Department of Pharmacy[1], Department of Cardiology[2], Second Hospital of Shandong University; School of Material Science and Engineering[3], Shandong University, Jinan; Heze Medical College[4], Heze; School of Pharmaceutical Sciences[5], Shandong University, Jinan, China

Bile salt liposomes for enhanced lymphatic transport and oral bioavailability of paclitaxel

Bin Zhang [1], Aiying Xue [2], Chen Zhang [3], Jinlong Yu [1], Wen Chen [4], Deqing Sun [1, 5]

Received October 28, 2015, accepted February 4, 2016

Deqing Sun, Department of Pharmacy, Second Hospital of Shandong University, 247 Beiyuan Street, Jinan 250033, China
deqingsunsu@163.com

Pharmazie 71: 320–326 (2016) *doi: 10.1691/ph.2016.5184*

Paclitaxel (PTX), a BCS class IV drug that is characterized by its poor solubility and is a substrate for P-glycoprotein, is one of the most widely used antineoplastic agents. However, oral administration of PTX for chemotherapy is highly challenging. The aim of this study was to develop bile-salt liposomes (BS-Lips) to enhance the absorption of PTX and thus improve its therapeutic outcome. The BS-Lips were prepared by the thin-film hydration method and characterized in terms of particle size and morphology. Drug release and in vitro stability in simulated gastrointestinal fluids and in media of different pH values were evaluated, as well as *in vivo* performance, including antitumor activity and pharmacokinetics in rats, with the plasma concentrations determined by a HPLC method. The PTX-loaded BS-Lips were successfully prepared with a diameter of approximately 150 nm and an entrapment efficiency of greater than 90 percent. Moreover, the BS-Lips were not affected by gastrointestinal enzymes or pH alternation, as evident from the unchanged particle size and the drug retained in BS-Lips after 6 h incubation. The insertion of bile salt into the lipid layer of liposomes increased the lymphatic transport of PTX by twofold. Importantly, BS-Lips increased the oral bioavailability of PTX by 2.5 and 4-fold, respectively, compared with conventional liposomes (Lips) and Taxol (free drug), thereby displaying a better inhibition of tumor growth that was similar to the group injected intravenously with Taxol. In conclusion, the BS-Lips represent promising vehicles for the oral delivery of PTX, thereby enabling an intravenous-to-oral switch for cancer chemotherapy.

1. Introduction

Palcitaxel (PTX) is one of the most widely used antineoplastic agents with a broad spectrum of antitumor activity against a wide range of cancer diseases, including leukemia, lung cancer, metastatic breast and refractory ovarian cancer (Dahmani et al. 2012; Jauhari and Dash 2006; Rowinsky et al. 1992). Currently, the drug is administered as an intravenous infusion in a mixture of Cremophor EL and alcohol because of its poor solubility in water. However, various side effects, including hypersensitivity reactions, hyperlipidaemia, neurotoxicity, allergic shock and altered pharmacokinetics, are incurred by such formulations, thereby greatly compromising the therapeutic effect (Agüeros et al. 2010; Sharma et al. 2015; Zabaleta et al. 2012). To improve the therapeutic index, a new PTX formulation for intravenous injection, a protein bound-PTX nanoformulation without the inclusion of toxic surfactants and organic solvents, was developed and approved by the FDA in 2005 (Koudelka and Turánek 2012; Sharma et al. 2015).

Abbreviations
Paclitaxel, PTX; bile salt liposomes, BS-Lips; conventional liposomes, Lips; P-glycoprotein, P-gp; polyoxyethylated castor oil, cremophor EL; encapsulation efficiency, EE %; Soybean phospholipid, SPC; Sodium deoxycholate, SDC; sodium lauryl sulfate, SLS; transmission electron microscope, TEM; polydispersity index, PI; dynamic light scattering, DLS; simulated gastric fluid, SGF; simulated intestinal fluid, SIF; peak plasma concentration, C_{max}; half-life,$T_{1/2}$; area under the plasma concentration–time curve up to the last time point, AUC; absolute bioavailability, F%; high-performance liquid chromatography, HPLC.

However, among the administration routes, oral delivery is considered to be most readily accepted route of drug administration, owing to its better compliance, flexibility in formulation and reduced cost (Luo et al. 2014; Yin et al. 2013). In particular, oral delivery of anticancer drugs for chemotherapy is an interesting topic in 21st century medicine as it may alter the current regimen of chemotherapy and provide improved patient care, comfort, reduction in healthcare costs and reduction in side effects (Mei et al. 2013; Narvekar et al. 2014; Schoener and Peppas 2012). However, the oral administration of anticancer drugs such as PTX, mitoxantrone, vinblastine, irinotecan and docetaxel is very challenging due to their low aqueous solubilities, poor intestinal permeabilities, metabolism by cytochrome P-450 enzyme and high level of P-gp efflux (Mazzaferro et al. 2013; Shapira et al. 2012; Thanki et al. 2013).

A nanocarrier is an efficient tool to encapsulate poorly soluble drugs and to protect therapeutic molecules, prolonging their blood circulation and changing the tissue distribution (Bertrand et al. 2014; Trickler et al. 2008). To achieve oral PTX delivery, several nanocarriers such as microemulsions (Nornoo et al. 2009; Pandita et al. 2011), liposomes (Pawar et al. 2014), polymeric micelles (Dahmani et al. 2012; Mo et al. 2011) and solid lipid nanoparticles (Baek and Cho 2015; Miglietta et al. 2000) have been reported. However, all of the reported nanocarriers have a low drug payload, poor stability in the gastrointestinal tract, low intestinal permeability, low bioavailability, or an unfavorable biocompatibility for oral PTX administration (Thanki et al. 2013). Thus, a new nanocarrier for oral PTX delivery is highly desired.

BS-Lips, vesicles that are prepared by incorporating bile salts such as deoxycholate (a non-ionic surfactant) into the lipid bilayers of the liposomes (Senior 2001), are promising carriers for the oral administration of biological macromolecular drugs such as insulin, vaccines and salmon calcitonin (Mann et al. 2006; Niu et al. 2012;

Song et al. 2005). Compared to Lips, BS-Lips are more competent for resistance to disruption by digestive enzymes and physiological bile salts in the gastrointestinal tract (Niu et al. 2012). Moreover, the incorporation of bile salt helps to promote the transit of liposomes through the gastrointestinal tract epithelia by a membrane-destabilizing effect through the interaction between the bile salt and the intestinal epithelia (Zabaleta et al. 2012). Thus, it is hypothesized that the BS-Lips possess great potential for solving the problems encountered in delivering PTX via the gastrointestinal tract.

Thus, in this study we show the use of BS-Lips for the oral delivery of PTX for the purpose of increasing its oral bioavailability and thus improving its therapeutic index. The aims of the present study were to (i) prepare the PTX-loaded BS-Lips, (ii) study their stability in simulated gastrointestinal fluids, (iii) investigate its pharmacokinetics in rats and examine the intestinal lymphatic transport and (iv) evaluate its antitumor activities in H22-bearing mice. In previous reports, BS-Lips were only used to deliver macromolecular drugs such as insulin or conventional insoluble drugs such as fenofibrate. To the best of our knowledge, this report is the first on the oral delivery of anticancer drugs for chemotherapy by using BS-Lips, which would greatly expand the application of BS-Lips in disease treatment. Importantly, the insertion of bile salt into the lipid layer of liposomes enhanced lymphatic transport of PTX by 2-fold and resulted in an increase in the oral bioavailability of PTX by 2.5 and 4-fold, respectively, compared with conventional liposomes and Taxol (free drug).

2. Investigations and results

2.1. Preparation and characterization of BS-Lips

The BS-Lips were fabricated [via] the thin-film hydration method. The impact of SDC/SPC molar ratios on EE (%) of PTX and particle size of BS-Lips is displayed in Fig. 1. When the ratio was changed from 1:1 to 1:3, an increase of EE (%) from 50% to 90% was observed; however, the EE (%) was not altered any more as the ratio was varied from 1:3 to 1:10 (Fig. 1 A). The particle size of BS-Lips was also impacted by SDC/SPC molar ratios, which BS-Lips with smallest diameter size were obtained at 1:4 ratio (Fig. 1 B). Thus, 1:4 of SDC/SPC ratio was selected as optimized formulation for BS-Lips.

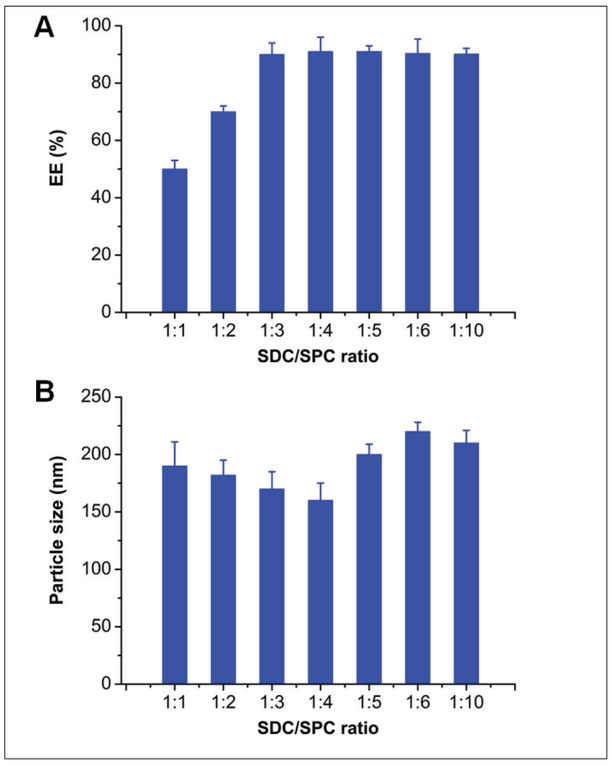

Fig.1: Influence of SDC/SPC molar ratios on (A) EE (%) and (B) particle size of BS-Lips (n = 3).

The diameter size/zeta-potential of BS-Lips was 154.3 nm/4.8 mV (Fig. 2 A), and the PI was 0.23, which was less than 0.3, thus indicating a homogeneous nanoparticle formulation (He et al. 2013). As shown in the TEM examination (Fig. 2 B), spherical particles with a particle size within the range of 100 to 200 nm were observed, therefore corresponding to those measured using DLS. The PTX drug was well encapsulated into the BS-Lips and the conventional Lips, with an EE (%) of greater than 90%.

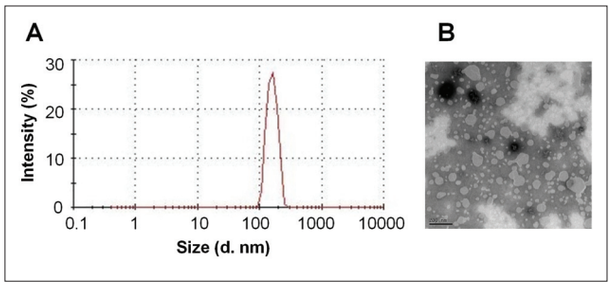

Fig. 2: (A) Particle size and size distribution by intensity and (B) TEM image of BS-Lips.

2.2. In vitro drug release

The PTX release from the PTX formulations is shown in Fig. 3. A sustained release profile over time was observed from BS-Lips and conventional Lips; however, the PTX release from the former was slower than from the latter, with around 50 percent of the PTX released from Lips by 36 h, while only 40 % of the drug were released from BS-Lips. It was shown previously that the insertion of a surfactant such as HS-15 or Cremophor-EL into the structure of the lipid bilayer would help to improve the *in vitro* stability of liposomes and thus reduce the release of drug (Ji et al. 2012). Bile salt is a nonionic surfactant, thus a similar effect on the stability of liposomes would be produced when it was inserted into the lipid bilayer.

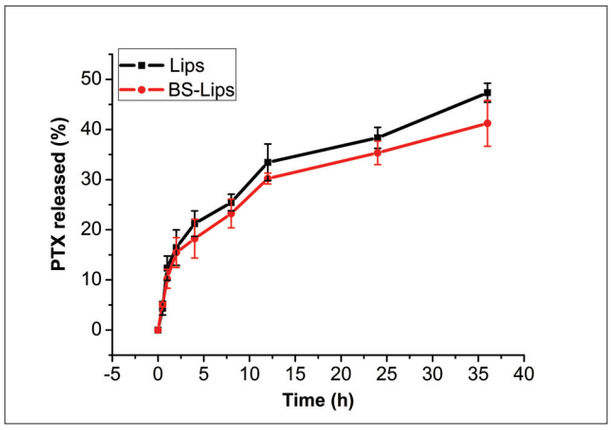

Fig. 3: In vitro drug release from conventional Lips and BS-Lips in pH 6.8 PBS containing 1% (w/w) SDS.

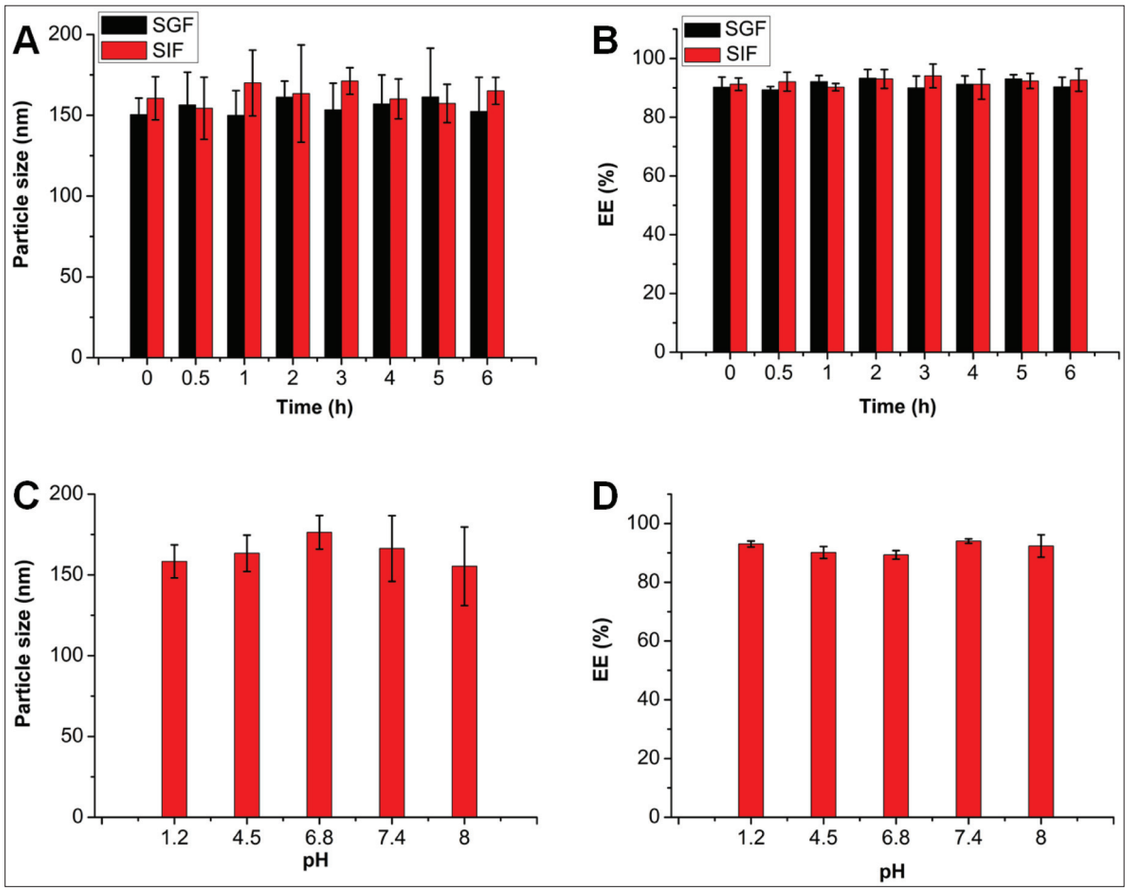

Fig. 4: Influence of (A and B) simulated gastrointestinal tract fluids and (C and D) pH on the particle size and retained drug in BS-Lips after 6 h incubation.

2.3. Stability study in simulated gastrointestinal fluids and media of different pH values

It is well known that the stability of nanoscale drug delivery systems is affected by the physicochemical environment of the gastrointestinal tract, which can reduce the amount of drug for transport across the intestinal epithelium and ultimately compromise the therapeutic outcome (Parmentier et al. 2011). Therefore, we performed a stability study of BS-Lips in simulated gastrointestinal fluids and in media of different pH values. The effects of SGF and SIF on the particle size and retained drug in BS-Lips are shown in Fig. 4 (A and B). The particle size and retained drug in BS-Lips were not influenced by both SGF and SIF as evident by the largely unaltered particle size and the high amount of retained drug (greater than 90 %) at the various time points. The influence of pH values on the particle size and retained drug in BS-Lips is shown in Fig. 4 (C and D). After 6 h of incubation in media at pH 1.2, 4.5, 6.8, 7.4 and 8.0, which simulate the pH conditions of the stomach, pylorus, small intestine, caecum and colon sections, respectively (McConnell et al. 2008; McDowell and McLeod 2007), there was no change in the particle size or the retained drug.

Table 1: Pharmacokinetic parameters of PTX post oral administration of BS-Lips, Lips and Taxol and intravenous injection of Taxol in rats (n=6)

Formulations	Tmax (h)	Cmax (μg/mL)	T1/2 (h)	AUC0–t (μg·h/mL)	F (%)
Taxol (intravenous injection)	0.083±0.00	8.51±2.08	2.73±0.45	6.45±1.25	–
Taxol (oral)	1.25±0.66	0.25±0.076	2.40±0.53	0.67±0.45	10.38
Lips (oral)	0.83±0.15	0.43±0.17	3.88±0.87	1.05±0.48	16.28
BS-Lips (oral)	0.83±0.15	0.70±0.36[a, b]	4.92±1.22	2.56±0.87[a, b]	39.69

[a] P<0.01 vs Lips (oral); [b] P<0.001 vs Taxol (oral)

2.4. Pharmacokinetics in rats

The plasma concentration profiles of PTX after a single intravenous administration of Taxol (free drug) at 10 mg/kg are depicted

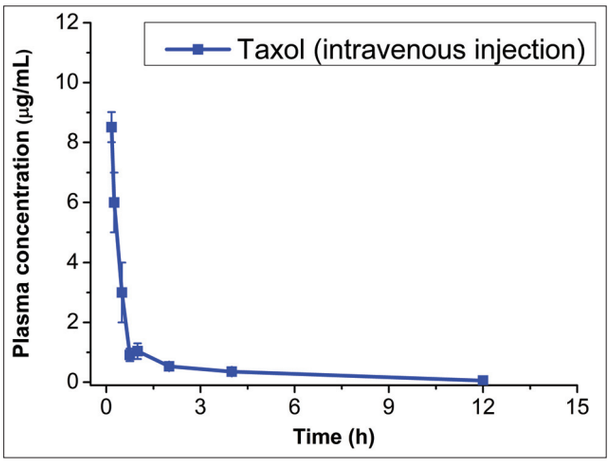

Fig. 5: Drug plasma concentration–time profile of PTX after intravenous injection of Taxol in rats. Data are represented as the mean ± S.D. (n = 6).

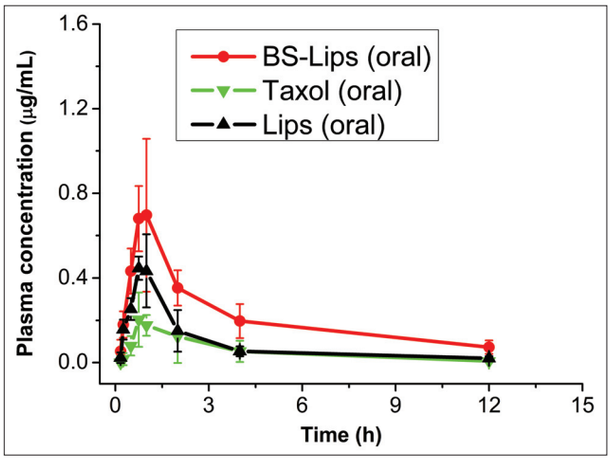

Fig. 6: Drug plasma concentration–time profile of PTX after oral administration of BS-Lips, Lips and Taxol in rats. Data are represented as the mean ± S.D. (n = 6).

in Fig. 5. The plasma PTX concentration declined rapidly within 2 h, and the drug was cleared rapidly. The C_{max} was approximately 8.51 μg/mL. The mean values obtained for AUC_{0-t} and $T_{1/2}$ were 6.45 μg·h/mL and 2.73 h, respectively (Table).

The plasma concentration–time curves and pharmacokinetic parameters of PTX after oral administration of conventional Lips, BS-Lips and Taxol in rats are shown in Fig. 6 and the Table. After an identical dose of PTX was administered to rats through the PTX formulations, the plasma concentration reached the maximum concentration at approximately 1 h and then decreased slowly.

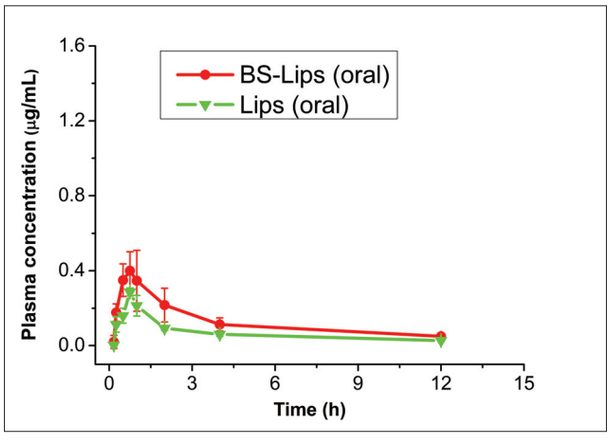

Fig.7: Drug concentration–time profiles of PTX in rats treated with cycloheximide or saline (control) after oral administration of BS-Lips and Lips in rats. Data are represented as the mean ± S.D. (n = 6).

Compared with Taxol, Lips and BS-Lips increased the C_{max} by more than 2- and 3-fold, respectively, and increased the AUC_{0-t} by approximately 1.5- and 4-fold, respectively, indicating enhanced absorption. Importantly, the C_{max} and AUC_{0-t} of BS-Lips were approximately 1.7- and more than 2.5-fold greater than those of Lips, respectively, which revealed that the insertion of bile salt into the lipid layer significantly improved the oral bioavailability of PTX. Moreover, the F% of BS-Lips was up to 39.7%, further confirming the better absorption of PTX.

2.5. *Intestinal lymphatic transport*

For a lipid formulation, lymphatic transport had significant impact on the oral bioavailability of a number of highly lipophilic drugs (Trevaskis et al. 2008). As indicated in Fig. 7, the plasma concentrations obtained from rats pretreated with a chylomicron flow blocker were significantly lower than that of control rats (Fig. 6), irrespective of the difference in formulations. It thus indicated that the intestinal lymphatic transport played an important role for the absorption of PTX. In detail, approximately 45% and 20% decrease in C_{max} and AUC_{0-t}, respectively, for Lips and approximately 55% and 60% reduction in C_{max} and AUC_{0-t}, respectively, for BS-Lips were observed. It thus indicated that the fraction of lymphatic absorption from the latter was greater than the former.

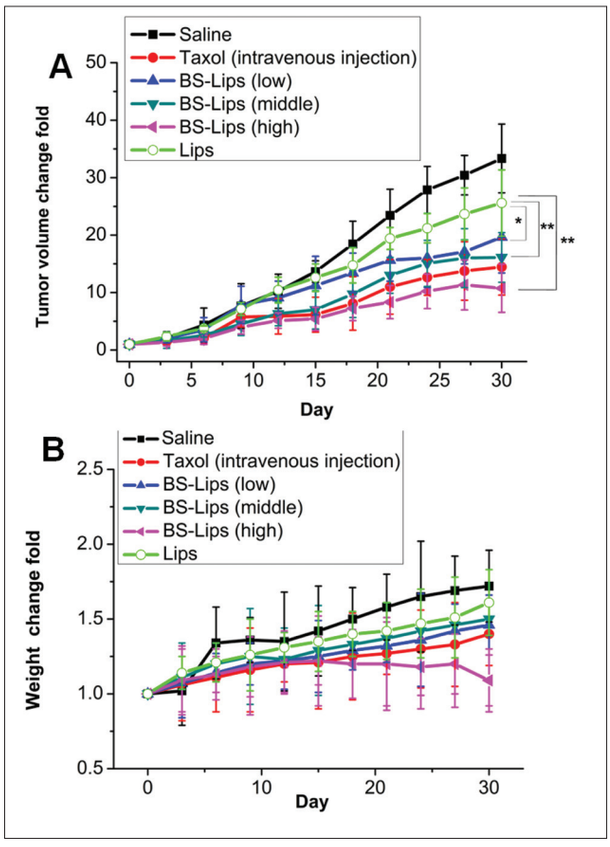

Fig. 8: (A) Tumor volume and (B) body weight change fold increases in H22-bearing mice after oral administration of BS-Lips at three doses and Lips or intravenous injection of Taxol every other day, with saline used as a control.

2.6. *In vivo antitumor study*

The *in vivo* antitumor activity of the PTX formulations was assessed in H22-bearing mice. When the average tumor volume in mice reached approximately 100 mm^3, treatments were carried out *via* intravenous injection of saline and Taxol and via oral administration of BS-Lips at three doses and of Lips. Saline was used as a control formulation. Body weight and tumor volume were examined every 3 days. The growth curves of the H22 tumors in mice after the treatment are displayed in Fig. 8 (A). The tumor volume from the saline group increased by approximately 35-fold after 30 days, indicating no antitumor effect. For the group injected with Taxol, the increase in tumor volume was approximately 14-fold, thus demonstrating that PTX significantly suppressed the tumor growth. The increase in tumor volume was approximately 25-fold for conventional Lips and 19-, 14- and 10-fold for the low, medium and high doses of BS-Lips, respectively. These results suggested that oral PTX in the forms of Lips and BS-Lips also inhibited the tumor growth significantly. However, BS-Lips, displaying a dose-dependent tumor growth inhibition, exhibited a better antitumor effect than the conventional Lips. Most importantly, a

similar inhibition of tumor growth was obtained from the groups that were orally administered BS-Lips and from the groups intravenously injected with Taxol at a dose of 10 mg/kg, further confirming the more effective inhibition of tumor growth by BS-Lips.

The changes in body weight of the mice treated with the various formulations are shown in Fig. 8 (B). All the groups, except for the group orally administered BS-Lips at a high dose, did not lose any body weight after administration, indicating the safety of the various formulations. However, the oral administration of BS-Lips at a high dose resulted in a significant decrease in body weight, indicating a serious side effect.

2.7. Conclusions

BS-Lips with a diameter of 150 nm were prepared for enhancing the absorption of PTX. The BS-Lips was able to maintain their integrity in simulated gastrointestinal fluid, enhance lymph PTX transport and thus increase oral bioavailability of PTX by 2.5 and 4-fold compared with conventional Lips and Taxol, respectively, thus resulting in better antitumor activity. In summary, the present BS-Lips are promising nanocarrier systems for the oral delivery of PTX, thereby achieving an intravenous-to-oral switch for cancer chemotherapy.

3. Discussion

It was demonstrated that the investigated BS-Lips remain intact in the presence of the gastrointestinal enzymes and pH alterations. Oral administration is an attractive route to deliver drugs incorporated in nanocarriers because of its ease of administration and patient compliance (Ensign et al. 2012; Gamboa and Leong 2013). However, drugs administered orally are challenged by an acidic environment, gastrointestinal enzymes, pH alterations and a tight monolayer of endothelial cells present throughout the gastrointestinal tract (Bakhru et al. 2013; Sakuma et al. 2001). The maintenance of intact nanoparticles is very critical for enhanced drug absorption (Niu et al. 2011, 2014). The enhanced stability of BS-Lips was related to the incorporation of bile salt into the lipid bilayer. Previous reports indicated that insertion of a surfactant into the structure of a lipid bilayer helps to maintain the integrity of liposomes by steric hindrance of the pancreatic enzymes or the membrane spanning structure that may increase the intermolecular forces in the membrane (Ji et al. 2012; Parmentier et al. 2011), thus reducing the drug leakage. Moreover, the membrane of BS-Lips would become more flexible due to the presence of bile salt in the lipid bilayer, which is beneficial for reducing the leakage of drug induced by membrane distortion in acidic medium (Hu et al. 2013). In fact, the present report was in line with a previous report that BS-Lips had good protection of insulin against enzymatic degradation by pepsin, trypsin and α-chymotrypsin in vitro and a sustained hypoglycemic effect for 24 h (Niu et al. 2012).

The insertion of bile salt into the lipid layer of liposomes could promote the lymphatic transport of PTX into systemic circulation. The uptake by lymph node macrophages was significantly affected by bilayer fluidity of liposomes, with being more susceptible to uptake by macrophages for increased bilayer fluidity (Oussoren et al. 1997). The BS-Lips are known to have increased membrane fluidity in contrast to conventional liposomes (Chen et al. 2011). Thus, BS-Lips were preferred to be uptaken by lymph node macrophages. Second, the surfactants, such as bile salt and Tween-80, could increase the permeability of intestinal membrane, promote the affinity between lipid particles and the intestinal membrane and open the tight junctions in the epithelial lining (Li et al. 2009; Sun et al. 2011). Additionally, intestinal administration of a small amount of bile salt could stimulate a rapid increase in biliary phospholipid output, thus resulting in an increase in lymph lipid transport (Trevaskis et al. 2005).

The oral bioavailability of PTX in the BS-Lips was markedly improved as compared to conventional Lips, resulting in better tumor growth inhibition. The oral bioavailability of P-gp drug substrates such as PTX that exhibit very poor absorption can be enhanced by their formulation into nanocarriers, probably as a result of active nanocarrier uptake by the enterocytes mediated by a transcytosis or endocytosis process (Attili-Qadri et al. 2013; Roger et al. 2009). Thus, the maintenance of intact of BS-Lips in the gastrointestinal tract was very important. BS-Lips maintained its integrity in SGF or SIF, thus increasing the chance that the nanocarriers were able to transit across the intestinal epithelium barrier. Moreover, this translocation was further enhanced by the incorporation of bile salt into the membrane, owing to that he presence of bile salt in the lipid bilayer may allow conventional liposomes to undergo a vesicle–micelle transition, producing mixed micelles that behave as good vehicles for drugs, especially insoluble ones, as well as one of the most important mesophases for absorption (Chen et al. 2009; Porter et al. 2007). For a lipid formulation, intestinal lymphatic transport plays a very important role in the absorption enhancement of lipophilic drugs since the drug can be delivered to systemic circulation without first-passing effect of liver; moreover, the lymphatic transport can be enhanced by the oral absorption of intact nanoparticles (He et al. 2015). Here, we confirmed that lymphatic absorption of PTX was enhanced significantly due to the addition of bile salt. We therefore ascribed the good absorption of PTX through BS-Lips to two main factors: the maintenance of BS-Lips integrity in the gastrointestinal tract and enhanced lymphatic absorption by the incorporation of bile salt into the formulation.

4. Experimental

4.1. Materials

PTX was from Cisen Pharmaceutical Co. Ltd. (Shandong, China). Taxol was obtained from Bristol-Myers Squibb (China) Investment Co. Ltd. (Shanghai, China). SPC (greater than 70% phosphatidylcholine) was from Taiwei Pharmaceutical Co. Ltd. (Shanghai, China). SDC, pepsin, pancreatin, cholesterol and SLS were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). H22 cells were from the Shandong Academy of Medical Sciences (Shandong, China). Fetal bovine serum and Dulbecco's Modified Eagle Medium were purchased from Invitrogen Life Technologies Inc. (California, USA). Male Wistar rats (200–250 g) and ICR mice (20–25 g) were from Vital River Laboratory Animal Technology Co. Ltd. (SCXK [JING] 20120001, Beijing, China). All other reagents used were of analytical grade.

4.2. Preparation of BS-Lips

The PTX-loaded BS-Lips were prepared by the thin-film hydration method previously reported by Chen et al (2009). Briefly, 10 mg of PTX, 334 mg of SPC and 50 mg of SDC were first dissolved in 10 mL of ethyl ether, followed by evaporation of the mixture under vacuum at 35 °C to form a thin film. A crude dispersion of liposomes was fabricated by adding 10 mL of phosphate buffer (pH 7.4) into the lipid film and hydrating for 20 min in a water bath at 30 °C. Subsequently, the particle size of BS-Lips was reduced by further homogenization using a high-pressure homogenizer (Niro-Soavi, S.p.A., Parma, Italy) at 200 bar for 15 cycles. The PTX-loaded Lips were prepared as described above, except for replacing the SDC with 10 mg of cholesterol.

4.3. Characterization of BS-Lips

4.3.1. Particle size and zeta-potential determination

The determination of the particle size, PI and zeta-potential of BS-Lips and Lips were performed on a 3000 HS Potential/Particle Sizer (Malvern Instruments, Malvern, UK). Three parallel measurements were carried out for each sample.

4.3.2. Transmission electron microscopy

One drop of the BS-Lips suspensions was placed onto on a copper grid and dried for 1 min at 25 °C after blotting off the excess sample with filter paper. Subsequently, one drop of phosphotungstic acid (2%, w/v, pH 6.5) was dripped onto the grid and maintained for 1 min for staining. Upon removal of the excess staining solution, the sample was air-dried for 15 min at 25 °C and then examined using TEM (H-700, Hitachi, Japan) with a field-emission gun at an acceleration voltage of 200 kV.

4.3.3. Encapsulation efficiency

The EE was estimated from the following formula: EE (%) = (PTX encapsulated in BS-Lips /total PTX added) × 100%. The drug content was measured by the HPLC system (Agilent 1200, USA) with a column (150 mm × 4.6 mm, Diamonsil™, Dikma). The mobile phase was a mixture of acetonitrile and water (70:30, v/v) pumped at a flow rate of 1.0 mL/min at 30 °C (Biswas et al. 2012). The sample injection volume was 20 μL, and the detection wavelength was 227 nm.

4.4. In vitro drug release

The drug release from BS-Lips or Lips was determined using a dialysis method described in a previous report (Bao et al. 2014). Briefly, 1 mL of sample was placed in a dialysis bag (MWCO = 10,000 Da) and then was immersed in 50 mL of PBS contained 1% SDS (w/v). The addition of SDS was for solubilizing the released drug. The release medium was stirred at a speed of 100 rpm at 37 °C in water bath. At each

predetermined time point, 1 mL of the external medium was withdrawn and replaced with equal volume of fresh medium. The amount of PTX was measured by the HPLC method described above. Triplicates of each sample were assessed.

4.5. Stability in simulated gastrointestinal fluids and media with different pH values

The stability study of PTX-loaded BS-Lips was performed in SGF (USP XXIII, pH 1.2, pepsin 0.32% w/v), in SIF (USP XXIII, pH 7.5, pancreatin 1% w/v) and in media with different pH values (pH 1.2, 4.5, 6.8 and 8.0). The media at pH 1.2 was 0.1 M HCl without pepsin, media at pH 4.5 was acetate buffer solution, and media at pH 6.8 or 8.0 was phosphate buffer solution. Briefly, 1 mL of PTX-loaded BS-Lips was mixed with 1 mL of the test fluids and then incubated for 6 h at 37 °C. Six hour later, the samples were centrifuged at 1,000 × g for 1 min to remove the insoluble particles. The particle size of the supernatant was determined by the 3000 HS Particle Sizer (Malvern Instruments, Malvern, UK), and the drug content was assayed by the HPLC method described above.

4.6. Pharmacokinetics in rats

The rats used in the experiments received care according to the Shandong University Unit for Laboratory Animal Medicine guidelines. The animal experiments were conducted using protocols approved by the Shandong University Institutional Animal Care and Use Committee.

The pharmacokinetics study of the PTX formulations, BS-Lips, Lips and Taxol were performed in Wistar rats. Twenty-four rats were fasted for 12 h and randomly divided into four groups (A, B, C and D, n=6). Groups A, B and C were orally administered the PTX formulations of BS-Lips, Lips and Taxol at PTX dose of 10 mg/kg, while group D was administered Taxol (free drug) via a tail vein injection at a dose of 10 mg/kg. At predetermined time points of 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 12 h, 0.5 mL of blood sample was sampled from the sinus jugularis and placed into heparinized tubes. Plasma samples were harvested by centrifugation (AnkeTGL-16G, Feige, Shanghai) for 5 min at 4,000 × *g* and stored at –20 °C until analysis.

4.7. Intestinal lymphatic transport

To examine the intestinal lymphatic transport, a chylomicron flow blocking rat model was used (Dahan and Hoffman 2005; Lind et al. 2008). Twelve rats were randomly divided into two groups (n=6) which were treated with an intraperitoneal injection of 3 mg/kg cycloheximide in saline (0.6 mg/mL). One hour later, BS-Lips and Lips were orally administered to the rats at the PTX dose of 10 mg/kg. The blood samples were collected as described above.

4.8. Sample preparation and analytical methods

The plasma concentration was assayed using an HPLC system (Agilent 1200, Agilent Technologies, USA) described in a previous report with minor modifications (Zhang et al. 2008). Briefly, 200 μL of plasma, 50 μL of internal standard (20 μg/mL diazepard in methanol) and 2 mL of tetra-butyl methyl ether were mixed in a plastic tube and vortexed for 60 s. The supernatant was dried with a stream of nitrogen at room temperature. Subsequently, the dried residue was reconstituted with 100 μL of mobile phase, vortexed for 5 min and centrifuged at 10, 000 × g for 5 min. Finally, 20 μL aliquots of the supernatant were injected into the HPLC system. The separation was performed on a RP-column (250 mm × 4.6 mm, Dikma) with a mobile phase of methanol/water (65:35, v/v) at 40 °C. The flow rate was 1.0 mL/min, and the detection wavelength was set at 227.0 nm using UV detection. The regression equations were applied for each experiment (R^2=0.9998). The method for the detection of drug concentration in plasma was specific, selective and accurate.

4.9. In vivo antitumor study

The *in vivo* antitumor activity of the PTX formulations was assessed in H22-bearing mice. The mice were injected subcutaneously in the right flank with 0.2 mL of cell suspension (2×10^7 cells). When the tumor volume reached 100 mm^3, the H22-bearing mice were randomly divided into 6 groups (A, B, C, D, E, F and G, n=9). Groups A and B were injected via the tail vein with saline and Taxol (free drug), respectively, while the other four groups were orally administered BS-Lips at three doses or Lips. For administration to the mice, a single dose of 10 mg/kg of PTX was used for Taxol and Lips, while three doses (5, 10 and 20 mg/kg of PTX) for BS-Lips were used. The administration interval was once every 3 days for 30 days. The volume of the tumor was monitored during the treatment period using the following formula: Tumor volume (mm^3) = $(a \times b^2)/2$, where a and b indicate the largest diameter and the smallest diameter, respectively. Additionally, the toxicities of the PTX formulations were assessed by monitoring the relative body weight every 3 days.

4.10. Data analysis

The pharmacokinetic parameters of PTX were calculated by a non-compartmental analysis with the DAS professional software version 2.0 (Chinese Pharmacology Society, Anhui, China). The data are expressed as the mean ± standard deviation. A two-sided t-test was conducted for the comparisons between the formulations. $P < 0.05$ indicates a significant difference.

Acknowledgments: This work was supported by the Fundamental Research Funds of Shandong University (2015JC012).

References

Agüeros M, Zabaleta V, Espuelas S, Campanero MA, Irache JM (2010) Increased oral bioavailability of paclitaxel by its encapsulation through complex formation with cyclodextrins in poly(anhydride) nanoparticles. J Control Release 145: 2–8.

Attili-Qadri S, Karra N, Nemirovski A, Schwob O, Talmon Y, Nassar T, Benita S (2013) Oral delivery system prolongs blood circulation of docetaxel nanocapsules via lymphatic absorption. PNAS 110: 17498–17503.

Baek JS, Cho CW (2015) Controlled release and reversal of multidrug resistance by co-encapsulation of paclitaxel and verapamil in solid lipid nanoparticles. Int J Pharm 478: 617-624.

Bakhru SH, Furtado S, Morello AP, Mathiowitz E (2013) Oral delivery of proteins by biodegradable nanoparticles. Adv Drug Deliv Rev 65: 811–821.

Bao QY, Zhang N, Geng DD, Xue JW, Merritt M, Zhang C, Ding Y (2014) The enhanced longevity and liver targetability of Paclitaxel by hybrid liposomes encapsulating Paclitaxel-conjugated gold nanoparticles. Int J Pharm 477: 408–415.

Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC (2014) Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev 66: 2–25.

Biswas S, Dodwadkar NS, Deshpande PP, Torchilin VP (2012) Liposomes loaded with paclitaxel and modified with novel triphenylphosphonium-PEG-PE conjugate possess low toxicity, target mitochondria and demonstrate enhanced antitumor effects in vitro and in vivo. J Control Release 159: 393–402.

Chen Y, Lu Y, Chen J, Lai J, Sun J, Hu F, Wu W (2009) Enhanced bioavailability of the poorly water-soluble drug fenofibrate by using liposomes containing a bile salt. Int J Pharm 376: 153–160.

Dahan A, Hoffman A (2005) Evaluation of a chylomicron flow blocking approach to investigate the intestinal lymphatic transport of lipophilic drugs. Eur J Pharm Sci 24: 381–388.

Dahmani FZ, Yang H, Zhou J, Yao J, Zhang T, Zhang Q (2012) Enhanced oral bioavailability of paclitaxel in pluronic/LHR mixed polymeric micelles: Preparation, in vitro and in vivo evaluation. Eur J Pharm Sci 47: 179–189.

Ensign LM, Cone R, Hanes J (2012) Oral drug delivery with polymeric nanoparticles: The gastrointestinal mucus barriers. Adv Drug Deliv Rev 64: 557–570.

Gamboa JM, Leong KW (2013) In vitro and in vivo models for the study of oral delivery of nanoparticles. Adv Drug Deliv Rev 65: 800–810.

He W, Lu Y, Qi J, Chen L, Hu F, Wu W (2013) Nanoemulsion-templated shell-cross-linked nanocapsules as drug delivery systems. Int J Pharm 445: 69–78.

He W, Yang K, Fan L, Lv Y, Jin Z, Zhu S, Qin C, Wang Y, Yin L (2015) Denatured globular protein and bile salt-coated nanoparticles for poorly water-soluble drugs: Penetration across the intestinal epithelial barrier into the circulation system and enhanced oral bioavailability. Int J Pharm 495: 9–18.

Hu S, Niu M, Hu F, Lu Y, Qi J, Yin Z, Wu W (2013) Integrity and stability of oral liposomes containing bile salts studied in simulated and ex vivo gastrointestinal media. Int J Pharm 441: 693–700.

Jauhari S, Dash AK (2006) A mucoadhesive in situ gel delivery system for paclitaxel. AAPS PharmSciTech 7: E53.

Ji X, Gao Y, Chen L, Zhang Z, Deng Y, Li Y (2012) Nanohybrid systems of non-ionic surfactant inserting liposomes loading paclitaxel for reversal of multidrug resistance. Int J Pharm 422: 390–397.

Koudelka Š, Turánek J (2012) Liposomal paclitaxel formulations. J Control Release 163: 322-334.

Li HL, Zhao XB, Ma YK, Zhai GX, Li LB, Lou HX (2009) Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. J Control Release 133: 238–244.

Lind ML, Jacobsen J, Holm R, Mullertz A (2008) Intestinal lymphatic transport of halofantrine in rats assessed using a chylomicron flow blocking approach: the influence of polysorbate 60 and 80. Eur J Pharm Sci 35: 211–218.

Luo C, Sun J, Du Y, He Z (2014) Emerging integrated nanohybrid drug delivery systems to facilitate the intravenous-to-oral switch in cancer chemotherapy. J Control Release 176: 94–103.

Mann JFS, Scales HE, Shakir E, Alexander J, Carter KC, Mullen AB, Ferro VA (2006) Oral delivery of tetanus toxoid using vesicles containing bile salts (bilosomes) induces significant systemic and mucosal immunity. Methods 38: 90–95.

Mazzaferro S, Bouchemal K, Ponchel G (2013) Oral delivery of anticancer drugs III: formulation using drug delivery systems. Drug Discov Today 18: 99–104.

McConnell EL, Fadda HM, Basit AW (2008) Gut instincts: Explorations in intestinal physiology and drug delivery. Int J Pharm 364: 213–226.

McDowell A, McLeod BJ (2007) Physiology and pharmacology of the brushtail possum gastrointestinal tract: Relationship to the human gastrointestinal tract. Adv Drug Deliv Rev 59: 1121-1132.

Mei L, Zhang Z, Zhao L, Huang L, Yang XL, Tang J, Feng SS (2013) Pharmaceutical nanotechnology for oral delivery of anticancer drugs. Adv Drug Deliv Rev 65: 880-890.

Miglietta A, Cavalli R, Bocca C, Gabriel L, Rosa Gasco M (2000) Cellular uptake and cytotoxicity of solid lipid nanospheres (SLN) incorporating doxorubicin or paclitaxel. Int J Pharm 210: 61-67.

Mo R, Jin X, Li N, Ju C, Sun M, Zhang C, Ping Q (2011) The mechanism of enhancement on oral absorption of paclitaxel by N-octyl-O-sulfate chitosan micelles. Biomaterials 32: 4609-4620.

Narvekar M, Xue HY, Eoh JY, Wong HL (2014) Nanocarrier for poorly water-soluble anticancer drugs--barriers of translation and solutions. AAPS PharmSciTech 15: 822-833.

Niu M, Lu Y, Hovgaard L, Guan P, Tan Y, Lian R, Qi J, Wu W (2012b) Hypoglycemic activity and oral bioavailability of insulin-loaded liposomes containing bile salts in rats: The effect of cholate type, particle size and administered dose. Eur J Pharm Biopharm 81: 265-272.

Niu M, Lu Y, Hovgaard L, Wu W (2011a) Liposomes containing glycocholate as potential oral insulin delivery systems: preparation, in vitro characterization, and improved protection against enzymatic degradation. Int J Nanomedicine 6: 1155-1166.

Niu M, Tan Y, Guan P, Hovgaard L, Lu Y, Qi J, Lian R, Li X, Wu W (2014) Enhanced oral absorption of insulin-loaded liposomes containing bile salts: A mechanistic study. Int J Pharm 460: 119-130.

Nornoo AO, Zheng H, Lopes LB, Johnson-Restrepo B, Kannan K, Reed R (2009) Oral microemulsions of paclitaxel: in situ and pharmacokinetic studies. Eur J Pharm Biopharm 71: 310-317.

Oussoren C, Zuidema J, Crommelin DJA, Storm G (1997) Lymphatic uptake and biodistribution of liposomes after subcutaneous injection.: II. Influence of liposomal size, lipid composition and lipid dose. Biochim Biophys Acta- Biomembranes 1328: 261-272.

Pandita D, Ahuja A, Lather V, Benjamin B, Dutta T, Velpandian T, Khar RK (2011) Development of lipid-based nanoparticles for enhancing the oral bioavailability of paclitaxel. AAPS PharmSciTech 12: 712–722.

Parmentier J, Becker MMM, Heintz U, Fricker G (2011) Stability of liposomes containing bio-enhancers and tetraether lipids in simulated gastro-intestinal fluids. Int J Pharm 405: 210–217.

Pawar AP, Vinugala D, Bothiraja C (2014) Nanocochleates derived from nanoliposomes for paclitaxel oral use: Preparation, characterization, in vitro anticancer testing, bioavailability and biodistribution study in rats. Biomed Pharmacother; doi:10.1016/j.biopha.2014.11.014

Porter CJ, Trevaskis NL, Charman WN (2007) Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov 6: 231–248.

Roger E, Lagarce F, Garcion E, Benoit JP (2009) Lipid nanocarriers improve paclitaxel transport throughout human intestinal epithelial cells by using vesicle-mediated transcytosis. J Control Release 140: 174–181.

Rowinsky EK, Onetto N, Canetta RM, Arbuck SG (1992) Taxol: the first of the taxanes, an important new class of antitumor agents. Semin Oncol 19: 646–662.

Sakuma S, Hayashi M, Akashi M (2001) Design of nanoparticles composed of graft copolymers for oral peptide delivery. Adv Drug Deliv Rev 47: 21–37.

Schoener CA, Peppas NA (2012) Oral delivery of chemotherapeutic agents: background and potential of drug delivery systems for colon delivery. J Drug Deliv Sci Technol 22: 459–468.

Senior K (2001) Bilosomes: the answer to oral vaccine delivery? Drug Discov Today 6: 1031-1032.

Shapira A, Davidson I, Avni N, Assaraf YG, Livney YD (2012) β-Casein nanoparticle-based oral drug delivery system for potential treatment of gastric carcinoma: Stability, target-activated release and cytotoxicity. Eur J Pharm Biopharm 80: 298–305.

Sharma S, Verma A, Venkatesh Teja B, Shukla P, Mishra PR (2015) Development of stabilized Paclitaxel Nanocrystals: In-vitro and in-vivo efficacy studies. Eur J Pharm Sci. 69: 51–60.

Song KH, Chung SJ, Shim CK (2005) Enhanced intestinal absorption of salmon calcitonin (sCT) from proliposomes containing bile salts. J Control Release 106: 298–308.

Sun M, Zhai X, Xue K, Hu L, Yang X, Li G, Si L (2011) Intestinal absorption and intestinal lymphatic transport of sirolimus from self-microemulsifying drug delivery systems assessed using the single-pass intestinal perfusion (SPIP) technique and a chylomicron flow blocking approach: Linear correlation with oral bioavailabilities in rats. Eur J Pharm Sci 43: 132–140.

Thanki K, Gangwal RP, Sangamwar AT, Jain S (2013) Oral delivery of anticancer drugs: Challenges and opportunities. J Control Release 170: 15–40.

Trevaskis N, Porter CH, Charman W (2005) Bile increases intestinal lymphatic drug transport in the fasted rat. Pharm Res 22: 1863–1870.

Trevaskis NL, Charman WN, Porter CJH (2008) Lipid-based delivery systems and intestinal lymphatic drug transport: A mechanistic update. Adv Drug Deliv Rev 60: 702–716.

Trickler WJ, Nagvekar AA, Dash AK (2008) A novel nanoparticle formulation for sustained paclitaxel delivery. AAPS PharmSciTech 9: 486–493.

Yin L, Qin C, Chen K, Zhu C, Cao H, Zhou J, He W, Zhang Q (2013) Gastro-floating tablets of cephalexin: Preparation and in vitro/in vivo evaluation. Int J Pharm 452: 241–248.

Zabaleta V, Ponchel G, Salman H, Agüeros M, Vauthier C, Irache JM (2012) Oral administration of paclitaxel with pegylated poly(anhydride) nanoparticles: Permeability and pharmacokinetic study. Eur J Pharm Biopharm 81: 514–523.

Zhang C, Qu G, Sun Y, Wu X, Yao Z, Guo Q, Ding Q, Yuan S, Shen Z, Ping Q, Zhou H (2008) Pharmacokinetics, biodistribution, efficacy and safety of N-octyl-O-sulfate chitosan micelles loaded with paclitaxel. Biomaterials 29: 1233–1241.