Pharmacokinetics and Antitumor Effect of Doxorubicin Carried by Stealth and Remote Loading Proliposome

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Purpose. The aim of the study was to prepare stealth and remote loading proliposome (SRP-L) to carry doxorubicin (DXR) and evaluate the pharmacokinetics, acute toxicity, and anticancer effect of DXR carried with SRP-L.

Methods. SRP-L was transparent solution. When SRP-L was injected into 0.9% NaCl aqueous solution containing DXR, liposomes formed and automatically loaded DXR (SRP-L-DXR). The long circulation of SRP-L-DXR was evaluated using the pharmacokinetics of SRP-L-DXR, cardiolipin liposomal DXR (CL-DXR) and free DXR (F-DXR). The acute toxicity and anticancer effect of SRP-L-DXR were evaluated in C57BL/6 mice and murine hystocytoma M5076 tumor model.

Results. The average diameter of SRP-L-DXR in pure water was 112.9 ± 8.6 (nm) and the encapsulation efficiency of SRP-L-DXR was 96.5 ± 0.2% in pure water, 95.5 ± 0.1% in 5% glucose and 98.01 ± 0.6% in 0.9% NaCl. The plasma concentration of SRP-L-DXR was much higher than those of F-DXR and CL-DXR. Compared with that of F-DXR, the SRP-L-DXR had lower acute toxicity and its anticancer effects depended upon the therapeutic treatment.

Conclusions. A novel proliposome (SRP-L) was developed, which could automatically load DXR and form SRP-L-DXR with excellent characteristics. SRP-L-DXR had lower acute toxicity but was not always more effective for the treatment of the ascitic M5076 than F-DXR.

KEY WORDS: stealth and remote loading proliposome; doxorubicin; pharmacokinetics; acute toxicity; anticancer effect.

INTRODUCTION

Doxorubicin (DXR) hydrochloride is one of the most widely used anticancer agents because of its broad spectrum of anticancer activity, reasonable therapeutic index, and intriguing biological and physicochemical actions. The major problem in cancer chemotherapy with DXR is the toxic responses, among of which cardiomyopathy is the most serious. To reduce the toxic responses and increase the anticancer effect of DXR, liposomes were selected to carry DXR as early as the late 70's (1). Liposomes are colloidal particles in which a lipid bilayer membrane, composed from self-assembled lipid molecules encapsulates part of the aqueous phase in which they are dispersed (2).

The mechanism responsible for the increased therapeutic effect of DXR involves a variety of factors. It is believed that the increased permeability of tumor vasculature is the main factor, which results in the accumulation of liposomal anticancer agents in tumor tissue (3,4). Therefore, the optimistic liposomal drug delivery system (DDS) has to meet the following requirements: 1. Particle size is small enough for liposomes to pass through the vasculature of cancer tissue and get in touch with tumor cells; 2. High encapsulation efficiency; 3. In vivo stable enough to bypass the uptake of mononuclear phagocytic system (MPS). At present, particle size distribution of DXR liposomes is controlled by extruders, encapsulation efficiency of DXR is increased by transmembrane ammonium sulfate gradient loading method and polyethylene glycol derivative of distearoylphosphatidyl ethanolamine (PEG-DSPE) is used to protect DXR liposomes (Stealth liposomes) circulating in the blood stream (5,6,7).

To simplify the preparation method of Doxil and meet the three requirements highlighted above, we designed a kind of stealth and remote loading proliposome (SRP-L). When the SRP-L was mixed with 0.9% NaCl aqueous solution containing DXR, liposomes formed and automatically loaded DXR (SRP-L-DXR). The SRP-L-DXR can circulate for a long period in the blood stream following i.v. administration. Oleic acid sodium salt was used to decrease the particle size of liposomes and increase the encapsulation efficiency of DXR, instead of extruders and ammonium sulfate gradient method.

The aim of the research was to evaluate the anticancer effects of SRP-L-DXR in the mouse murine ascitic histiocytoma M5076 tumor model and study the pharmacokinetics of SRP-L-DXR through the comparison with those of F-DXR and cardiolipin liposomal DXR (CL-DXR). The formula of SRP-L is different from Doxil and the commercial empty liposomes. Cardiolipin liposome was one of the typical conventional liposomes used to carry DXR and therefore was selected in the comparison experiment although it was confirmed that it could not increase the anticancer effect of DXR (8,9). DXR is sometimes directly injected into the bladder to kill cancer cells there. Therefore, the ascitic M5076 tumor model and i.p. injection of SRP-L-DXR was selected to evaluate the direct cancer cell killing effect of DXR.

MATERIALS AND METHODS

Chemicals, Animals, and Tumor

Soybean phosphatidylcholine (SPC) was kindly supplied by Fuda Pharmaceutical Company (Shanghai, China), polyethylene glycol derivative of distearoylphosphatidyl ethanolamine (PEG-DSPE, mean molecular weight of PEG: 2000) from NOF Co. Ltd. (Tokyo, Japan) and oleic acid sodium salt from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Cholesterol, cardiolipin and stearylamine were purchased from Sigma (St. Louis, MO.). DXR hydrochloride was obtained from Kyowa Hakko Kogyo

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ABBREVIATIONS: stealth and remote loading proliposome, SRP-L; doxorubicin, DXR; doxorubicin carried with stealth and remote loading proliposome, SRP-L-DXR; cardiolipin liposomal doxorubicin, CL-DXR; free doxorubicin, F-DXR; polyethylene glycol derivative of distearoylphosphatidyl ethanolamine, PEG-DSPE; soybean phosphatidylcholine, SPC; zeta potential, ζ; encapsulation efficiency, E.E.; increase in life span; ILS; systemic clearance, CL; distribution volume at steady state, Vss; mean residence time, MRT; half life, $T_{1/2}$.

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Co. Ltd. (Tokyo, Japan). Chemicals for HPLC were of HPLC grade and the other chemicals were of analytical grade.

The animals used were 18–20 g male C57BL/6j mice and supplied by Tokyo animal experiment center (Japan). All mice used in the study were raised and kept in SPF animal laboratory.

The murine histiocytoma M5076 tumor cells were supplied by the Cancer Chemotherapy Center of Japanese Foundation for Cancer Research (Tokyo, Japan). After 4 transplant generations, the tumor cells were used in this study.

Preparation of SRP-L and CL-DXR

Preparation of SRP-L was based upon the ethanol injection method (10). The required amounts of SPC, cholesterol, PEG-DSPE and oleic acid sodium salt were dissolved in mixed solvent of ethanol:glycerin (3:1, v/v), sterilized by filtration, filled into ampule (2 ml/ampule) and sealed after oxygen was driven out with aseptic nitrogen gas. Each ampule contained 200 mg SPC, 40 mg PEG-DSPE, 30 mg oleic acid sodium salt and cholesterol was used to carry 30 mg DXR hydrochloride. The whole preparation procedure was finished in clean bench and all materials were pyrogen-free and sterilized. When the proliposome was injected into 0.9% NaCl, 5% glucose or pure water containing DXR hydrochloride, liposomes formed and automatically loaded DXR.

Cardiolipin liposomes were prepared as described in literature (11). Briefly, 5.6 μmole cardiolipin was complexed to 11.2 μmole DXR in methanol solution and then the mixture was evaporated to dryness under N_2 gas. To this dried mixture were then added 28.5 μmole SPC, 19.5 μmole cholesterol and 11.1 μmole stearylamine in chloroform solution. The mixture was evaporated to dryness under N_2 gas. The dried lipids were resuspended in 6 ml of 0.01 M phosphate buffer with 0.85% NaCl (pH 7.4) and sonicated in ice water 10 min. 3 times. The free DXR (F-DXR) was separated from the liposomal form by supercentrifugation method.

Characterization of SRP-L-DXR

The particle size distribution and the zeta potential (ζ) (mv) of SRP-L-DXR were determined using the laser light scattering instrument (ELS800, Otsuka Electronics, Japan) and the dynamic light scattering method. The shape of SRP-L-DXR was observed using the scanning electronic microscope (Model, JSM-T200, JEOL Co. Ltd., Tokyo, Japan). The encapsulation efficiency of SRP-L-DXR was determined using the Sephadex G50 column method. The SRP-L-DXR was separated from the F-DXR using the Sephadex G50 column and different mobile phases including 0.9% NaCl, 5% glucose and pure water. The DXR in the free fraction and the SRP-L-DXR fraction was determined using the HPLC method (12) described in "pharmacokinetics." The encapsulation efficiency was calculated according to the following equation:

$$\text{Encapsulation Efficiency} = (A_1/A_t) \times 100\%,$$

where A_1 was the amount of DXR in the liposomal form and A_t was the amount of total DXR.

Acute Toxicity and Anticancer Effect

To evaluate the acute toxicity of SRP-L-DXR, the mice used were inoculated 1×10^6 M5076 tumor cells per mouse i.p. and divided by 20 mice per group. The tumor cells were removed from normal donor mice under the shortly acting methoxyflurane inhalation anaesthetic state. Five days later after inoculation, the mice were administered F-DXR and SRP-L-DXR i.v. in different doses. The acute toxicity of F-DXR and SRP-L-DXR was reflected in the survival rate of the mice of each group.

To evaluate the antitumor effect of SRP-L-DXR, the mice were inoculated the M5076 tumor cells using the method above and divided by 10 mice per group. When the treatment was i.v. injection in a single dose five days after inoculation, two doses were established, 5.6 and 8.3 mg DXR/kg, respectively. When the treatment was i. p., the mice were administered SRP-L-DXR and F-DXR ten days after inoculation and the doses were 2.8 and 5.6 mg DXR/kg, respectively. Increase in life span (ILS) was calculated according to the following equation: ILS = (T/C − 1) × 100%, where T and C are the median survival time of treated mice and control mice, respectively and statistically analyzed using the non-parametric Williams-Wilcoxon test.

Pharmacokinetics

C57BL/6 mice (male, 18–20 g) were selected to evaluate the pharmacokinetics of SRP-L-DXR. The mice were fasted 12 hr with only water allowed and three mice were used for each time point. DXR of different formulations was administered i. v. through the tail vein and the dose was 5 mg DXR/kg. The blood samples were collected following the decapitation method and immediately centrifuged at 4000 rpm for 10 min. The plasma was separated and kept at −20°C. The extraction of DXR from plasma and the determination of DXR were carried out according to previous reports (12). The HPLC system composed of LC-10AS pump (Shimadzu Co., Ltd., Japan), SIL-10A auto injector (Shimadzu Co., Ltd., Japan), RF-10AXL fluorescence detector (Ex = 470 nm, Em = 585 nm, Shimadzu, Japan) and a YMC-Pack ODS-A, 150 × 6.0 mm, I.D., S-5 μm, 120A column (YMC Co., Ltd., Japan). The mobile phase was 1/15M KH_2PO_4:CH_3CN = 75:25 (V/V, pH = 4.16, adjusted with H_3PO_4) and the flow rate was 1.0 ml/min. The concentration of DXR in each sample was determined with a constructed calibration curve. Data were analyzed with the nonlinear least-squares data fitting program (13).

RESULTS

Characterization of SRP-L-DXR

The average diameter, encapsulation efficiency and zeta potential of SRP-L-DXR in different dilutent are shown in Table I. The results in Table I indicate that the average diameter of

Table I. Average Diameter (A.D.), Encapsulation Efficiency (E.E.), and Zeta Potential (ζ) of SRP-L-DXR in Different Dilutent

Dilutent	A.D. (nm)*	E.E.(%)*	ζ (mv)*
H_2O	112.9 ± 8.6	96.5 ± 0.2	−44.2 ± 10.6
5% glucose	115.2 ± 9.4	95.5 ± 0.1	−29.6 ± 2.2
0.9%NaCl	129.0 ± 1.9	98.1 ± 0.6	−13.9 ± 9.2

* Mean (n = 3) ± standard deviation.

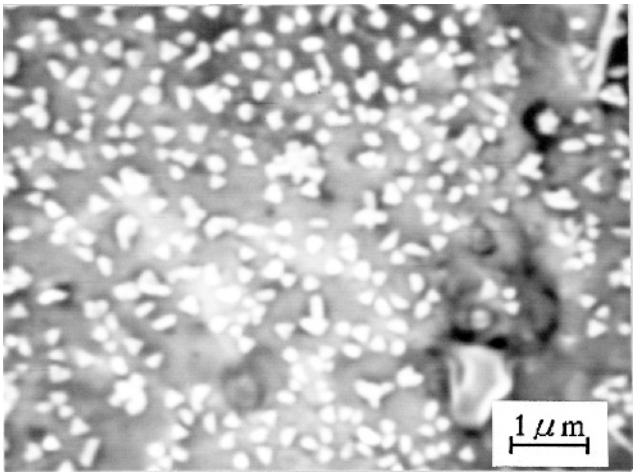

Fig. 1. Scanning electronic photographs of SRP-L-DXR. The bar represents 1 μm.

SRP-L-DXR was 112.9 ± 8.6 (nm) in pure water, 115.2 ± 9.4 (nm) in 5% glucose and 129.0 ± 1.9 (nm) in 0.9% NaCl (n = 3). The encapsulation efficiency of SRP-L-DXR was 96.5 ± 0.2% in pure water, 95.5 ± 0.1% in 5% glucose, 98.1 ± 0.6% in 0.9% NaCl. The zeta potential of SRP-L-DXR was −44.2 ± 10.6 (mv) in pure water, −29.6 ± 2.2 (mv) in 5% glucose and −13.9 ± 9.2 (mv) in 0.9% NaCl. The shape of SRP-L-DXR is shown in Fig. 1. Figure 1 indicates that the particle size distribution of SRP-L-DXR is homogenous.

Toxicity and Antitumor Effects

The acute toxicity of F-DXR and SRP-L-DXR is presented in Table II. Results in Table II indicate that the acute toxicity of SRP-L-DXR was significantly decreased compared with that of F-DXR. In addition, the maximal tolerance dose of SRP-L-DXR was close to 15.0 mg/kg and the maximal tolerance dose of F-DXR was close to 8.3 mg/kg.

The anticancer effect of F-DXR and SRP-L-DXR is represented in Tables III–IV. The results in Table III indicate that in the ascitic M5076 tumor model, at the dose of 5.6 mg/kg administered i.v., the ILS of F-DXR group was 34.9% while the ILS of SRP-L group was 64.6% and at the dose level of 8.3 mg/kg, the ILS of F-DXR group was 39.8% while the ILS of SRP-L group was 72.4%. At both doses, the ILS of SRP-L group was significantly increased compared with that of the F-DXR group. The results in Table IV indicate that in the ascitic M5076 tumor model, at the dose of 2.8 mg/kg administered i.p., the ILS of the F-DXR group was 140.5% while the ILS of the SRP-L-DXR group was 84.6% and at the dose of 5.6 mg/kg the ILS of the F-DXR group was 217.1% while the ILS of the SRP-L-DXR group was 150.8%. Therefore, the direct cancer cell killing effect of SRP-L-DXR was significantly decreased compared with that of F-DXR.

Table II. Death Rates of Ascitic M5076 Tumor-bearing C57 BL/6 Mice within 10 Days Following Administration of F-DXR and SRP-L-DXR in Different Doses Five Days after Inoculation

	Death rates (%)	
Dose (mg DXR/kg)	F-DXR	SRP-L-DXR
5.6	0	0
8.3	10	0
11.3	30	0
15.0	100	10
17.5	100	30

Table III. Antitumor Effect of SRP-L-DXR in the Tumor Model of Ascitic M5076

Agent	Dose of DXR[a] (mg/kg)	MST[b] (days ± SD)	ILS[c] (%)	Lethal toxicity (%)
Control	—	18.1 ± 0.7	—	0
F-DXR	5.6	24.6 ± 0.9	34.9	0
	8.3	25.3 ± 0.9*	39.8	10
SRP-L-DXR	5.6	29.8 ± 2.8	64.6**	0
	8.3	31.2 ± 1.4	72.4***	0

[a] Single i.v. starting 5 days after inoculation through the tail vein of the mice.
[b] Mean survival time of mice of each group.
[c] Increase of life span of each group.
* One animal died within 10 days and not included in the calculation.
** $p < 0.01$, compared with F-DXR (5.6 mgDXR/kg).
*** $p < 0.01$, compared with F-DXR (8.3 mgDXR/kg).

Pharmacokinetics

Plasma clearance of DXR of different formulations following i.v. of a dose of 5 mgDXR/kg through the tail vein of C57BL/6 mice is shown in Fig. 2. The plasma concentration-time curves of DXR were best fitted to triexponential decay curves. Pharmacokinetic parameters of DXR of different formulations are shown in Table V. The CL (systemic clearance) and the Vss (distribution volume at steady state) of SRP-L-DXR were 0.1 (1 hr^{-1} kg^{-1}) and 15.4 (1 kg^{-1}), while those of F-DXR were 2.2 (1 hr^{-1} kg^{-1}) and 156.2 (1 kg^{-1}) and those of CL-DXR were 0.5 (1 hr^{-1} kg^{-1}) and 40.2 (1 kg^{-1}). The AUC (area under the plasma concentration—time curve from 0 time to infinity) of SRP-L-DXR was 39.7 (μg ml^{-1} hr), while those of F-DXR and CL-DXR were 2.2 (μg ml^{-1} hr) and 10.0 (μg ml^{-1} hr), respectively. The MRT (mean residence time) of SRP-L-DXR was 122.3 (hr) while those of F-DXR and CL-DXR were 69.8 (hr) and 80.5 (hr), respectively. However, the $T_{1/2\gamma}$ (half-life of γ phase) of both SRP-L-DXR and CL-DXR was

Table IV. Direct Cancer Cell Killing Effect of F-DXR and SRP-LDXR in Ascitic M5076 Tumor Model

Agent	Dose of DXR[a] (mg/kg)	MST[b] (days ± SD)	ILS[c] (%)	P[d]
Control	—	17.5 ± 1.08	—	—
F-DXR	2.8	42.1 ± 4.45	140.5*	<0.01
	5.6	55.5 ± 5.35	217.1**	<0.01
SRP-L-DXR	2.8	32.3 ± 3.67	84.6	<0.01
	5.6	43.9 ± 5.88	150.8	<0.01

[a] Single i.p. starting 10 days after the inoculation of ascitic M5076.
[b] Mean survival time of each group of mice.
[c] Increase in life spans of each group of mice.
[d] Compared with control.
* $P < 0.01$, compared with SRP-L-DXR (2.8 mg/kg).
** $P < 0.01$, compared with SRP-L-DXR (5.6 mg/kg).

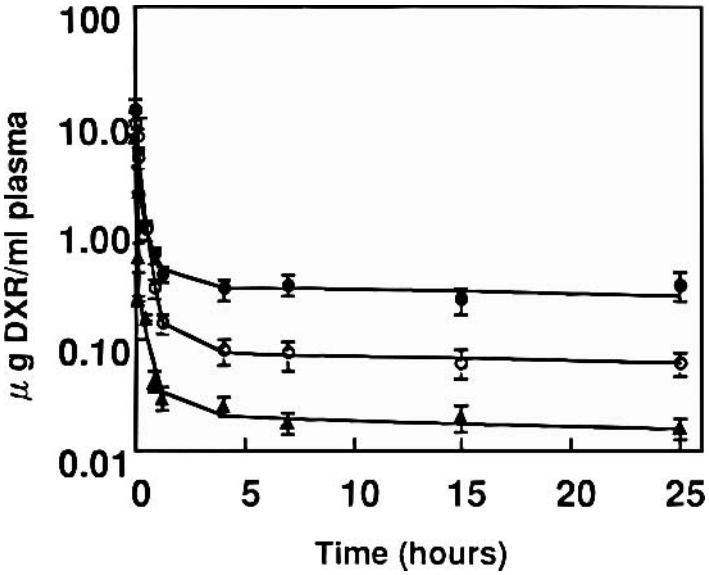

Fig. 2. Plasma concentration-time curves of F-DXR (▲), CL-DXR (◯), and SRP-L-DXR (●) (mean ± SD, n = 3). Sampling time was 0.017, 0.083, 0.167, 0.5, 0.833, 1.167, 4, 7, 15, and 25 (hr) following i.v. at a dose of 5 mg DXR/kg.

70.4 (hr) in this experiment, while that of F-DXR was 59.5 (hr). These results indicate that SRP-L-DXR was more stable and circulated for a longer time in the blood circulating system than F-DXR and CL-DXR.

DISCUSSION

The concept of proliposome was introduced into liposome technology more than 10 years ago with the aim to overcome the disadvantages of liposomal DDS (14). Conventional proliposome can be stored in dry and sterilized state, which can then be dispersed/dissolved by adding water before use to form an isotonic multilamellar liposomal DDS suitable for i.v. or other administration routes. In conventional proliposome, there are three main problems: large particle size distribution, low encapsulation efficiency and short circulation time in the blood stream following i.v. injection. To overcome the problems above, we combined the proliposome technology, the sterically stabilized liposome technology and remote-loading technology together and developed a novel kind of proliposomal DDS, i.e., stealth and remote loading proliposome (SRP-L). In our SRP-L, PEG-DSPE was also used to stabilize liposomes while circulating in the blood stream.

Table V. Pharmacokinetic Parameters[a] of F-DXR, CL-DXR, and SRP-S-DXR

Parameters	F-DXR	CL-DXR	LRP-L-DXR
CL (1 hr^{-1} kg^{-1})	2.2	0.5	0.1
AUC (μg ml^{-1} hr)	2.2	10.0	39.7
V_1 (1 kg^{-1})	0.3	0.5	0.3
V_2 (1 kg^{-1})	1.1	0.1	0.5
V_3 (1 kg^{-1})	154.7	39.5	14.6
V_{ss} (1 kg^{-1})	156.2	40.2	15.4
$T_{1/2}$ α (min)	0.9	8.6	2.0
$T_{1/2}$ β (min)	14.9	56.5	17.3
$T_{1/2}$ γ (min)	59.5	70.4	70.4
MRT (hr)	69.8	80.5	122.3

[a] The following parameters were included: CL, clearance; AUC, area under the plasma concentration-time curve of DXR; V_1, V_2, and V_3 are the apparent distribution volumes of the first, second, and third compartments, respectively; Vss, steady state apparent distribution volume; $T_{1/2}$ α, $T_{1/2}$ β, and $T_{1/2}$ γ are the half-lives of the α, β, and γ phases, respectively; MRT is the mean residence time of DXR in the bloodstream.

SRP-L is transparent lipid solution and different from Doxil, and the commercial empty liposomes used to carry DXR. In our SRP-L, oleic acid sodium salt and glycerin were used to reduce the particle size of liposomes, which was much easier than many other reported methods, such as sonication (15), French pressure cell (16) and filtration using extruders (17). At the same time, oleic acid sodium salt could increase the negative zeta potential of liposomes and therefore the liposomes could automatically load positively charged DXR, which was a novel kind of remote-loading technology and different from the ammonium sulfate gradient method (18) and cardiolipin method (11). The ammonium sulfate gradient method (used in the preparation of Doxil) included the following steps: 1. liposomes were prepared in ammonium sulfate solution by ethanol injection method; 2. liposomes containing ammonium sulfate were separated by supercentrifugation method; 3. then liposomes were resuspended in 10% glucose solution; 4. the liposomes were then incubated for 1 hr at 60°C. The cardiolipin method is shown in "Material and Methods." Therefore, the preparation method of SRP-L was much easier than that of Doxil and the conventional cardiolipin liposomes.

This study shows that when SRP-L was mixed with 0.9% NaCl, 5% glucose or pure water containing DXR, liposomes formed and automatically loaded DXR. The particlesize distribution was within the range of 80–200 nm and the encapsulation efficiency was close to 100%. In the SRP-L-DXR, oleic acid sodium salt has negative charge and can be combined with DXR of positive charge, which results in the high encapsulation

efficiency. Oleic acid sodium salt has higher HLB (hydrophile-lipophile balance) value than that of phospholipid and therefore the HLB value of the SRP-L-DXR membrane is increased when oleic acid sodium salt is added, which maybe results in the small particle size distribution of the SRP-L-DXR. Compared with the conventional cardiolipin liposomes and the stealth liposomes, the preparation process is much easier and therefore the cost will be significantly reduced. Our SRP-L is suitable for the DXR clinically used and can be used to carry other positive charged anticancer agents (at least 17 kinds of anticancer agents are positive charged ones).

The present work shows that the acute toxicity of SRP-L-DXR was significantly decreased compared with that of F-DXR. In the toxicity experiment, the ascitic M5076-bearing C57BL/6j mice were used to evaluate the acute toxicity of SRP-L-DXR because the DXR tolerance dose of tumor-bearing mice will be different from that of non-tumor bearing mice. Clinically used DXR is often administered at multiple doses. Under this condition, the long circulation can induce the superposition of DXR in the heart or other organs, which will result in the increase of the acute toxicity of DXR (19). Therefore, further research work is needed to evaluate the acute toxicity of SRP-L-DXR when administered at multiple doses.

The therapeutic experiments indicate that the anticancer effects of SRP-L-DXR administered i.v. were significantly increased compared with that of F-DXR in the ascitic M5076 tumor model. The permeability of the vasculature of cancer is much higher than that of normal tissue and the vasculature of cancer tissue will become more leaky with the development of cancer. Under the condition of long circulation, more SRP-L-DXR can pass through the vasculature of cancer tissue and kill cancer cells, which will result in the increased anticancer effects of DXR (20). The therapeutic experiments indicate that when administered i.p., the anticancer effect of SRP-L-DXR was significantly decreased compared with that of F-DXR in the ascitic M5076 tumor model (Table IV). Therefore, SRP-L decreased the direct cancer cell killing effect of DXR. SRP-L-DXR was not the first choice compared with F-DXR for bladder injection therapy.

The results of the pharmacokinetics indicate that the CL of SRP-L-DXR from plasma was significantly reduced compared with that of F-DXR and CL-DXR (Table V). Therefore, SRP-L-DXR can circulate in the blood stream for a longer period after i.v., which can result in greater accumulation of SRP-L-DXR in cancer tissue directly and increase the anticancer effect of DXR. The pharmacokinetic experiment indicates that the CL of CL-DXR was significantly reduced and the AUC and the MRT of CL-DXR were significantly increased compared with those of F-DXR, which was also reported by other authors (11). However, the anticancer effect of CL-DXR was significantly decreased compared with that of F-DXR when the tumor model was not within the mononuclear phagocytic system (MPS) (19,21). In addition, the $t_{1/2\alpha}$ and $t_{1/2\beta}$ of SRP-L-DXR and CL-DXR were less than 1 hour. Therefore, the long circulation seems not to be the only factor responsible for the increase of anticancer effect of SRP-L-DXR.

The cancer cells might be killed by SRP-L-DXR by two mechanisms. One is that DXR was released from SRP-L-DXR and then killed cancer cells. Another is that SRP-L-DXR was taken by cancer cells and then the cancer cells were killed. In the later mechanism, we inferred that when carried by SRP-L, the uptake of DXR by the cancer cells was decreased compared with that of F-DXR, which is supported by the result that the direct cancer cell killing effect of SRP-L-DXR was significantly decreased compared with that of F-DXR. In addition to the long-circulation mechanism, we infer that because the SRP-L-DXR is protected by PEG-DSPE against the destruction of MPS, a bigger liposomal DXR depot might form in the blood circulating system compared with those of F-DXR and CL-DXR (without protection) and therefore more free DXR can be released from the depot and accumulate in cancer tissue to kill cancer cells. Further research work is needed to confirm which mechanism above is more important.

From the discussion above, we come to the conclusions: A novel proliposome (SRP-L) was developed, which could automatically load DXR and form SRP-L-DXR with excellent characteristics; SRP-L-DXR had lower acute toxicity but was not always more effective for the treatment of the ascitic M5076 than F-DXR.

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