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ORIGINAL ARTICLE

Characterization and pharmacokinetics of a novel pirarubicin liposome powder

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Abstract

Background: Pirarubicin (THP), an analogue of doxorubicin, has exhibited promising activities against acute leukemia, malignant lymphoma, and several solid tumors. However, the cumulative cardiotoxicity limits its wide application in chemotherapy. *Method*: To provide an alternative strategy for reducing the cardiotoxicity, a novel THP liposome powder (L-THP), comprising distearoylphosphatidylcholine, distearoylphosphatidylglycerol, cholesterol, and lactose was appropriately prepared based on the physicochemical properties of THP. And L-THP was characterized and evaluated. Comparative studies on pharmacokinetic and biodistribution behaviors between L-THP and commercialized THP injection were performed in normal mice through intravenous administration. *Results*: When L-THP was reconstituted in a proper amount of normal saline for injection, it had a mean diameter of around 220.0 nm, a zeta potential of about −33.0 mV, and a high THP entrapment efficiency of more than 93.1%. Pharmacokinetics study showed that heart accumulation of THP could be reduced by 81.2% for L-THP. *Conclusion*: These results suggest that our L-THP might greatly reduce the cardiotoxicity, thus improving the therapeutic index of THP. Meanwhile, further preclinical studies are warranted to define the cardiotoxicity and the therapeutic efficacy of L-THP.

Key words: *Cardiotoxicity; characterization; liposome powder; pharmacokinetics; pirarubicin*

Introduction

Liposomes, as a colloidal drug-delivery system, possess several distinct advantages such as their biocompatibility, targeting ability, and high encapsulation yields of hydrophobic drugs. By manipulating drug release characteristics, liposomes can modulate in vivo behavior of the entrapped drug in the hydrophobic core, thus lowering the drug-related toxicity and improving the therapeutic index of drugs[1–4]. Actually, liposome products (e.g., Doxil^{TM}, Amphotericin B liposomes), which are well known for their high therapeutic index and low side effects have created a huge market value.

Pirarubicin (4′-*o*-tetrahydropyranyldoxorubicin, THP, structure shown in Figure 1) is a commonly used anthracycline against several solid tumors, acute leukemia, and malignant lymphoma[5]. When THP crosses the cellular membrane by passive diffusion, it can intercalate into the base pairs of DNA, thus inhibiting the replication process of cells[6]. It has been reported that THP can induce much less cardiotoxicity than doxorubicin in animal models[7]. However, studies on women with metastatic breast cancer have indicated that THP can cause a significant decrease in the left ventricular ejection fraction and full-blown congestive heart failure at the cumulative dose of 460 and 500 mg/m^2, respectively[8]. Meanwhile, for elderly patients with non-Hodgkin's lymphoma, THP may cause severe cardiac dysfunction at the cumulative dose of 360 mg/m^2 [9]. Therefore, concern is needed regarding the cumulative cardiotoxicity of THP. Strategies for limiting or preventing anthracycline-induced cardiotoxicity have advanced in at least three different directions: (i) synthesis of analogues of the natural compounds: second- and third-generation anthracyclines; (ii) clinical use of cardioprotective agents; and (iii) development of

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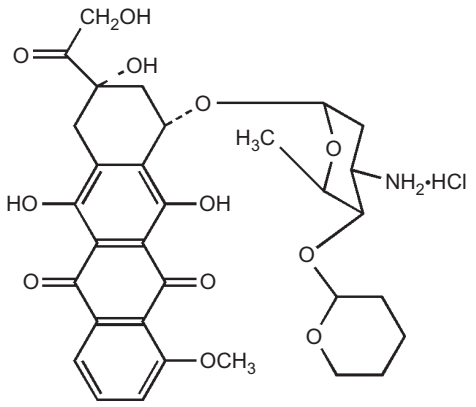

Figure 1. The molecular structure of THP.

tumor-targeted formulations[10]. For the third strategy, liposome drug-delivery-based system exhibits promising future perspectives by limiting the uptake of anthracyclines in the heart tissue while improving the tumor penetration. For instance, the cardiotoxicity of doxorubicin, whose structure is similar to THP can be greatly reduced when encapsulated into the liposomes[11]. Therefore, the prevailing liposome drug-delivery system may provide an alternative solution to the cardiotoxicity of THP.

Distearoylphosphatidylcholine (DSPC) has some unique physicochemical properties, such as a higher transition temperature, resulting in a reduced phospholipid tail movement in bilayers. Therefore, it has gradually been used as the major bilayer component in the liposome formulation in recent years[12,13]. It has also been reported in the literature that liposomes containing DSPC as the major phospholipids are more stable than analogous liposomes containing dipalmitoyl phosphatidylcholine (DPPC)[14]. Meanwhile, due to the rigid structure, DSPC is less flexible than soybean and yolk phospholipids. Therefore, DSPC will surely have different effects on the in vitro behavior of THP liposomes, such as the entrapment efficiency, particle size, zeta potential, and stability. Moreover, it is widely accepted that lipid compositions of liposomes can play a vital role on the in vivo kinetics and biodistribution behavior of encapsulated drugs[15,16]. However, little research was done on the effect of DSPC on the in vitro properties of liposomes or the in vivo behavior of THP.

In this paper, a novel pirarubicin liposome powder (L-THP) was prepared by an appropriate method based on the solubility of THP in different dispersion mediums, and DSPC was selected as the main phospholipid in the liposome formulation. In vitro properties of L-THP, such as the entrapment efficiency, particle size distribution, zeta potential, morphology, and long-term stability were evaluated. Further, different pharmacokinetic and biodistribution behaviors between L-THP and commercialized THP injection (F-THP) were investigated after intravenous injection into normal mice.

Materials and methods

Materials

DSPC (99.5% purity), distearoylphosphatidylglycerol (DSPG, at least 98.5% purity) were purchased from the Lipoid GmbH (Ludwigshafen, Germany). Pirarubicin hydrochloride (THP, 99.5% purity) was obtained from Shenzhen Olympic Star Pharmaceutical Co., Ltd. (Guangdong, China). Cholesterol (Chol) and ethanol were obtained from Beijing Chemical Reagents Company (Beijing, China). Lactose was purchased from Beijing Jingqiu Chemical Industrial Co., Ltd. (Beijing, China). These materials were used without further purification. All organic solvents were of analytical grade. Pathogen-free male mice (20–22 g) were purchased from Animal Experiment Center of Medicine College in Peking University (Beijing, China). Ethics approval was gained from the Institutional Animal Care and Use Committee of Tsinghua University (Beijing, China).

THP solubility measurements

The solubility of THP in water, phosphate buffer (pH 7.4) and 16.0 (w/w) hydroethanolic solution were determined by the solubility method at room temperature and 37°C[17]. In brief, an excess amount of THP was added to 2 mL solvent, the resulting suspensions were vigorously shaken for 72 hours in the oscillation incubator (Haierbin Donglian Electronic Co., Ltd., Harbin, China), and then left to equilibrate. For each group, 20 μL of the saturated supernatant fluid was withdrawn at the predominated time (24 hours, 48 hours and 72 hours). And the corresponding concentrations of THP were further determined by high-performance liquid chromatography (HPLC).

Preparation of L-THP

L-THP was prepared from DSPC, DSPG, Chol, and THP (DSPC:DSPG:Chol:THP = 27:3:10:4, in weight ratio). All the lipids and THP were dissolved in a proper amount of ethanol. Then a fixed amount of water was added. The homogenous solution was formed after the process of microfluidizer (MFIC^{TM} Corporation, Newton, MA, USA). A THP liposome suspension was obtained after the removal of ethanol. Before lactose was added, it was processed again with the microfluidizer. After proper freeze-thawing cycles, it was finally lyophilized.

Measurement of particle size distribution and zeta potential of THP liposomes

The particle size distribution and zeta potential of L-THP were determined by using a dynamic laser light scattering instrument (Zetasizer 3000HS; Malvern Instrument Ltd., Worcs, Britain). L-THP was dissolved with a proper amount of normal saline for injection (concentration of THP was kept at ~1.0 mg/mL), then the particle size distribution and zeta potential of L-THP were determined at room temperature, respectively.

Morphology of L-THP

A proper amount of L-THP was frozen in liquid nitrogen, fractured immediately and vacuum-dried. The cross sections of liposome powder were coated with gold. After affixed with a double conducted graphite tape to an aluminum stub, they were placed into a vacuum chamber. Then samples were imaged by Hitachi SEM S-3000N (Hitachi, Tokyo, Japan). After all the images were collected, the operating mode of scanning electron microscope (SEM) was then switched to electron probemicro-analyzer (EPMA) (silicon solid state detector, Model 7021-H; Horiba, Japan).

Entrapment efficiency and drug loading amounts of L-THP

Liposome suspension was formed when L-THP was added to a proper amount of normal saline for injection. The entrapment efficiency (EE) of L-THP was defined as the ratio of the encapsulated amount of THP in the liposome to the amount of THP in the liposome suspension. The supernatant was separated from the liposome suspension through ultracentrifugation techniques (Beckman Coulter-Optima^{TM} L-100XP, Brea, CA, USA) where the operation mode was 40,000 rpm, 3 hours, 4°C. Both the supernatant and liposome suspension were equally diluted with ethanol and concentrations of THP in the supernatant and liposome suspension were measured by RP-HPLC. The percentage of EE was calculated according to the following equation:

$$\text{EE}\ (\%) = (C_{\text{total}} - C_{\text{free}})/C_{\text{total}} \times 100. \quad (1)$$

where C_{total} is the concentration of THP in the liposome dispersion, and C_{free} represents the concentration of THP in the supernatant.

Stability measurements of L-THP

The stability of L-THP was evaluated by determination of the average particle size and polydispersity index of liposome suspension in time. A detailed procedure was described in the particle size measurement section above.

Pharmacokinetic and biodistribution studies

A hundred and sixty pathogen-free mice (weight ranges from 20.0 to 22.0 g) were used for in vivo pharmacokinetic and biodistribution studies, and the protocol was duly approved by the Institutional Animal Care and Use Committee of Tsinghua University (Beijing, China). L-THP was dispersed in the normal saline for injection, while F-THP was dispersed in water. All mice were fasted for a whole night before the experiment. They were divided into two groups (n = 80): one group was intravenously administrated with L-THP at a dose of 20.0 mg THP/kg body weight, the other group was administrated with F-THP at the same dose by intravenous injection through the tail vein (i.v.). For each group, 10 mice were used at each predetermined time after i.v. injection (7, 10, 15, 30, 45, 60, 90, and 120 minutes). Blood samples were collected from the retro-orbital plexus under mild ether anesthesia and transferred into heparinized microcentrifuge tubes. Mice were sacrificed and tissues (heart, lung, spleen, liver, and kidney) were immediately collected. Each tissue was washed with normal saline and cleaned with filter paper before extraction and analysis.

Plasma was gained from the blood samples by centrifugation at 1776 × g for 10 minutes at 4°C. To 60 μL of Internal Standard (IS) daunomycin (~5 μg/mL) 300 μL of plasma was added and vortexed for 60 seconds. Extraction was done by adding 3.6 mL of chloroform-methanol (3:1, v/v), vortexed for 3 minutes, and centrifuged at 4000 rpm for 30 minutes at 4°C. Finally, the organic layer was separated and organic solvents were removed in a sample concentrator (Techne; Labway Science Development Ltd., Beijing, China). The residue was reconstituted in 100 μL of ethanol and analyzed using an in vivo HPLC method. For the tissues, 1 mL normal saline was added before the extraction and tissue homogenates were obtained through the treatment of a hand-held homogenizer (FSH-2; Jiangsu Jintan Ronghua Instrument Co., Ltd., Jintan, China) at 10,000 rpm for 1 minute. Then 60 μL of IS was added to the resulting tissue homogenate and vortexed for 2 minutes. Tissue extraction was almost the same as that of plasma, except the extract solvent was 5 mL of chloroform-methanol (4:1, v/v).

HPLC assay for THP

THP was quantified in vitro by HPLC methods. The HPLC system was composed of an LC 2010A pump (Shimadzu Co., Ltd., Kyoto, Japan), a SIL-10A auto sampler, and UV variable detector (Shimadzu Co., Ltd.,

Table 1. THP solubility (mg/mL) in different vehicles at room temperature and 37°C, respectively, and values reported are mean ± SD, $n = 3$.

Solvent	THP solubility at room temperature (mg/mL)	THP solubility at 37°C (mg/mL)
Phosphate buffer pH 7	6.3 ± 0.2	10.7 ± 0.4
Distilled water	8.0 ± 0.6	24.2 ± 1.3
16% (w/w) hydroethanolic solution	28.4 ± 1.5	36.4 ± 2.7

Kyoto, Japan). Separations were performed on a Lichrospher 100 RP-18e column (4 mm × 250 mm) (Merck & Co., Inc., Darmstadt, Germany). The column temperature was kept at 30°C and λ was fixed at 254 nm. The mobile phase was acetonitrile and 0.1 M acetate ammonium (pH 4.0 was adjusted by acetic acid) = 35:65 (v/v), and the flow rate was 1 mL/min. The analytical method was validated in terms of specificity, linearity, reproducibility, and precisions of intraday and interday. The limit of quantification was 0.2 μg/mL.

The in vivo HPLC assay for THP was very similar to the in vitro HPLC assay; besides that a flow rate of 0.8 mL/min and daunomycin (IS) and a C18 guard column (Agilent, Neuherberg, Germany) were used. The limit of detection and quantification for THP was 70.0 and 250.0 ng/mL, respectively. The analytical method was validated in terms of specificity, linearity, reproducibility, precisions of intraday and interday, and recovery value. The RSD of intra- and inter-day were both less than 5.0% over the selected range, and the recovery values of THP and IS were about 95.0–105.0%.

Pharmacokinetic analysis

The pharmacokinetic parameters associated with each mouse were estimated by compartment and noncompartment methods. Nonlinear regression analysis showed that the best model fitting experimental data was a two-compartment open model with first-order input and output from the central compartment. The observed highest plasma or tissue concentration and the corresponding sampling time were defined as C_{max} and t_{max}, respectively. The area under the concentration-time curve ($AUC_{0-\infty}$) was calculated by using the linear trapezoidal rule, and extrapolated to infinity by dividing the last measurable concentration by the terminal rate constant of elimination determined by log-linear regression. The terminal log-linear portion of the plasma or tissue concentration-time curve was defined by least-squares regression of at least three terminal concentrations that yielded the smallest residual mean square error. All the calculations were performed in the Drug and Statistics (DAS) software (ver.2.0) (Drug Evaluation Center of Shanghai University of Traditional Chinese Medicine, Shanghai, China), which is frequently used in the pharmacokinetic analysis[18]. The data were presented as means ± SD.

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2000 Analysis Tool Pack and S-Plus 2000 (MathSoft Inc. Seattle, WA, USA). Statistical differences of the mean values of $AUC_{0-\infty}$ for plasma and tissue between F-THP and L-THP were tested using the two-sided Student t-test with α set to 0.01. A P-value <0.01 was considered statistically significant.

Results and discussion

Solubility of THP

An appropriate dispersion system is very important in the liposome drug-delivery system, as the solubility of drugs in the dispersion medium has a vital role in the entrapment capacity and particle size of liposomes and consequently, the in vivo pharmacokinetic and biodistribution behaviors of drugs[17,19]. Results of THP solubility in different dispersion mediums at room temperature and 37°C are presented in Table 1. It can be observed that the solubility of THP in hydroethanolic solution was about 3.7-folds that of distilled water, which is in good accordance with literature[20]. The high solubility of THP in 16% (w/w) hydroethanolic solution suggests that hydroenthanolic solution is an ideal dispersion medium for preparing THP liposomes. Although THP can be easily dissolved in chloroform, methanol, ether, or other toxic organic solvents should be avoided in the liposome drug-delivery system.

Particle size distribution and zeta potential of L-THP

It is well recognized that size distribution is an important topic in the pharmaceutical application of liposomes[21]. For instance, the optimal size range for parenteral administration is between 70 and 400 nm. Within this size range, liposomes favor the accumulation of drugs into certain target organs such as liver, spleen, and bone marrow. Besides, they also have a good stability in the blood, thus displaying predictable drug-release rates. Moreover, liposomes of more than 400 nm have a greater tendency to aggregate[22]. Data of the particle-size distribution of L-THP are shown in Table 2. It can be observed that the average particle size of THP liposomes was ~220 nm, indicating that L-THP is suitable for parenteral

Table 2. Particle-size distribution, zeta potential, and entrapment efficiency of L-THP, which were prepared from DSPC:DSPG:Chol:THP = 27:3:10:4 (w/w) at lactose:lipid = 5:1 (w/w), and values reported are mean ± SD, $n = 3$.

Batch no.	Average particle size (nm)	Polydispersity index	Zeta potential (mV)	Entrapment efficiency (%)
080529	222 ± 8	0.16 ± 0.05	−33.8 ± 0.2	96.1 ± 0.1
080603	230 ± 10	0.22 ± 0.03	−33.9 ± 0.1	93.5 ± 0.4
080610	224 ± 12	0.28 ± 0.05	−34.0 ± 0.1	97.5 ± 0.3

administration. The low polydispersity index of ~0.2 also suggests that L-THP tends to aggregate less.

It is noted in literature that the instability problem of liposomes should be attributed to the collisions and eventual merging of liposomal membranes of two or more liposomes[23], and it is also widely accepted that increasing the repulsive interactions of liposomes can reduce the collision frequency. Therefore, liposomes composed of charged polar lipids, such as those carrying higher electronic charges, are expected to be more stable than liposomes composed of neutral lipids[24]. In this paper, a small amount of DSPG (DSPC:DSPG = 9:1, w/w) was added to make the whole liposome drug-delivery system more stable. The zeta potential value of L-THP was around –33.8 mV, further indicating that L-THP will be very stable. However, the amount of DSPG is limited as it would result in bigger liposomes (data not shown).

Morphology study on L-THP

The morphology of L-THP was observed through a scanning electron micrograph (Figure 2). It could be seen that L-THP appeared to be buried in a glassy matrix of lactose under the lyophilized state. Sucrose, glucose, lactose, and trehalose were lyophilized protective adjuvants in the former experiment. Lactose was finally selected for its better lyophilized effect and lower price; moreover, it is also very safe as it is an excipient approved by FDA. Therefore, it has been widely used in the preparation of liposome powder[25].

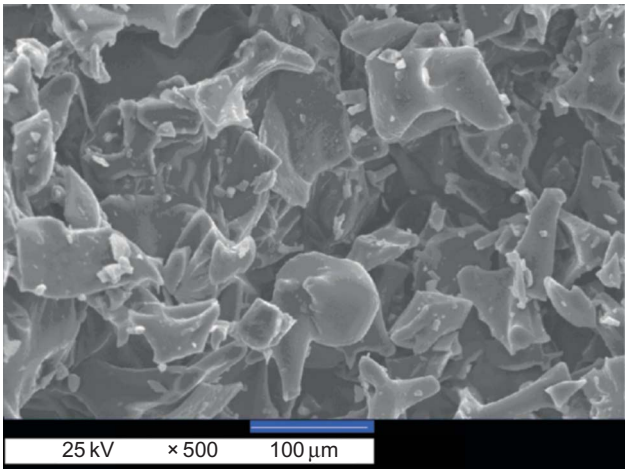

Figure 2. Scanning electron micrograph of L-THP, which was prepared from DSPC:DSPG:Chol:THP = 27:3:10:4 (w/w) at lactose:lipid = 5:1 (w/w) ($n = 3$).

Entrapment efficiency and drug-loading amount of L-THP

Due to the unique closed structure and physicochemical properties of liposomes, a wide variety of functional components can be encapsulated into the interior of liposomes or incorporated into the lipid bilayer membrane or adhered to the vesicles. Entrapment efficiency, a very important parameter in the liposome drug-delivery system, is closely related to the liposome preparation method and formulation[26]. It can be seen in Table 2 that L-THP has a higher entrapment efficiency (>93.1%), which should be attributed to our liposome preparation method and formulation.

During the liposome preparation, microfluidizers were used twice for different purposes. It can be found in literature that the principle of the microfluidic process is that the cross-section area of deep channels can result in a more homogenous velocity and shear force profile, thus facilitating the formation of homogenity[27]. It was first employed in the formation of homogeneous solutions between lipids and THP in the hydroethanolic system. In this procedure, the microfluidic process is favorable to the formation of homogenous droplets, which can self-assemble into liposomes during the solvent removal process, thus benefiting the formation of uniform liposomes. Previously, it was used to reduce the particle size and improve the entrapment efficiency of L-THP, since larger liposomes can be cut down and rearranged in the reaction channel. Therefore, the microfluidic process can play a vital role in improving the entrapment efficiency. Meanwhile, the freeze-thawing process might also improve the particle-size distribution and increase the entrapment efficiency of L-THP, because the Brownian motion can promote the rearrangement of liposomes whether in the freezing or in the thawing stage.

In our previous paper, the interaction between THP and DSPC or DSPG was investigated through calorimetric and spectroscopic studies together with a quantum calculation based on molecular modeling[28]. We found that the acyl chains of DSPC bilayers were significantly disturbed in the presence of THP. This phenomenon suggests that THP can be more easily encapsulated into

Table 3. Stability test for THP liposomes: average particle size and polydispersity index over time, and values reported are mean ± SD, $n = 3$.

Days after preparation	Average particle size (nm)	Polydispersity index
0	218 ± 4	0.18 ± 0.1
1	222 ± 6	0.19 ± 0.2
7	220 ± 5	0.20 ± 0.1
14	222 ± 8	0.20 ± 0.2
30	226 ± 6	0.21 ± 0.2
90	230 ± 7	0.22 ± 0.3
180	226 ± 12	0.22 ± 0.2
365	238 ± 10	0.23 ± 0.2

the bilayers of DSPC and hence changing the mobility of DSPC bilayers. Meanwhile, strong interactions between THP and the polar heads of DSPG might limit the further access of THP into the phospholipids bilayers, indicating that THP might tend to adhere to the vesicles of DSPG. However, the huge amount of DSPC in the liposome formulation implies that most of THP can be encapsulated into the lipid bilayer membrane. Therefore, the former study not only sheds light on the possible location of THP in the liposomes but also confirms the high entrapment efficiency of L-THP from the calorimetric and spectroscopic aspects.

Stability of L-THP

Table 3 presents the results of the average size and polydispersity index of L-THP, in which three batches were kept at room temperature and measured at various time intervals after preparation. It can be seen that the average size and poly index of liposomes did not change significantly over time, and no obvious structural changes were observed during the 1-year period. This suggests that L-THP will be very stable during the storage as compared to the liposome suspensions. Besides, liposome powder is also an appropriate dosage for shipment. The stability test for 2 years of L-THP still continues.

Pharmacokinetic and biodistribution behaviors of L-THP

As the concentrations of THP in the liver were very low for L-THP and F-THP, the results of THP in the liver were not listed in Figure 4 and Table 4 (data not shown). The short circulation time of THP in the plasma for L-THP (in Figure 3) showed a rapid uptake of the drug when encapsulated into the liposomes. Furthermore, the $AUC_{0-\infty}$ value of THP in the plasma (in Table 4) for L-THP was 127.3 ± 7.2 mg min/L ($P < 0.01$) whereas it was 175.6 ± 13.4 mg min/L for F-THP. These results indeed show that the bioavailability of THP in the blood

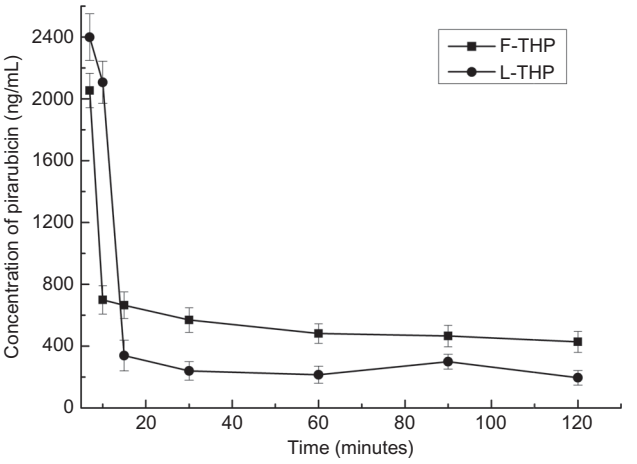

Figure 3. Plasma concentration of THP over time after intravenous injection of F-THP (–■–) and L-THP (–●–)at a dose of 20.0 mg THP/kg body weight, and the data represents the mean ± SD ($n = 10$).

Table 4. Pharmacokinetic parameters of plasma and tissues for F-THP and L-THP after intravenous injection in mice at a dose of 20 mg THP/kg body weight, and values reported are mean ± SD, $n = 10$.

Plasma and tissues	$AUC_{0-\infty}$ for F-THP (mg min/L)	$AUC_{0-\infty}$ for L-THP (mg min/L)
Plasma	127.3 ± 7.2	175.6 ± 13.4*
Heart	717.4 ± 56.7	134.4 ± 10.1*
Spleen	6228.8 ± 450.5	21,775.6 ± 789.2*
Lung	14,291.4 ± 2319.3	18,492.0 ± 3413.4
Kidney	215.8 ± 16.2	441.4 ± 33.4

*$P < 0.01$ versus F-THP.

stream could not be improved when encapsulated into the liposomes. However, it could be obviously observed from Table 4 that the overall bioavailability of THP in the plasma and tissue could still be greatly improved when entrapped into the liposomes. It was noted in Figure 4 that THP had a rapid uptake and short circulation time in the spleen, whereas it had a prolonged circulation time in the lung when encapsulated into liposomes, which is well consistent with literature[29,30].

It was indicated in Table 4 that the biodistribution behavior of THP was greatly modulated when encapsulated into the liposomes. For instance, the accumulation of THP in the heart could be significantly reduced when THP was encapsulated into the liposomes, as the $AUC_{0-\infty}$ value of THP in heart significantly decreased from 717.4 ± 56.7 mg min/L of F-THP to 134.4 ± 10.1 mg min/L of L-THP ($P < 0.01$). It is widely accepted that anthracycline accumulation in the heart can be closely related to its cardiotoxicity. The unifying mechanism of anthracycline-induced cardiotoxicity is the reactive oxygen species (ROS) overproduction and the formation of C-13 alcohol metabolites. In this hypothesis, after one-electron reduction, the quinine moiety of

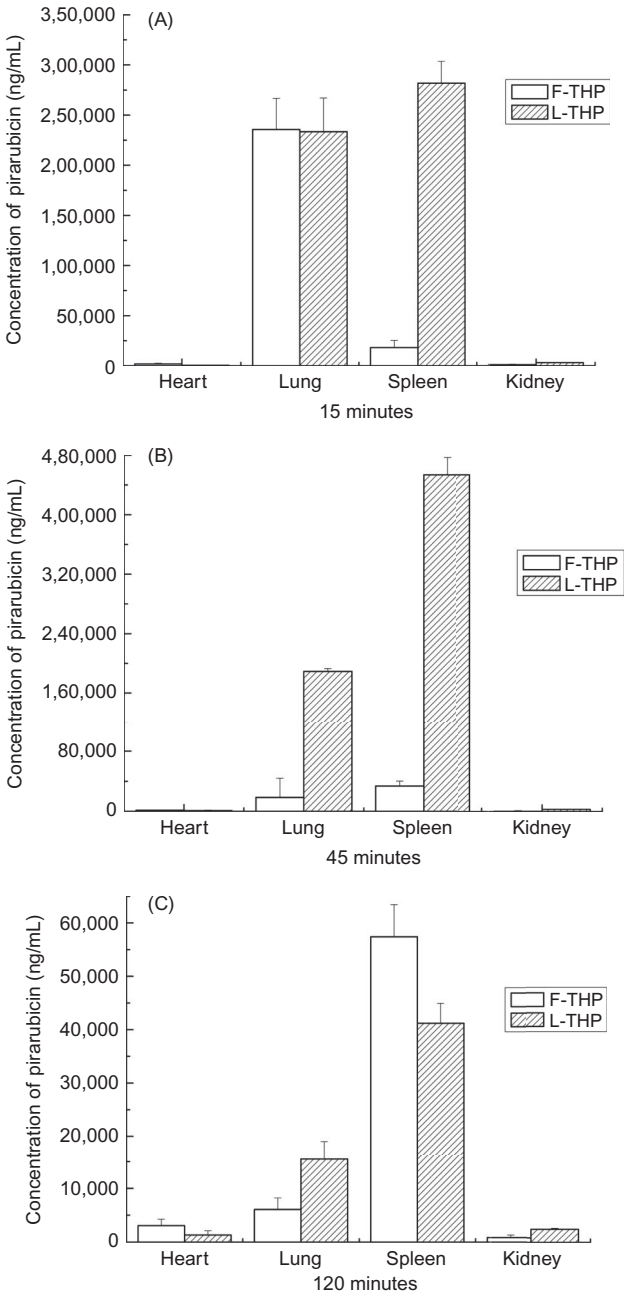

Figure 4. (A) Distribution of THP in tissues of mice at 15 minutes after intravenous injection of F-THP and L-THP, and the data represents the mean ± SD (n = 10); (B) Distribution of THP in tissues of mice at 45 minutes after intravenous injection of F-THP and L-THP, and the data represents the mean ± SD (n = 10); (C) Distribution of THP in tissues of mice at 120 minutes after intravenous injection of F-THP and L-THP, and the data represents the mean ± SD (n = 10).

anthracyclines can result in a semiquinone radical, which can form H_2O_2 and result in lipid peroxidation, thus increasing the ROS concentration over the physiologic level. The C-13 metabolites of anthracyclines, formed through two-electron reduction, will cause chronic myocardial damage[10]. Therefore, it could be concluded that the high accumulation of anthracycline in the heart indicates severe cardiotoxicity. For instance, it is known that THP is less cardiotoxic than doxorubicin, for the accumulation of THP in the heart was lower than that of doxorubicin[31,32]. Then, the decreased accumulation of THP in the heart for L-THP shows that the cardiotoxicity of THP can be greatly reduced when encapsulated into the liposomes.

It can also be observed in Table 4 that THP was mainly accumulated in the lung and spleen for F-THP, which corresponds well with literature[5,31,33]. Meanwhile, THP was enriched in the spleen when encapsulated into the liposomes, as the $AUC_{0-\infty}$ value in the spleen reached 21,775.6 ± 789.2 mg min/L for L-THP ($P < 0.01$) whilst it was 6228.8 ± 450.5 mg min/L for F-THP. This result should be ascribed to the phenomenon that liposomes are found to be plagued by rapid opsonization and thus more easily taken up in the reticuloendothelial system (RES) cells located mainly in liver and spleen[4,34]. The $AUC_{0-\infty}$ value of THP in the lung for F-THP was comparable to that of L-THP, which might suggest that L-THP can still be widely used in the chemotherapy of lung carcinoma. Pharmacokinetic and biodistribution results show that the therapeutic index of THP could be enhanced when encapsulated into liposomes. However, different in vivo behaviors of THP when encapsulated into the liposomes should be ascribed to liposome formulation, as the lipid compositions of liposomes also have a great influence on pharmacokinetic and biodistribution behaviors of the encapsulated drug[16,35].

Conclusions

A novel pirarubicin liposome powder, comprising DSPC, DSPG, cholesterol, and lactose, was appropriately prepared based on the physicochemical property of THP. In vitro evaluation suggests that L-THP has a high entrapment efficiency as well as a better particle-size distribution and lower zeta potential. L-THP was also found to be very stable over a period of 1 year. Meanwhile, in vivo tests show that when THP encapsulated into the liposomes, its cardiotoxicity can be greatly reduced while its therapeutic index can be improved. This paper further suggests that the lipid compositions of liposomes can play a vital role for the in vitro and in vivo behavior of the encapsulated drug. Therefore, physicochemical properties of lipids and encapsulated drugs should be taken into account in liposome preparation. Meanwhile, further preclinical studies are warranted to define the toxicity and the therapeutic efficacy of L-THP. This paper provides not only an alternative liposome preparation technique but also comprehensive insights and guidance for the development of anthracycline liposomes.

Declaration of interest

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