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ORIGINAL ARTICLE

Synthesis of a novel polymer bile salts-(polyethylene glycol)$_{2000}$-bile salts and its application to the liver-selective targeting of liposomal DDB

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Abstract

Purpose: The objective of this study was to achieve a sustained and targeted delivery of liposome to the liver, by modifying the phospholipid [phosphatidylcholine (PC)/cholesterol (10 : 1) liposomes with a novel polymer bile salts-(polyethylene glycol)$_{2000}$-bile salts (BP_2B). *Methods*: First, we generated a novel BP_2B by *N,N*′-dicyclohexylcarbodiimide/4-dimethylaminopyridine esterification method and confirmed by Fourier transform infraredand ^{1}H-NMR spectra. Second, we prepared the BP_2B-modified liposomes (BP_2BL) that included BP_2B, and the effect of the weight ratios of BP_2B/PC on entrapment efficiency was investigated and BP_2B/PC = 3% (w/w) was determined as the optimum ratio for the 4,4′-dimethoxy-5,6,5′,6′-bi (methylenedioxy)-2,2′-bicarbomethoxybiphenyl liposomes. And then, the ability of the liver target of BP_2BL was studied by calculating the targeted parameters. *Results and Discussion*: All the results revealed that the introduction of polyoxyethylene chains could control interactions of bile salt moieties on liposome surfaces with the receptor compared with traditional liposomes (CL), marking BP_2BL as a suitable carrier for hepatic parenchymal cell-specific and sustained targeting. It was suggested that liposomes containing such novel BP_2B have great potential as drug delivery carriers for the liver-selective targeting that has targeted and sustained drug delivery.

Key words: *Bile salts-(polyethylene glycol)$_{2000}$-bile salts; DDB; liposome; liver targeting; targeted parameters*

Introduction

The liver diseases occur frequently in clinical practice. However, many drugs used in clinic for hepatitis, liver fibrosis, cirrhosis, and hepatoma lack organ selectivity, which leads to low drug concentration in liver[1]. It is well known that the liver consists of hepatocytes (hepatic parenchymal cells), Kupffer cells, and endothelial cells (hepatic nonparenchymal cells), and many fatal diseases such as chronic hepatitis, enzyme deficiency, and hepatoma occur in hepatocytes. However, most of particulate delivery systems are easily taken up within seconds or minutes after injection because of phagocytosis by the reticuloendothelial system, including Kupffer cells of the liver and macrophages of the spleen[2]. It does represent a major barrier to deliver drugs to hepatocytes.

In recent years, the hepatic targeted drug delivery system gets a rapid progress with the development of targeting drug delivery system. The hepatic targeted drug delivery system selectively distributes drugs to the pathological parts of the liver, which not only enhances their bioavailability but also reduces the side effects of targeting. Several approaches for the targeted delivery of drugs to the liver cells for the treatment of various microbial and viral diseases have been explored[3,4]. The coefficient of the liver target unceasingly increases with the use of prodrug, liposome[5–7], emulsion, and so on. Prodrug is taken up readily by the hepatocytes[8], and the following fast

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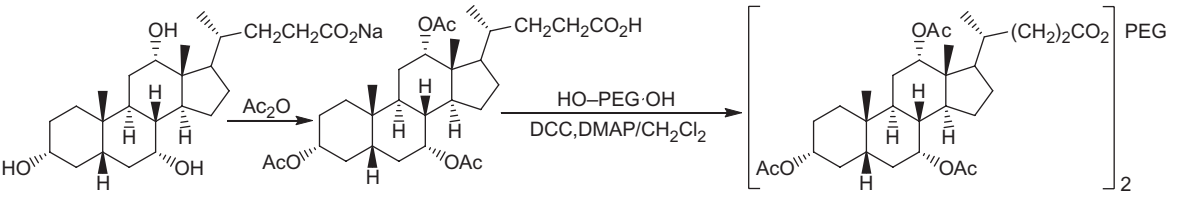

Figure 1. Chemical reaction scheme for preparing the BP_2B conjugate.

excretion, however, would cause a short duration of drug exposure. And also the decomposition of prodrug is influenced by the carboxy-lesterase in vivo, resulting in the significantly individual variation[9,10]. Liposome and emulsion can improve the coefficient of the liver targeting and prolong the circulation of drug; however, there still remain some shortcomings: instability in plasma, non-specific association with the extracellular matrix, and so on. This situation has imposed severe limitations on the use of these systems for therapeutic drug delivery.

The receptor-mediated drug targeting is a promising approach to selective drug delivery. And bile salt-coated liposomes can successfully be delivered to the liver[11]. But the recognition of bile salt-coated liposomes by the receptors is highly efficient; the liposomes can be rapidly delivered to the targeting cells. Therefore, the concentration–time profile in the target could be rapidly switched to the elimination phase, which in turn would result in a short duration of drug exposure[12].

It is well known that lipoplexes or polyplexes can be coated with polymers such as poly(ethylene glycol) (PEG), which introduces a steric barrier on their surfaces. These PEGylated lipoplexes or polyplexes exhibit a reduction of interaction with opsonins and/or nonspecific interactions with reticuloendothelial system cells[13–15]. However, the PEG coating may also reduce the particle–cell membrane interactions that are needed for cell uptake, the consequence of which would lead to a decrease in drug uptake. On the contrary, the stealth liposome mainly improves the concentration of the liver by depending on the prolonged circulation of drug in vivo, thus it may decrease the liver targeting. There are many reports that conjugate the ligand at the distal end of PEG spacer arm linked to polylysine, to galactose, or to a lipid[16–19]. These targeting PEG–lipids were incorporated either in liposomes or in lipoplexes. And these polymers are not only very complicated in synthesis but also too expensive to bring into industrialization.

Taken together, based on the above results, the objective of our study was to design a novel polymer that is cheap and simple in synthesis and could control delivery of the liposomes through a receptor-mediated process. For this purpose, first, we synthesized a novel polymer bile salts-$(PEG)_{2000}$-bile salts (BP_2B), in which we assumed that PEG coating retards bile salts receptor-mediated uptake of bile salts liposomes[20]. Second, the BP_2B surface-modified liposomes (BP_2BL) were prepared, and their physicochemical characteristics in comparison to unmodified CL were determined within this study. Finally, to examine the feasibility of BP_2B coating CL to achieve sustained and targeting drug delivery system, we carried out animals experiment and studied the liver targeting profiles of CL and BP_2BL.

Bifendate is widely used in China for the treatment of chronic hepatitis by lowering alanine transaminase in patients[21–23]. The structure of bifendate [4,4′-dimethoxy-5,6,5′,6′-bi (methylenedioxy)-2,2′-bicarbomethoxybiphenyl, DDB] is shown in Figure 1. Currently, there are only oral preparations on market because DDB is insoluble in water, which results in low bioavailability. However, for acute hepatitis patients and those with decreased liver functions after surgical operations, parenteral dosages would provide the best benefits for them. To prepare the DDB solution suited for intravenous (i.v.) injection, several research groups had tried to improve the solubility of DDB in water[24,25]. In this study, DDB was selected as a model drug and encapsulated in BP_2B liposomes with phosphatidylcholine (PC) and cholesterol (Chol). The hepatocyte-targeting potential of the DDB entrapped in liposomes (BP_2BL) was also investigated and evaluated.

Materials and methods

Chemicals

N,*N*-Dicyclohexylcarbodiimide (DCC) was obtained from Sinopharm Chemical Reagent Co., Ltd. The chemical, 4-dimethylaminopyridine (DMAP), was purchased from the Zhejiang Xianju Pharmaceutical and Chemical Experimental Plant. Chol (China Medicine Shanghai Chemical Reagent Corporation, China) and succinic anhydride (Shanghai Chemical Reagent Co., Ltd., China) were used. PEG with Mn 2000 g/mol was precipitated from Xilong Chemical Reagent Corporation. CH_2Cl_2 was refluxed over P_2O_5 and then distilled. Bifendate material (DDB, 99.0% purity) was supplied by Zhejiang Hisoar Pharmaceutical Company Ltd. All other reagents were of analytical reagent (AR).

Structural analysis

IR spectra of acetyl bile ester, PEG_{2000}, and the reaction products were recorded on a Nicolet Avatar 370 DTGS spectrophotometer (KBr disk). ^{1}H-NMR was performed on a Bruker (AVACE) AV-500 using $CDCl_3$ as a solvent.

Methods

Synthesis of BP_2B

The synthesis of BP_2B involved two steps of chemical modification on bile salts by esterification, one with acetic anhydride to obtain acetyl bile ester, and then with PEG to obtain sufficiently hydrophilic terminal according to Figure 1.

Synthesis of triacetyl bile ester. Sodium cholate (17.2 g, 40 mmol), acetic acid (5 mL), and acetic anhydride (172 mL) were charged into a round bottom flask. A slight excess of acetic anhydride was used to ensure that hydroxy terminals were completely reacted. After refluxing the reaction mixture for 10 hours, the solvent was removed under reduced pressure. The resulting crude product was dissolved in CH_2Cl_2 (200 mL) and then was washed with water (200 mL × 2) on a separating funnel and dried overnight with anhydrous sodium sulfate. After filtered next day, the filtrate was dried under reduced pressure. The residue was purified by column chromatography on silica gel H (dichloromethane:methanol = 100:1, v/v). Thin-layer chromatography analysis (TLC) R_f = 0.47 (CH_2Cl_2: methanol:acetic acid = 50:1:0.05) visualized with iodine vapor showed more polar spot. ^{1}H-NMR ($CDCl_3$, ppm) δ:0.74 (s, 3H, CH_3), 0.83 (d, 3H, CH_3, J = 6.45), 0.92 (s, 3H, CH_3), 1.10–2.39 (m, 27H), 2.03 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 4.58 (m, 1H, CH), 4.91 (d, 1H, CH, J = 2.57), 5.09 (s, 1H, CH); IR(γ/cm):3442, 2945, 2874, 1736, 1379, 1248, and 1026; and ESI(-)/70eV:533.55 $[M-H]^-$, 557.30 $[M+Na]^+$.

Synthesis of BP_2B conjugates. The BP_2B was done in principle as described by Bülbül et al., Pütz et al., and Harris et al.[11,20,26] PEG_{2000} 7.49 g (3.75 mmol), triacetyl bile ester 4.0 g (7.49 mmol), and toluene (250 mL) were charged into a round bottom flask, the mixture was refluxed with water bath apparatus until no water drip, and the solvent was removed under reduced pressure and redissolved in anhydrous CH_2Cl_2 (250 mL). Then DCC 1.54 g (7.49 mmol) and DMAP 0.1 g were added into the solution and stirred at room temperature for 48 hours. After filtered, the filtrate was washed with saturated aqueous solution of sodium bicarbonate (200 mL × 3) and with saturated salt water (200 mL × 3). Then the solution was dried by anhydrous sodium sulfate overnight. After filtered, the solvent were removed under reduced pressure. The product was further purified by column chromatography on silica gel H using dichloromethane/methanol (50:1, v/v) as an eluant. A single spot by TLC analysis R_f = 0.68 (CH_2Cl_2:methanol:acetic acid = 10:1:0.05) was visualized with iodine vapor. ^{1}H-NMR ($CDCl_3$, ppm) δ: 0.73 (s, 3H, CH_3), 0.82 (m, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.02–2.38 (m, 27H), 2.05 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 3.50–4.30 (m, 80H), 4.57 (m, 1H, CH), 4.90 (s, 1H, CH), 5.08 (s, 1H, CH); IR(γ/cm): 2885, 1734, 1468, 1360, 1344, 1281, 1244, 1148, 1117, 1061, 1026, 964, 843, and 530.

Liposome preparation

CL and BP_2BL were prepared according to a modification of the method of Bangham et al.[27] In brief, lipid components and DDB were dissolved in a chloroform/methanol mixture (4:1) and the mixture was perfectly dispersed by sonication. Then the mixture was dried to be a thin film in the rotary evaporation apparatus under vacuum in water bath at 37°C for 1 hour. The resulting film was hydrated with glucose solution (5%) at 25°C for 2 hours to make a coarse lipid suspension. After the liposome suspension was extruded at 4°C in the homogenizer (AVESTIN Co., Ottawa, Canada), the DDB-loaded liposomes were stored at 4°C for future investigations. The effect of the mount of BP_2B (BP_2B/PC = 0%, 1.5%, 3.0%, 5.0%, 10.0%, 15.0%, 20.0%, w/w) in the liposome on entrapment efficiency (EE) was studied. The determination of size and zeta potential of the liposomes were performed by dynamic light scattering (Zetasizer 3000HSA, Malvern Instruments Ltd., Worcestershire, UK). The shape of the liposomes was observed by the transmission electron microscope (H-7000 Hitachi, Japan) at an accelerating voltage of 75 kV. DDB concentrations were determined by measurement of absorbance at 278 nm (SPD-20A, Shimadzu Co. Ltd., Tokyo, Japan) after dissolving the liposomes in methanol[28].

In vitro release experiments

Because of the insolubility of DDB in water, it is difficult to select a suitable dissolution medium. The 'flow-through' (USP apparatus 4) method permits constant optimal sink conditions because of the continuous flow of fresh solvent. So the in vitro release profile of CL was subjected to study using the 'flow-through' method on a semiautomated flow-through dissolution–dialysis system (SOTAX CE 7smart, SOTAX Co., Ltd., Basel, Switzerland), which is a modified version of the dissolution–dialysis assembly of Shah and Sheth[29]. In brief, the sample was placed in a dialysis bag and then transferred to a small volume cell (internal diameter 22.6 mm) through which the solvent (pH 7.4 phosphate-buffered saline) is passed with 4.0 mL/min constant 'flow through' rate at a bath temperature of 37°C. The eluate was filtered upon leaving the cell and then collected in 4.0 mL automatically using the Win SOTAX Advanced Dissolution

Software at preset intervals over a period of 24 hours, and then manually compensated by fresh phosphate-buffered saline at each sampling point. The concentrations of DDB in dissolution medium were analyzed by high-performance liquid chromatography (HPLC). The release percentage was calculated according to the following equation:

$$\text{Drug release (\%)} = \frac{M \text{ release}}{M \text{ total}} \times 100\%$$

where M release is the amount of DDB released from DDB liposomes into dialysis medium at scheduled intervals, and M total is the total amount of DDB in DDB liposomes.

Pharmacokinetics in blood

Twelve Wistar rats (male, 180–220 g), which were purchased from the Experimental Animal Center of China Pharmaceutical University, were divided randomly into two groups and fasted for 24 hours with free access to water. CL or BP_2BL were injected into rats (0.9 mg/kg) through tail vein at a single dose. After i.v. injection, the rats were anesthetized with ether and a heparinized capillary was then inserted into the eyeground veins to get 0.5 mL blood into a plastic centrifuge tube at the time intervals of 5, 10, 15, 30, 45, 60, 90, 120, 240, and 480 minutes, respectively. About 0.5 mL blood was collected from the retro-orbital plexus in heparin-coated tubes and centrifuged at 1000 × *g* for 5 minutes at 4°C to get the plasma. All samples were immediately frozen at −20°C until analysis.

Liver-targeting profiles of DDB liposomes

Kunming mice (male, 8 weeks old, weighing 20 ± 2 g) were purchased from the Experimental Animal Center of China Pharmaceutical University. Randomly, 108 mice were divided into two groups, each with 54 mice. Mice were anesthetized with pentobarbital sodium (40 ± 60 mg/kg) and the CL or BP_2BL was injected into mice (1.3 mg/kg) through tail vein at a single dose. After administration, the mice were killed at the time intervals of 3, 5, 10, 15, 30, 45, 60, 90, and 120 minutes, respectively. At each sampling time, six mice were killed and their hearts, livers, lungs, kidneys, and brains were removed, washed with saline, blotted dry, and weighed. All samples were kept at −20°C until analysis.

The changes of drug content as a function of time in each tissue or plasma were recorded to calculate the area under the curve (AUC). Three targeting parameters [the intake rate (Re) and the targeting efficacy (Te)] were chosen in this study to evaluate the liver-targeting characteristics of DDB liposomes[30].

Results and discussion

Synthesis of BP_2B

The main objective of this study was to obtain the novel derivatives of bile acid that bile salts may be served as ligands to improve the liver targeting of liposomes and more easily to be obtained on an industrial scale compared to bile-phospholipids.

First, to protect three hydroxyl groups of bile acid, sodium cholate was refluxed in acetic anhydride in the presence of acetic acid to obtain triacetyl bile ester. BP_2B conjugate was synthesized by esterifying with PEG in the presence of DCC and DMAP at room temperature.

To detect whether esterification occurred, the FTIR and the ^{1}H-NMR were measured and their spectra of triacetyl bile ester, PEG_{2000}, and BP_2B were displayed, respectively. In the FTIR spectra, it was observed that the carbonyl stretching vibrations of ester linkage for triacetyl bile ester (Figure 1b) and BP_2B (Figure 1c) were located at 1735.71 and 1733.78 cm^{-1}, respectively, which were absent in the original PEG_{2000} (Figure 2a). These results indicated that the ester was formed by the reaction between the hydroxyl group in PEG_{2000} and the carboxylic group of triacetyl bile ester. The fingerprint regions at 1114.36 and 1116.64 cm^{-1} were corresponded to –C–O–C– stretching vibration in PEG_{2000} and BP_2B, respectively. All these results confirmed that Chol had been reacted into place on the PEG_{2000} backbone. The ^{1}H-NMR spectrum of the BP_2B was also resolved in Figure 3c. The signals at 0.73–2.23 ppm were attributed to the fragment of triacetyl bile ester. The 18-, 19-, and 21-position methyl protons in triacetyl bile ester found at 0.73, 0.81, and 0.92 ppm, respectively. The 3-, 7-, and 12-position acetyl protons in triacetyl bile ester found at 2.05, 2.09, and 2.14 ppm, respectively. The signals at 3.61–3.78 ppm were attributed to the repeating units in PEG_{2000}. These results further indicated that the esterification had definitely occurred.

Liposome preparation

The effect of BP_2B/PC weight ratios on EE was shown in Figure 4. It could be easily seen that the EE was significantly influenced by the mount of BP_2B. The EE of DDB liposome was decreased rapidly when the BP_2B/PC weight ratios were above 3.0%. Especially, the EE decreased to 20.35 ± 6.74% when the BP_2B/PC weight ratio was 20.0%. On the contrary, it is well known that the Te was higher with the increased amount of bile salts[31]. Also, the results of the mount of BP_2B/PC effect on the size, zeta potential, and polydispersity

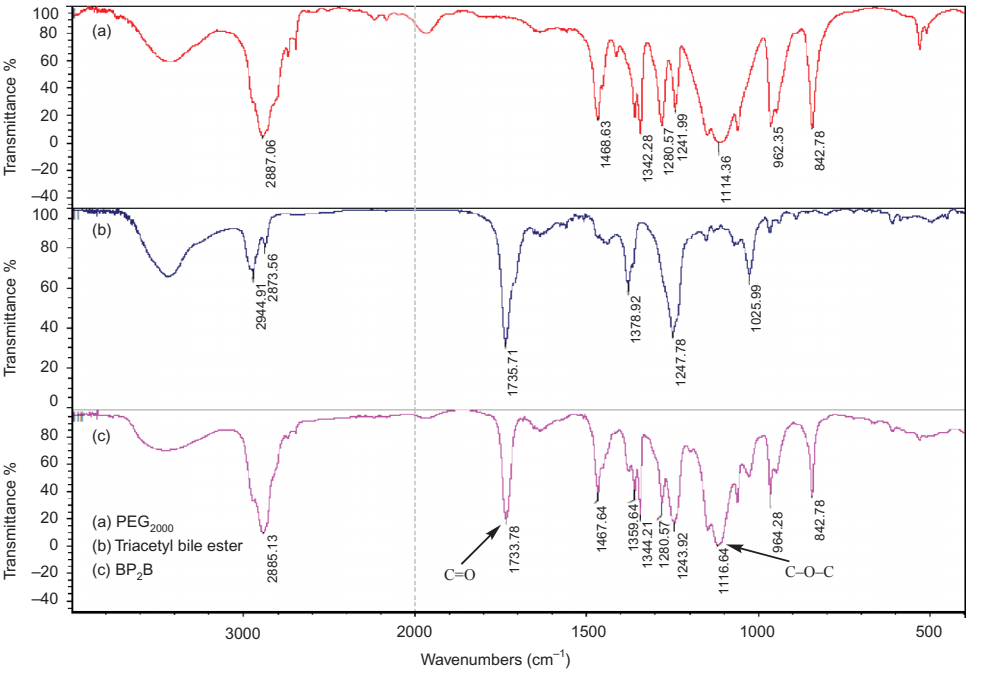

Figure 2. FTIR transmittance spectra for (a) PEG_{2000}, (b) Triacetyl bile ester, and (c) BP_2B.

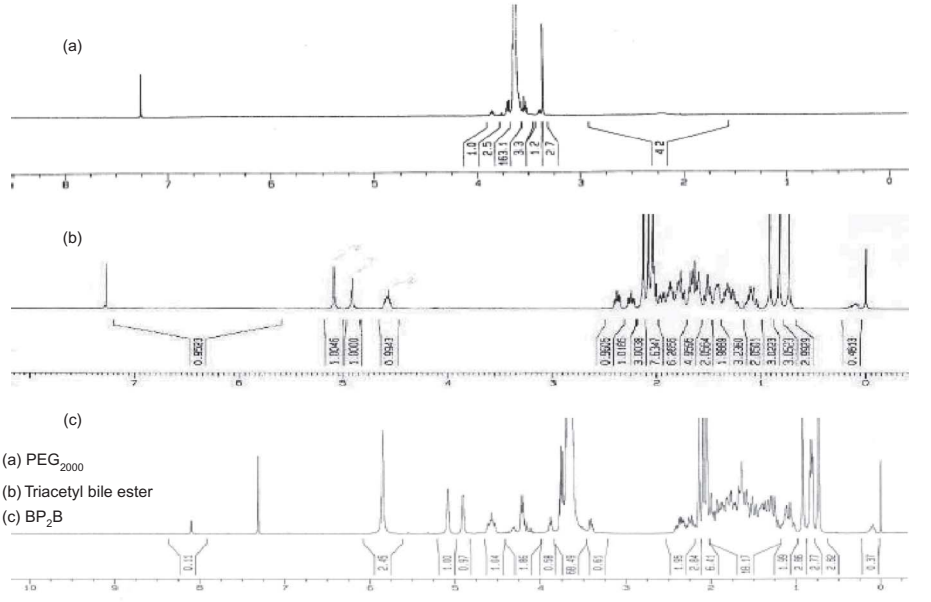

Figure 3. ^{1}H-NMR spectrum for (a) PEG_{2000}, (b) Triacetyl bile ester, and (c) BP_2B.

index (PI) of BP_2BL were shown in Figure 5. From the results we could conclude that the size, zeta potential, and PI of BP_2BL decreased with the increasing of BP_2B/PC. To keep high EE and Te of DDB liposome simultaneously, the BP_2B/PC weight ratio of 3.0% was selected in the end.

Physicochemical properties of CL and BP_2BL

The physicochemical properties of the optimal CL and BP_2BL formulation were studied. As shown in Figure 6, the shape of liposomes was round or oval by the transmission electron microscope (40,000). The EE of

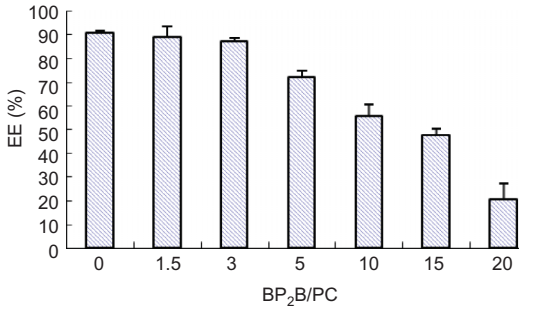

Figure 4. The effect of the ratios of BP_2B/PC on the EE of BP_2BL.

CL and BP_2BL were 90.65 ± 4.22% and 86.89 ± 4.62%, respectively. The average vesicle diameters of CL and BP_2BL were 309 ± 17 and 274 ± 14 nm; the zeta potentials were −24.9 ± 2.03 and −22.21 ± 7.03 mV; and the PIs were 0.337 ± 0.041 and 0.273 ± 0.017, respectively as shown in Figure 7.

In vitro release experiments

The in vitro drug release profiles without fetal calf serum (FCS) were shown in Figure 8. From the results we could see that without the existence of FCS, the release of DDB from liposomes was less than 40% in 24 hours. And the release of BP_2BL was slower than that of CL. It was possible because the bile salts could lower bilayer permeability by reducing the free volume created by the thermal motion of the fatty-acyl chains through inserting the lipid bilayer[32].

Pharmacokinetics in blood

The mean plasma concentration–time profiles of CL and BP_2BL following i.v. injection were shown in Figure 9 and the major pharmacokinetic parameters were listed in Table 1.

Table 1 shows that BP_2BL gave $t_{1/2\beta}$ of 222.92 ± 65.01 minutes as terminal elimination half-life, compared with 120.74 ± 10.23 minutes for CL, indicating a longer elimination half-life for DDB in modified liposome formulation. It appears that DDB in BP_2BL could be given intravenously with a long duration of action because of high drug and stability. The AUC and Cl values confirmed this trend. BP_2BL resulted in 1.44 times increase in AUC, significantly increased from 27,210.81 ± 241.56 to 39,130.53 ± 387.9 min·ng/mL ($P < 0.05$), and 13.7% decrease in Cl compared with CL ($P < 0.05$). In addition, mean residence time (MRT) of BP_2BL was longer than that of CL although statistically not significant ($P > 0.05$). All these results show a signification change in drug pharmacokinetic parameters through modifying the liposome by using BP_2B.

Liver-targeting profiles of CL and BP_2BL

Lots of studies have demonstrated that the phospholipid-conjugated bile salts can enhance the uptake of liposomes by hepatocytes[8–11]. And PEG is known to prolong the liposome in vivo through reducing the interactions of liposomes with biological components[33–35]. Therefore, to demonstrate whether BP_2BL can alter the distribution and MRT of the drug entrapped in liposomes, the in vivo fate of DDB was evaluated after administration of CL or BP_2BL to mice.

The mean liver concentration–time curves of CL and BP_2BL were protracted as shown in Figure 10. From the results we could also see that the release of DDB from BP_2BL in the liver was slower than that of CL, which means that the BP_2B could prolong the release of DDB from liposomes.

A comparative liver-targeting study was conducted between CL and BP_2BL to understand the magnitude of change in redistribution profiles of the drug when administered in BP_2BL. The AUC of different deliveries in each tissue or plasma was calculated. The AUC and the targeting parameters were shown in Table 2.

Re was defined as $Re = AUC_{BP2BL}/AUC_{CL}$. If the values of Re is greater than 1, the tissue is exposed to drug to a greater extent by the BP_2BL. In our study, the value of Re for the liver was 1.45 in the case of BP_2BL, indicating

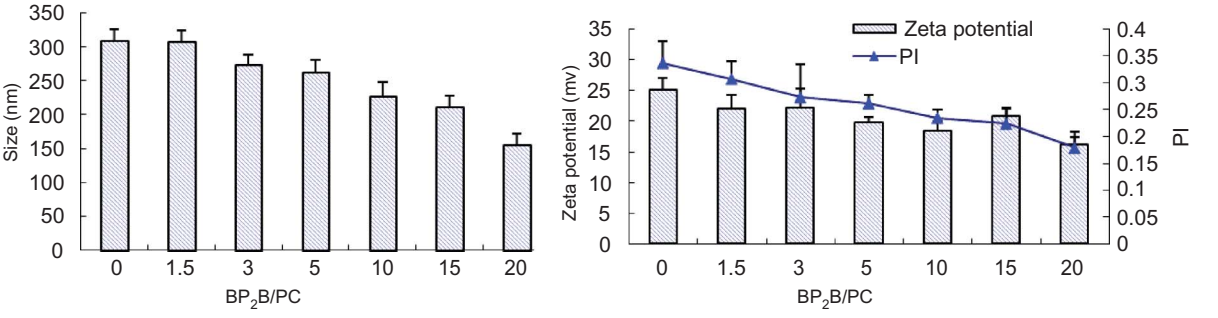

Figure 5. The effect of the ratios of BP_2B/PC on the profile of BP_2BL.

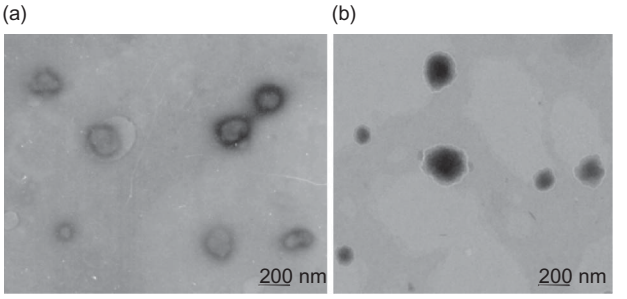

Figure 6. Transmission electron microscope photographs of CL (a) and BP_2BL (b).

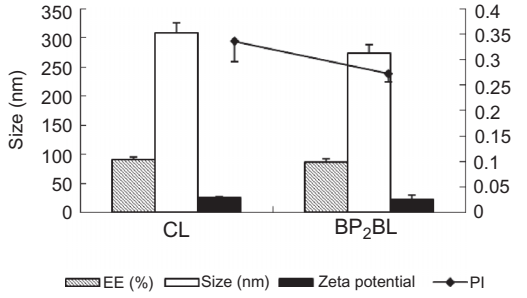

Figure 7. Comparative profiles of CL and BP_2BL.

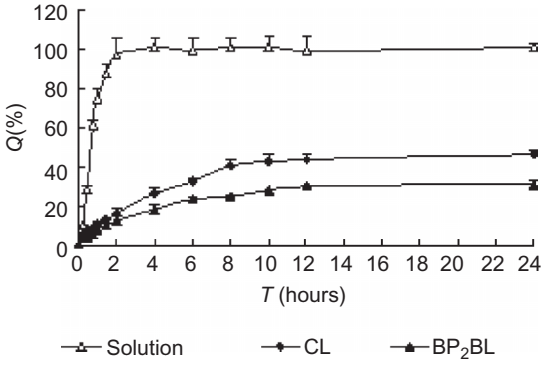

Figure 8. Dynamic release profiles of the solution, CL and BP_2BL ($n = 3$).

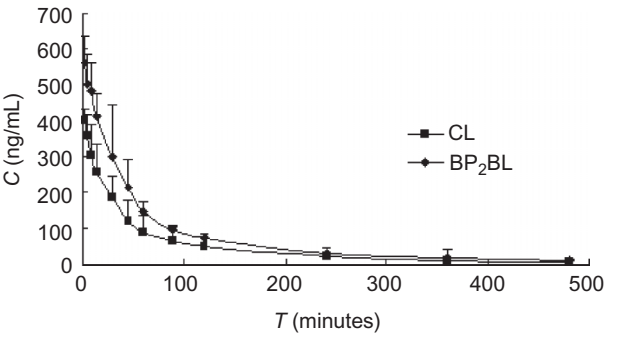

Figure 9. The concentration–time curves of DDB in plasma of rats after i.v. administration of DDB in different formulations (n = 6, mean ± SD).

Table 1. Pharmacokinetic parameters of DDB after intravenous (i.v.) injection of BP_2BL or CL in rats ($n = 6$).

Compartmental parameters	Parameter values ± SD	
	CL	BP_2BL
MRT_{0-T} (minutes)	127.11 ± 18.93	157.06 ± 22.24
AUC_{0-T} (min·ng/mL)	27,210.81 ± 241.56	39,130.53 ± 387.99*
Cl [mg/min/(ng/mL)]	3.88E–5±2.58E–6	5.33E–6 ± 1.53E–6*
$t_{1/2\beta}$ (minutes)	120.74 ± 10.23	222.92 ± 65.01**
C_{max} (ng/mL)	360.32 ± 55.45	564.59 ± 76.69*

Values are expressed as mean ± SD, *$P < 0.05$, **$P < 0.01$.

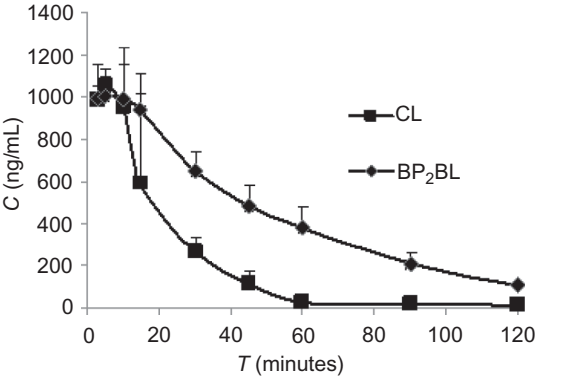

Figure 10. The concentration–time curves of DDB in liver tissues of mice after i.v. administration of 1.3 mg/kg DDB in different formulations ($n = 6$, mean ± SD).

that the exposure of the DDB to the liver was significantly increased by entrapment in BP_2BL. Therefore, it could be concluded that BP_2BL was more efficient to deliver DDB to the liver as compared to CL.

Re provides a good indication about the relative efficacy of two delivery systems in reference to one tissue, but it does not provide any information about the efficacy of a given delivery system through the ratio of targeted tissue to nontargeted tissue distribution of drug. Thus, to further demonstrate the efficiency of a delivery system against the nontargeted tissue, we defined that the values of Te were determined by the following equation, $Te = AUC_{liver\ target}/AUC_{untargeted}$. From the results we could see that the Te values of the liver to other tissues for liposomal DDB increased significantly.

In summary, the BP_2BL showed the largest value of AUC for the liver. The high distribution of the drug in the livers with BP_2BL formulations may be explained by the receptor-ligand binding. This indicated that the BP_2BL used in this study was preferable for targeting to the liver than other tissues compared to CL.

Table 2. Liver targeting parameters of CL and BP_2BL after intravenous (i.v.) administration in mice ($n = 6$).

Samples	$AUC_{0-\infty}$ [(ng/mL)·min]			Te	
	CL	BP_2BL	Re	CL	BP_2BL
Plasma	21,088.9 ± 319.01	43,926.4 ± 591.04*	2.08 ± 0.21	2.64±0.32	1.84 ± 0.02
Heart	3433.86 ± 121.42	3831.23 ± 192.93	1.12 ± 0.15	16.18±1.93	21.04 ± 1.36*
Liver	55,570.8 ± 392.84	80,606.2 ± 634.22**	1.45 ± 0.09	1	1
Lung	11,193.11 ± 219.03	12,630.2 ± 241.49	1.13 ± 0.04	4.96±0.48	6.38 ± 0.32
Kidney	9446.08 ± 129.11	5377.49 ± 212.58*	0.57 ± 0.01	5.88±0.51	14.99 ± 0.63**
Brain	4772.94 ± 99.13	1113.69 ± 102.35**	0.23 ± 0.01	11.64 ± 0.99	72.38 ± 3.39**

$Re = (AUC)_{BP_2B}/(AUC)_{CL}$, $Te = (AUC)_{\text{liver targeted}}/(AUC)_{\text{untargeted.}}$ $^*P < 0.05$, $^{**}P < 0.01$.

Conclusions

In this study, we have synthesized a novel PEG-conjugated bile salt (BP_2B) with simple and cheap method and successfully modified liposomes of DDB-utilized BP_2B. The physicochemical characterization of BP_2BL prepared by PC/Chol, with or without BP_2B modification, included an investigation of their properties, such as zeta potential, size, and morphology. The images of transmission electron microscope (TEM) indicated that all these liposomes were spherical in shape and homogeneous in size. The results of the release profiles of CL and BP_2BL in vitro were indicated that BP_2BL was slower than that of CL.

The studies of pharmacokinetics in blood of CL and BP_2BL had shown that the values of AUC, C_{max}, $t_{1/2\beta}$, and MRT of BP_2BL were increased greatly and the Cl of BP_2BL was decreased compared with CL. All these results show that the BP_2BL could sustain release in vivo through modifying the liposome by using BP_2B.

To evaluate the liver targetability of CL and BP_2BL, we have studied the tissue distribution and calculated the targeting parameters. All these results showed that the incorporation of BP_2B (BP_2B/PC = 3%) into liposomes could be a great potential as drug delivery carriers for liver targeting that has targeted and sustained drug delivery just as PEGylated galactosylated liposomes[36].

However, the liver consisted of hepatocytes (hepatic parenchymal cells), Kupffer cells, and endothelial cells (hepatic nonparenchymal cells), and the hepatocyte is our target tissue for drug therapy. So to confirm whether the BP_2B-LP can improve the hepatocytes cell uptake, the factors that affected the hepatocytes cell uptake and so on will be pursued in our future work.

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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