and 4 days later (arrow) were treated with STEALTH® liposomal doxorubicin of various mean diameters: ●, saline control; ▲, 100 nm; ■, 157 nm; ♦, 255 nm. The results represent the mean ± S.D. of 6–10 mice from two pooled experiments.

4. Discussion

The biodistribution of STEALTH® liposomes has long been known to be size dependent, and the dermal localization of long-circulating liposomes has been previously described [9,11,27,35,36]. However, this is the first systematic investigation to compare the accumulation of liposomes in tumors versus cutaneous tissues (skin and paws). Results of this study indicate that time to peak levels of liposome accumulation was delayed in cutaneous tissues relative to tumor tissue, and time to peak levels was not dependent on liposome size.

Liposome levels peaked in tumor tissue at or before 24 h, whereas levels in skin and paws peaked at 48 h. If a liposome system can be engineered that leaks its drug contents after tumor levels peak, but before skin levels peak, it might lead to lower levels of drug in the skin, potentially reducing the likelihood of PPE. The higher uptake of liposomes into paws compared to skin suggests that there may be a pressure-dependent extravasation of liposomes into paws. Pressure would be exerted on the paws as the mice walk around the cage, feed, groom, etc. Although we did not apply external pressure on the mice's skin to mimic human skin under pressure (e.g., belt lines), our paw data may reflect the human condition where PPE lesions are found on the hands and feet. Thus, following the clinical administration of long-circulating liposomal DXR, it may be necessary to limit activity to avoid this pressure-dependent accumulation of liposomes. Indeed, some clinicians prescribe bed rest for patients on Caelyx therapy for a short period of time following drug administration. Addi-

tionally, patients on Caelyx are counseled to avoid situations that will increase blood flow to the skin (e.g., hot baths) in an effort to reduce the number of liposomes localizing in the skin.

Tumor to skin and tumor to paw ratios did not change for the various sizes of liposomes. This demonstrates that altering liposome diameter does not decrease liposome accumulation into skin or paws without a proportional decrease in tumor accumulation. This is a surprising finding given that tumor blood vessels are reported to be leakier than normal capillaries (skin and paws), due to fenestrations and gaps in the endothelium that can range in size from 380 to 780 nm [25,37]. This would suggest that even though there are large gaps in the tumor vasculature, there is also a size cutoff for particles that can pass through these gaps. Recent work by Hobbs et al. [25] demonstrated that the pore sizes in tumor blood vessels were dependent upon the tumor model used and on the anatomical location of tumor implantation. Most tumors implanted subcutaneously exhibited a pore size range of 380–780 nm (as large as 1.2–2 μm in one tumor type); pore size was smaller in the cranial microenvironment. The authors also identified that pore size range is heterogeneous in any given tumor, and that, for a particle or liposome to penetrate the pore, its diameter should be much smaller than that of the pore. Because pore size is dependent upon tumor type and location, using a "leakier" tumor may have produced a difference in the tumor to skin ratios for the various sizes of liposomes. Ishida et al. further demonstrated the concept of pore size cutoff in recent experiments with the murine C26 colon carcinoma implanted subcutaneously. The authors showed a size-dependent accumulation of STEALTH® liposomes that was independent of blood liposome concentrations [34]. This was inferred from the observation that increasing the blood levels of 400-nm-diameter STEALTH® liposomes (achieved following splenectomy) did not increase liposome accumulation into tumors. In other words, the odds of any particle passing through a pore increases as the particle size decreases relative to the diameter of the pore.

In the current study, we hypothesize that the 4T1 tumor may have a pore size cutoff of approximately 250 nm, as liposome accumulation dropped off significantly for the largest size of liposomes. Skin and paws accumulated substantially lower concentrations of liposomes than did tumors, but tumors also exhibited a similar fall-off in accumulation for the larger liposomes. The lower levels of liposome accumulation in skin and paws are consistent with the explanation that blood vessels in these tissues are "tighter" than those found in solid tumors.

The data from the therapeutic experiments show a dependence of therapeutic activity on liposome size, with smaller liposomes (100 and 157 nm) being more efficacious than larger liposomes (255 nm). This is probably due to higher tumor levels of the smaller liposomes, and is consistent with work from other laboratories using doxorubicin or annamycin [38,39]. Although the 100-nm size of Caelyx is considered optimal for therapeutic activity, based on our data, a modest increase in size would probably not result in a decrease in therapeutic activity and may also not reduce the incidence of cutaneous toxicities such as PPE. However, a small increase to 150 nm would result in higher drug to lipid ratios, which may offer a cost saving in liposome preparation.

Several factors are likely responsible for the decrease over time in tumor to skin and tumor to paw ratios. First, the concentrations of liposomes in skin and paws peak later than that in tumors. As skin and paw concentrations increase, their ratios to tumor concentrations will decrease if they are accumulating liposomes to a greater degree than tumors at later time points. Secondly, tumors continue to grow over the course of the study, with weights ranging from 0.20 g at 24 h after injection to 0.34 g at 96 h after injection. If the tumor grows faster than the rate of accumulation of liposomes in the tumor, the concentration of liposomes (cpm per mg tissue) will decrease.

This study provides some guidance into potential strategies that may be used to avoid PPE. The increased accumulation of liposomes into paws over skin may reflect a pressure-dependent extravasation of liposomes, suggesting that bed rest and a reduction in daily activities may help to reduce PPE in the hands and feet. These findings provide a potential strategy to reduce the incidence of PPE, for example, by engineering liposome formulations that allows for the release of drug from liposomes after maximal tumor accumulation occurs, but before skin levels plateau, either by altering drug leakage rates or triggering drug release (e.g., hyperthermia or programmable fusogenic vesicles) [40–42]. Ongoing experiments are being conducted to test this hypothesis.

Acknowledgements

The technical assistance of Elaine Moase, Tatsuhiro Ishida, Debbie Iden, and Christian Hansen is gratefully acknowledged. G.J.R.C. is the recipient of an Alberta Heritage Foundation for Medical Research graduate studentship. This work was supported by ALZA Corporation (Mountain View, CA) and the Canadian Institutes for Health Research (UOP 48092).

References

[1] D.W. Northfelt, B.J. Dezube, J.A. Thommes, R. Levine, J.H. Von Roenn, G.M. Dosik, A. Rios, S.E. Krown, C. DuMond, R.D. Mamelok, J. Clin. Oncol. 15 (1997) 653–659.
[2] F. Muggia, J.D. Hainsworth, S. Jeffers, P. Miller, S. Groshen, M. Tan, L. Roman, B. Uziely, L. Muderspach, A. Garcia, A. Burnett, F.A. Greco, C.P. Morrow, L.J. Paradiso, L.-J. Liang, J. Clin. Oncol. 15 (1997) 987–993.
[3] M.R. Ranson, J. Carmichael, K. O'Byrne, S. Stewart, D. Smith, A. Howell, J. Clin. Oncol. 15 (1997) 3185–3191.

[4] G. Berry, M. Billingham, E. Alderman, P. Richardson, F. Torti, B. Lum, A. Patek, F.J. Martin, Ann. Oncol. 9 (1998) 711–716.
[5] E. Samantas, H. Kalofonos, H. Linardou, C. Nicolaides, N. Mylonakis, G. Fountzilas, P. Kosmidis, D. Skarlos, Ann. Oncol. 11 (2000) 1395–1397.
[6] A.N. Gordon, J.T. Fleagle, D. Guthrie, D.E. Parkin, M.E. Gore, A.J. Lacave, J. Clin. Oncol. 19 (2001) 3312–3322.
[7] E. Rivera, V. Valero, L. Syrewicz, Z. Rahman, F.L. Esteva, R.L. Theriault, M.M. Rosales, D. Booser, J.L. Murray, R.C.J. Bast, G.N. Hortobagyi, J. Clin. Oncol. 19 (2001) 1716–1722.
[8] T. Safra, S. Groshen, S. Jeffers, D.D. Tsao-Wei, L. Zhou, L. Muderspach, L. Roman, C.P. Morrow, A. Burnett, F. Muggia, Cancer 91 (2001) 90–100.
[9] T.M. Allen, C.B. Hansen, F. Martin, C. Redemann, A. Yau-Young, Biochim. Biophys. Acta 1066 (1991) 29–36.
[10] D. Papahadjopoulos, T.M. Allen, A. Gabizon, E. Mayhew, K. Matthay, S.K. Huang, K.D. Lee, M.C. Woodle, D.D. Lasic, C. Redemann, F.J. Martin, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 11460–11464.
[11] A. Gabizon, D. Goren, A.T. Horowitz, D. Tzemach, A. Lossos, T. Siegal, Adv. Drug Del. Rev. 24 (1997) 337–344.
[12] M. Lotem, A. Hubert, O. Lyass, M.A. Goldenhersh, A. Ingber, T. Peretz, A. Gabizon, Arch. Dermatol. 136 (2000) 1474–1480.
[13] J.J. Lokich, C. Moore, Ann. Intern. Med. 101 (1984) 798–800.
[14] K.B. Gordon, A. Tajuddin, J. Guitart, T.M. Kuzel, L.R. Eramo, J. VonRoenn, Cancer 75 (1995) 2169–2173.
[15] M.L. Hensley, B. Hoppe, L. Leon, P. Sabbatini, C. Aghajanian, D. Chi, D.R. Spriggs, Gynecol. Oncol. 82 (2001) 464–469.
[16] C.J. Fabian, R. Molina, M. Slavik, S. Dahlberg, S. Giri, R. Stephens, Invest. New Drugs 8 (1990) 57–63.
[17] A.M. Lopez, L. Wallace, R.T. Dorr, M. Koff, E.M. Hersh, D.S. Alberts, Cancer Chemother. Pharmacol. 44 (1999) 303–306.
[18] D.M. Vail, R. Chun, D.H. Thamm, L.D. Garrett, A.J. Cooley, J.E. Obradovich, Clin. Cancer Res. 4 (1998) 1567–1571.
[19] A. Gabizon, R. Isacson, E. Libson, B. Kaufman, B. Uziely, R. Catane, C.G. Ben-Dor, E. Rabello, Y. Cass, T. Peretz, A. Sulkes, R. Chisin, Y. Barenholz, Acta Oncol. 33 (1994) 779–786.
[20] O. Lyass, A. Hubert, A. Gabizon, Clin. Cancer Res. 7 (2001) 3040–3046.
[21] M. Amantea, M.S. Newman, T.M. Sullivan, A. Forrest, P.K. Working, Hum. Exp. Toxicol. 18 (1999) 17–26.
[22] A.N. Gordon, C.O. Granai, P.G. Rose, J. Hainsworth, A. Lopez, C. Weissman, R. Rosales, T. Sharpington, J. Clin. Oncol. 18 (2000) 3093–3100.
[23] J.W. Cowens, P.J. Creaven, W.R. Greco, D.E. Brenner, Y. Tung, M. Osto, F. Pilkiewicz, R. Ginsberg, N. Petrelli, Cancer Res. 53 (1993) 2796–2802.
[24] F. Yuan, M. Leunig, S.K. Huang, D.A. Berk, D. Papahadjopoulos, R.K. Jain, Cancer Res. 54 (1994) 3352–3356.
[25] S. Hobbs, W.L. Monsky, F. Yuan, W.G. Roberts, L. Griffith, V.P. Torchillin, R.K. Jain, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 4607–4612.
[26] R.M. Abra, C.A. Hunt, Biochim. Biophys. Acta 666 (1981) 493–503.
[27] T.M. Allen, C. Hansen, J. Rutledge, Biochim. Biophys. Acta 981 (1989) 27–35.
[28] E.F. Sommerman, P.H. Pritchard, P.R. Cullis, Biochem. Biophys. Res. Commun. 122 (1984) 319–324.
[29] E.M. Bolotin, R. Cohen, L.K. Bar, S.N. Emanuel, D.D. Lasic, Y. Barenholz, J. Liposome Res. 4 (1994) 455–479.
[30] G.R. Bartlett, J. Biol. Chem. 234 (1959) 466–468.
[31] C.J. Aslakson, F.R. Miller, Cancer Res. 52 (1992) 1399–1405.
[32] E. Moase, W. Qi, T. Ishida, Z. Gabos, B.M. Longenecker G.L. Zimmermann, L. Ding, M. Krantz, T.M. Allen, Biochim. Biophys. Acta 1510 (2001) 43–55.
[33] T.M. Allen, C.B. Hansen, L.S.S. Guo, Biochim. Biophys. Acta 1150 (1993) 9–16.
[34] O. Ishida, K. Maruyama, K. Sasaki, M. Iwatsuru, Int. J. Pharm. 190 (1999) 49–56.
[35] A. Gabizon, D. Papahadjopoulos, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 6949.
[36] A. Gabizon, D. Papahadjopoulos, Biochim. Biophys. Acta 1103 (1992) 94–100.
[37] H. Hashizume, P. Baluk, S. Morikawa, J.W. McLean, G. Thurston, S. Roberge, R.K. Jain, D.M. McDonald, Am. J. Pathol. 156 (2000) 1363–1380.
[38] L.D. Mayer, L.C. Tai, D.S. Ko, D. Masin, R.S. Ginsberg, P.R. Cullis, M.B. Bally, Cancer Res. 49 (1989) 5922–5930.
[39] Y.Y. Zou, Y.H. Ling, S. Reddy, W. Priebe, R. Perezsoler, Int. J. Cancer 61 (1995) 666–671.
[40] S.K. Huang, P.R. Stauffer, K. Hong, J.W. Guo, T.l. Phillips, A. Huang, D. Papahadjopoulos, Cancer Res. 54 (1994) 2186–2191.
[41] D. Needham, G. Anyarambhatla, G. Kong, M.W. Dewhirst, Cancer Res. 60 (2000) 1197–1201.
[42] G. Adlakha-Hutcheon, M.B. Bally, C.R. Shew, T.D. Madden, Nat. Biotechnol. 17 (1999) 775–779.