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ORIGINAL ARTICLE

Ammonium sulfate gradient loading of brucine into liposomes: effect of phospholipid composition on entrapment efficiency and physicochemical properties in vitro

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Abstract

Background: Brucine, the major active alkaloid constituent extracted from traditional Chinese herbal medicine *Nux vomica*, had been found to possess remarkable antitumor, analgesic, and anti-inflammatory activities. In this study, we attempted to encapsulate brucine into liposomes to improve its therapeutic effects. The entrapment efficiency (EE) and the stability of liposomes are two key factors associated with the therapeutic effects of liposomal drugs. We developed a novel liposome-based brucine formulation that was composed of soybean phosphatidylcholine (SPC) and hydrogenated soybean phosphatidylcholine (HSPC). *Method*: The liposomes with different phospholipid composition were characterized for their EE, vesicle size, drug release profile, and leakage in vitro. *Results*: The molar ratio of HSPC/SPC = 1:9 was determined as the optimum ratio. Compared with conventional liposomes composed of only SPC or HSPC, EE of the brucine-loaded novel liposomes was increased markedly, especially at high drug/lipid molar ratios. The results of drug release showed that the novel liposomes were more stable than the conventional SPC liposomes in the presence of fetal calf serum. In addition, the results of the leakage experiments revealed that the novel liposomes also had better stability in phosphate buffer solution (PBS) with respect to drug retention. Although the conventional HSPC liposomes is more stable than the novel liposomes, the novel liposomes composed of 10% HSPC and 90% SPC may still have promising application potential because HSPC is much more expensive than SPC. *Conclusion*: Taken together, efficient encapsulation of brucine into the novel liposomes, their improved stability, and the price of phospholipids indicate that the novel liposomes may act as promising carriers for active alkaloids such as brucine.

Key words: *Ammonium sulfate gradient; brucine; entrapment efficiency; hydrogenated soybean phosphatidylcholine; liposomes; soybean phosphatidylcholine*

Introduction

Brucine, an amphipathic weak base, is a white, odorless, crystalline solid with a molecular weight of 394.45 (Figure 1; formula: $C_{23}H_{26}N_2O_4$). The solubility of brucine in pH 7.4 phosphate buffer solution (PBS) is 1.988 mg/mL.

Strychnos nux-vomica L. (Loganiaceae) is grown extensively in southern Asian countries. *Nux Vomica*, the dried seed of this plant, has been used clinically for improving blood circulation, relieving rheumatic pain, and treating cancer over a long time. Alkaloids have been proved to be the main bioactive components responsible for the pharmacological and toxic effects exerted by *Nux Vomica* to a great extent. Sixteen alkaloids have been isolated and identified from *Nux Vomica*[1], among which brucine takes up about 20.6–27.4%[2].

Jun Chen and Aihua Lin authors contribute equally to this work.

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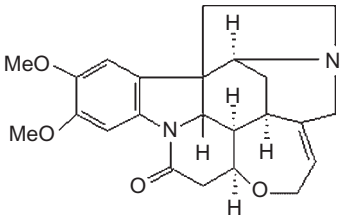

Figure 1. Chemical structure of brucine.

In our previous studies, the total alkaloid fraction from *Nux Vomica* was shown to remarkably suppress tumor cells' growth[2,3]. To identify the alkaloid that is mainly responsible for the anti-tumor effect of *Nux Vomica* as well as its mechanisms, the cytotoxic effects of brucine, strychnine, isostrychnine, and brucine N-oxide on HepG2 cells were compared[4]. The results demonstrated that brucine exhibited the strongest inhibitory effect on HepG2 cells proliferation. Further study revealed that Ca^{2+}- and Bcl-2-mediated mitochondrial pathways were involved in brucine-induced HepG2 cell apoptosis[5].

Analgesic and anti-inflammatory properties of brucine had been intensively investigated[6]. Brucine revealed significant protective effects against the thermic and chemical stimuli in hot-plate test and writhing test. In formalin test, brucine caused significant inhibitory effects on both early and late phase of pain stimulus, which suggested that central and peripheral mechanism are involved in the pain modulation.

Unfortunately, the potential use of brucine is severely limited because of its violent toxicity[7]. Until now, brucine has not been used clinically or being researched in clinical trials.

Liposomes have been proved to be the proper carriers for the delivery of brucine. Deng et al. studied the effects of free and liposomal brucine on inhibiting transplanted tumor growth and prolonging their survival time in mice[8]. The anti-tumor activity was evaluated by tumor inhibitory ratio for S180 and Heps. And the results showed that the inhibitory effect of brucine liposome is much stronger than that of brucine solution. In addition, liposomal brucine was also proved to be safe, effective, and promising formulation for analgesic and anti-inflammatory effects[9].

As a qualified pharmaceutical product, the liposomal formulation should have a high-drug–lipid ratio and entrapment efficiency (EE). But brucine has a low affinity to lipid membranes and tends to move into exterior aqueous compartment. At the drug/lipid molar ratio of about 0.12, the EE of brucine liposomes was only 71.95%[9]. Gradients of ammonium sulfate in liposomes have been successfully used to obtain active loading of amphipathic weak bases into the aqueous compartment of liposomes[10]. In our laboratory, this approach was applied to encapsulate brucine inside liposomes. The EE value was slightly increased[11], and the satisfactory EE (>80%) could not be obtained if the drug/lipid molar ratio increased above 0.14. As a high-EE value is the foundation for achieving sustained release, attenuation of toxic drugs and effectively transporting drugs, the low-lipid affinity behavior of brucine has made the development of a liposome-based brucine formulation more challenging.

Recently, a novel liposome-based drug delivery system capable of incorporating large amounts of hydrophobic substances has been invented[12]. The composition of the novel liposomes at least comprised first and second phospholipids, drugs, and other additives such as cholesterol, antioxidants, and so on. According to the invention, the phase transition temperature of the first phospholipids (Tg1) is from 40°C to 70°C and preferably from 50°C to 65°C. The phase transition temperature of the second phospholipids (Tg2) is from −30°C to 20°C and preferably from −20°C to 4°C. The drug delivery temperature (T1) and storage temperature (T2) are chosen at the specified ranges subject to the order of Tg1 > T1 > T2 > Tg2. The drug delivery temperature is usually about 37°C, whereas the storage temperature is optionally from 4°C to 25°C. Therefore, under the drug delivery temperature or the storage temperature, the first phospholipids having higher phase transition temperature form the gel state phase, and the second phospholipids having lower-phase transition temperature form the liquid–crystal phase. Thus, this novel liposome composition results in coexistence of multiple discontinuous immiscible phases (gel state phases and liquid–crystal phases) occurring on bilayer membrane of liposomes when the liposomes are delivered or stored. The phase boundary barrier between the regions of immiscible phases is able to reduce lateral movement and aggregation of the hydrophobic drugs, thereby stabilizing the liposomes. As a result, the existence of lateral phase-separated phospholipid regions is advantageous for incorporating large amount of hydrophobic molecules such as 9-nitrocamptothecin (9-NC) into the phospholipids bilayer of liposomes. Furthermore, after 9-NC was encapsulated into novel liposomes, pharmacokinetic results revealed an increase in area under the plasma concentration–time curve (AUC) and a decrease in distribution volume of 9-NC following intravenous administration to rats[13]. Increased stability in plasma may account for the improved pharmacokinetic behavior of the novel liposomes.

Until now, however, the reported preparation of the novel liposomes is only limited to the method of thin film hydration; other currently used liposome preparation techniques, such as ammonium sulfate gradient loading method, have not been reported for the preparation of the novel liposomes. Moreover, whether hydrophilic

drugs as well as amphipathic compounds can be encapsulated into the novel liposomes is still to be investigated.

In this article, as a means to obtain efficient drug loading, we assessed the feasibility of novel liposomes composed of two phospholipids, namely soybean phosphatidylcholine (SPC) and hydrogenated soybean phosphatidylcholine (HSPC), to encapsulate brucine by the ammonium sulfate gradient loading method. The physicochemical characteristics of the brucine-loaded novel liposomes were also described in detail and compared with conventional liposomes composed of only SPC or HSPC.

Materials and methods

Materials

SPC and HSPC were purchased from Lipoid Corp. (Ludwigshafen, Germany). The 98% purity of the lipid was verified by thin-layer chromatography. Cholesterol was purchased from Huixing Biochemistry Reagent Company (Shanghai, China). Brucine was obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Sephadex G-50 was purchased from Pharmacia Biotech (Uppsala, Sweden). High-performance liquid chromatography (HPLC)-grade acetonitrile was obtained from Tedia Company Inc. (Fairfield, OH, USA). Fetal calf serum (FCS) was purchased from Equitech-Bio Inc. (Kerrvile, TX, USA). All other reagents were of analytical grade.

Methods

Liposome preparation

The liposomes were prepared by the ammonium sulfate gradient loading method as described previously[10]. Briefly, SPC and cholesterol at a molar ratio of 3:1 were dissolved in 2 mL ethanol and then injected into the solution of 5 mL 200 mM ammonium sulfate under magnetical stirring at 25°C. The ethanol was evaporated odorless under vacuum, and then water was added to adjust the volume of final liposomes suspension to 5 mL. To get homogenous liposome suspensions, liposomes were ultrasonically treated for 3 minutes, using a JY92-II probe ultrasonicator (Xinzhi Biotechnology Co. Ltd., Ningbo, China) equipped with a tapered microtip. The resulted liposomes were filtered through a 0.22-μm micropore filter (Shanghai Institute of Pharmaceutical Industry, Shanghai, China) to remove the titanium fragments. The transmembrane ammonium sulfate gradient was created by four consecutive dialysis exchanges (2 hours at a time) against 20 volumes of PBS (pH = 7.4) under room temperature. Brucine in powder form was added to liposomes at 25°C for 20 minutes.

For the preparation of liposomes composed of cholesterol and lipids (HSPC or the mixture of HSPC and SPC), namely the novel liposomes and the conventional HSPC liposomes, the temperature for ethanol injection and drug loading was increased from 25°C to 60°C (above the phase transition temperature of HSPC). Other compositions and procedures were the same as those of the conventional SPC liposomes.

Entrapment efficiency

The EE was calculated by the percentage of brucine encapsulated into liposomes relative to the total amount of brucine (encapsulated and free) in liposome suspension.

To determine the total amount of brucine in liposome suspension, 0.5 mL brucine liposome was disrupted by the addition of 2 mL ethanol–isopropanol (1:4, v/v) to form a clear solution. The concentration of brucine was assayed by UV spectroscopy (TU1800S, Puxi Instrument Company, Beijing, China) at 264 nm compared to a standard curve.

To completely remove unentrapped drug, brucine liposome was passed through a Sephadex G-50 column (1 × 27 cm) equilibrated with PBS (pH 7.4). The resulted PBS fraction containing the liposomes free of nonentrapped brucine was collected and the encapsulated brucine concentration was determined as described above.

To ensure that separation using a gel chromatography was realized, 1.75 mg/mL brucine novel liposome suspensions composed of HSPC/SPC = 1:9 and 1.75 mg/mL brucine solution were eluted on Sephadex G-50 column (1 × 27 cm). Fractions of 2 mL were collected and the amount of brucine in all fractions was determined by UV spectroscopy as described above.

If significant nonentrapped brucine was retained on the column, the determination of EE was no longer trustworthy. Brucine solution with the concentration of 1.75 mg/mL was eluted on the column and the eluted fractions were collected together and the amount of brucine was determined by UV spectroscopy. The recovery was calculated by the percentage of brucine in the eluted fractions relative to the amount of brucine solution.

Vesicle size measurement

The particle size of the brucine-loaded liposome was measured by dynamic light scattering, using a Zetasizer 3000 (Malvern Instrument, Malvern, UK). A sample of the formulation (0.25 mL) was diluted with distilled water and the intensity-weighted size was averaged from 10 runs.

HPLC analysis of brucine

A Shimadzu HPLC System (Kyoto, Japan) consisting of an LC-20AT pump and a SPD-20A UV–Vis detector was used for the assay of brucine. The mobile phase consisted of acetonitrile and buffer (10 mM sodium heptane sulfonate and 20 mM potassium dihydrogen phosphate, adjusted to pH 2.8 with 10% phosphonic acid). The ratio of acetontrile : buffer (v/v) was adjusted to

24:76. Separation was carried out at 35°C using a reverse phase C_{18} column (Lichrospher, 5 μm, 4.6 × 250 mm, Hanbang Corp., Huaian, China). The detection wavelength was 264 nm and a flow rate of 1.0 mL/min was employed. A sample volume of 20 μL was injected.

Calibration curves of brucine in PBS were linear in the range of 0.4–12.8 mg/L. The regression coefficient was 0.9994. Within-day and between-day precisions of brucine were no more than 1.50%.

Drug release in vitro

The in vitro release of brucine-loaded liposomes was analyzed according to the published method[9,13]. Unentrapped drug was removed from brucine-loaded liposome over a Sephadex G-50 column as described above before in vitro release test. Then brucine liposome suspension (drug content: 1.5 mg) was placed in a dialysis bag (Wanqing Corp., Nanjing, China) with a molecular weight cutoff of 10,000. The dialysis bag was suspended in 100 mL isotonic PBS (pH 7.4), which was constantly stirred at 37°C on a TP-3 magnetic stirrer with thermostat (Xinlian Electronic Instrumental Factory, Nanjing, China). The stirrer was operating at 500 rpm. At scheduled time intervals, 1 mL of PBS sample was withdrawn and assayed for brucine content by HPLC. The volume of dissolution medium was maintained at 100 mL throughout the experiment. To evaluate the effect of serum albumin on drug release from liposomes, brucine liposome suspension combined with isometric FCS was placed in the dialysis bag.

Leakage experiments in vitro

The in vitro leakage assays were carried out according to the method described previously[14] with minor modification. The concentration of brucine liposomes with the drug-to-lipid molar ratio of 0.14 and 0.28 was 1.75 and 3.5 mg/mL, respectively. Briefly, brucine liposomes with different phospholipids compositions were mixed with isotonic PBS (pH 7.4) at a ratio of 1:9 (v/v). The resulting suspension was incubated at 37°C. At various time points, aliquots were withdrawn for the determination of brucine retention. The samples collected at specified time points were passed through a Sephadex G-50 column (1 × 27 cm) to separate the liposomal brucine from the leaked drug. The concentration of liposomal brucine was assayed as described above. The drug retention following incubation was calculated by dividing the drug/phospholipid ratio at indicated time point following incubation by the initial drug/phospholipid ratio.

Acute toxicity test

Male ICR mice, weighing 20–25 g on the day of testing, were allowed to acclimatize in the laboratory 1 week prior to use. Brucine was dissolved in pH 7.4 PBS and 1.75 mg/mL brucine solution was obtained. Brucine novel liposomes with the composition of HSPC/SPC = 9:1 was at the concentration of 3.5 mg/mL and the EE was about 80%. The maximal and minimal lethal doses were preliminarily estimated. The dosages for determination of LD_{50} were then calculated according to the ratio of dose for each group and intravenously administered to five dosage groups (10 mice each). All mice were observed for general symptoms. The number of dead mice was recorded. LD_{50} values and 95% confidence limits were calculated using the Bliss method.

Results

The method to determine entrapment efficiency

The elution profiles of brucine liposomes with different phospholipid composition on Sephadex G-50 column are shown in Figure 2. The former parts must be related to liposomes, and the latter parts must be related to the nonentrapped brucine. It had been demonstrated that

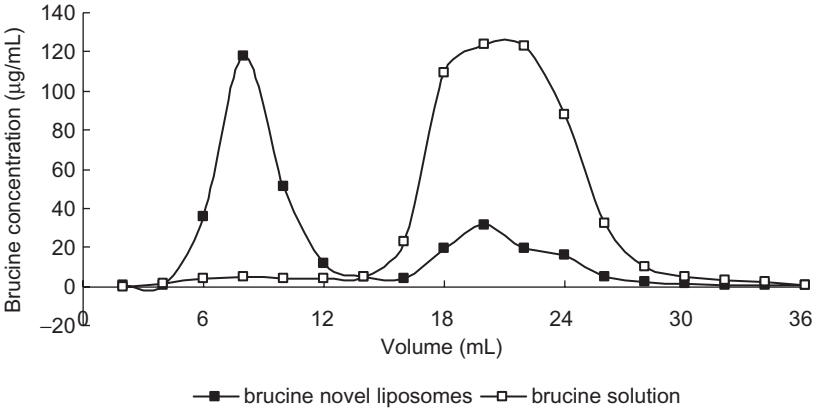

Figure 2. Elution profiles of 1.75 mg/mL brucine novel liposomes and brucine solution on Sephadex G-50 column.

the liposomal brucine was completely separated from the nonentrapped brucine.

The recovery of brucine for brucine solution eluted on the column was in the range of 96.85–99.84%.

Effect of phospholipid composition on encapsulation efficiency

The effect of HSPC/SPC molar ratios on brucine EE into liposomes at drug/lipid molar ratio of 0.32 is shown in Figure 3. It could be easily observed that the EE was significantly influenced by HSPC/SPC molar ratio and high EE was achieved with HSPC/SPC molar ratio between 1:1 and 1:18. There was no statistical difference in the EE values with ratios of 1:1–1:18 when the statistical analysis was performed by means of Student's *t*-test. The liposomes composed of HSPC/SPC at a molar ratio of 1:9 exhibited the highest EE.

Furthermore, brucine was encapsulated into novel liposomes composed of HSPC/SPC at a molar ratio of 1:9 and conventional liposomes composed of only SPC or HSPC with different drug/lipid molar ratios. The resulted EE is shown in Figure 4. While the drug/lipid molar ratio was higher than 0.28, the novel liposomes possessed obviously higher EE compared to the conventional liposomes. At the drug/lipid molar ratio of 0.56, the EE of brucine-loaded novel, SPC, and HSPC conventional liposomes were 64.0 ± 0.8%, 44.1 ± 2.8%, and 34.2 ± 2.6%, respectively. However, when the drug/lipid molar ratio decreased to 0.14, the effect of phospholipid composition on EE was no longer significant.

To compare the pharmaceutical characterization of brucine-loaded liposomes composed of different phospholipid composition, the drug/lipid molar ratio was set at 0.14 to obtain the same EE. In addition, because it also possessed the same EE, the pharmaceutical characterization of brucine-loaded novel liposomes at the drug/lipid molar ratio of 0.28 was also investigated to determine the effect of drug/lipid molar ratio on the properties of brucine-loaded novel liposomes.

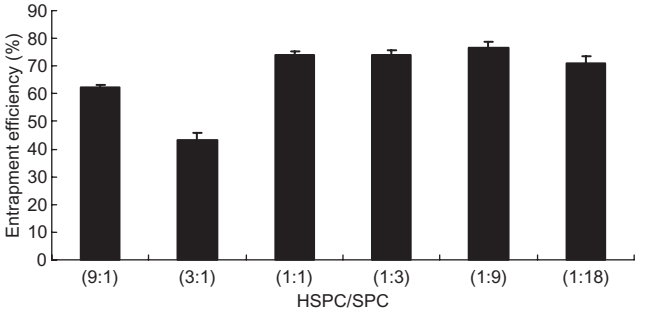

Figure 3. Effect of HSPC/SPC molar ratios on entrapment efficiency of brucine-loaded novel liposomes (drug-to-lipid molar ratio is 0.32). The entrapment efficiency is the mean of three runs ± SD for each HSPC/SPC molar ratio.

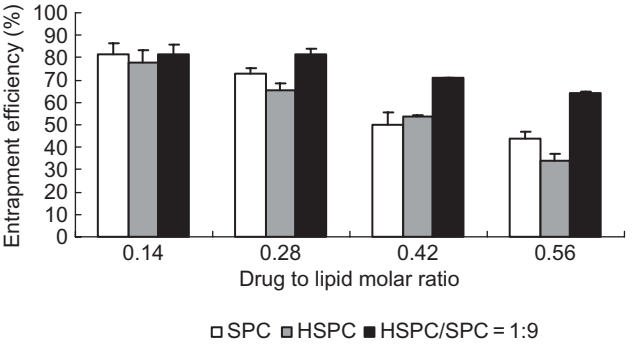

Figure 4. Effect of drug-to-lipid molar ratios on entrapment efficiency of brucine-loaded novel liposomes (HSPC/SPC = 1:9) and conventional liposomes composed of only SPC or HSPC. The entrapment efficiency is the mean of three runs ± SD for each drug-to-lipid molar ratio.

Effect of phospholipid composition on physicochemical properties

Sizes of liposomes

The intensity-weighted particle sizes of brucine-loaded novel and conventional liposomes are shown in Figure 5. It could be observed that the size of novel liposomes was larger than that of conventional liposomes. The average particle size of brucine-loaded novel liposomes was in the range of 147–159 nm, whereas the size range of conventional liposomes was 114–122 nm. Polydispersity index was always lower than 0.15, indicating that liposome population was homogenous in size.

Drug release in vitro

The in vitro drug release profiles are shown in Figures 6 and 7. Without the existence of FCS, the release of brucine from liposomes was less than 4% in 10 hours.

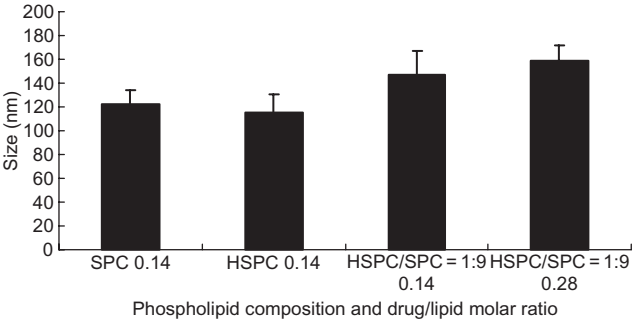

Figure 5. Sizes measured for brucine-loaded liposomes with different phospholipid composition and drug/lipid molar ratio. The sizes are the mean of three runs ± SD for each phospholipid composition.

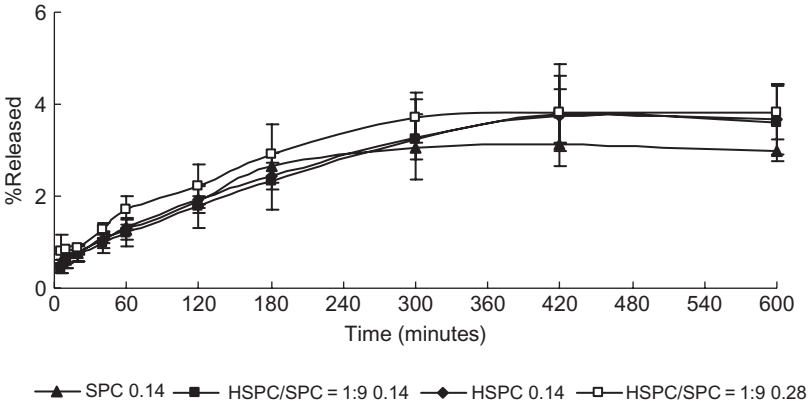

Figure 6. In vitro release profiles of brucine from liposomes with different phospholipid compositions and drug-to-lipid molar ratios in the presence of PBS ($n = 3$).

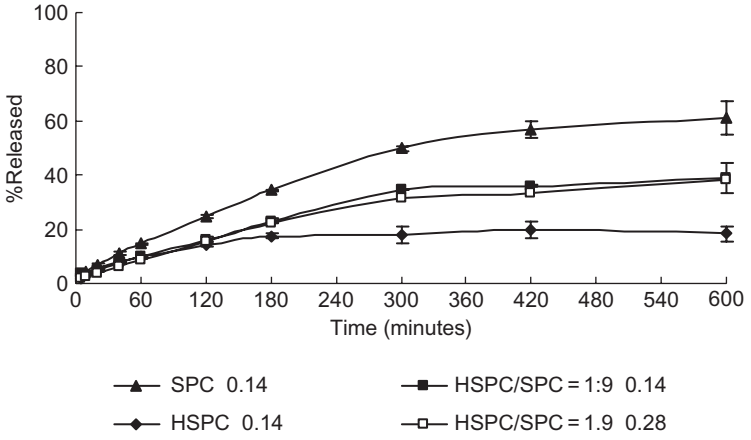

Figure 7. In vitro release profiles of brucine from liposomes with different phospholipid compositions and drug-to-lipid molar ratios in the presence of fetal calf serum ($n = 3$).

There was no difference among brucine-loaded liposomes with different phospholipid composition and drug/lipid molar ratio. However, with the existence of FCS, the release of brucine was accelerated dramatically and a large extent of drug release was observed. Figure 7 indicates that the retention of the loaded brucine in the liposomes was significantly dependent on phospholipid composition. Compared with the conventional SPC liposomes, the novel liposomes in which 10% HSPC was combined with 90% SPC was proved to improve drug retention significantly. Only 38 ± 5% encapsulated brucine had been released from the novel liposomes and 61 ± 6% released from the conventional SPC liposomes in 10 hours. The conventional HSPC liposomes had a minimal release of encapsulated brucine. Moreover, the release of brucine from the novel liposomes did not vary with the drug/lipid molar ratio ranged from 0.14 to 0.28.

Leakage experiments in vitro

The in vitro leakage profiles of brucine from different liposomes are shown in Figure 8. It was obvious that the leakage of liposomal brucine was markedly affected by both phospholipid composition and drug-to-lipid molar ratio. For conventional liposomes composed of only HSPC, rapid drug leakage was detected between 8 and 24 hours and then the leakage was hardly observed. Compared with SPC liposomes, the brucine retention in the novel liposomes was significantly improved. Furthermore, it seemed that the stability of the novel liposomes was decreased with the increase of drug/lipid molar ratio.

Acute toxicity test

Table 1 shows a comparison of the acute toxicity of free and liposomal brucine. The intravenous LD_{50} of brucine solution was 12.16 mg/kg while the brucine novel

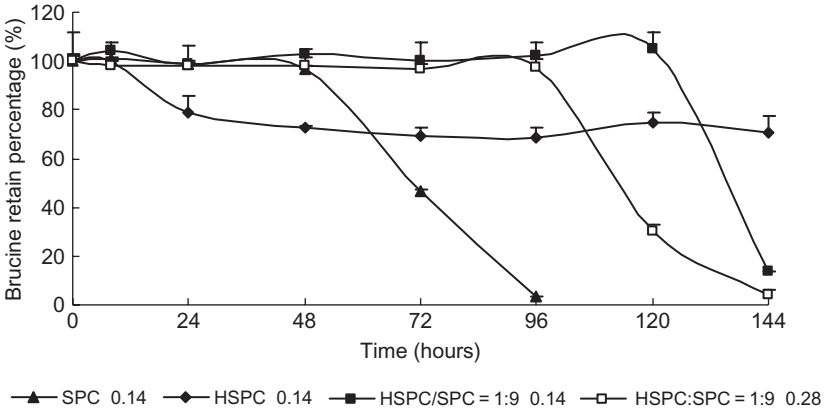

Figure 8. In vitro leakage profiles of brucine from liposomes with different phospholipid composition and drug-to-lipid molar ratios ($n = 3$).

Table 1. Acute toxicity comparison of free and liposomal brucine in mice.

Samples	Concentraion (mg/mL)	LD_{50} (mg/kg)[a]
Brucine solution	1.75	12.16 (10.98–13.46)
Brucine novel liposomes	3.5	25.64 (23.66–27.80)

[a]Numbers within parentheses indicate the 95% confidence limits for the statistics.

liposomes were less toxic with LD_{50} of 25.64 mg/kg, approximately twofold higher than the value of free brucine.

Discussion

In order for brucine-loaded liposomes to be used as a drug delivery system in vivo, they must be designed in such a way that they are stable in the blood for an extended period of time, allowing the liposomes to reach a target cell, prior to the release of their content. For this purpose, we focused on the lipid composition to inhibit the penetration and permeation of the drug molecules through the lipid bilayer, especially with the presence of serum.

The stability of brucine-loaded liposomes with different phospholipid composition was examined by drug release kinetics and leakage experiments in vitro. The results of drug release test with the presence of PBS revealed that nearly 100% of the encapsulated drug (>96%) was retained inside the liposomes in 10 hours and there was no difference among brucine-loaded liposomes with different phospholipid composition. One possible explanation for the good drug retention is that brucine forms a gel-like precipitate with ammonium sulfate as doxorubicin does[15] because of the lower solubility of the brucine sulfate salt formed in the intraliposomal aqueous phase.

To compare the effect of phospholipid composition, the stability of the brucine-loaded liposomes was further investigated by the leakage experiments at 37°C for 7 days. Compared with conventional SPC liposomes, the stability of the novel liposomes was significantly improved. The brucine-loaded conventional HSPC liposomes had the best stability in spite of the existence of slight rapid leakage in 24 hours. The result is not surprising, as the rate of drug leakage is dependent on liposome composition: liposomes formed from high-phase transition lipids release less rapidly than liposomes formed from low-phase transition. Moreover, for the first time, the results indicated that the stability of novel liposomes composed of 10% HSPC and 90% SPC might be between the conventional liposomes composed of only HSPC and SPC. In our previous report[13], the release rate of the encapsulated 9-NC from novel liposomes was accelerated compared with the conventional SPC liposomes. As brucine was actively loaded into the inner aqueous phase and 9-NC was encapsulated in the bilayer membrane of liposomes, the different position of encapsulated drug in liposomes might account for the inconsistent stability evaluation results of the novel liposomes. For brucine, the inclusion of 10% HSPC to the total phospholipids is required to achieve less leakage compared with conventional SPC liposomes.

It is well known that serum components destabilize liposomal membranes[16]. Phospholipid exchange and transfer to lipoproteins, mainly high-density lipoprotein, destabilizes and disintegrates liposomes, with subsequent loss of content. Therefore, with the presence of FCS, the release of brucine was significantly increased. The drug release rate from the novel liposomes was much lower than that of conventional SPC liposomes. Only 38 ± 5% encapsulated brucine had been released from the novel liposomes and 61 ± 6% released from the conventional SPC liposomes in 10 hours. The results suggested that the novel liposomes resist the destruction effect of serum albumin, which was in good accordance with our

previous report[13]. Therefore, compared with conventional SPC liposomes, the stability of novel liposomes containing brucine in blood circulation in vivo may be improved correspondingly. Although the conventional HSPC liposomes have a minimal release of encapsulated brucine, the novel liposomes composed of 10% HSPC and 90% SPC may have promising application potential instead of conventional HSPC liposomes because HSPC is much more expensive (about 10-fold) than SPC.

The phase transition temperatures of HSPC and SPC are 50°C and –20°C, respectively. Thus, the two immiscible phases, namely gel state phases and liquid-crystal phases, coexist in bilayer membrane of the novel liposomes. The phase boundary barrier between the regions of immiscible phases is able to reduce lateral movement and aggregation of the hydrophobic drugs, thereby stabilizing the liposomes[12]. The theory provides a rationale for the significantly increased drug-loading potential of the novel liposomes containing hydrophobic drugs such as paclitaxel and 9-NC which are encapsulated in bilayer membrane.

Because brucine is encapsulated into the inner aqueous medium of liposomes, the above explanation might not completely account for the increased stability and EE of brucine. It has been known for some time that the maximal release of low-molecular-weight liposome contents occurs at a temperature higher than the gel-to-liquid crystalline transition. It is likely that the inclusion of 10% HSPC is not sufficient to significantly change physical state of the whole lipid membranes. However, the included HSPC might create to some extent gel state phase areas. The penetration and permeation of the drug molecules through the resulted gel state phase areas is thus inhibited compared with the liquid–crystal phases created by SPC. Therefore, the drug leakage of the novel liposomes is decreased compared with the conventional SPC liposomes. The addition of 10% HSPC also results in the increase of the rigidity of the bilayer. Since the increase of rigidity helps to avoid the absorption of serum albumin in the liposomes[17], the increased stability of the novel liposomes in FCS is expectable. However, elaborate studies (such as the determination of the effect of HSPC/SPC ratio on the stability of the novel liposomes) should be required to gain definite proof of this hypothesis. Further studies are also needed in order to gain insight into the in vivo behavior of the novel liposomes.

The size of the novel liposomes was increased about 20% compared with the conventional SPC or HSPC liposomes. Consequently, the trapped volume of the vesicles might be larger than the conventional liposomes, which might partly account for the increased EE of the novel liposomes.

Vesicle size is critical for liposome localization in solid tumors, namely the enhanced permeability and retention effect. On the basis of animal models, there is a cut-off size of 400 nm for particle extravasation in tumors[18]. It has been suggested that a liposome diameter of about 100 nm is likely to be an optimal size[19]. However, the pore size of tumor vessels varies depending on the type of tumor, the site of the tumor growth, and the degrees of tumor growth and regression. Therefore, it is difficult to determine that the slight increase of size of brucine novel liposomes leads to the different enhanced permeability and retention effect.

Until recently, little research about the characterization of novel liposomes composed of an unsaturated phospholipid and a saturated phospholipid has been reported. In this article, the EE and physicochemical properties in vitro of the novel liposomes and of the corresponding conventional liposomes were compared. The results revealed that the novel liposomes possessed both high EE and stability compared with that of conventional SPC liposomes.

Conclusions

A major problem in the pharmaceutical application of liposomes is their efficient and stable loading with drugs. In this article, we successfully obtain novel liposomes containing brucine by adding 10% HSPC into a formulation composed of SPC and cholesterol. Compared with the conventional SPC liposomes, the brucine-loaded novel liposomes which possess both high EE and stability would meet pharmaceutical demands for clinical use. Considering the price of HSPC and SPC, the novel liposomes may have much developing potential compared to that of the conventional HSPC liposomes.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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