Formulation and Evaluation of Self-Emulsifying Drug Delivery Systems of Larotaxel for Oral Administration

W MINGLI, J SHAN, Y TIAN[1], G JINGXIN, H HAIBING, W YANJIAO, Z YU* AND T XING

Department of Pharmaceutics, School of Pharmacy, [1]School of Functional Food and Wine, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, PR China

Mingli *et al.*: An Oral Self-Emulsifying Formulation of Larotaxel

The objective of the present study was to develop a self-emulsifying drug delivery system of larotaxel and evaluate its *in vitro* and *in vivo* performance. The prepared larotaxel loaded self-emulsifying drug delivery system spontaneously formed microemulsion with a mean particle size of 115.4 nm when mixed with 100-fold water under gentle agitation. *In vivo* pharmacokinetics study of larotaxel loaded self-emulsifying drug delivery system demonstrated 5.19-fold enhancement in oral bioavailability compared to larotaxel-Sol. Furthermore, intestinal bio-distribution studies revealed that the intestinal residence time of larotaxel loaded self-emulsifying drug delivery system was dramatically extended in comparison to larotaxel-Sol. Lymphatic transport studies showed that cycloheximide, a chylomicron secretion blocker, would impede oral absorption of larotaxel loaded self-emulsifying drug delivery system, which confirmed that lymphatic route was involved in the absorption of larotaxel loaded self-emulsifying drug delivery system. What is more, *in vivo* antitumor experiment proved that the antitumor activity of oral larotaxel loaded self-emulsifying drug delivery system was significantly superior to oral larotaxel-Sol, which was close to intravenous larotaxel-Sol. In conclusion, self-emulsifying drug delivery system is a promising vehicle for the oral delivery of larotaxel.

Key words: Larotaxel, self-emulsifying drug delivery system, oral bioavailability, antitumor

Cancer remains one of the most life-threatening diseases with significant increases in morbidity and mortality[1]. Currently, many treatment modalities have been applied for tumors, such as chemotherapy, surgery, laser therapy, molecular targeted therapy[2], photothermal therapy[3] and emerging immunotherapy[4]. Among these options, conventional chemotherapy still remains a vital treatment approach for most cancer cases due to its effective tumor killing effect[5-7]. Larotaxel (LTX) is a modified new-generation taxane compound, which exert their cytotoxic effect as the current taxanes by binding to tubulin to promote the formation of stable microtubules and prevent their depolymerization[8,9]. LTX has a much lower affinity for P-glycoprotein than docetaxel and it is more effective than paclitaxel in treatment of resistant cancers[10-13]. Nevertheless, clinical application of LTX is mainly hindered by its poor water solubility and there are urgent needs to develop a suitable drug delivery system for delivering LTX[14]. The oral drug delivery is one of the most acceptable routes of administration due to its safety, high compliance, and cost-effectiveness[15]. However, oral delivery of taxanes is hampered by its poor oral bioavailability owing to their practical insolubility in water and high affinity to drug efflux transporters in the gastrointestinal tract[16,17]. Self-Emulsifying Drug Delivery Systems (SEDDS) refers to a formulation consists of oil, surfactant and co-surfactant/co-solvent, which will disperse into oil-in water emulsion in the fluid of gastrointestinal tract upon oral administration. The excipients contained in the SEDDS tend to dissolve hydrophobic drugs and inhibit drug efflux mediated by P-glycoprotein expressed in the gut membrane and thus improve oral bioavailability[18,19]. SEDDS has exhibited

*Address for correspondence
E-mail: pharmzy@163.com

significant potentials for improving oral delivery efficiency of different drugs[20,21]. In the present study, a novel LTX-SEDDS was prepared and systematically evaluated, including particle size, Zeta potential, emulsifying ability, oral bioavailability, intestinal biodistribution, absorption mechanism and *in vivo* anti-tumor efficacy of LTX-SEDDS. LTX was purchased from Yantai Badian Pharmacy Research Co., Ltd. Monoolein was supplied by Gattefossé. Tricaprylin was obtained from Sigma Chemical Co. Tween 80 was purchased from Shanghai Shenyu chemical Chemical Co. Ltd. Tert-Butyl methyl ether was obtained from Sinopharm Chemical Reagent Co. LTD. All other chemicals and reagents were of analytical or chromatographic grade. Sprague-Dawley rats (180-220 g) and Balb/c mice (18-22 g) were provided by the Animal Laboratory Center of Shenyang Pharmaceutical University. All of the animal experiments in this work were performed according to the Guidelines for the Care and Use of Laboratory Animal approved by the Ethics Committee of Animal Experimentation of Shenyang Pharmaceutical University. Statistical analysis of data was performed using GraphPad Prism 8. A difference of p<0.05 was considered to be statistically significant. The larotaxel self-emulsifying drug delivery systems was prepared as follows. 10 mg LTX and 0.5 g tricaprylin were dissolved in adequate methylene chloride under ultrasound. Then, methylene chloride was evaporated under reduced pressure at 30° for 1 h. After that, 0.1 g monoolein and 0.4 g Tween 80 were added and dissolved thoroughly. The residual methylene chloride was removed completely by vacuum evaporation. The mean particle size of LTX-SEDDS after emulsification was 115.4 nm with small polydispersity index of 0.197 (fig. 1A). The Zeta potential of LTX-SEDDS after emulsification was around -13.0 mV (fig. 1B), which is beneficial to prevent particles aggregate and maintain the stability of LTX-SEDDS. Furthermore, LTX-SEDDS could completely emulsified and form stable microemulsion in different media (including distilled water, simulated gastric fluid and simulated intestinal fluid) within 3 min. It is speculated that LTX-SEDDS has high adhesiveness in the gastrointestinal tract[22], which is good for the absorption of drugs. To investigate the intestinal retention effect of LTX-SEDDS, the bio-distribution of the gastrointestinal tract was studied by *ex vivo* imaging using DiR as near infrared probe. SEDDS was labeled with DiR *via* hydrophobic interaction

and was prepared according to the preparation method of LTX-SEDDS by replacing LTX with DiR. DiR-Sol of 2 mg/ml was set as the control group. Then, DiR labeled SEDDS and DiR-Sol was administered to Balb/c mice orally at DiR dose of 2 mg/kg, respectively. At 0.5 h, 2 h, 4 h, and 6 h after administration, the mice were sacrificed to collect stomach and small intestines for *ex vivo* imaging using excitation wavelength of 740 nm and emission wavelength at 790 nm. As shown in fig. 2, fluorescence intensity of DiR-Sol rapidly attenuated with only weak DiR fluorescence observed in the end of the ileum and colon at 2 h after administration. While DiR-SEDDS showed stronger fluorescence intensity than DiR-Sol throughout all segments of the intestine and still exhibited strong fluorescence at 4 h after administration. The results revealed that SEDDS could adhere to the gastrointestinal tract and prolong residence time of cargos in the intestine, which is beneficial to improve oral absorption of larotaxel. It is reported that self-micro emulsion drug delivery system could promote lymphatic transport to deliver therapeutic agent into systemic circulation[23,24]. This leads to avoidance of first-pass metabolism and enhancement of oral bioavailability of taxanes[25,26]. We studied the lymphatic transport of LTX-SEDDS by using cycloheximide to inhibit the secretion of chylomicrons. Cycloheximide is a protein synthesis inhibitor and does not affect other absorption pathways except for lymphatic transport. Sprague-Dawley rats (180-220 g) were divided randomly into two groups of five animals each, which were fasted overnight and had free access to water. Group I was intraperitoneally injected with high dose of cycloheximide (3 mg/kg) to prevent the secretion of chylomicrons from the enterocyte. 1 h after intraperitoneal injection, all rats in Group I and Group II were given LTX-SEDDS (20 mg/kg) *via* oral gavage. Blood samples were collected from orbital vein at 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h and 24 h post-administration into heparinized tubes. Plasma was separated and stored at -20° until Ultra Performance Liquid Chromatography- tandem Mass Spectrometry (UPLC-MS/MS). The typical pharmacokinetic parameters were calculated by DAS 2.0 software. As shown in fig. 3A and Table 1, after oral administration of LTX-SEDDS, a distinct absorption phase was observed with the plasma AUC 0-24 h of 892.429±279.785 μg/l.h. However, the absorption of LTX in the LTX-SEDDS group with intraperitoneal injection of cycloheximide (3 mg/kg)

was significantly declined with the plasma AUC 0-24 h of 64.759±19.951 µg/l.h. These results indicated that LTX-SEDDS could promote oral absorption of LTX through lymphatic transport pathway, and oral absorption of LTX-SEDDS decreased significantly when lymphatic pathway was blocked. In order to systematically evaluate the oral bioavailability, the pharmacokinetic behaviors of LTX-SEDDS were assessed in SD rats, and LTX-Sol was set as control group. LTX-SEDDS and LTX-Sol both equivalent to 40 mg/kg of LTX were given separately to two groups of rats (n=4) by intragastric administration. About 0.3 ml blood samples were taken from orbital vein at predetermined time points of 15 min, 30 min,1 h, 2h, 4 h, 6 h, 8 h, 12 h and 24 h post-administration. Plasma samples were extracted with tert-Butyl methyl ether and the concentrations of drugs were determined by a validated UPLC-MS/MS method. The mean larotaxel plasma concentration-time profiles and main pharmacokinetic parameters are presented in fig. 3B and Table 2, respectively. AUC 0-24 h (1896.517±485.276 µg/l.h) of LTX-SEDDS was 3.4-fold higher than that of LTX-Sol and the relative bioavailability of LTX-SEDDS was 519.32 %. The results of pharmacokinetic study proved the advantageous of LTX loaded SEDDS in promoting intestinal absorption of hydrophobic LTX.

The significantly improved oral bioavailability might be ascribed to the following aspects. The excipients in SEDDS increased the solubility of LTX. SEDDS could circumvent P-glycoprotein mediated efflux to improve absorption of LTX in intestinal. SEDDS could effectively adhere to the intestinal, which was confirmed in the intestinal retention experiment. SEDDS could promote lymphatic transport to deliver LTX into systemic circulation and avoid first-pass hepatic metabolism to increase oral bioavailability. Inspired by the results of pharmacokinetics study, it could be speculated that oral LTX-SEDDS might possess superior *in vivo* antitumor ability. The 4T1-tumor-bearing mice were selected to investigate the *in vivo* anti-tumor potential of LTX-SEDDS. Twenty four 4T1-tumor-bearing mice were randomly divided into four groups(n=6), and then treated with Saline, intravenous injection of LTX-Sol (5 mg/kg), oral administration of LTX-Sol (20 mg/kg) and LTX-SEDDS (20 mg/kg), respectively. All the mice were treated on d 0, d 2, d 4, d 6 and d 8 and the tumor volume was measured every other day. As shown in fig. 4A, the tumor volume of the mice treated with Saline and oral administration of LTX-Sol grew quickly, demonstrating oral administration of LTX-Sol could not inhibit the growth of tumors.
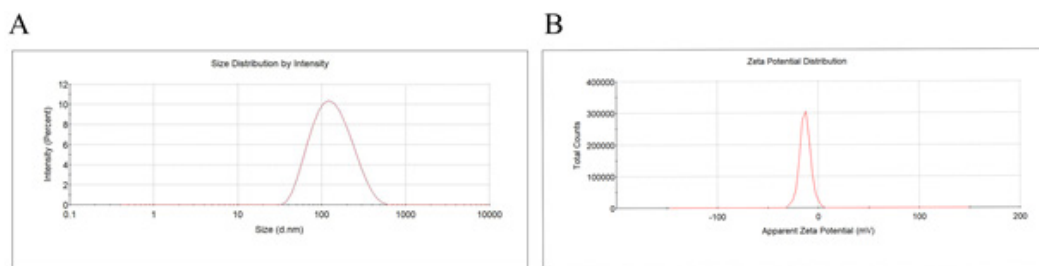

Fig. 1: Characterization of LTX-SEDDS; (A): Particle size of LTX-SEDDS after emulsification and (B): Zeta potential distribution of LTX-SEDDS after emulsification
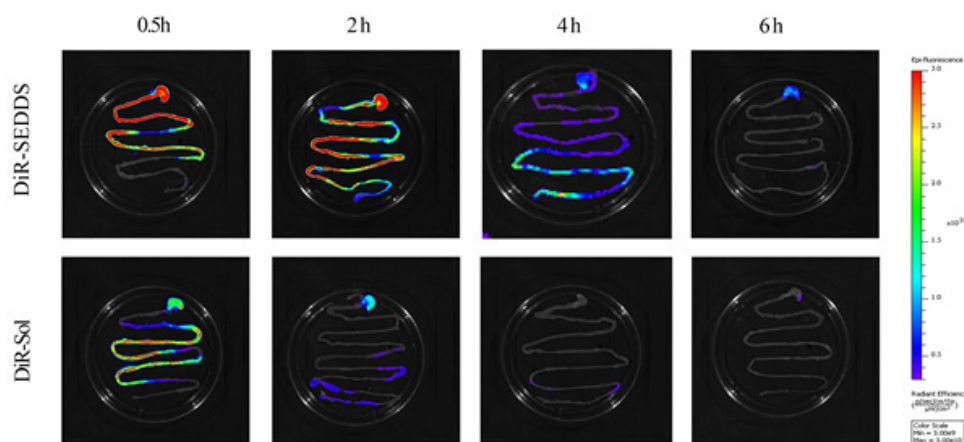

Fig. 2: Ex vivo imaging of DiR-SEDDS and DiR-Sol in the stomach and small intestine of mice harvested at 0.5, 2, 4 and 6 h after oral administration
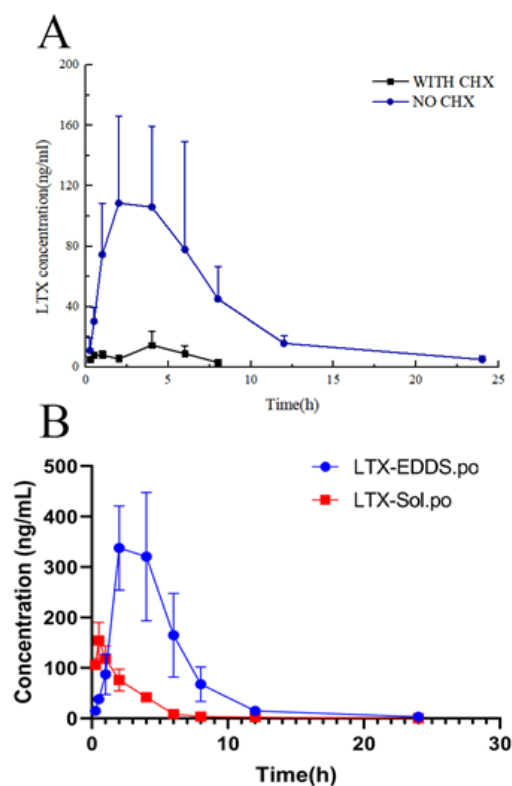
Fig. 3: Results of pharmacokinetic studies; (A): Pharmacokinetic profiles of oral administration of 20 mg/kg LTX-SEDDS to non-treated and cyclo-heximide-treated rats (mean±SD, n=5) and (B): Pharmacokinetic profiles of oral LTX-Sol (40 mg/kg); oral LTX-SEDDS (40 mg/kg) (mean±SD, n=4); (A) (): with CHX; (): no CHX; (B) (): LTX-EDDS.po and (): LTX-Sol.po

TABLE 1: PHARMACOKINETIC PARAMETERS OF ORAL ADMINISTRATION OF 20 mg/kg LTX-SEDDS IN NON-TREATED RATS AND CYCLOHEXIMIDE-TREATED RATS, MEAN±SD, n=5

Parameters	LTX-SEDDS	LTX-SEDDS with cycloheximide
AUC (0-24) (μg/l.h)	892.429±279.785	64.759±19.951
AUC (0-∞) (μg/l.h)	908.87±261.143	76.181±19.04
C^{max} (μg/l)	143.682±59.898	13.805±5.5
T^{max} (h)	3.4±1.949	3.25±1.5
$T_{1/2}$ z (h)	3.808±2.34	2.628±1.035
Vz(l/kg)	302.872±277.135	2217.069±1348.061
MRT(0-t) (h)	6.243±0.879	3.838±0.478

TABLE 2: PHARMACOKINETIC PARAMETERS OF ORAL LTX-SOL (40 mg/kg) AND ORAL LTX-SEDDS (40 mg/kg), MEAN±SD, n=4

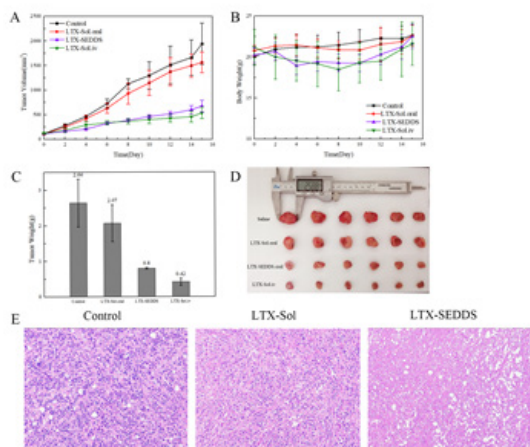| Parameters	LTX-SEDDS.po	LTX-Sol.po
AUC (0-24 h) (μg/l.h)	1896.517±485.276	347.973±30.968
C_{max} (μg/l)	409.725±73.499	119.14±24.376
T_{max} (h)	2.5±1	1.25±0.5
T1/2 z (h)	3.154±0.522	1.731±0.418
MRT(0-t) (h)	4.668±0.765	2.639±0.163
Frbs	539.12 %	-

Fig. 4: In vivo evaluation of 4T1 xenografts in Balb/c mice; (A): Changes of tumor volumes of 4T1 xenografts in Balb/c mice treated with Saline, LTX-Sol(oral), LTX-SEDDS (oral) and LTX-Sol (i.v.); (B): Changes of body weight of 4T1-bearing mice after various treatment; (C): Weight of excised tumor; (D): Photos of excised tumors and (E): H&E-stained tumor slice images of mice after various treatment (mean±SD, n=6)

By contrast, the oral LTX-SEDDS group presented remarkable inhibition efficacy toward tumor growth. At the end of the treatment, mice were sacrificed and tumors were dissected and weighted. Tumor weight and the photographs of the tumors were shown in fig. 4C and fig. 4D, respectively. Both oral LTX-SEDDS and intravenous LTX-Sol exhibited effective tumor inhibition. What is more, the Tumor Inhibition Rates (TIRs) of the oral LTX-SEDDS group was 69.69 %, which is 3.2-fold higher than that of oral LTX-Sol group with 21.59 %. Furthermore, histological examination of hematoxylin and eosin staining tumor tissue section indicated that the tumors treated with oral LTX-SEDDS exhibited obviously enhanced cell death compared with oral LTX-Sol (fig. 4E). In addition, oral LTX-SEDDS group exhibited slight body weight loss during treatment as intravenous injection LTX-Sol (fig. 4B), which was attributed to the systemic toxicity caused by the high bioavailability of LTX-SEDDS, and the body weight of mice returned to original level after the last dose. In conclusion, the LTX loaded SEDDS was successfully prepared, and it exhibited excellent *in vivo* performance in terms of oral delivery. SEDDS could prolong the residence time of LTX in the gastrointestinal tract and facilitate lymphatic transport compared with LTX-Sol. Notably, LTX-SEDDS exhibited a 5.19-fold higher oral bioavailability than that of LTX-Sol. It is worth noting that oral administration of LTX-SEDDS presented remarkable *in vivo* therapeutic efficacy with tumor inhibition rates of 69.69 %. LTX-SEDDS have good clinical application prospects for maintenance therapy of cancers due to the convenience of oral administration, which is beneficial to increase the compliance of patients and ease the pressure of medical institutions during the COVID-19 pandemic.

Conflict of interests:

The authors declared no conflict of interests.

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