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The influence of the route of administration and liposome composition on the potential of liposomes to protect tissue against local toxicity of two antitumor drugs

C. Oussoren [a,*], W.M.C. Eling [b], D.J.A. Crommelin [a], G. Storm [a], J. Zuidema [a]

[a] *Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University; PO Box 80 082, 3508 TB Utrecht, The Netherlands*

[b] *Department of Medical Microbiology, University Hospital Nijmegen, Nijmegen, The Netherlands*

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Abstract

The present paper reports on the influence of the route of administration and liposome stability on the protective effect of liposome encapsulation of two model antitumor agents, mitoxantrone and doxorubicin. The results demonstrate that liposome encapsulation can protect surrounding tissue from the cytotoxic effects of the drugs after subcutaneous (sc) and intramuscular (im) administration. The route of administration is an important factor influencing tissue damage. Liposomal mitoxantrone caused much less tissue irritation after im injection than after sc injection. Liposome stability is also an important factor. Liposomes composed of 'fluid-state' phospholipids only delayed the damaging effects of doxorubicin when injected sc. Liposomes with a more rigid nature were much more effective in preventing local tissue damage over a longer period of time when administered sc. Results suggest that slow release of liposome-associated drugs may eventually cause severe local tissue damage. The incorporation of the hydrophilic lipid derivative distearoylphosphatidylethanolamine-poly(ethyleneglycol) (PEG-PE) had no apparent effect on the protective effect of liposomes after sc administration. © 1998 Elsevier Science B.V.

Keywords: Liposome; Antitumor drug; Subcutaneous; Intramuscular; Local toxicity; Protective effect

1. Introduction

Peripheral tumors often form metastases in regional lymph nodes [1]. To achieve effective therapy, it is important to deliver antineoplastic agents selectively to these lymphatic metastases. After intravenous administration, drug delivery to lymph nodes is generally low, because of insufficient transfer of the drug from the blood circulation into the lymphatic system. Local injection (e.g., subcutaneous (sc), intradermal (id), intraperitoneal (ip) and intramuscular

Abbreviations: Chol, cholesterol; DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; EPC, egg-phosphatidylcholine; EPG, egg-phosphatidylglycerol; id, intradermal; im, intramuscular; ip, intraperitoneal; PEG-PE, poly(ethyleneglycol)-distearoylphosphatidylethanolamine; sc, subcutaneous; TL, total lipid

* Corresponding author. Fax: +31 30 2517839; E-mail: c.oussoren@far.ruu.nl

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(im)) of high concentrations of antineoplastic agents may be considered as a relatively effective therapeutic method since part of the injected drug can be taken up by the lymphatic capillaries and subsequently transported to regional lymph nodes. However, many antineoplastic agents have vesicant properties, i.e. they cause severe tissue damage upon local injection. Therefore, local administration of the free drug is often not feasible.

Liposomes have received considerable attention as drug carriers to target drugs to regional lymph nodes, after local injection [2–4]. Local injection of liposome-encapsulated drugs has important advantages over the use of free drug. Relatively high drug concentrations in regional lymph nodes may be achieved as compared to administration of the free drug [5–8]. Another important advantage relates to the observation that the entrapment of highly irritating drugs in liposomes protects surrounding tissue from the direct cytotoxic effect of the drug after sc, id, ip, and im injection [9–16]. Exposure of tissue to the free drug is potentially affected by the rate of clearance of the drug from the injection site and release rate of the drug from the liposomes. The route of administration is an important factor determining disappearance of liposomal drug from the injection site [17]. In addition, as drug release strongly depends on liposome stability, the liposome lipid composition may also influence the protective effect of liposomes. Steric stabilization of the liposome surface by incorporation of a lipid derivative of poly(ethyleneglycol) (PEG), distearoylphosphatidylethanolamine (PEG-PE), stabilizes liposomes in vivo by reducing interactions with components of the biological environment [18]. Therefore, the protective effect of sterically stabilized liposomes on the local toxicity of the encapsulated drug is of interest as well.

In the present report, the influence of the route of administration and liposome lipid composition on the protective effect of liposomes against local toxicity of two model antitumor drugs, mitoxantrone and doxorubicin, is described. To study the influence of the route of administration, mice were either sc or im injected with liposome-encapsulated mitoxantrone. To study the effect of liposome stability, mice were sc injected with doxorubicin encapsulated in 'fluid-state' liposomes composed of unsaturated phospholipids or with doxorubicin encapsulated in 'gel-state' liposomes composed of saturated lipids. The influence of steric stabilization of the liposome surface was studied by incorporation of PEG-PE into the liposome bilayer, in both fluid-state and gel-state liposomes.

2. Materials and methods

2.1. Chemicals

Egg-phosphatidylcholine (EPC), egg-phosphatidylglycerol (EPG), dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) were donated by Lipoid GmbH (Ludwigshafen, FRG). Distearoylphosphatidylethanolamine-poly(ethyleneglycol)2000 (PEG-PE) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Doxorubicin was donated by Pharmachemie BV (Haarlem, The Netherlands). Mitoxantrone was obtained from Lederle GmbH (Wolfratshausen, FRG). Cholesterol (Chol) and 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (Hepes) were obtained from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Preparation of liposomes

Small mitoxantrone-liposomes (mean size about 110 nm, pd < 0.4) composed of EPC:EPG:Chol (molar ratio 10:4:4) were prepared by the 'thin-film' method. Briefly, a mixture of the appropriate amounts of lipids was dissolved in an mixture of chloroform/methanol (4:1 v/v) and evaporated to dryness by rotation under reduced pressure at 40°C. After flushing the lipid film with nitrogen for at least 20 min, the film was hydrated in a sterile solution of mitoxantrone (2 mg/ml) dissolved in an isotonic acetate buffer (pH 5). Liposomes (145 mM total lipid (TL)) were sized by sonication until a mean size of about 0.1 μm was obtained. To obtain a more homogeneous dispersion, liposomes were extruded through a 0.2 μm polycarbonate membrane filter (Nuclepore; Costar, Cambridge, MA, USA). The amount of non-encapsulated, free mitoxantrone was found to be negligible when separated from the liposome formulation by centrifugation in a Centrisart I concentration tube (Sartorius AG, Göttingen, FRG).

Small doxorubicin-liposomes (mean size varying between 85 and 110 nm, pd < 0.2) of several compositions were prepared by the 'ammonium sulfate gradient' method as described by Haran et al. [19]. Briefly, a thin film of the appropriate lipids, prepared as described above, was hydrated in an ammonium sulfate solution (240 mM). Liposomes (120 mM TL) were sized by extruding the liposome dispersion sequentially through two 0.1 and 0.05 μm polycarbonate membrane filters under nitrogen pressure. The external medium of the liposomes was replaced by applying the liposomes onto a Sephadex G-25M column (PD-10; Pharmacia, Woerden, The Netherlands) and subsequent elution with Hepes/glucose buffer (10 mM Hepes, 1 mM EDTA, 270 mM glucose, pH 7.4). Then liposomes were incubated with doxorubicin (about 15 μmol TL/mg doxorubicin) for 30 min at 60°C. Non-encapsulated doxorubicin was removed using the cation exchange resin Dowex 50WX-4 (Serva, Heidelberg, FRG) [20]. Empty liposomes (i.e. devoid of doxorubicin) were prepared in a similar way, except that liposomes were now incubated with Hepes/glucose buffer without doxorubicin.

2.3. Liposome characterization

Lipid phosphate was determined by the colorimetric method of Rouser [21]. Mitoxantrone was determined spectrometrically in 1% Triton-X100 in an isotonic acetate buffer (pH 5) at 608 nm. The amount of doxorubicin was determined fluorimetrically after destruction of the liposomes with acidified ethanol (94%, 0.3 M HCl; excitation wavelength 490 nm, emission wavelength 591 nm). Mean particle size was determined by dynamic light scattering at 25°C with a Malvern 4700 system using a 25 mW He–Ne laser (NEC, Tokyo, Japan) and the automeasure version 3.2 software (Malvern, Malvern, UK). As a measure of particle size distribution of the dispersion, the system reports a polydispersity index (pd). This index ranges from 0.0 for an entirely monodisperse up to 1.0 for a polydisperse dispersion.

2.4. Animal experiments

Six weeks old female Swiss mice (mitoxantrone) or C57 Black mice (doxorubicin) from the animal facility of the University of Nijmegen with an approximate body weight of 25 g were used. Animals received standard laboratory chow and water ad libitum. The animal experiments adhered to the "Principles of Laboratory Animal Health Care".

To study the influence of the route of administration on the protective effect of liposomes, mice (3 animals per group) were injected sc into the neck region or im into the gastrocnemius muscle with a single injection (0.1 ml) of either non-encapsulated, free mitoxantrone (0.2 or 0.02 mg) dissolved in an isotonic acetate buffer (pH 5) or liposome-encapsulated mitoxantrone (0.2 mg mitoxantrone, 14 μmol TL). As a control mice were injected sc or im with an isotonic acetate buffer (pH 5). To study the influence of liposome lipid composition, mice were injected sc into the neck region with a single injection (0.1 ml) of doxorubicin (0.1 or 0.01 mg) dissolved in Hepes/glucose buffer or a single dose of doxorubicin (0.1 mg) encapsulated in liposomes (about 1 μmol TL) consisting of several lipid compositions. As a control mice were injected sc with a single dose of empty liposomes composed of EPC:EPG:Chol (10:1:4). A control experiment on tissue reaction after im injection of empty liposomes was not performed since local tissue damage was not observed in a similar experiment as reported by Kadir et al. [16]. Mice were killed by cervical dislocation 1 or 7 days after injection. The site of injection was exposed and examined for macroscopic evaluation. Then, in case of sc injection, skin at the sc injection site and, in case of im injection, the gastrocnemius muscle were collected. Tissue samples were left in a 4% formol solution prior to dehydration in alcohol and subsequent paraffin embedding. Tissue blocks were cut into 3 series of serial 5 μm sections of 20 sections each and 50 μm between the subsequent series and deparaffinized prior to staining with the Goldner (Masson-Trichrome) stain. The sections were evaluated by light microscopy. The lightmicrographs shown should be considered as typical examples of the evaluated sectioned material.

3. Results

3.1. Influence of the route of administration

The influence of the route of administration on the protective effect of liposomes against the vesicant

Table 1
Tissue irritation after sc and im administration of free and liposomal mitoxantrone

Formulation	Drug dose (mg)	Tissue reaction 1 day post-injection	Tissue reaction 7 days post-injection
sc administration			
Acetate buffer	–	No tissue damage	ND [a]
Free mitoxantrone	0.02	ND	Mild tissue damage and repair
Free mitoxantrone	0.20	Severe inflammation and necrosis of muscle cells	Inflammation, necrosis of skin and muscle tissue, and repair
Liposomal mitoxantrone [b]	0.20	Slight inflammation and minimal tissue damage	Inflammation, necrosis of skin and muscle tissue, and repair
im administration			
Acetate buffer	–	No tissue damage [c]	ND
Free mitoxantrone	0.02	ND	Slight inflammation, mild muscle cell damage and repair
Free mitoxantrone	0.20	Severe inflammation and necrosis of muscle cells	Severe inflammation, necrosis of muscle cells, and repair
Liposomal mitoxantrone [b]	0.20	Minimal tissue reaction	Minimal tissue reaction

[a] Not determined.
[b] Mitoxantrone was encapsulated in small (0.1 μm, pd < 0.4) liposomes, composed of EPC:EPG:Chol (molar ratio 10:4:4).
[c] Occasional haemorrhage along needle track.

properties of an encapsulated model antitumor drug was studied 1 and 7 days after sc and im administration of free and liposome-encapsulated mitoxantrone. Results are presented in Table 1.

Sc administration of 0.2 mg free mitoxantrone resulted in an inflammatory response developing rapidly at the injection site. One day post-injection, edema, extensive infiltration by inflammatory cells and necrosis were observed (Fig. 1(A)). Seven days post-injection, inflammatory cells and skin damage were still present, but repair by formation of connective tissue was also observed (Fig. 1(B)). A 10-fold lower dose of mitoxantrone caused a much less severe tissue response; seven days after sc administration of 0.02 mg mitoxantrone a slight inflammatory response and repair were observed (Table 1). A single sc injection of 0.2 mg liposomal mitoxantrone caused a much less intense inflammatory response as compared to 0.2 mg free mitoxantrone 1 day after injection (Fig. 2(A)). However, severity of the tissue reaction increased and 7 days after administration of 0.2 mg liposomal mitoxantrone, edema and an extensive inflammatory response were observed (Fig. 2(B)). At this time-point, tissue damage was as severe as tissue damage 7 days after injection of 2.0 mg free mitoxantrone.

Im injection of 0.2 mg free mitoxantrone caused a severe inflammatory response and necrosis of muscle cells one day post-injection (Fig. 3(A)). Seven days post-injection the inflammatory response had progressed and muscle cell damage was more extensive than 1 day post-injection (Fig. 3(B)). A 10-fold lower dose (0.02 mg) of free mitoxantrone resulted in a much less intense inflammatory response 7 days after im administration (Table 1). In contrast to 0.2 mg free mitoxantrone, an equal dose of liposomal mitoxantrone induced virtually no inflammatory response 1 and 7 days after im injection (Fig. 4). Seven days

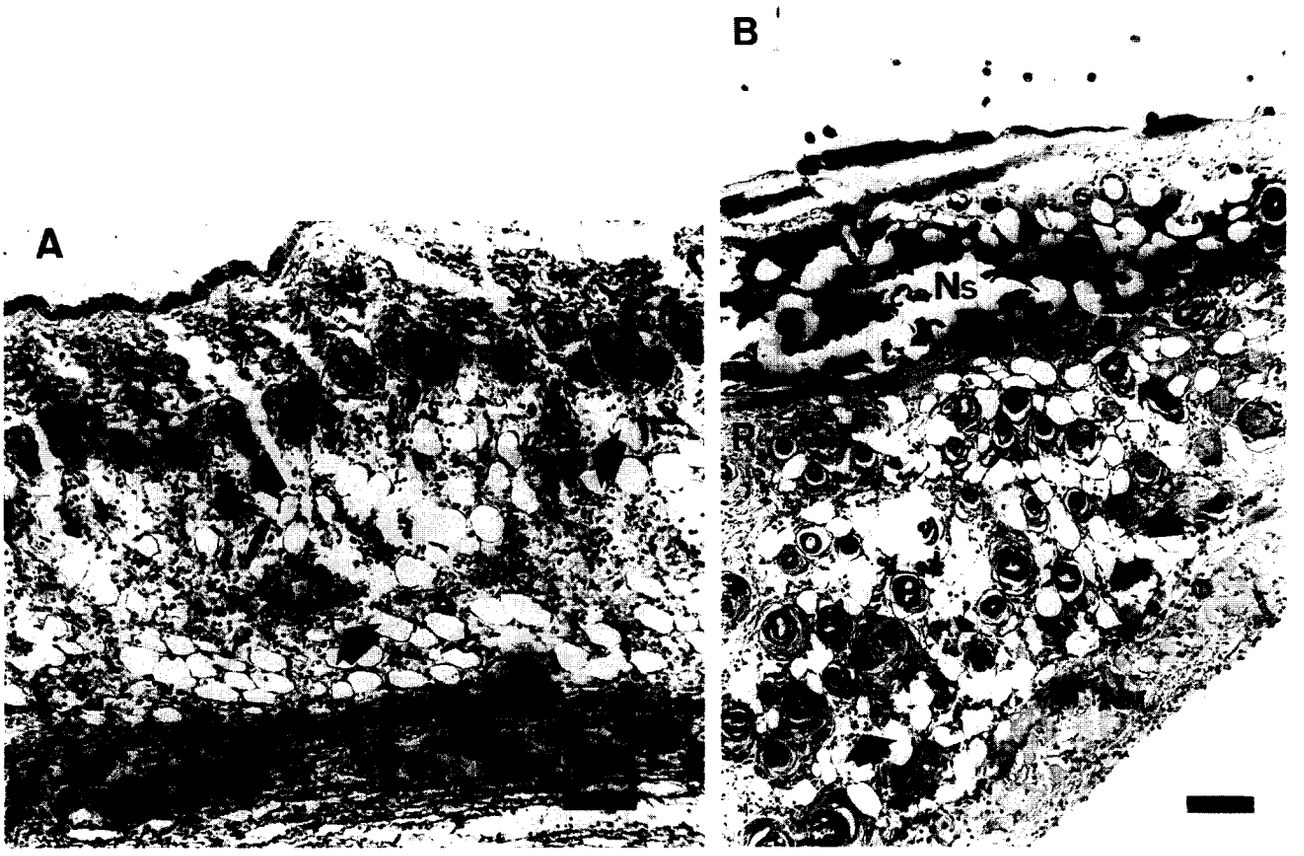

Fig. 1. Lightmicrograph of a skin section from a mouse given a single sc injection of 0.2 mg free mitoxantrone. (A) One day post-injection; extensive infiltration (arrows) and necrosis of muscle cells (Nm) (bar = 100 μm). (B) Seven days post-injection; necrotic skin (Ns), necrotic muscle cells (Nm), renewed epithelium (R) and slight inflammation (arrows) (bar = 100 μm).

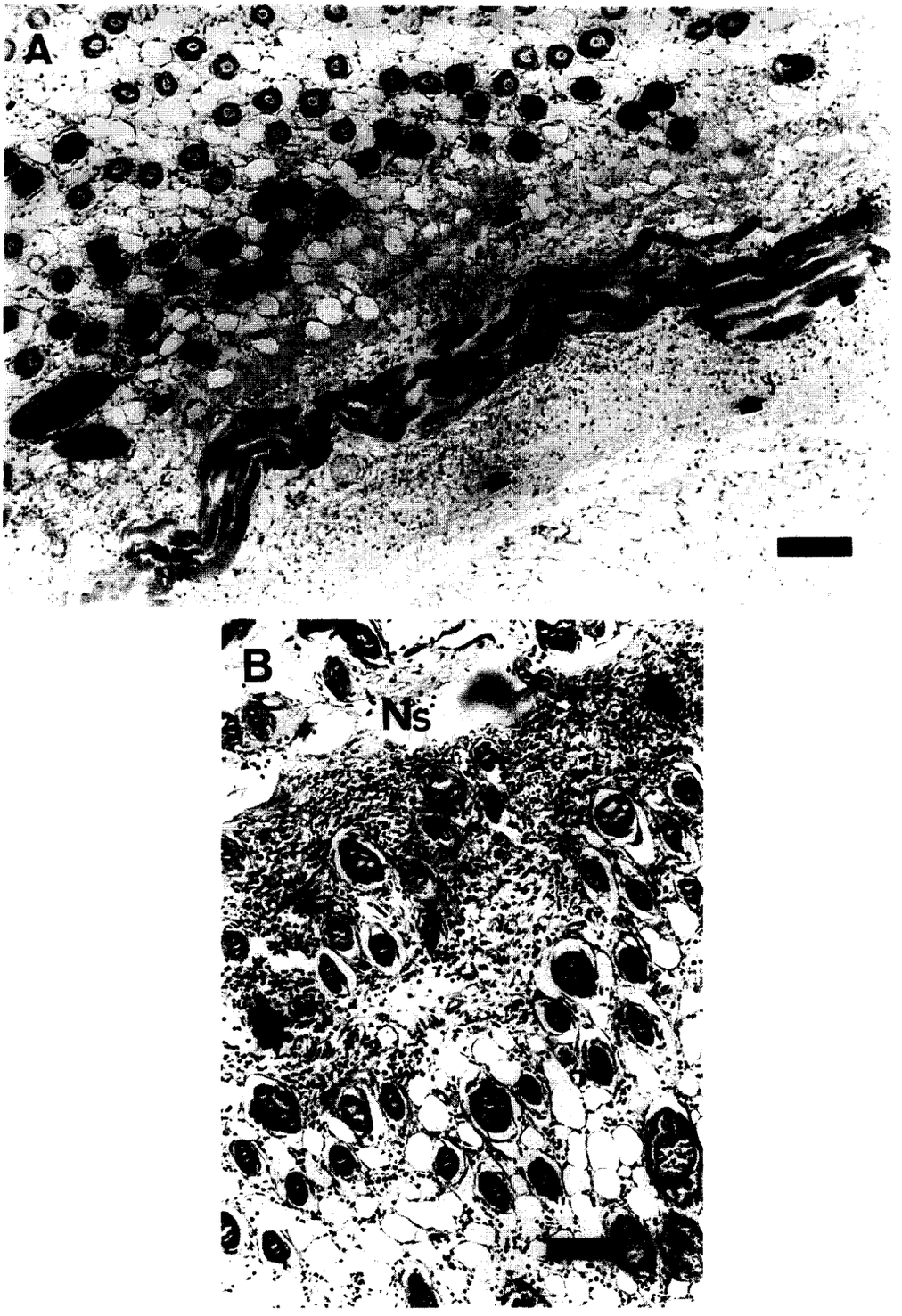

Fig. 2. Lightmicrograph of a skin section from a mouse given a single sc injection of 0.2 mg mitoxantrone encapsulated in small (0.1 μm) liposomes. (A) One day post-injection; slight inflammatory response (arrows) and no muscle cell (M) damage (bar = 100 μm). (B) Seven days post-injection; extensive inflammatory response (arrows) and necrosis of skin tissue (Ns) (bar = 100 μm).

Table 2
Tissue reaction after sc administration of free and liposomal doxorubicin

Formulation/lipid composition (molar ratio)	Drug dose (mg)	Tissue reaction 1 day post-injection	Tissue reaction 7 days post-injection
EPC:EPG:Chol (10:1:4)	–	No tissue reaction	ND [a]
Free doxorubicin	0.01	Modest inflammation	Modest inflammation
Free doxorubicin	0.10	Severe inflammation and necrosis of muscle cells	Severe tissue damage and necrosis of muscle cells
EPC:Chol (2:1)	0.10	Slight inflammation, minimal tissue damage	Necrosis of dermis and muscle cells
EPC:Chol:PEG-PE (1.85:1:0.15)	0.10	Slight inflammation, no tissue damage	Necrosis of dermis and muscle cells
DPPC:Chol (2:1)	0.10	Slight inflammation, no tissue damage	Modest inflammation, no tissue damage
DPPC:Chol:PEG-PE (1.85:1:0.15)	0.10	Slight inflammation, no tissue damage	Modest inflammation, minimal tissue damage

Small (0.1 μm, pd < 0.2) liposomes of several compositions were injected at a dose of about 1 μmol TL.
[a] Not determined.

after administration of liposomal mitoxantrone, loose connective tissue was observed at the site of injection and in all animals tissue reaction was even less than after administration of a 10-fold lower dose of free mitoxantrone.

3.2. Influence of liposome stability

The influence of liposome stability on the protective effect of liposomes against dermal toxicity was studied 1 and 7 days after sc administration of free doxorubicin and doxorubicin encapsulated in liposomes with relatively 'fluid' bilayers and liposomes with more 'rigid' bilayers. Liposomes with the more fluid bilayers were composed of EPC:Chol (2:1, molar ratio) with or without PEG-PE (5 mol%). Liposomes which are expected to be more stable against release of the entrapped drug, rigid liposomes, were composed of DPPC:Chol (2:1, molar) with or without PEG-PE (5 mol%). Results of histological examinations of surrounding tissue are given in Table 2.

Free doxorubicin at a dose of 0.1 mg, caused severe and rapid damage to surrounding tissue. One day post-injection, extensive infiltration by inflammatory cells was observed in the epithelial and adipose layers (Fig. 5(A)). Cells were severely damaged and necrotic areas were observed. Seven days post-injection tissue damage was still severe. Moreover, the area of inflammation was slightly increased (Fig. 5(B)). A 10-fold lower dose of doxorubicin caused much less tissue response as only few inflammatory cells were observed 1 and 7 days after injection (Table 2). In control animals receiving empty liposomes, no adverse tissue reaction was observed 1 day post-injection.

Tissue response was much milder 1 day after sc administration of 0.1 mg liposome-encapsulated doxorubicin as compared to the free drug. A modest infiltration of inflammatory cells was observed when 0.1 mg doxorubicin was encapsulated in EPC:Chol and EPC:Chol:PEG-PE liposomes 1 day post-injection (Table 2, Fig. 6). Tissue damage was less than seen after administration of 0.1 mg free doxorubicin as no gross lesions were observed. Inflammation was restricted to the dermis and was not observed in the underlying muscle layer. Surprisingly, 7 days post-injection, the inflammatory responses had progressed and necrotic areas were observed in the dermis and underlying muscle layer in all animals (Fig. 6(B)). Skin toxicity appeared to be even more severe when compared to skin lesions 7 days after administration of 0.1 mg free doxorubicin. Administration of doxorubicin encapsulated in the more rigid DPPC:Chol and DPPC:Chol:PEG-PE liposomes induced a mild, but prolonged inflammatory response. Although numerous inflammatory cells were observed at both observation time-points, tissue damage was minimal (Table 2, Fig. 7). Compared to day 1, the inflamma-


A
B
Nm
Nm

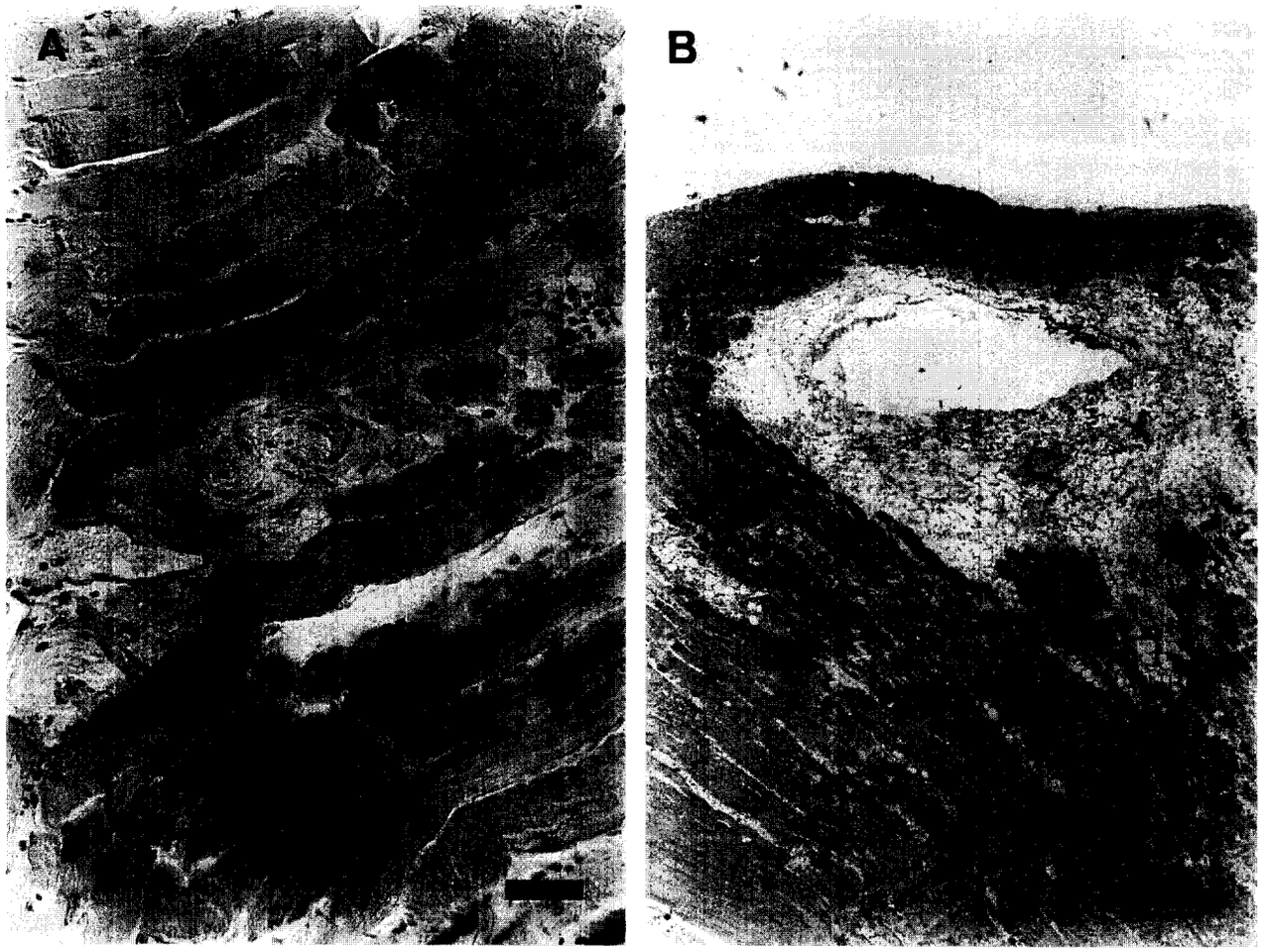

Fig. 4. Lightmicrograph of a skin section from a mouse given a single im injection of 0.2 mg mitoxantrone encapsulated in small (0.1 μm) liposomes. (A) One day post-injection; virtually no inflammation or muscle cell (M) damage (bar = 40 μm). (B) Seven days post-injection; no muscle cell damage (M), loose connective tissue (C) with little inflammatory response (bar = 400 μm).

tory response was only slightly increased at 7 days post-injection.

4. Discussion

Several papers have appeared that demonstrate the potential of liposomes to protect surrounding tissue against the local toxic effects of highly irritating agents after sc and im administration. [9,11–13,16]. The influence of the route of administration and the liposome lipid composition on the protective effect of liposomes have received minimal attention until now. This paper reports on the influence of these two factors potentially affecting the protective effect of liposome encapsulation against the vesicant properties of two model anti-tumor drugs. The effect of the route of administration was studied after sc and im administration of mitoxantrone-containing liposomes,

Fig. 3. Lightmicrograph of a skin section from a mouse given a single im injection of 0.2 mg free mitoxantrone. (A) One day post-injection; inflammation (arrows) and necrosis of muscle cells (Nm) (bar = 40 μm). (B) Seven days post-injection; severe inflammatory response (arrows) and extensive muscle cell damage (Nm) (bar = 40 μm).

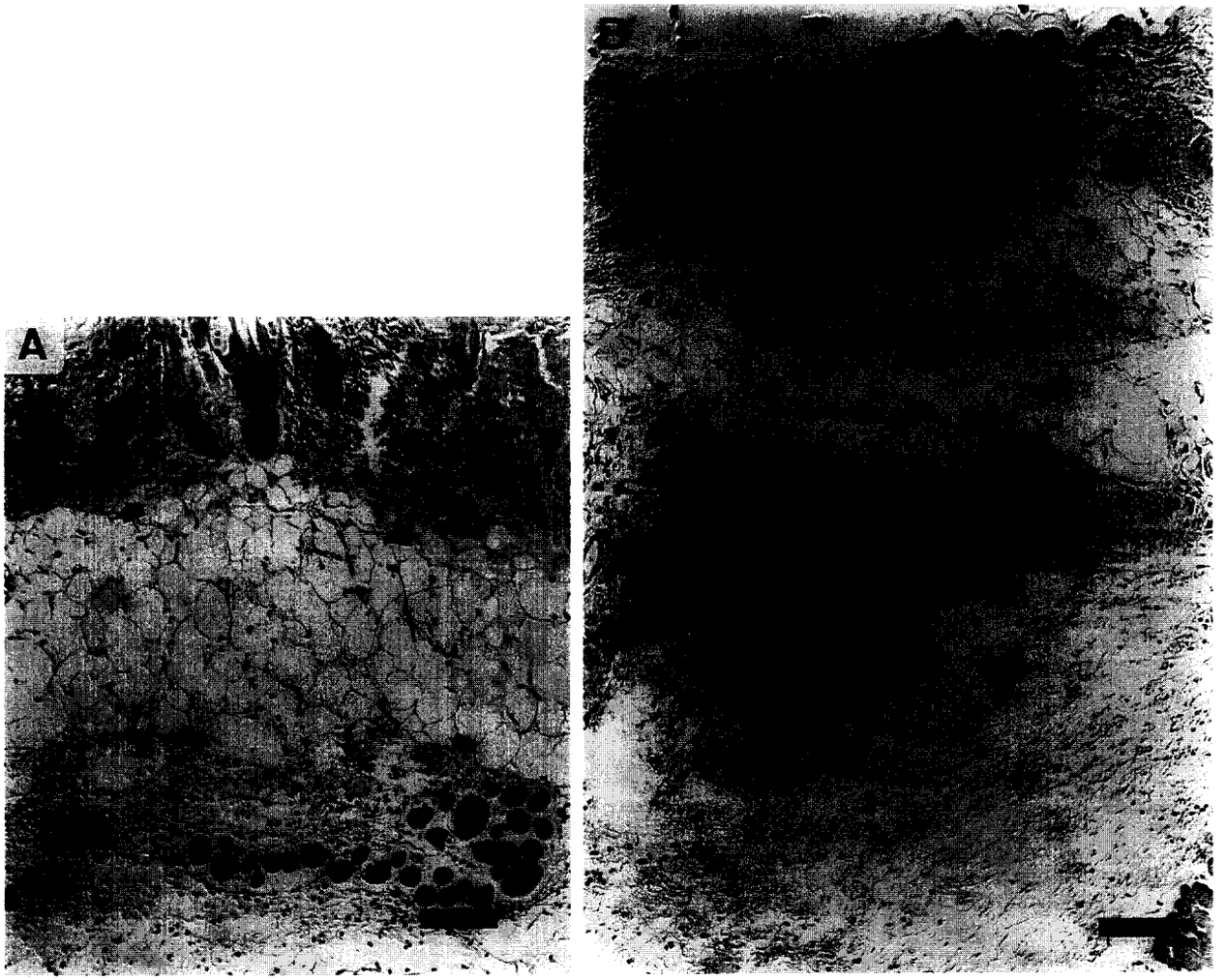

Fig. 5. Lightmicrograph of a skin section from a mouse given a single sc injection of 0.1 mg free doxorubicin. (A) One day post-injection; extensive inflammation (arrows) in both skin epithelium and muscle layer, and necrotic muscle cells (Nm) (bar = 100 μm). (B) Seven days post-injection; inflammatory cells (arrows) and necrosis of muscle cells (Nm), almost complete disappearance of muscle layer (bar = 100 μm).

the effect of liposome lipid composition was studied after sc administration of doxorubicin-containing liposomes of several lipid compositions.

Mitoxantrone is an anthracenedione derivative with a spectrum of clinical activity similar to the anthracycline doxorubicin [22]. Local tissue damage of mitoxantrone has been reported only occasionally and is usually less severe than tissue damage caused by local administration of doxorubicin [10]. However, under the chosen conditions, we observed severe tissue damage after sc administration of 0.2 mg free mitoxantrone. Doxorubicin has proven to be highly toxic to surrounding tissue when administered locally [9–12]. Presumably, local tissue damage is caused by a direct cytotoxic effect of the drug. Both drugs have a high affinity for cellular DNA resulting in inhibition of DNA synthesis and cell death [23,24]. With respect to skin injury, there have been speculations that doxorubicin released from dying cells in the dermis induces damage and death in neighbouring cells, only to be released again, thus forming a repetitive cycle. This would account for the progressive nature of the tissue damage [9,25]. The results presented here indicate that mitoxantrone and doxo-

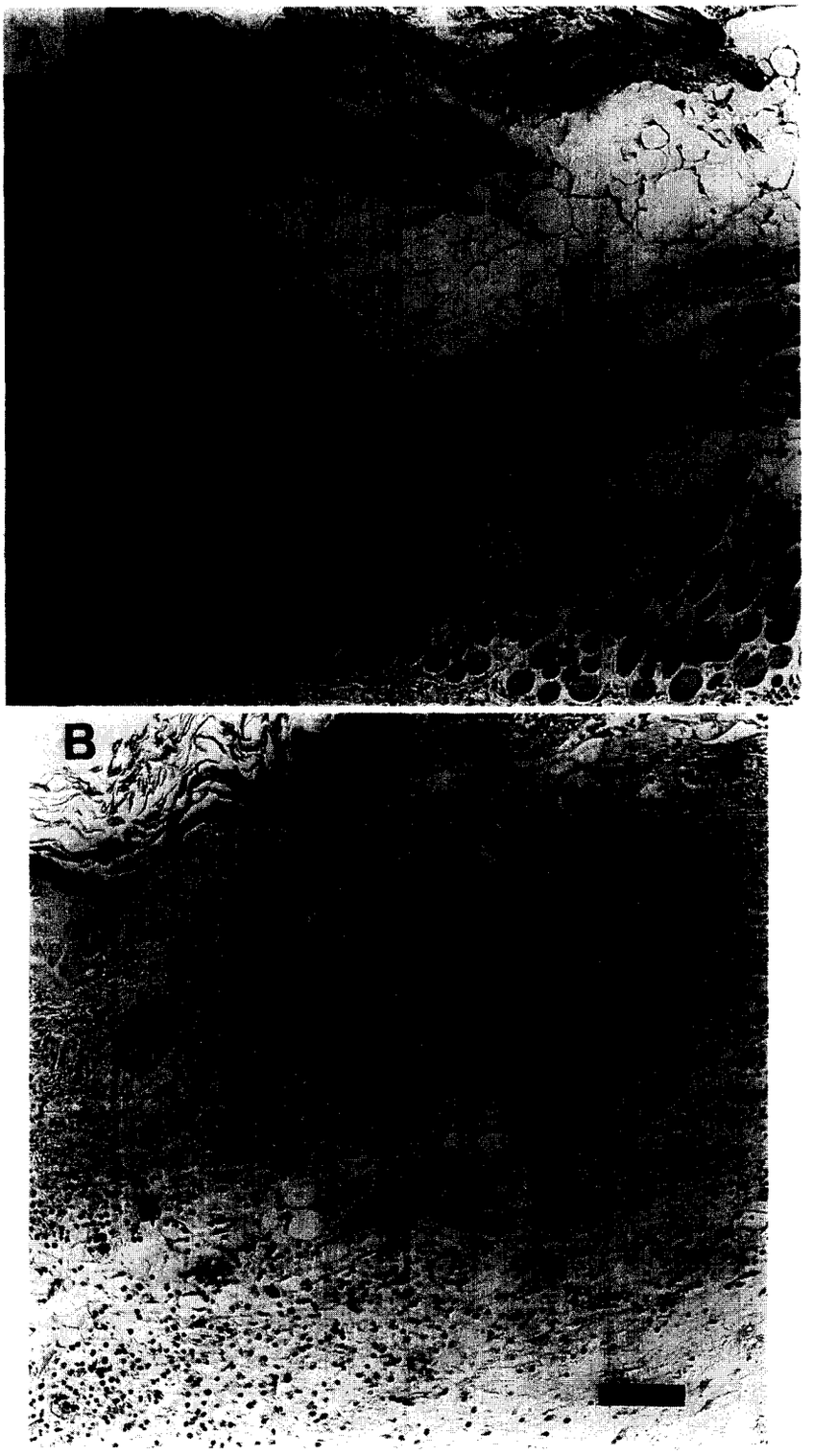

Fig. 6. Lightmicrograph of a skin section from a mouse given a single sc injection of 0.1 mg doxorubicin encapsulated in fluid-state liposomes composed of EPC:Chol. (A) One day post-injection; modest infiltration of inflammatory cells (arrows) no muscle cell damage (M) (bar = 100 μm). (B) Seven days post-injection; inflammatory response (arrows) and necrotic areas in skin (Ns) and necrosis of muscle cells (Nm), almost complete disappearance of muscle layer (bar = 100 μm).

rubicin at the doses tested, are both progressively vesicant and cause similar patterns of tissue damage when injected sc.

Liposome encapsulation of mitoxantrone reduced local toxicity after sc and im administration. Local tissue damage was strongly dependent on the route of

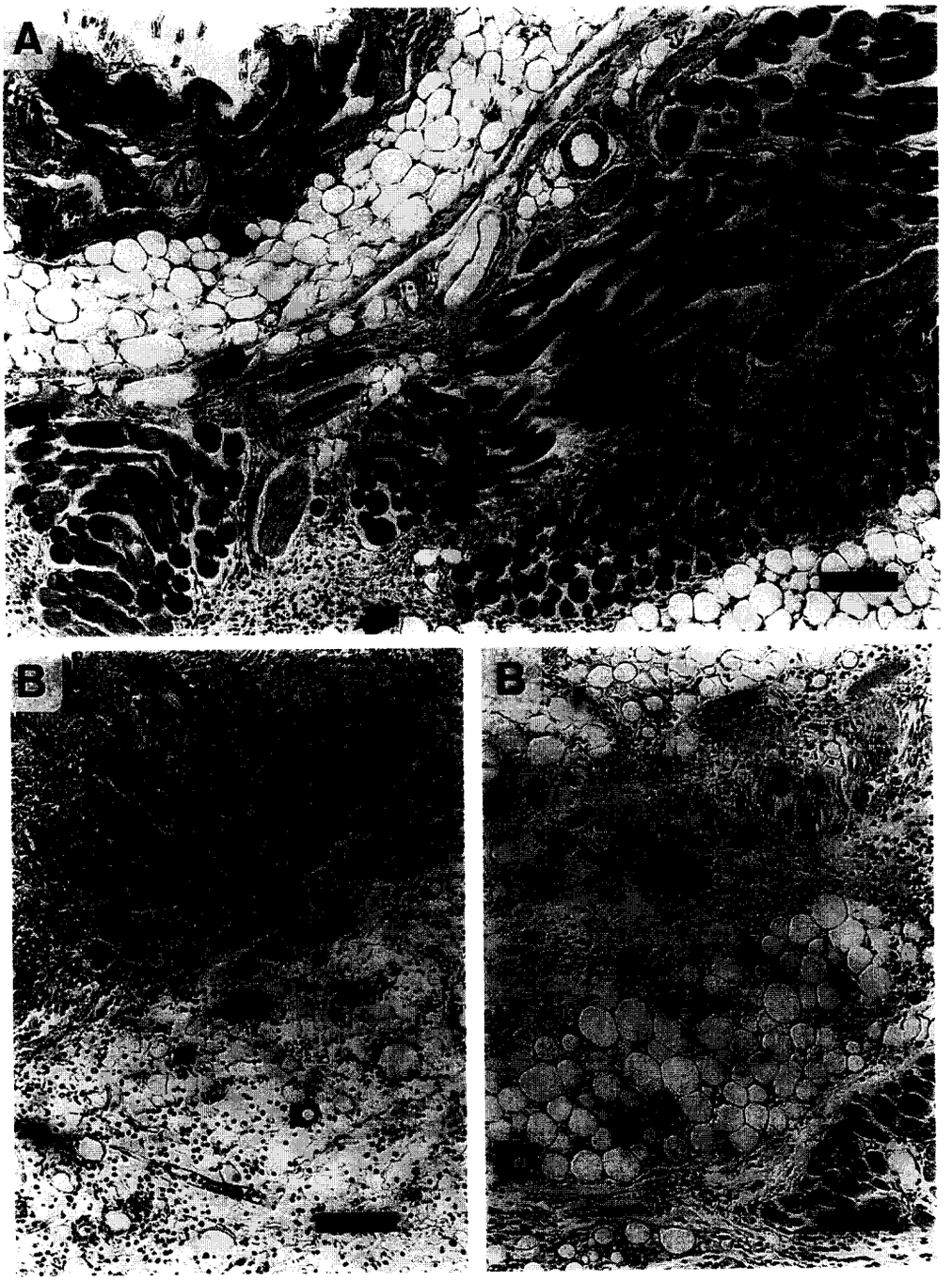

Fig. 7. Lightmicrograph of a skin section from a mouse given a single sc injection of 0.1 mg doxorubicin encapsulated in rigid liposomes composed of DPPC:Chol. (A) One day post-injection; mild infiltration of inflammatory cells (arrows), no muscle cell damage (M) (bar = 100 μm). (B) Seven days post-injection, inflammation (arrows), but no damage in epithelium (E) or muscle layer (M) (bar = 100 μm).

administration. Initially, surrounding tissue was protected efficiently against the toxic effects of the encapsulated mitoxantrone after injection via both routes of administration. However, seven days post-injection remarkable differences were observed in the protective effect of liposomes following sc and im injection. At this time-point, encapsulation of liposomes dramatically reduced toxic effects after im administration but not after sc administration. The observed differences between the protective effect after sc and im administration may be ascribed to the more complete and faster clearance of injected material after im injection than after sc administration [17]. Differences in the rate of clearance from the im and sc injection site are attributed to a richer supply of blood in muscle tissue and increased blood flow through muscles during body movement of the animal [17,27]. Released drugs may be cleared rapidly from the im injection site. As surrounding tissue will only be exposed to very low concentrations of the free drug, tissue damage will be negligible. In contrast, the prolonged inflammatory response observed following sc administration of mitoxantrone- and doxorubicin-containing liposomes may be the result of a large proportion of the injected liposome dose remaining at the injection site for a long period of time [3]. Lack of clearance from the injection site may result in accumulation of the released drug and thus in prolonged exposure of surrounding tissue to high concentrations of the cytotoxic drug.

It is of interest to study the ability of liposomes to reduce vesicant properties of encapsulated antitumor drugs in relation to the liposome lipid composition after sc administration. The protective effect of liposomes on the vesicant properties of doxorubicin has been reported previously [9–12]. However, little attention has been paid to liposome stability. The present results demonstrate that liposome stability is an important factor determining the protective effect of liposomes. Initially, 1 day after sc injection, all liposome compositions protected surrounding tissue against the damaging effects of mitoxantrone and doxorubicin. However, seven days after administration, marked differences were observed. At this time-point, doxorubicin encapsulated in EPC:Chol liposomes was at least as toxic as an equal dose of the free drug, whereas doxorubicin encapsulated in DPPC:Chol liposomes induced only mild inflammatory response. As the more rigid membranes of DPPC:Chol liposomes generate more stable liposomes with regard to leakage of the liposome content as compared to liposomes composed of EPC:Chol [26], the stronger protective effect of DPPC:Chol may be attributed to slower release of the encapsulated drug as compared to the 'fluid' EPC:Chol liposomes. These results are in agreement with the observation on tissue damage caused 7 days after sc injection of mitoxantrone encapsulated in liposomes which were also composed of 'fluid-state' lipids.

The effect of inclusion of PEG-PE into the liposomes was negligible. Apparently, exposure of surrounding tissue to drug is not significantly influenced by steric stabilization of the liposome surface.

It is tempting to speculate on the basis of the results presented here that encapsulation of doxorubicin in liposomes with more rigid bilayers could be used safely when treatment consists of local administration. However, it should be realized that even in the case of rigid liposomes damage to local tissue may be severe after prolonged exposure to a low, but persistent dose of vesicant antineoplastic agents, such as mitoxantrone or doxorubicin, slowly released from liposomes. Therefore, it is of critical importance to assess long-term local toxicity of liposome-encapsulated mitoxantrone or doxorubicin before designing treatment protocols on the basis of local administration of these drugs.

In conclusion, liposomes can protect surrounding tissue from the toxic effects of the encapsulated antineoplastic agents mitoxantrone and doxorubicin. The route of administration is an important factor influencing tissue damage. The protective effect of liposomes on local toxicity of mitoxantrone is much stronger after im than after sc administration. This observation is attributed to the faster and more complete clearance of the vesicant drug after im injection as compared to sc injection. Furthermore, it was demonstrated that the protective effect of liposomes on the vesicant properties of doxorubicin is stronger for liposomes with a more rigid nature. Although liposomes with more fluid bilayers initially protect surrounding tissue from direct dermal toxicity of the encapsulated drug, slow release may result in prolonged and persistent exposure to low doses of the toxic drug and may be a serious cause of local tissue damage. The incorporation of PEG-PE had no appar-

ent effect on the protective effect of liposomes after sc administration.

References

[1] G. Poste, L.J. Fidler, The pathogenesis of cancer metastasis, Nature 283 (1980) 139–149.

[2] H.M. Patel, Fate of liposomes in the lymphatics. in: G. Gregoriadis (Ed.), Liposomes as drug carriers, Wiley, New York, 1988, pp. 51–62.

[3] C. Oussoren, J. Zuidema, D.J.A. Crommelin, G. Storm, Lymphatic uptake and biodistribution of liposomes after subcutaneous injection. II. Influence of liposomal size, lipid composition and lipid dose, Biochem. Biophys. Acta 1328 (1997) 261–272.

[4] A. Tümer, C. Kirby, J. Senior, G. Gregoriadis, Fate of cholesterol-rich liposomes after subcutaneous injection into rats, Biochim. Biophys. Acta 760 (1983) 119–125.

[5] A.J. Jackson, Intramuscular absorption and regional lymphatic uptake of liposome-entrapped inulin, Drug Metab. and Dispos. 9 (1981) 535–540.

[6] H. Konno, T. Tadakuma, K. Kumai, T. Takahashi, K. Ishibiki, O. Abe, S. Sakaguchi, The antitumor effect of adriamycin entrapped in liposomes on lymph node metastases, Jpn. J. Surg. 20 (1990) 424–428.

[7] J. Khato, J.E. Priester, S. Sieber, Enhanced lymph node uptake of melphalan following liposomal entrapment and effect on lymph node metastasis in rats, Cancer Treat Rep. 66 (1982) 517–527.

[8] T. Yaguchi, M. Yamauchi, H. Takagi, N. Ohishi, K. Yagi, Effect of sulfatide-inserted liposomes containing entrapped adriamycin on metastasized cells in lymph nodes, J. Clin. Biochem. Nutr. 9 (1990) 79–85.

[9] J.A.E. Balazsovits, L.D. Mayer, M.B. Bally, P.R. Cullis, M. McDonell, R.S. Ginsberg, R.E. Falk, Analysis of the effect of liposome encapsulation on the vesicant properties, acute and cardiac toxicities and antitumor efficacy of doxorubicin, Cancer Chemoth. Pharm. 23 (1989) 81–86.

[10] R.T. Dorr, D.S. Alberts, H.G. Chen, Experimental model of doxorubicin extravasation in the mouse, J. Pharmacol. Meth. 4 (1980) 237–250.

[11] A.A. Gabizon, O. Pappo, D. Goren, M. Chemla, D. Tzemach, A.T. Horowitz, Preclinical studies with doxorubicin encapsulated in polyethyleneglycol-coated liposomes, J. Liposome Res. 3 (1993) 517–528.

[12] E.A. Forssen, Z. Tökes, Attenuation of dermal toxicity by liposome encapsulation, Cancer Treat. Rep. 67 (1983) 481–484.

[13] N.L. Boman, V.A. Tron, M.B. Bally, P.R. Cullis, Vincristine-induced dermal toxicity is significantly reduced when the drug is given in liposomes, Cancer Chemother. Pharmacol. 37 (1996) 351–355.

[14] G. Delgado, R.K. Potkul, J.A. Treat, G.S. Lewandowski, J.F. Barter, D. Forst, A. Rahman, A phase I/II study of intraperitoneally administered doxorubicin entrapped in cardiolipin liposomes in patients with ovarian cancer, Am. J. Obstet. Gynecol. 160 (1989) 812–819.

[15] E.A. Forssen, Z.A. Tökes, Use of anionic liposomes for the reduction of chronic doxorubicin-induced cardiotoxicity, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 1873–1877.

[16] F. Kadir, W.M.C. Eling, D. Abrahams, J. Zuidema, D.J.A. Crommelin, Tissue reaction after intramuscular injection of liposomes in mice, Int. J. Clin. Pharm. Ther. Toxicol. 30 (1992) 374–382.

[17] F. Kadir, W.C.M. Eling, D.J.A. Crommelin, J. Zuidema, Influence of injection volume on the release kinetics of liposomal chloroquine administered subcutaneously or intramusculary to mice, J. Control Release 17 (1991) 277–284.

[18] M.C. Woodle, D.D. Lasic, Sterically stabilized liposomes. Biochim. Biophys. Acta 1113 (1992) 171–199.

[19] G. Haran, R. Cohen, L.K. Bar, Y. Barenholz, Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphiphatic weak bases, Biochim. Biophys. Acta 1151 (1993) 201–205.

[20] G. Storm, L. Van Blooys, M. Brouwer, D.J.A. Crommelin, The interaction of cytostatic drugs with adsorbents in aqueous media, Biochim. Biophys. Acta 818 (1985) 343–351.

[21] G. Rouser, S. Flusher, A. Yamamoto, Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots, Lipids 5 (1970) 494–496.

[22] I.E. Smith, Mitoxantrone (Novantrone), A review of experimental and early clinical studies, Cancer Treat. Rev. 10 (1983) 103–115.

[23] W.G. Foye, O. Vajragupta, S.K. Sengupta, DNA-binding specificity and RNA polymerase inhibitory activity of bis(aminoalkyl)anthraquinones and bis(methylthio)vinylequinone iodides, J. Pharm. Sci. 71 (1982) 253–257.

[24] W. Foster, E. Stutter, Interaction of anthracycline antibiotics with biopolymers, 9. Comparative study of the interaction kinetics of daunomycin, adriamycin and iremycin with DNA, Int. J. Biol. Macromol. 6 (1984) 114–124.

[25] S.D. Averbuch, G. Gaudiano, T.H. Kock, N.R. Bachan, Doxorubicin induced skin necrosis in the swine model, protection with a novel radical dimer, J. Clin. Oncol. 4 (1986) 94–99.

[26] J. Senior, Fate and behaviour of liposomes in vivo: A review of controlling factors, Ther. Drug Carrier Syst. 3 (1987) 123–193.

[27] J.R. Casley-Smith, An electron microscopic study of injured and abnormally permeable lymphatics, Ann. New York Acad. Sci. 116 (1964) 803–830.