Neovascular Targeting Therapy: Paclitaxel Encapsulated in Cationic Liposomes Improves Antitumoral Efficacy[1]

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ABSTRACT

Purpose: Cationic liposomes have been shown to selectively target tumor endothelial cells. Therefore, the encapsulation of antineoplastic drugs into cationic liposomes is a promising tool to improve selective drug delivery by targeting tumor vasculature. It was the aim of our study to evaluate tumor selectivity and antitumoral efficacy of paclitaxel encapsulated in cationic liposomes in comparison with the free drug paclitaxel (Taxol®) *in vivo*.

Experimental Design: Experiments evaluating tumor selectivity were carried out in male Syrian golden hamsters bearing the amelanotic hamster melanoma A-Mel-3 in dorsal skinfold preparations. Growth of tumor cells was observed after s.c. inoculation (day 0). On days 5, 7, 9, 12, 14, and 16, animals were treated by continuous i.v. infusion over 90 min with 5% glucose, Taxol®, unloaded cationic liposomes, or paclitaxel encapsulated into cationic liposomes (LipoPac), respectively (lipid dose, 150 mg/kg body weight; paclitaxel dose, 5 mg/kg body weight). Tumor volumes and presence of regional lymph node metastases were quantified.

Results: Vascular targeting of rhodamine-labeled cationic liposomes was maintained after encapsulation of paclitaxel as revealed by *in vivo* fluorescence microscopy (ratio of dye concentration, tumor:normal tissue = 3:1). The s.c. tumor growth revealed a remarkable retardation of tumor growth after treatment with LipoPac (1.7 ± 0.3 cm^3). In contrast, control tumors showed exponential tumor growth [tumor volume at the end of the observation period (mean $\pm$ SE): 5% glucose, 17.7 ± 1.9 cm^3; unloaded cationic liposomes, 10.0 ± 1.6 cm^3; Taxol®, 10.7 ± 1.7 cm^3]. In addition, the appearance of regional lymph node metastases was significantly delayed by treatment with paclitaxel encapsulated into cationic liposomes in comparison with all other groups.

Conclusions: The data suggest that cationic liposomes are a powerful tool for selective and efficient drug delivery to tumor microvessels. This may serve as proof of the concept of neovascular tumor targeting therapy by cationic liposomes.

INTRODUCTION

Denekamp (1) has proposed vascular targeting as a new therapeutic concept based on the destruction of tumor microvasculature. Subsequently, tumor cells die as a consequence of impaired supply of nutrients and accumulation of metabolites. Due to their high rate of proliferation, tumor endothelial cells reveal susceptibility against cytotoxic drugs (2). Furthermore, tumor endothelial cells are considered to be genetically stable and thus less prone to drug resistance. Selective delivery of cytotoxic drugs to tumor endothelium may be an approach to establish vascular targeting for tumor therapy.

Chemotherapy with conventional paclitaxel (Taxol®; Bristol-Myers Squibb, New York, NY) is currently applied against a broad spectrum of advanced human cancers, including ovarian and breast cancer, non-small cell lung carcinoma, melanoma, and head and neck cancer (for review, see Ref. 3). Nevertheless, Taxol® is prone to toxicity and the development of drug resistance. Due to its low aqueous solubility, the hydrophobic paclitaxel has to be dissolved for clinical use in Cremophor EL, a mixture of the polyoxyethylated castor oil and dehydrated ethanol. This solvent can cause serious side effects in humans such as hypersensitivity reactions and neurotoxicity (4).

New approaches to package paclitaxel are currently under investigation. Of considerable interest as a drug delivery system for lipophilic drugs are liposomes, *i.e.*, spherical vesicles comprised of a lipid membrane with an internal aqueous compartment. First data from clinical Phase I studies using liposomal paclitaxel are encouraging with respect to reducing toxic side effects (5). Nevertheless, liposomal formulations containing paclitaxel have been reported thus far to modify pharmacokinetics, but not to improve antitumoral activity (6).

Cationic liposomes have been described to selectively target angiogenic endothelial cells in tumors (7). This property enables selective drug delivery to tumor endothelial cells. Therefore, the aim of our study was to prove the concept of vascular targeting using cationic liposomes as a vehicle for the cytotoxic drug paclitaxel. In comparison with Taxol®, we quantified tumor accumulation and antitumoral efficacy of paclitaxel encapsulated in cationic liposomes (LipoPac) *in vivo*. In addition, the effect of the drugs on regional lymph node metastases was evaluated.

MATERIALS AND METHODS

Preparation of Liposomes. DOTAP,[4] DOPC, and Rh-DOPE (Avanti Polar Lipids Inc., Alabaster, AL) were used for synthesis of the liposomes. Pure paclitaxel (Synopharm, Barsbüttel, Germany) was used for encapsulation into cationic liposomes, whereas the conventional paclitaxel formulation (Taxol®; Bristol-Myers Squibb) was diluted in 5% glc (B. Braun, Melsungen, Germany).

Cationic liposomes with a total lipid content of 50 mM were prepared by the lipid film method followed by several cycles of extrusion. For paclitaxel-containing liposomes, 0.25 mmol of DOTAP, 0.235 mmol of DOPC, and 0.012 mmol of paclitaxel were dissolved in 15 ml of chloroform (Merck, Darmstadt, Germany). For fluorescence microscopy experiments, 0.1 mmol of DOTAP, 0.089 mmol DOPC, 0.006 mmol of paclitaxel, and 0.005 mmol of Rh-DOPE were dissolved in chloroform. For control experiments, cationic liposomes without paclitaxel were prepared by dissolving 0.25 mmol of DOTAP and 0.25 mmol of DOPC in 15 ml of chloroform. The respective mixture was gently warmed to 40°C in a round-bottomed flask, and the solvent was evaporated under vacuum in a rotary evaporator until a thin lipid film was formed. Solvent traces were eliminated by drying the film at 5 millibars for 60 min. Multilamellar liposomes formed spontaneously upon addition of 10 ml of 5% glc (w/v) to the flask. The suspension was left overnight to allow swelling of the liposomes. Next, the suspension was extruded five times in a 10-ml extruder (Northern Lipids Inc., Vancouver, British Columbia, Canada) with a thermobarrel thermostated at 30°C. The pore size of the polycarbonate membrane (Osmonics, Minnetonka, MN) was 200 nm. The resulting suspension was stored at 4°C under argon.

Analysis of Liposomes. The particle size of the liposomes was analyzed by photon correlation spectroscopy using a Malvern Zetasizer 3000 (Malvern Instruments, Herrenberg, Germany). Typically, suspensions exhibited a $Z_{average}$ of 180–200 nm.

Lipid and paclitaxel concentrations were determined by high-performance liquid chromatography using an UV-visible detector (205 nm for lipids, 227 nm for paclitaxel). The separation and quantitation of the components were carried out using a C8 LiChrospher 60 RP-select B column (250 × 4 mm, 5-μm particle size) with a C18 precolumn. Aliquots of the samples were diluted 1:3 with tetrahydrofuran (J. T. Baker, Phillipsburg, NJ) before measurement.

Based on a water solubility of paclitaxel of at most 11.5 μM (8) and on the measured paclitaxel concentration in the described liposomal formulations (1.2 mM), at most 1.0% of paclitaxel was not incorporated in the liposomes. The stability of the formulations was checked regularly; in particular, the absence of needle-like paclitaxel crystals (9) was assured in each formulation intended for use in the described animal studies.

In a functional assay, the content of fluorescence-labeled lipids in the liposomes was further quantified by fluorescence spectroscopy using a fluorescence detector (Jobin-Yvon, Grasbrunn, Germany). Aliquots of samples were diluted in chloroform, and analysis was carried out at a fluorophore-specific wavelength (excitation, 560 nm; emission, 580 nm).

Transparent Skinfold Chamber Preparation. Experiments were carried out using male Syrian golden hamsters (40–50 g bw; Charles River, Sulzbach, Germany) in accordance with institutional and governmental guidelines. The animals were housed in single cages and had free access to tap water and standard laboratory food (ssniff; Spezialdiaeten GmbH, Soest, Germany) throughout the entire experiments.

To permit quantitation of tumor and normal tissue pharmacokinetics of fluorescence-labeled liposomes loaded with paclitaxel, a transparent chamber preparation was surgically implanted into the dorsal skin of the animals as described previously in detail (10, 11). After a recovery period of 24 h from anesthesia and microsurgery, chamber preparations were inoculated with 2 μl of dense tumor cell suspension (~2×10^5 cells) of the A-Mel-3 amelanotic melanoma of the hamster (12). One day before infusion of the drug, permanently indwelling fine polyethylene catheters (PE10; inner diameter, 0.28 mm) were implanted into the right jugular vein. All surgical procedures were performed under anesthesia with ketamine (100 mg/kg bw, i.p.; Ketavet; Parke-Davis, Berlin, Germany) and xylazine (10 mg/kg bw, i.p.; Rompun; Bayer, Leverkusen, Germany).

***In Vivo* Fluorescence Microscopy for Quantitation of Pharmacokinetics.** *In vivo* fluorescence microscopy was performed after 6–7 days of tumor growth (mean diameter of tumors, 4–5 mm), when functional tumor microcirculation was well established. For detection of fluorescence induced by rhodamine liposomes in the tumor and surrounding host tissue, the awake chamber-bearing hamster ($n = 3$) was immobilized in a Perspex tube on a specially designed stage (Effenberger, Munich, Germany) under a Leitz microscope (Type 307–143003/514660; Leitz, Munich, Germany). Autofluorescence of the surrounding tissue was determined and subtracted from respective fluorescence images. At 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min after start of liposome administration (i.v. infusion over 90 min; dosage, 5 mg paclitaxel/kg bw), fluorescence was registered in tumor and surrounding host tissue as described previously (13). In brief, fluorescence images were digitized on-line and analyzed by an image analysis system (KS400; Zeiss, Oberkochen, Germany). Fluorescence values are given as a percentage of the solid reference fluorescence signal (percentage standard) inserted into each chamber preparation (Impregum F; ESPE, Seefeld, Germany). Fluorescence in tumor and adjacent normal tissue was quantified in ROI of approximately 50 × 50 μm² by densitometric measurement. ROI were selected and set in the epi-illumination image of each chamber preparation before injection of fluorescence-labeled liposomes. Thus, ROI were created regardless of fluorescence localization, and they did not encompass larger blood vessels. The ROI were identical for all observation times.

Localization of Paclitaxel Encapsulated in Cationic Liposomes. In separate experiments ($n = 3$) using higher magnification, we performed *in vivo* high-resolution fluorescence

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microscopy to determine the exact localization of fluorescence-labeled LipoPac in the above-mentioned chamber preparation at the same dose level. Distribution of the fluorescence-labeled drug was determined 0, 30, 60, 90, and 120 min after the start of a 90-min continuous infusion in microvessels of tumor and surrounding normal tissue.

s.c. Tumor Growth and Analysis of Metastases. The dorsal skin of hamsters (70 $\pm$ 5 g bw) was shaven and chemically depilated under general anesthesia. A-Mel-3 tumor cells (4–6 $\times$ 10^6) were suspended in a 10-μl volume of RPMI 1640 (Biochrom, Berlin, Germany) and injected s.c. into the lumbosacral region of the dorsal skin. The longer (*l*) and shorter (*w*) perpendicular axes and the height (*h*) of each tumor nodule were measured, and tumor volume was calculated according to the following formula (14).

$$V_t = 0.837 \times l \times w \times h$$

Metastases of the animals were determined by daily palpation of axillar and inguinal lymph nodes and finally verified at autopsy.

Treatment Protocol. Twenty-four h before the start of treatment, permanently indwelling fine polyethylene catheters (PE10) were implanted into the right jugular vein. On days 5, 7, 9, 12, 14, and 16 after tumor cell implantation, four groups of animals were treated (*n* = 6 animals/group). Paclitaxel encapsulated in cationic liposomes was i.v. given continuously over 90 min. The cationic liposomes contained 1 mg/ml paclitaxel, yielding a dose of 5 mg paclitaxel/kg bw.

Control animals received Taxol® (5 mg/kg bw), unloaded cationic liposomes, or just the solvent 5% glc, respectively. For all groups the same injection volumes and infusion parameters were used as for paclitaxel in cationic lipsomes (5 ml/kg bw). To adjust injection volumes, Taxol® was diluted in 5% glc, yielding a paclitaxel concentration of 1 mg/ml. Moreover, unloaded cationic liposomes were used at the same lipid dose (50 mM, 150 mg/kg) as paclitaxel-containing liposomes. Animals were sacrificed on day 23 after tumor cell inoculation by an overdose of pentobarbital (Nembutal; Sanofia-Leva, Hannover, Germany).

Statistical Analysis. Results are presented as mean $\pm$ SE. Data were evaluated using the Friedman repeated measures ANOVA on ranks and Kruskal-Wallis ANOVA on ranks, respectively (SigmaStat; Jandel Scientific, San Rafael, CA).

Analysis of the onset of metastatic disease, *i.e.*, comparison of metastasis-free intervals, was performed according to the Kaplan-Meier method, and the differences among the groups were compared for statistical significance using the Cox *F* test (Statistica; StatSoft Inc., Tulsa, OK). *P*s < 0.05 were considered significant.

RESULTS

Liposome Pharmocokinetics by *in Vivo* Fluorescence Microscopy. To test tumor selectivity of cationic liposomes after encapsulation of paclitaxel, pharmacokinetics of fluorescence-labeled liposomes was determined. Representative fluorescence images of tumor and normal tissue in the transparent chamber preparation at different times are depicted in Fig. 1A.

We could clearly demonstrate that cationic liposomes maintain their ability to selectively target tumor tissue after encapsulation of paclitaxel: quantitation by image analysis revealed maximum fluorescence intensity in tumor tissue at 120 min until 240 min after start of administration (Fig. 1*B*). The ratio between tumor and normal tissue indicates an up to 4-fold selectivity for the tumor in comparison with surrounding normal tissue (Fig. 1*C*). High fluorescence signals in tumor tissue were maintained up to the end of the observation period (360 min).

Localization of Paclitaxel Encapsulated in Cationic Liposomes. *In vivo* fluorescence microscopy demonstrated that the intratumoral endothelial lining rather than tumor tissue itself is the target for LipoPac (Fig. 2): fluorescence of labeled paclitaxel-containing liposomes is restricted to intratumoral microvessels and is not detectable in the tumor extravascular compartment. Endothelial deposition in tumor vessels increased substantially during infusion time (Fig. 2, *A* and *B*). In contrast, the affinity of LipoPac was essentially smaller for microvessels in normal tissue compared with microvessels in tumor tissue (Fig. 2*D*).

s.c. Tumor Growth. Antitumoral efficacy of paclitaxel encapsulated into cationic liposomes was evaluated in s.c. growing A-Mel-3 tumors. Animals tolerated the treatment well, gained weight, and did not show significant differences in body weight over the observation period of 23 days.

On day 7 after tumor cell inoculation (*i.e.*, 2 days after first treatment), tumor growth was already significantly delayed in all treatment groups compared with control animals receiving the solvent only (5% glc). Quantitation of subsequent tumor growth revealed a growth delay of 2.7 $\pm$ 1.1 days for Taxol® and 3.2 $\pm$ 0.8 days for unloaded cationic liposomes in comparison with 5% glc controls, respectively (Fig. 3). Remarkably, treatment with paclitaxel encapsulated into cationic liposomes reduced tumor growth even further. This resulted in a growth delay of 9.9 $\pm$ 0.8 days in comparison with Taxol®, 9.4 $\pm$ 0.8 days in comparison with unloaded cationic liposomes, and 12.6 $\pm$ 0.8 days in comparison with 5% glc controls.

At the end of the observation period, tumor volumes in animals treated with paclitaxel encapsulated into cationic liposomes were significantly smaller than those in any other group (5% glc, 17.7 $\pm$ 1.9 cm^3; unloaded cationic liposomes, 10.0 $\pm$ 1.6 cm^3; Taxol®, 10.7 $\pm$ 1.7 cm^3; LipoPac, 1.7 $\pm$ 0.3 cm^3; *P* < 0.05; Figs. 3 and 4).

Onset of Metastases. The remarkable effect on retardation of tumor growth was further substantiated by analysis of onset of regional lymph node metastasis (Fig. 5). Lymph node metastases (axillar or inguinal) were first encountered on day 9 after tumor cell inoculation in 5% glc-treated control animals. Three days later, palpable metastases were found in all animals of this group. Comparably, at day 12, 66% of the animals treated with Taxol® or unloaded cationic liposomes were still free of palpable lymph nodes, but lymph node metastasis was noted in all animals of both groups 2 days later (day 14).

In contrast, regional lymph node metastasis was absent in animals treated with LipoPac until day 16 (*P* < 0.05).

Overall, in animals treated with LipoPac, it took 21 days until all of the animals presented with palpable lymph node metastases. This corresponds to a prolongation of disease-free state of 9 days compared with 5% glc-treated animals and an increase of 7 days compared with animals treated with unloaded-liposomes or Taxol®.
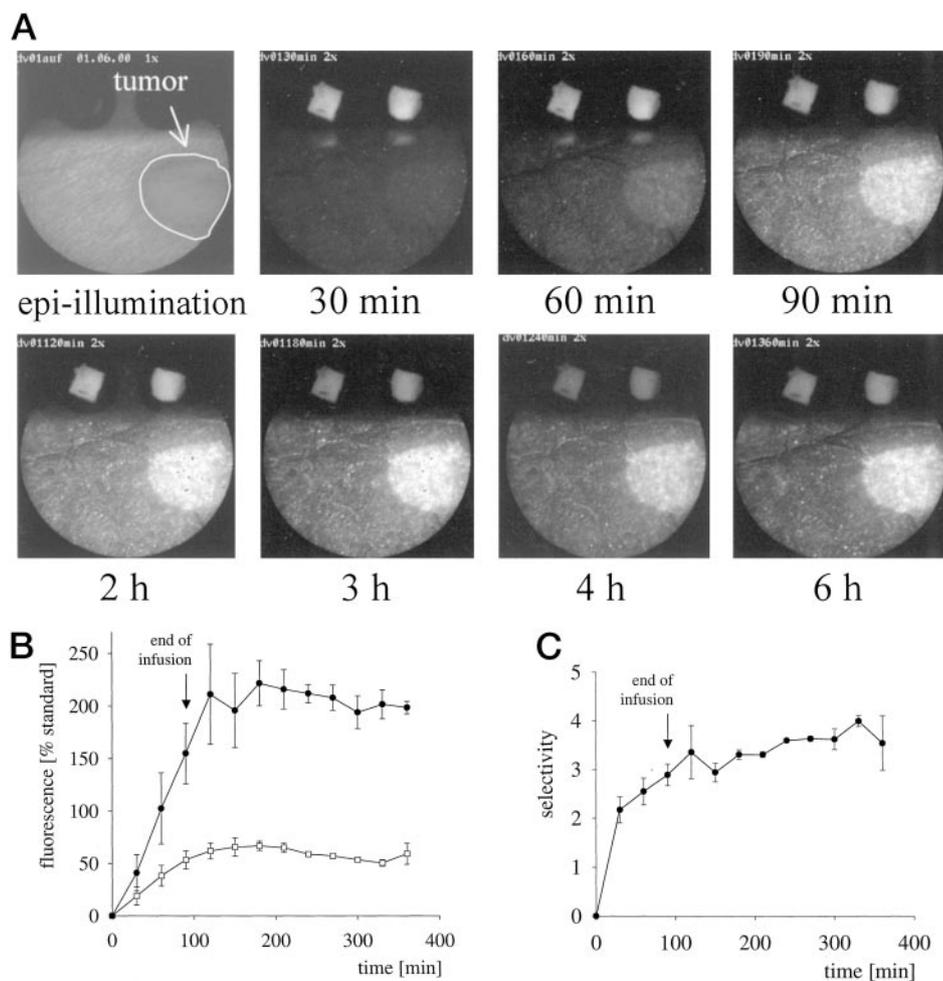
Fig. 1 *In vivo* fluorescence microscopy of fluorescence-labeled cationic liposomes after encapsulation of paclitaxel. *A,* fluorescent signal seen in an A-Mel-3 tumor in the transparent skinfold preparation before and 30 min, 60 min, 90 min, 2 h, 3 h, 4 h, and 6 h after a continuous, 90-min, i.v. injection of paclitaxel encapsulated into cationic rhodamine-labeled liposomes. *B,* quantitation of fluorescence signal of LipoPac in tumor (●) and surrounding normal tissue (□): cationic liposomes keep their ability to target tumor tissue after encapsulation of paclitaxel. High fluorescence signals in tumor tissue were maintained up to the end of the observation period (360 min). *C,* the fluorescence ratio between tumor and normal tissue yielded an up to 4-fold selectivity for the tumor in comparison with surrounding normal tissue; $n = 3$; mean $\pm$ SE.

DISCUSSION

In cancer chemotherapy, increased attention is currently focused on the development of drug delivery systems for systemic application with the aim of enhancing the selectivity of agents by enhancing their accumulation at the site of disease. *In vivo*, the endothelial wall prevents many systemically circulating agents with *in vitro* activity from reaching the tumor cell at adequate concentrations. Therefore, the target may have to be redefined; Denekamp (1) suggested targeting the accessible microvasculature feeding tumor cells. A suitable carrier with a microvascular targeting property are cationic liposomes that have been shown to accumulate selectively in angiogenic tumor endothelial cells (7).

Here we demonstrated that cationic liposomes retain their known ability to accumulate in tumor blood vessels also after encapsulation of the cytotoxic drug paclitaxel. Treatment with this liposomal formulation significantly increased the antitumoral efficacy of the drug. Therefore, chemotherapeutic drug delivery targeted to the tumor microvasculature by means of cationic liposomes appears to be a promising new strategy for cancer chemotherapy.

Paclitaxel is a chemotherapeutic drug used clinically, for

example, in advanced ovarian or metastatic breast cancer (3). Limitations of clinical administration thus far are the dose-limiting toxicity and aqueous insolubility of paclitaxel. However, the first preliminary clinical trials using other, noncationic liposomal formulations of paclitaxel have shown better tolerability with comparable antitumoral efficacy (5). There are also approaches to synthesize water-soluble paclitaxel prodrugs introducing polyethylene glycol ester conjugates (15). These compounds are reported to reduce toxicity (16) or may even improve antitumoral efficacy (17). But all these concepts lack the essential idea of distinct tumor targeting. Consequently, further attempts to develop not only water-soluble but in particular tumor-targeting derivatives of paclitaxel have been pursued by attaching peptides onto the chemical structure of paclitaxel intended to bind to receptors on the tumor cell surface: this resulted in an improved antitumoral effect on NCI-H1299 tumor cells *in vitro* (18). These findings indicate that a targeting concept of chemotherapy with paclitaxel may improve the potential of the drug.
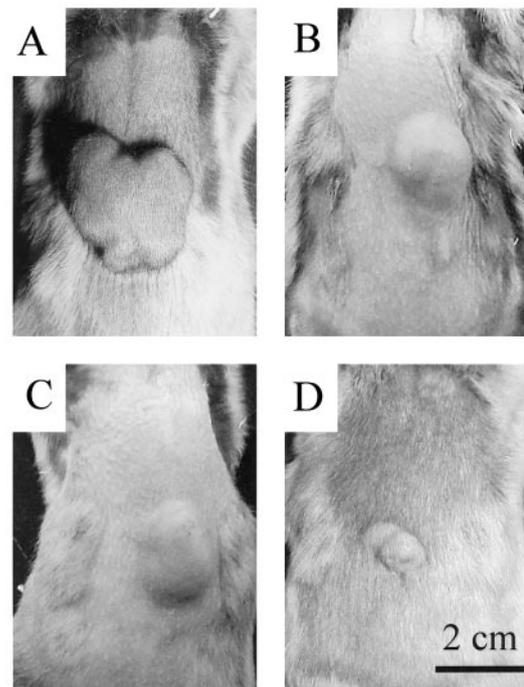
In our study, we chose a very unique property of tumors as target: the proliferation rate of tumor endothelial cells, which is 50–1000-fold higher compared with the proliferation rates of

Fig. 2 Vascular targeting of paclitaxel encapsulated in cationic liposomes. High-resolution *in vivo* fluorescence microscopy reveals fluorescence of labeled paclitaxel-containing liposomes restricted to intratumoral microvessels. No label was detected in the tumor extravascular compartment. Endothelial deposition increased significantly during the 90-min infusion (*arrows*). *A,* status after 30 min of continuous infusion; *B* and *C,* status at the end of the infusion. *D,* in contrast, the affinity of labeled liposomes was essentially smaller for microvessels in normal tissue (30 min after end of infusion). *Arrows* and *dotted line* indicate tumor margin.
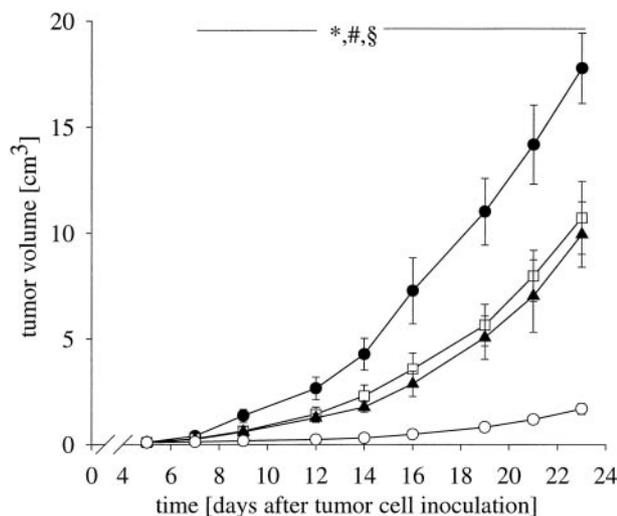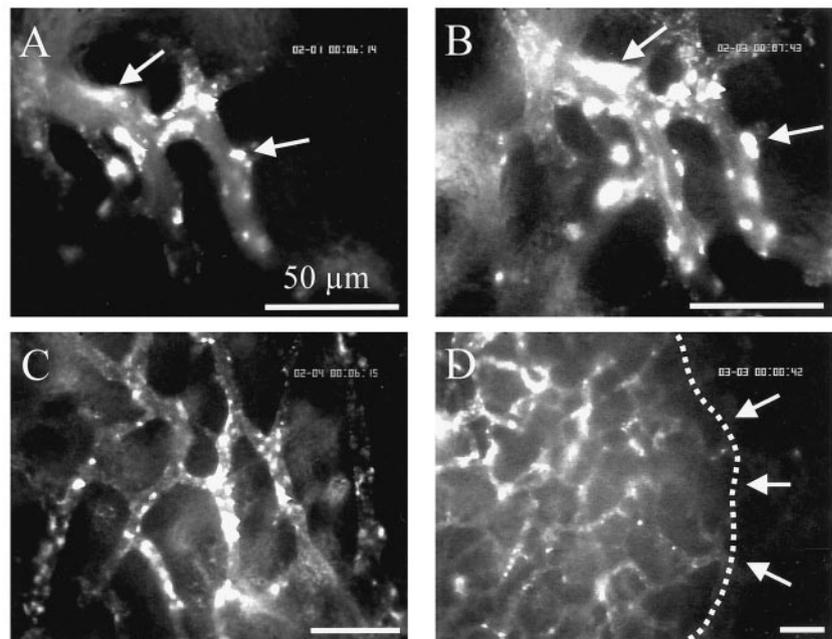
Fig. 3 Tumor growth of s.c. inoculated A-Mel-3 tumor cells. The length and width of each tumor were measured using calipers, and mean tumor volumes were calculated for each group. Tumor growth was significantly delayed after treatment with liposomal paclitaxel compared with controls. 5% glc, ●; unloaded cationic liposomes, ▲; Taxol®, □; LipoPac, ○.*, $P < 0.05$ *versus* prior day of measurement; #, $P < 0.05$ *versus* 5% glc control; §, $P < 0.05$, LipoPac *versus* all other groups; $n = 6$/group; mean ± SE.

Fig. 4 Representative A-Mel-3 tumors on day 16 after tumor cell inoculation. *A,* 5% glc; *B,* unloaded cationic liposomes; *C,* Taxol®; *D,* LipoPac.

normal endothelial cells (1, 2). The mechanisms by which cationic liposomes selectively target activated tumor endothelium are still hypothetical, ranging from receptor-mediated endocytosis (7) to charge-dependent binding and uptake by a potentially altered glycocalyx of tumor microvessels. Microvascular targeting was highly reproducible in our tumor model (19). Moreover, after encapsulation of paclitaxel, cationic liposomes

maintained preferential targeting selectivity for tumor vessels as compared with surrounding normal tissue. As a consequence, the antimitotic drug paclitaxel was delivered effectively to tumor vascular endothelial cells, impairing their proliferative and vital activity. In comparison with Taxol®, which targets mitosis
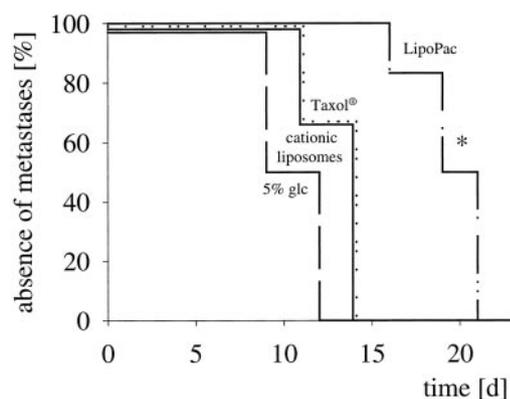
Fig. 5 Kaplan-Meier analysis of the metastasis-free interval. Onset of metastasis was significantly delayed in animals treated with paclitaxel encapsulated into cationic liposomes; ∗, $P < 0.05$. 5% glc, *dashed line*; unloaded cationic liposomes, *solid line*; Taxol®, *dotted line*; LipoPac, *dashed and dotted line*.

of tumor cells, the improved effect of the cationic liposomal preparation is based on attacking the vascular compartment of the tumor.

At low magnification, we clearly demonstrated that LipoPac targets tumor tissue with a selectivity of up to 4-fold compared with surrounding normal tissue. By analyzing in detail the deposition of fluorescence-labeled LipoPac at higher magnification, it was demonstrated that LipoPac does not accumulate in the tumor interstitium but is restricted to tumor microvasculature. This indicates that the intratumoral endothelial cell lining rather than the extravascular tumor tissue is the target of LipoPac.

It is noteworthy that tumor vessel targeting by cationic liposomes does not necessarily mean that the greatest fraction of a drug applied systemically ends up in the tumor. However, intratumoral microvascular accumulation of LipoPac resulted in dense coverage of the tumor vessel surface as compared with normal tissue microvessels. Along these lines, very high effective drug levels were reached locally at tumor microvasculature. In addition to specific rheologic conditions of tumor microcirculation (*e.g.,* sluggish, irregular blood flow), the structural difference in charge between activated neovascular tumor endothelium and nonactivated endothelium in normal tissues appears to be quite profound and thus crucial for tumor vessel selectivity (20, 21). Nevertheless, distinct differences in differentiation of the endothelial lining in various organs (*e.g.,* kidney, spleen, and liver) may influence the degree of uptake of cationic liposomes and thus may differ from tumor surrounding normal tissue that was used as a reference in this study. A detailed analysis of whole body distribution of LipoPac was not subject of this investigation, although there are data available about cationic liposomes (22). However, in accordance with our own findings (19), Campbell *et al.* (23) recently demonstrated that cationic charge determines the distribution of liposomes between the vascular and the extravascular compartment of tumors rather than influencing whole body distribution.

Taken together, these findings favor neovascular targeting rather than alteration of whole body redistribution as the crucial

mechanism underlying the observed therapeutic efficacy of LipoPac.

Using the concept of vascular targeting, Huang *et al.* (24) have reported complete tumor remissions: they induced tumor infarction by targeting artificially transferred markers on tumor endothelial cells, thus directing procoagulant truncated tissue factor to these cells. Although this approach is experimental, these observations clearly stress the potential of vascular targeting tumor therapy. Comparably, tubulin-binding agents exert significant antivascular effects *in vivo*, as has been reported for combretastatin A-4 (25) and ZD6126 (26). Recently, there have been further reports concerning immunological vascular targeting using fusion proteins of an antibody fragment targeting the angiogenic ED-B domain of fibronectin after linkage to various cytokines (for review, see Ref. 27).

Interestingly, unloaded cationic liposomes demonstrated a slight antitumoral effect. It is well known that cationic substances may be therapeutically active (28). Although paclitaxel was sufficiently stably encapsulated into liposomes, an additive effect of free paclitaxel and unloaded cationic liposomes cannot be ruled out for the observed therapeutic efficacy of LipoPac.

In addition, in our experiments, the delay of metastatic onset in animals treated with LipoPac correlates with tumor growth delay. Many investigators argue that the number of cells available to form metastases is proportional to the size of the primary tumor. Therefore, a delay in metastasis may not be due to an inhibition of metastasis formation by the drug action itself. In case of the A-Mel-3 tumor, previous experiments with local tumor therapy have shown that the appearance and growth of metastases are not directly dependent on the primary tumor burden but are strongly related to the time after s.c. tumor inoculation (29). Thus, we interpret the observed delay of incidence of palpable lymph node metastases as a consequence of LipoPac treatment *per se* rather than as a consequence of primary tumor size. Furthermore, the delayed onset of melanoma metastases in our experiment is seen to be indicative that an antivascular mechanism is likely to be involved because both metastatic spread and growth of metastases depend on tumor vascularization (30, 31).

The targeting property of cationic liposomes provides a very high (up to 4-fold) tumor selectivity, and the liposomal preparation was well tolerated in our experiments. Therefore, the risk of serious adverse side effects should be low. Moreover, cationic liposomes offer the possibility to encapsulate a large variety of chemotherapeutic substances that might yield additional efficacy when selectively delivered to the tumor vasculature. LipoPac is currently entering clinical Phase Ib/II trials.

Our data clearly show that treatment with the chemotherapeutic substance paclitaxel encapsulated into cationic liposomes resulted in a significant retardation of tumor growth and delay in the onset of metastatic disease in comparison with the conventional paclitaxel formulation (Taxol®). Cationic liposomes therefore appear to be an efficient drug delivery system for vascular targeting tumor therapy.

ACKNOWLEDGMENTS

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