

Targeting Chemotherapy to Solid Tumors with Long-circulating Thermosensitive Liposomes and Local Hyperthermia

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The effectiveness of the combination of long-circulating, thermosensitive liposomes and hyperthermia is described. Small-sized, thermosensitive liposomes that encapsulate doxorubicin (DXR-PEG-TSL (SUV)) have a prolonged circulation time and are extravasated to targeted solid tumors *in vivo*, where they preferentially release the agent in an anatomical site subjected to local hyperthermia. Liposomes were prepared by the incorporation of amphipathic polyethyleneglycol (PEG) to prolong their circulation time. DXR-PEG-TSL (SUV) was retained longest and was accumulated most efficiently in solid tumors in Balb/c mice. The combination of DXR-PEG-TSL (SUV) and hyperthermia at the tumor sites 3 h after injection, gave high concentrations of doxorubicin in tumor tissue and resulted in more effective tumor retardation and increased survival time. A large amount of DXR-PEG-TSL (SUV) was extravasated into the tumors during circulation for 3 h after injection, suggesting that the encapsulated drug was released into the interstitial spaces of the lesions by local hyperthermia. This system is expected to be clinically valuable for the delivery of a wide range of chemotherapeutic agents in the treatment of solid tumors.

Key words: Targeting chemotherapy — Hyperthermia — Thermosensitive liposomes — Long-circulating liposomes — Polyethyleneglycol

Liposomes have been proposed as useful drug carriers in targeted drug delivery systems and are under investigation in several therapeutic fields.¹⁾ To achieve maximum targeting, liposomes should remain in the systemic circulation for a long time; however, formulations of liposomes used previously were rapidly removed from the blood stream by the reticuloendothelial system (RES).^{2,3)} It has recently been shown that newly developed liposomes, containing the monosialoganglioside (G_{M1})⁴⁾ or amphipathic polyethyleneglycol (PEG),⁵⁻⁷⁾ are not readily taken up by macrophages in the RES and that they remain in the circulation for a relatively long time. More importantly, these so-called long-circulating liposomes (100–200 nm in mean diameter) were able to traverse the endothelium of blood vessels in solid tumors and were extravasated into the interstitial spaces, resulting in significantly greater accumulation compared with conventional liposomes.^{8,9)} Generally, the capillary permeability of the endothelial barrier in newly vascularized tumors is significantly greater than that of healthy tissue.¹⁰⁻¹²⁾ Under such conditions, liposomes of small size and longer half-life in the circulation will have more opportunity to traverse the tumor capillaries. Thus, in terms of drug delivery to solid tumors, these long-circulating liposomes offer several advantages over customary liposomal formulations. PEG

is particularly useful because of its ease of preparation, relatively low cost, controllability of molecular weight and ease of attachment to lipids or proteins, including antibodies. For these reasons, many recent studies have focused on the use of liposomes with surface-associated PEG.^{4,13)}

Liposomes can be designed to release their contents preferentially in an anatomical region subjected to local hyperthermia (thermosensitive liposomes).^{14,15)} We have reported that the combination of long-circulating thermosensitive liposomes entrapping doxorubicin (DXR) and the application of local hyperthermia at the tumor site showed a significantly enhanced antitumor effect.^{16,17)} Although ordinary thermosensitive liposomes have drawbacks, such as low stability and short half-life in the circulation, the newly developed thermosensitive liposomes overcame these by the incorporation of G_{M1} or PEG. However, these liposomes were so large that they could not be extravasated into solid tumor tissue; they could only release DXR in the circulation in the region of the tumor. Since DXR itself can diffuse out of blood vessels and reach solid tumor tissue effectively, an enhanced antitumor effect was obtained in this system. DXR is an anthracycline antibiotic used in the treatment of a variety of human cancers. It is a most potent drug showing a strong association between dose and antitumor response; thus, in order to intensify its antitumor effect, it is necessary to deliver a large amount of DXR into the interstitial spaces of solid tumors.

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We therefore investigated the accumulation of thermosensitive liposomes in solid tumors and the effective release of drug from them in the interstitial spaces in tumor tissue, using much smaller long-circulating thermosensitive liposomes which incorporated DXR. We also examined the antitumor activity of these liposomes in combination with local hyperthermia.

MATERIALS AND METHODS

Materials Dipalmitoyl phosphatidylcholine (DPPC) (COATSOME MC-6060), distearoyl phosphatidylcholine (DSPC) (COATSOME MC-8080) and distearoyl phosphatidylethanolamine (DSPE) (COATSOME ME-8080) were kindly donated by NOF Corp., Tokyo. NOF also provided a monomethoxy polyethyleneglycol succinimidyl succinate (PEG-OSu) (SUNBRIGHT VFM-4101) of average molecular weight 2000. ^{67}Ga was from New England Nuclear Japan. DXR and deferoxamine (DF) were purchased from Ciba Geigy, Kobe, and Kyowa Hakko, Tokyo, respectively. Cholesterol (CH) and triethylamine were purchased from Wako Pure Chemical, Osaka. An amphiphathic PEG (DSPE-PEG) was synthesized systematically by combination of DSPE with PEG-OSu, as described previously by Maruyama *et al.*¹⁸⁾

Liposome preparation and DXR encapsulation Liposomes were prepared from DPPC/DSPC/CH (7:2:1, m/m) and an appropriate amount of DSPE-PEG by the reverse-phase evaporation (REV) and extrusion. The lipid mixture (5 mg in total lipids) was dissolved in 600 μl of isopropyl ether/chloroform (1:1, v/v) and 300 μl of 300 mM citric acid (pH 4.0) was added. Liposomes were formed by the REV procedure and extruded more than ten times through polycarbonate filters (Nuclepore, Nomura Science, Tokyo) to control size. Liposomes sized to 100–120 nm or 200–230 nm in mean diameter by extrusion, were referred to as small unilamellar vesicles (SUV) or large unilamellar vesicles (LUV), respectively. The extrusion was performed at 50°C in a thermostatic extrusion device (EXTRUDER, Lipex Biomembrane, Canada). Liposome size was measured by dynamic laser light-scattering measurements using a ELS-800 particle analyzer (Otsuka Photonics, Tokyo). If necessary, ^{67}Ga -DF was added for determination of liposomal biodistribution or integrity of liposomes *in vivo*. The encapsulation of DXR into liposomes was done under conditions based on the report of Mayer *et al.*^{19,20)} The exterior pH of liposomes was adjusted to 7.8 with 1.0 N NaOH, creating a pH gradient across the lipid membrane (acid inside vs. basic outside). This liposome suspension was stood at 60°C for 3 min and then combined with preheated DXR solution (60°C for 3 min) at a DXR-to-lipid ratio of 0.2 (w/w). This admixture was heated at 60°C for 10 min with intermittent vortex mixing and chromatographed on a BioGel A1.5m column (2

cm \times 20 cm, Bio-Rad). The DXR-entrapping liposomes were finally sterilized by passage through a membrane filter of 0.45 μm pore size. DXR-trapping efficiency was estimated by dissolving the liposomes in acidified ethanol (0.3 N HCl in 50% ethanol) and measuring the fluorescence intensity at 470 nm (excitation) and 585 nm (emission).

Thermosensitivity of liposomes Temperature-dependent release profiles of DXR from liposomes were estimated by incubation with 20% (v/v) fetal bovine serum-saline at various temperatures (36–46°C) for 5 min. The released DXR was separated from the liposomal suspension by ultrafiltration and assayed by fluorescence spectrometry as described under doxorubicin encapsulation. A Centrisart (molecular weight cut-off 20 000, Sartorius, 3000 rpm, 20 min) was used for this procedure. The absorption of DXR on the membrane was less than 2%.

Biodistribution and stability *in vivo* Mouse colon carcinoma 26 cells (1×10^5 cells) were inoculated into a hind foot of Balb/c mice (male, 7 weeks old, weighing 22–25 g), and the tumor was allowed to grow for approximately 8 days, when the mean of its length and width was 8 mm. DXR-liposomes labelled with ^{67}Ga -DF (35 kBq and 600 μg lipids) were injected into the tail vein of tumor-bearing mice at a dose of 5 mg DXR/kg. At selected times post-injection, the animals were lightly anesthetized, bled via the retro-orbital sinus and killed by cervical dislocation. Organs were excised and counted for ^{67}Ga using a γ counter. Scintillation counting was done by the procedure described previously.¹⁶⁾ The weight of total blood was assumed to be 7.3% of the body weight.²¹⁾ Contamination with blood in each organ was corrected by examining the distribution of ^{51}Cr -labelled erythrocytes.¹⁶⁾ Statistical significance of differences was determined by using Student's *t* test.

***In vivo* studies with hyperthermia** Hyperthermia treatment was carried out with two different schedules at 5 min (schedule A) or 3 h (schedule B) after drug administration. Free DXR or liposomal DXR was injected via the tail vein at a dose of 5 mg DXR/kg. Mice bearing Colon 26 tumors were anesthetized with sodium pentobarbital. Local hyperthermia was induced with a radiofrequency oscillator (RF-hyperthermia HEH-100, Omron, Kyoto); a temperature of 42°C was continued for 20 min. The apparatus used in this study was modified to accommodate small animals. Five minutes after completion of hyperthermia treatment, blood was collected from the retro-orbital sinus, and major organs were excised. A 0.1 g portion of tissue, or the whole tissue if it weighed less than 0.1 g, was used for measurement of DXR concentration. Samples were homogenized and extracted with butanol/toluene (1:1, v/v), and the extracts were assayed by HPLC.¹⁶⁾

Tumoricidal effect Tumor-bearing mice were randomly assigned to groups of 10. Treatment was started when the

tumor reached a diameter of 8 mm (=Day 0). The dose per injection was 5 mg DXR/kg body weight. Local hyperthermia at 42°C for 20 min was applied under schedule A or schedule B as described above. Tumor volumes were determined as reported previously.²²⁾ Survival time was recorded in days after treatment with local hyperthermia.

RESULTS

Encapsulation of DXR and thermosensitivity Encapsulation of DXR into liposomes was achieved by the use of a pH gradient. Plain SUV and LUV showed over 95% trapping efficiency. The inclusion of 3 mol% of DSPE-PEG somewhat decreased the DXR encapsulation rate (Table I). The preparation process was unaffected by liposomal size; however, the inclusion of 6 mol% DSPE-PEG greatly decreased the trapping efficiency (Table I). Liposomes without PEG caused aggregation during storage at 4°C for

1 day. No aggregation was observed, even after 7 days, with PEG-containing liposomes, apparently due to the surface coating by PEG molecules. SUV and LUV containing 3 mol% of DSPE-PEG were employed in subsequent experiments.

The temperature-dependent release of DXR from SUV or LUV containing 3 mol% of DSPE-PEG was examined in 20% (v/v) fetal bovine serum-saline. As shown in Fig. 1, 91.2% or 71.0% of entrapped DXR was released from LUV with 3 mol% of PEG or plain LUV at 43°C, respectively. Both types of SUV showed lower release, in particular at higher temperatures. The release rate of DXR from PEG-SUV or plain SUV at 43°C was 51.0% or 49.2%, respectively. All liposomes tested were less leaky at temperatures below 37°C. These results indicate that DXR was released from liposomes in a temperature-dependent manner, and that the inclusion of 3 mol% of PEG did not interfere with DXR release. However, DXR release was markedly influenced by liposomal size. Hereafter, ther-

Table I. Lipid Composition, Size and Encapsulating Efficiency in Thermosensitive Liposomes

Liposome	Lipid composition	Ratio (m/m)	Size (mean±SD)	Encapsulating efficiency of DXR (%)
DXR-TSL (LUV)	DPPC/DSPC/CH	7:2:1	223±32	98
DXR-PEG-TSL (LUV)	DPPC/DSPC/CH/DSPE-PEG	7:2:1:0.31	202±30	95
DXR-PEG-TSL (SUV)	DPPC/DSPC/CH/DSPE-PEG	7:2:1:0.64	198±38	60
DXR-TSL (SUV)	DPPC/DSPC/CH	7:2:1	114±22	95
DXR-PEG-TSL (SUV)	DPPC/DSPC/CH/DSPE-PEG	7:2:1:0.31	110±23	90
DXR-PEG-TSL (SUV)	DPPC/DSPC/CH/DSPE-PEG	7:2:1:0.64	111±20	68

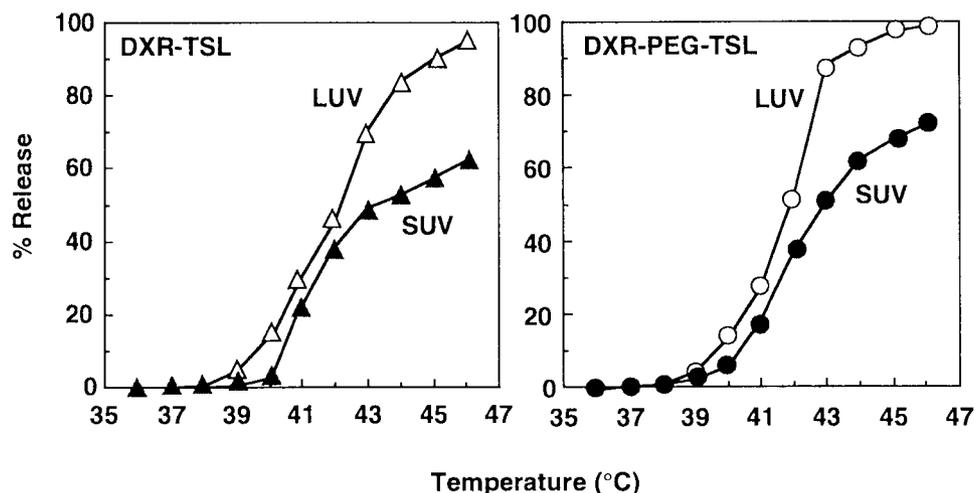


Fig. 1. Effect of inclusion of amphipathic PEG and liposomal size on temperature-dependent release of DXR from thermosensitive liposomes. DXR-TSL (DPPC/DSPC/CH, 7:2:1 m/m, (Δ) LUV, (▲) SUV) and DXR-PEG-TSL (DPPC/DSPC/CH/DSPE-PEG, 7:2:1:0.31 m/m, (○) LUV, (●) SUV) were incubated in 20% (v/v) fetal bovine serum-saline at various temperatures (36–46°C) for 5 min. DXR concentration was 10 μg/ml.

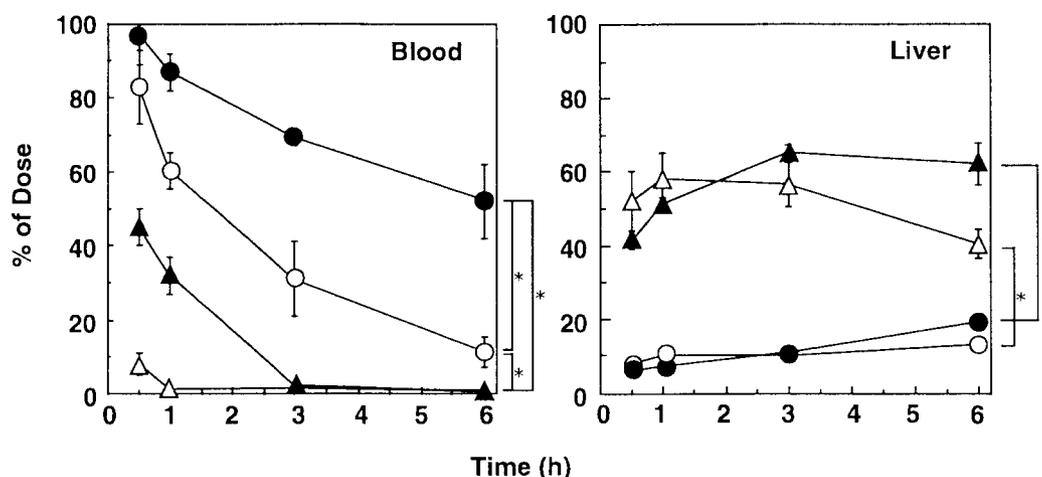


Fig. 2. Time-course of blood residence and RES (liver) uptake of (Δ) DXR-TSL (LUV), (\circ) DXR-PEG-TSL (LUV), (\blacktriangle) DXR-TSL (SUV) and (\bullet) DXR-PEG-TSL (SUV) in mice bearing Colon 26 solid tumors without hyperthermia. Tumor-bearing mice were injected i.v. with 0.2 ml of ^{67}Ga -labelled liposomes. Data are expressed as mean \pm SD ($n=3-5$). * $P<0.001$.

mosensitive SUV and LUV encapsulating DXR are referred to as DXR-thermosensitive liposomes (SUV) (DXR-TSL (SUV)) and DXR-thermosensitive liposomes (LUV) (DXR-TSL (LUV)), respectively, and those containing 3 mol% DSPE-PEG are referred to as DXR-PEG-TSL (SUV) and DXR-PEG-TSL (LUV), respectively.

Biodistribution of thermosensitive liposomes encapsulating DXR in tumor-bearing mice Thermosensitive liposomes were labelled with ^{67}Ga -DF and injected into mice via the tail vein. Biodistribution was measured up to 6 h after injection. The results are summarized in Figs. 2 and 3. DXR-TSL (LUV) and DXR-TSL (SUV) were rapidly cleared from the circulation and concomitantly accumulated in the RES (liver), showing that liposomes of this lipid composition and size range are readily taken up by the RES. The clearance of DXR-TSL (SUV) was slower than that of DXR-TSL (LUV), which corresponded to a difference of 100 nm in mean liposomal diameter. On the other hand, the inclusion of 3 mol% of DSPE-PEG significantly increased the blood concentration of TSL over a long period, and decreased RES uptake. More importantly, only DXR-PEG-TSL (SUV), which showed the most prolonged circulation, accumulated in solid tumor tissue with high efficiency (Fig. 3).

Blood and tissue-associated DXR concentrations were measured at the terminal time points of two different schedules (schedule A or B), with or without local hyperthermia for 20 min after injection of thermosensitive liposomes. As shown in Fig. 4, blood DXR after administration of DXR-PEG-TSL (LUV) or DXR-PEG-TSL (SUV) was significantly increased as compared with that after DXR-TSL (LUV), DXR-TSL (SUV) or free DXR. DXR-TSL (SUV) and DXR-TSL (LUV) showed high

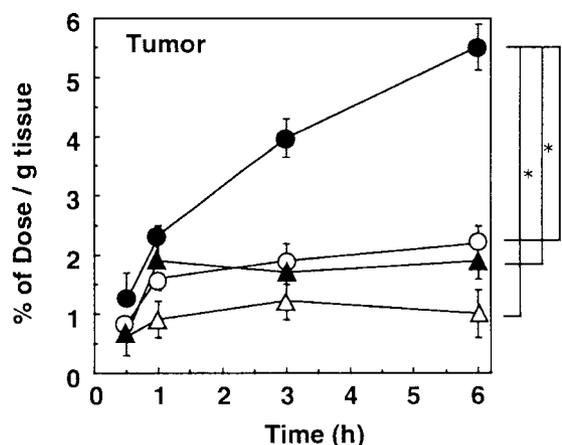


Fig. 3. Time-course of tumor accumulation of (Δ) DXR-TSL (LUV), (\circ) DXR-PEG-TSL (LUV), (\blacktriangle) DXR-TSL (SUV) and (\bullet) DXR-PEG-TSL (SUV) in mice bearing Colon 26 solid tumors without hyperthermia. Tumor-bearing mice were injected i.v. with 0.2 ml of ^{67}Ga -labelled liposomes. Data are expressed as mean \pm SD ($n=3-5$). * $P<0.001$.

RES uptake, whereas DXR-PEG-TSL (SUV) and DXR-PEG-TSL (LUV) showed less uptake. There was no marked difference in the accumulation of DXR in the heart among the free drug and liposomal formulations. When hyperthermia was applied, blood concentrations of DXR decreased most with DXR-PEG-TSL (SUV), which indicated that DXR was released from PEG-TSL *in vivo* in a thermosensitive manner, followed by rapid clearance of the released DXR. There were no marked differences in tissue concentrations of DXR between groups with and

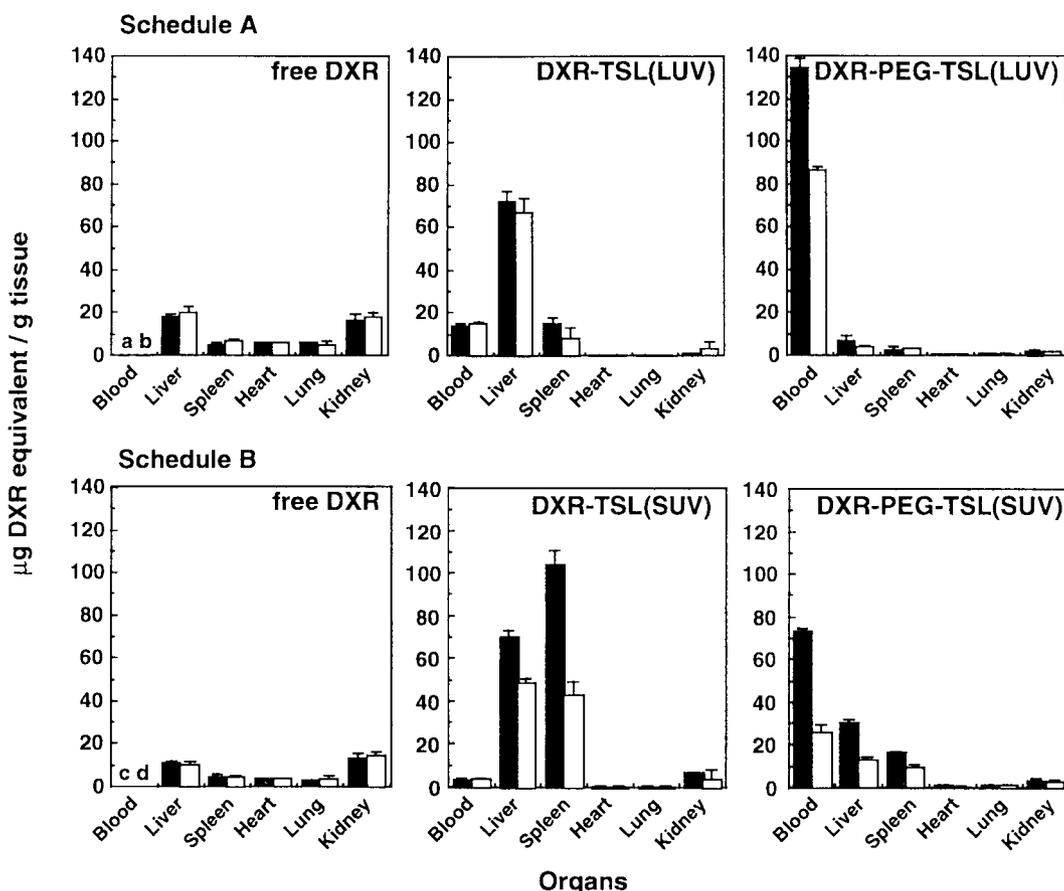


Fig. 4. Tissue DXR levels after administration of free DXR or DXR-thermosensitive liposomes to mice bearing Colon 26 solid tumors with (□) or without (■) local hyperthermia at different times after injection (schedule A or B). DXR solution or liposomal DXR was injected via the tail vein at a dose of 5 mg/kg. Local hyperthermia at 42°C was applied at the tumor site for 5 min (schedule A) or 3 h (schedule B) after drug administration and was continued for 20 min. DXR concentrations in major organs were determined at 5 min after termination of hyperthermia. a, b, c and d are 0.072, 0.071, 0.023 and 0.023 µg/ml, respectively. Data are expressed as mean±SD (n=3–5).

without hyperthermia. Thus, except for hyperthermia-induced release of DXR in blood, the tissue distribution characteristics of DXR reflected the liposome distribution.

The tumor-associated DXR concentrations with or without hyperthermia are summarized in Fig. 5. Without hyperthermia, there was no marked difference in tumor accumulation of DXR at 30 min after injection between DXR-TSL (LUV) and DXR-PEG-TSL (LUV). Hyperthermia treatment with schedule A supplied higher amounts of DXR to the tumor tissue. In this case, DXR concentrations were determined at 5 min after termination of hyperthermia. The value of 9.8 µg/g tissue was obtained from the system of DXR-PEG-TSL (SUV), and this was 5.0-fold higher than that of DXR-TSL (LUV) and hyperthermia, and 6.7-fold higher than that of free drug solution and hyperthermia. These concentrations with the liposomal

systems were, of course, much higher than those obtained without hyperthermia. Since amphipathic PEG prolongs the circulation time of liposomes, thereby increasing the amount of liposomes which pass the heated site, the amount of DXR released from PEG-TSL at that site is expected to be higher than that from TSL.

On the other hand, the system of DXR-PEG-TSL (SUV) and hyperthermia at 3 h after injection (schedule B) showed the highest DXR concentration in solid tumor, 12.2 µg/g tissue. In the case of no hyperthermia, a relatively high DXR concentration (7.9 µg/g tissue) in solid tumors was obtained from DXR-PEG-TSL (SUV) at 3 h after injection. Thus DXR concentrations in tumor tissue were increased 1.5-fold after hyperthermia treatment for 20 min. Based on this result and those shown in Fig. 3, intact DXR-PEG-TSL (SUV) appeared to pass fairly

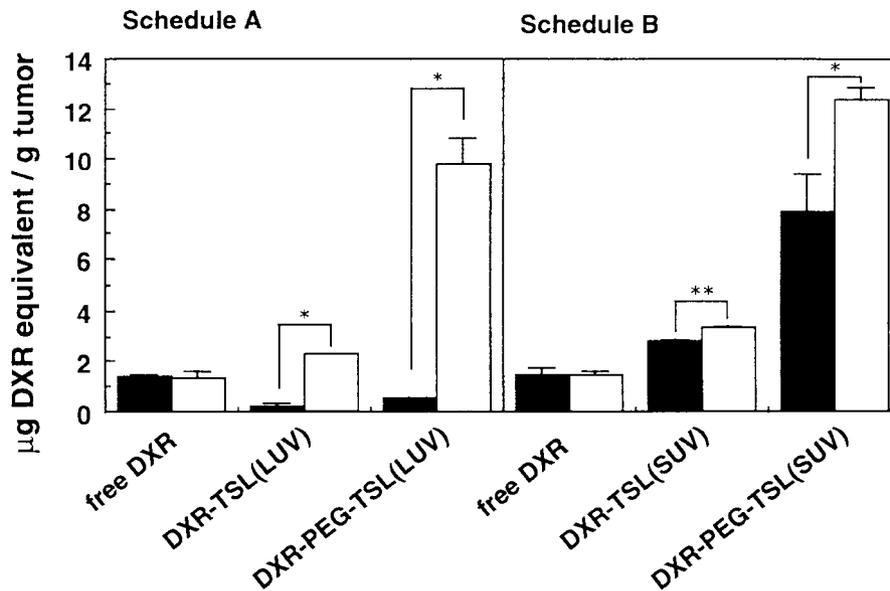


Fig. 5. Tumor concentrations of DXR after administration of free DXR or DXR-thermosensitive liposomes to mice bearing Colon 26 solid tumors, with (□) or without (■) local hyperthermia at the tumor site. For details, see the legend to Fig. 4. * $P < 0.001$, ** $P < 0.01$.

readily through the leaky tumor endothelium by passive convective transport. Furthermore, the greater part of injected DXR-PEG-TSL (SUV) still remained in blood at 3 h after injection and passed the heated site, due to the prolonged circulation time resulting from the DSPE-PEG coating. Thermosensitive release from circulating PEG-TSL (SUV) and the extravasated complex presumably resulted in this higher DXR concentration in tumors.

Tumoricidal effect The antitumor activities of free DXR and thermosensitive liposomal DXR with hyperthermia are summarized in Fig. 6. The apparatus used in hyperthermia was specially modified for small animals and was able to heat locally the tumor region inoculated in a hind foot of mice. No morphologic change was seen in the tumor region during hyperthermia treatment (42°C , 20 min). Hyperthermia alone (without administration of drugs) caused retardation of tumor growth but gave no improvement in survival time, compared with controls. The system of free drug (5 mg DXR/kg) and hyperthermia was ineffective, and gave the same result as heating alone. On the other hand, both schedule A and B, which are combinations of systemic liposomal DXR injection and local hyperthermia, effectively retarded tumor growth and increased survival time. Results with long-circulating DXR-TSL were significantly better. DXR-PEG-TSL (SUV) and hyperthermia in schedule B showed more effective tumor retardation (and concomitantly increased survival time) than did DXR-PEG-TSL (LUV) and hyperthermia in schedule A. These results correspond well with

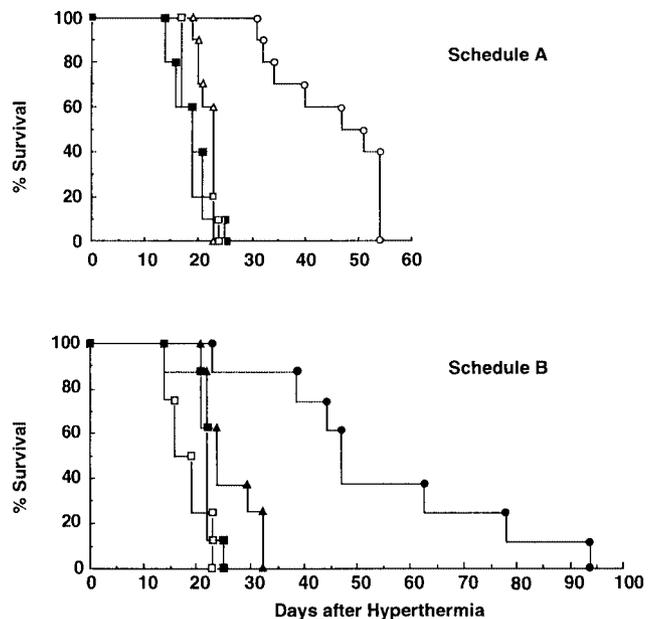


Fig. 6. Survival curve of mice bearing Colon 26 solid tumors after i.v. injection of 5 mg DXR/kg of free DXR or DXR-thermosensitive liposomes and hyperthermia with schedule A or B. For schedule A or B, local hyperthermia at 42°C for 20 min was applied at the tumor site at 5 min or 3 h after drug administration, respectively. □ saline, ■ free DXR, △ DXR-TSL (LUV), ○ DXR-PEG-TSL (LUV), ▲ DXR-TSL (SUV), ● DXR-PEG-TSL (SUV).

those in Figs. 3 and 5. They show clearly that DXR incorporated in small-sized long-circulating thermosensitive liposomes in combination with hyperthermia for an appropriate time, sufficient to permit extravasation to the tumor tissue, causes a marked improvement in therapeutic efficacy, by inhibiting tumor growth and prolonging survival.

DISCUSSION

Although numerous anticancer drugs have been developed, solid tumors in general respond poorly to treatment. There is a need to find ways by which anticancer drugs can be selectively targeted to tumors. Solid tumors generally possess the following pathophysiological characteristics, (a) hypervascularity; (b) incomplete vascular architecture; (c) secretion of vascular permeability factors that stimulate extravasation within the cancer; (d) little drainage of macromolecules and particles, which results in their long-term retention in cancer tissue.¹⁰⁻¹² These characteristics of solid tumors are believed to be the basis of the so-called EPR effect (enhanced permeability and retention effect). Thus, the permeability of the endothelial barrier in newly vascularized tumors is increased compared with that of healthy tissues. Normal tissues outside the RES are generally known to have continuous and non-fenestrated vascular endothelia, and extravasation of liposomes is very limited.

Generally, extravasation of circulating molecules into tissues is a function of local blood flow and microvascular permeability. In this study, we designed long-circulating thermosensitive liposomes that can be carried to the targeted solid tumour *in vivo*. It is possible that small-sized liposomes with prolonged circulating half-lives could predominantly pass through the leaky tumor endothelium by passive convective transport.^{23, 24} It is conceivable that PEG-TSL (SUV) could also take advantage of the EPR effect to ensure that the drug stays longer in the vicinity of the tumor. Indeed, small-sized DXR-PEG-TSL (SUV) were able to traverse the endothelium of blood vessels in tumors and pass into interstitial spaces during circulation for 3 h after injection, as shown in Fig. 3. As demonstrated in Fig. 5 (right panel) and Fig. 6 (bottom panel), the highest DXR concentration in tumor tissue and a significantly increased survival time were achieved under schedule B. These results indicate that since a much larger amount of DXR-PEG-TSL (SUV) would be extravasated into tumor tissue, the incorporated drug was released from the extravasated liposomes in the interstitial spaces of solid tumors by local hyperthermia. Furthermore, large amounts of DXR-PEG-TSL (SUV) are still circulating in tumor capillaries 3 h after injection, when hyperthermia is applied. These processes are illustrated in Fig. 7. Thus, the EPR effect and hyperthermia caused a marked improvement in therapeutic efficacy, inhibiting the tumor growth.

On the other hand, such extravasation occurs less readily with DXR-PEG-TSL (LUV). Liposomes with thermosensitivity and long-circulating half-lives, however, have a greater chance to release entrapped drug at a hyperthermic site. The present results clearly indicate that the use of DXR-PEG-TSL (LUV) in combination with local hyperthermia could deliver a higher drug concentration to the solid tumor (left panel in Fig. 5) and resulted in superior therapeutic efficacy as compared with the PEG-lacking thermosensitive liposomal system used in the past (upper panel in Fig. 6). Although there was no difference in the release rate of DXR at 42°C between DXR-TSL (LUV) and DXR-PEG-TSL (LUV), the drug concentration in the tumor provided by DXR-PEG-TSL (LUV) was 3–3.5 times higher than that by TSL (LUV). A much larger amount of long-circulating TSL (LUV) would pass the heated site during hyperthermia for 20 min compared with TSL lacking PEG. Therefore a larger amount of DXR would be released in capillaries of the tumor mass from long-circulating TSL by hyperthermia, and would enter the tumor tissue by simple diffusion. Since DXR itself can diffuse out of blood vessels and reach the solid tumor tissue effectively, a much-enhanced antitumor effect was obtained in this system. DXR is a drug showing a strong association between dose and antitumor response. Further, it is known that DXR cytotoxicity is enhanced at elevated temperature.²⁵ Therefore, the hyperthermic effect, in com-

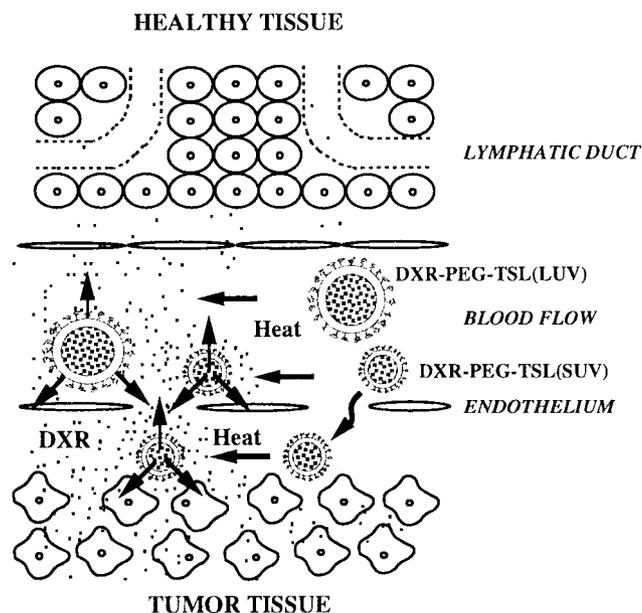


Fig. 7. Schematic diagram of extravasation of small-sized, long-circulating thermosensitive liposomes through capillaries and DXR release from long-circulating thermosensitive liposomes by local hyperthermia at the tumor site.

bination with the thermal sensitization of DXR and its higher concentration in the tumor mass, most probably delivered lethal damage to the tumor cells.

The mechanism by which PEG prolongs the circulation time of liposomes has not been fully clarified. However, increased hydrophilicity²⁶⁾ and/or steric barriers on the liposomal surface (3) might be important. A hydrophilic surface may prevent or reduce the interactions of liposomes with serum constituents, resulting in an enhanced stability of liposomes in the blood and a reduced rate of RES interaction. The inclusion of PEG endowed thermosensitive liposomes with a long-circulation character and did not interfere with the temperature-dependent DXR release.

In summary, we have designed a new preparation of long-circulating, thermosensitive liposomes which can be extravasated to a solid tumor *in vivo*. The use of long-circulating thermosensitive liposomes and local hyperthermia resulted in a higher drug distribution to the tumor and

more effective retardation of tumor growth than the usual thermosensitive liposomal system. This system is expected to be clinically useful, not only for thermal chemosensitization of DXR, but also for the delivery of a wide range of chemotherapeutic agents in the treatment of solid tumors.

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