

Intestinal lymphatic transport study of antitumor lead compound T-OA with liposomes

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Abstract: Intestinal lymphatic transport has been proved to have contribution to oral absorption of some highly lipophilic drugs. T-OA, 3 β hydroxyolea-12-en-28-oic acid-3,5,6-trimethylpyrazin-2-methylester, has been reported to have anti-cancer activity. However, T-OA's poor solubility and difficulty to be absorbed cause low oral bioavailability. This work aims to investigate the influence of T-OA liposomes on intestinal lymphatic transport with rat model. T-OA liposomes were prepared by freeze-drying method, and particle size, zeta potential and entrapment efficiency of T-OA liposomes were detected to evaluate liposomes. Conscious restrained rat model was selected to evaluate intestinal lymphatic transport. The particle size, zeta potential and entrapment efficiency of T-OA liposomes were (184.05 \pm 10.93) nm, (-21 \pm 0.85) mV and (93.24 \pm 2.25) %, respectively. The cumulative amounts in mesenteric lymph of T-OA liposomes and T-OA suspension within 12 h were (921.39 \pm 19.73) μ g and (332.31 \pm 21.39) μ g (n=6), respectively. Experimental results showed that T-OA liposomes could significantly promote T-OA's intestinal lymphatic transport and enhance its oral bioavailability.

Keywords: Intestinal lymphatic transport, antitumor lead compound T-OA, liposomes, conscious restrained rat model, oral bioavailability.

INTRODUCTION

Intestinal lymphatic system has been shown to be a contributor to the absorption of a number of orally administered highly lipophilic drugs. There are lots of advantages of drugs transportation through the intestinal lymphatic system, such as avoid hepatic first-pass effect to enhance oral bioavailability, improve drugs' pharmacokinetic properties and lymphatic targeting. For drug with strong first-pass effects, intestinal lymphatic transporting is helpful to decreasing the first-pass effects (Yanez *et al.* 2011, Trevaskis *et al.* 2008, O'Driscoll 2002). There are three ways by which drugs can get into lymphatic system, via the paracellular route with the aid of absorption enhancers, the M cells and gut-associated lymphoid tissues (GALT) and transcellular route in association with the triglyceride core of the chylomicrons (Sun *et al.* 2011, Yáñez *et al.* 2011). The third route has been considered as the major mechanism of lipophilic drugs' lymphatic delivery when the drugs were formulated with lipid-based vehicles (O'Driscoll 2002, Trevaskis 2008, Chai and Tao 2008).

There are many models for studying oral preparations' intestinal lymphatic transport, including *in vitro* models and *in vivo* models (Edwards *et al.* 2001, Cai and Li 2013a). Chylomicron flow blocking model and lymph intubation model are *in vivo* models, while Caco-2 cell model is the most popular *in vitro* model. The advantage

of chylomicron flow blocking model is that it doesn't require a lymph duct-cannulation. However, the impact of blocking chylomicron flow in lipid processing and synthesis has to be fully characterized. For instance, Pluronic-L81 lowers plasma VLDL (very low density lipoprotein) and low-density lipoprotein cholesterol and reduces lipid and apoprotein secretion (Podlich *et al.* 1996). The adverse effects induced by intestinal chylomicron flow inhibitors Pluronic-L81 and colchicine treatment and the apparent influence on other absorption pathways as implied by the d-xylose loading test and the altered linear terminal slope, indicate that it is not an appropriate model to mimic the mesenteric lymph duct cannulation model (Dahan and Hoffman 2005). Lymph intubation is a more direct and effective model and anesthetized rats model is the most commonly used model. Compared with anesthetized rats model, conscious restrained rat model has many advantages, such as smaller trauma, animals' condition closer to physiological state, faster lymph flow, longer lymphatic fluid collection time and more accurate experimental results. Therefore, conscious restrained rat model was selected to study intestinal lymphatic transport of antitumor lead compound T-OA in this study.

T-OA (C₃₈H₅₈O₃N₂) was selected as a model drug to study intestinal lymphatic transport. T-OA is an anti-cancer lead compound. It is synthesized by tetramethylpyrazine (TMP) and oleanolic acid (OA), both

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of which are extracted from Chinese herbal medicines. T-OA's pharmacodynamics experiments showed that it can significantly inhibit a variety of cancer cells' proliferation, avoid pulmonary metastasis of liver cancer cells strain LM3 invasion, induce apoptosis in cancer cells, inhibit angiogenesis in CAM model and exhibit good activity in inhibiting tumour S180 model mice (Wang P, *et al.* 2013). Mice were given maximum single-day dose of 6 g/kg and observed for 14 consecutive days to evaluate T-OA's oral acute toxicity, and the result showed that the drug has low toxicity and high safety (Lei *et al.* 2012, Xu 2014, Wang *et al.* 2013). T-OA has been reported to have antitumor activity, but its poor solubility causes the low oral bioavailability. Preliminary pharmacokinetic studies showed that the oral bioavailability of T-OA is less than 10%, which limited its application. This study attempted to improve its oral bioavailability by embedding T-OA in liposomes .

In recent years, because of liposome's advantages, such as its target ability, sustained-release, toxicity reduction and drug stability improvement, several researches studied liposomes as a novel drug delivery system. Due to the special structure of the liposomes, it can entrap water-soluble and lipophilic drugs. In this way, it can improve the solubility and the oral bioavailability of drugs. Studies about liposomes showed that as lipid-based delivery systems, liposomes could enhance intestinal lymphatic transport of drug (Ling *et al.* 2006). T-OA is suitable for the preparation of liposomes since it's lipophilic. This article attempts to prepare T-OA liposomes to promote T-OA's intestinal lymphatic transport and enhance its oral bioavailability.

MATERIALS AND METHODS

T-OA and T-GA used in the study were synthesized in Beijing University of Chinese Medicine. Cholesterol was purchased from Amresco, Inc. (OH, USA). Soybean phospholipid was purchased from Lipoid (Germany). Trehalose was used as freeze-dried protective agent; acetonitrile was Chromatographic grade (Thermo Fisher Scientific, USA) and all other reagents were of analytical grade. Tert-butyl alcohol (injection grade, batch number DC4DF64301) was provided by Merck Chemical Technology (Shanghai) Co., Ltd.

Animals

Specific pathogen-free, male Sprague -Dawley rats weighing 210-250g obtained from the Sibeifu (Beijing) Biotechnology Co., Ltd were used in the experiment. All animals were housed in temperature-controlled quarters (24±1°C) with a 12 h/12 h light/dark cycle. Food and water were available ad libitum. All animal procedures were performed according to animal care protocols approved by the Ethics Committee of Beijing University of Chinese Medicine in accordance with the National

Institute of Health Guide for the Care and Use of Laboratory Animals. (Beijing, China, certificate No. Kj-dw-18-20151111-01)

The synthetic route and Log P's determination of T-OA

The anti-tumour lead compounds T-OA is synthesized by tetramethylpyrazine (TMP) and oleanolic acid (OA). The synthetic route and T-OA's structural formula are shown in fig. 1 (Xu, 2014).

The partition coefficient of T-OA was determined by shake-flask method (Guo *et al.* 2011, Zhang *et al.* 2006, Wang *et al.* 2005). Appropriate amount of T-OA was dissolved in octanol solution which was saturated by purified water, as solution A. Transferred 0.5mL solution A to a 10mL eppendorf tube (EP tube), added 5mL octanol saturated water solution in the EP tube, incubated in 37°C water bath and shook with 100rpm for 24h. The aqueous phase was separated from solution by centrifuging under 3000 rpm for 10 min. The drug content in aqueous phase was detected by Ultra Performance Liquid Chromatography (UPLC), the concentration was equilibrium solubility in aqueous phase, recorded as C_w. The concentration of T-OA in solution A was recorded as C. The partition coefficient was calculated by follow formula.

$$P=(C-10C_w)/C_w$$

Preparation of T-OA liposomes

T-OA liposomes were prepared by freeze-drying method (Yang *et al.* 2008, Li *et al.* 2016). 48mg of soybean phospholipid, 6 mg cholesterol and 5.4 mg T-OA were dissolved in 1.5 mL Tert-butanol to get solution A. 9 mg of trehalose was dissolved in 4.5mL purified water and got solution B. Solution A and solution B were mixed, filtered through 0.22µm microporous filter membrane. Filtrate was divided in penicillin bottles with equal volume and pre-frozen for 13 h in -80°C refrigerator, followed by froze for 24 hours in Freeze-drying machine to obtain T-OA proliposomes for further utilization.

Physicochemical characterization of T-OA liposomes

Particle size and zeta potential of the liposomes were measured by Zeta sizer Nano ZS analyser (Malvern Instruments Co., Worcestershire, UK). 80µL of re-dissolved liposomes were diluted 100-fold with ultra-pure water. The dynamic light scattering data was collected using a helium laser as the light source and mean results were provided by photon correlation spectroscopy (PCS).

Total drug content was detected with UPLC by disrupting 100µL of re-dissolved liposomes using 900µL acetonitrile. For determining the entrapment efficiency, re-dissolved liposomes were centrifuged at 2000 rpm for 5min. 100µL supernatant liquid was taken out and broken with 900 acetonitrile, drug content of supernatant liquid was the drug who encapsulated in liposomes

$$\text{Entrapment Efficiency} = \frac{\text{drug content encapsulated in liposomes}}{\text{total drug content}} \times 100\%$$

In vitro drug release study

The release of drug from T-OA liposomes was investigated by dialysis bag method (Levy and Benita 1990), and T-OA suspensions were used as the control group. 3 mL release medium (0.5% SDS solution) was placed in dialysis bag (MWCO 8000-10,000, Sigma) and immersed bag in 100 ml release medium. T-OA liposomes and suspensions (contain the same content of T-OA) were poured into the outer dissolution medium maintained at 37°C and stirred at a rate of 100rpm. Aliquots of dissolution medium were withdrawn inside the dialysis bag at different time intervals and were replaced with the same volume of fresh medium to maintain the sink conditions. The content of T-OA was detected by UPLC and calculated cumulative release percentage of T-OA (Lin *et al.* 2014).

Establishment of conscious restrained rat lymph cannulation models

Healthy SD rats were fasted for 12 h with free access to water. 1.0~1.5mL of olive oil was given to rats by oral gavage one hour before surgery to identify the mesenteric lymph duct quickly and clearly during the surgical procedure. Anesthetized rat with sodium pentobarbital did abdominal incision of which the length is about 4cm and the mesenteric lymphatic vessel, a milky white duct, would be found on the right side and parallel to the upper edge of the kidney. Connective tissue and blood vessels around were separated gently to make mesenteric lymphatic vessels fully exposed. The PE-50 tube was inserted into the distal end of the lymphatic duct. The milky white lymph liquid would be observed in the tube after cannulate (Porter *et al.* 1996, Hauss *et al.* 1998, Gong *et al.* 2011, Zhang 2012, Cai *et al.* 2016b). After surgery, rats was restricted to freedom within a rat cage and fasted with free access to water.

Bioavailability studies

Administration and sampling

Twelve rats (male, 210-250g) were divided into two groups, the T-OA liposomes group and T-OA suspension group. The animals were administrated 15-18 h after surgery. Animals in T-OA liposomes were administrated with T-OA liposomes powder suspended in water (equivalent to 150mg/kg of T-OA). Rats in the latter group were administrated 150mg/kg of T-OA suspended in 0.5% sodium dodecyl sulphate (SDS). The lymph samples were collected to a heparinized eppendorf tube (EP tube) per hour, while the blood samples were withdrawn from the subclavian vein in heparinized EP tube at the same time interval. The lymph samples were stored in -20°C immediately. The blood samples were centrifuged immediately at 4000rpm for 10 min to separate plasma and plasma samples were stored in -20°C until analysed.

Lymph samples treatment

10 µL of T-GA solution (as the internal standard) and 1mL acetonitrile were added into 100µL of lymph liquid. The samples were vortexed (Qilinbeier® VORTEX-5 Vortex Mixer, Beijing southeast Yicheng Laboratory Equipment Co., Ltd.) for 4 min and centrifuged at 15000rpm for 10 min (G16 medical high-speed centrifuge, Baiyang Centrifuge Factory). The supernatant was withdrawn to another EP tube and the residue was further added in 0.5 mL acetonitrile to extract drugs as mentioned above. The supernatants obtained from double extraction were united to evaporate by organomation (Beijing tongtai technology development co., LTD). The residues were dissolved by 100 µL of acetonitrile, solutions were vortexed and centrifuged at 15000 rpm for 10 min. Five microliters of the supernatant were injected in the UPLC system for quantification.

Plasma samples treatment

10µL of T-GA (as the internal standard) was added to 200µL plasma and vortexed for 1 min. Then added 1mL ethyl acetate in plasma, vortexed for 4min and centrifuged at 15000 rpm for 10min. The supernatants were transferred to another EP tube and the remaining precipitations were added 0.5mL ethyl acetate to extract drugs again as mentioned above. The supernatants of double extraction were united to dry up by organomation. The residues were re-dissolved by 100µL of acetonitrile, solutions were vortexed for 1 min and centrifuged for 10 min at 15000 rpm. Samples were analysed by UPLC.

UPLC analysis

UPLC analyses of T-OA *in vitro* and in lymph samples were performed using a Waters ACQUITY UPLC series with binary solvent management system, on-line degasser, automatic sampler and photo-diode array (Waters Co., USA). Chromatography was recorded on a ACQUITY UPLC® BEH C18 reversed-phase column (2.1mm×50mm, particle size 1.7µm) (Waters Co., USA).

The analysis of T-OA *in vitro* was as follows. The injection volume was µL. The mobile phase consisted of a mixture of acetonitrile (solvent A) and water (solvent B), the ratio between solvent A and B is 98:2 (v/v). The flow rate and column temperature were kept constant at 0.4 mL/min and 30°C, respectively.

The analysis of T-OA in lymph was as follow. The injection volume was 5µL. The mobile phase consisted of a mixture of acetonitrile (solvent A) and 0.5% formic acid in water (solvent B), the ratio between solvent A and B is 90:10 (v/v). The flow rate and column temperature were kept constant at 0.4 mL/min and 30°C, respectively.

The intra-day and inter-day precision and accuracy at three different concentrations are summarized in table 1. Stability of lymph sample at two different concentrations (5.85 and 83.52µg/mL) was expressed as RSD, which ranged from 0.8% to 1.05% summarized in table 2.

Table 1: Intra- and inter-day precision of T-OA in lymph (mean \pm SD, n=5)

Concentration level	Intra-day		Inter-day	
	Ai/As	Precision (RSD %)	Ai/As	Precision (RSD %)
Low	0.56 \pm 0.01	2.57	0.59 \pm 0.03	4.84
Middle	1.00 \pm 0.04	3.96	1.03 \pm 0.06	5.48
High	2.61 \pm 0.06	2.27	2.45 \pm 0.19	7.94

Table 2: Recovery and stability of T-OA in lymph (mean \pm SD, n=5)

Concentration level	Recovery		stability	
	Cobs / C the	Precision (RSD %)	Ai/As	Precision (RSD %)
Low	102.75 \pm 0.03	3.19	0.61 \pm 0.01	0.8
Middle	101.42 \pm 0.05	4.76		
High	97.27 \pm 0.02	2.01	2.77 \pm 0.33	1.05

The results indicated that the present method was reliable and reproducible for quantitative T-OA in biological samples.

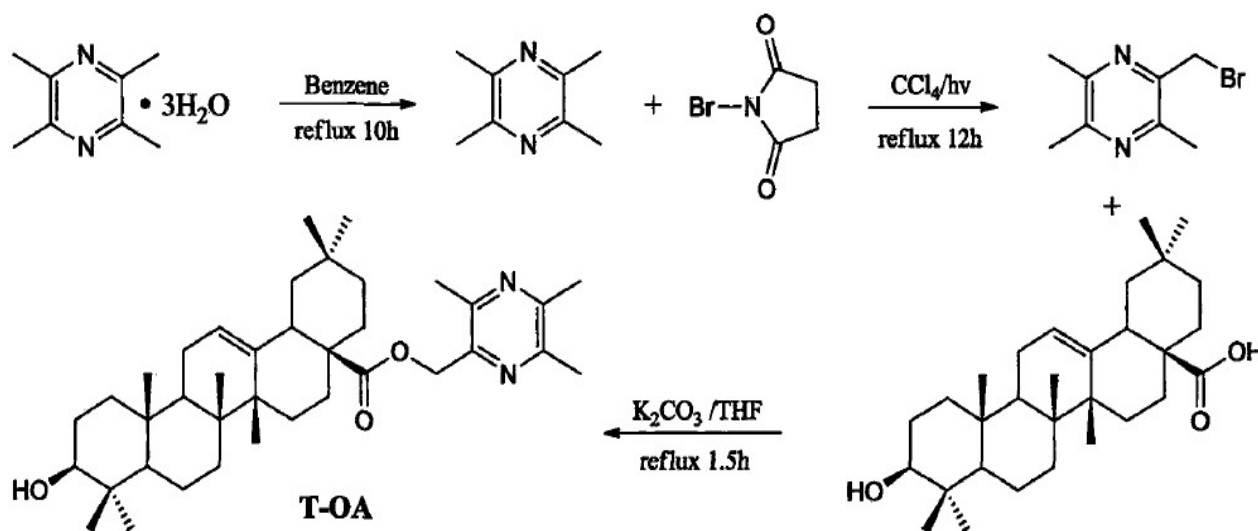


Fig. 1: Synthetic route and T-OA's structural formula

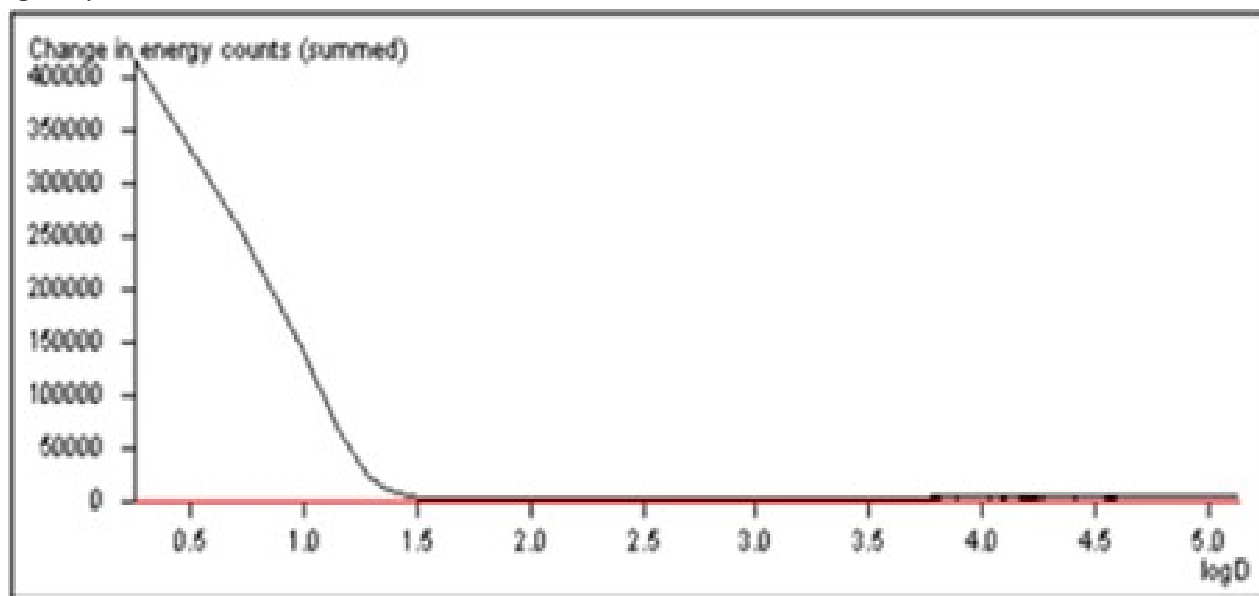


Fig. 2: Result of T-OA oil and water partition coefficient determined by LDA method

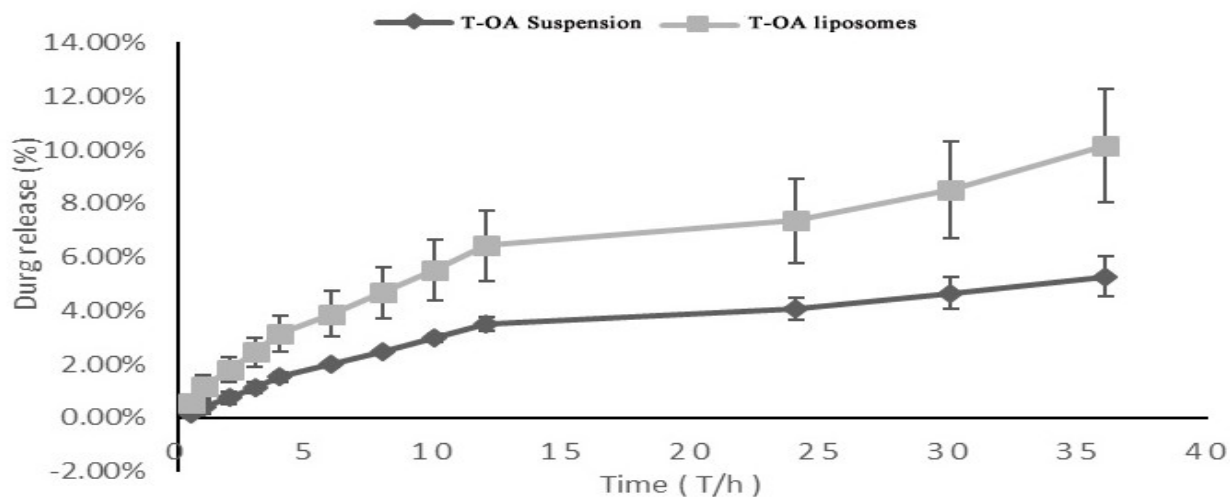


Fig. 3: *In vitro* release of T-OA liposomes and T-OA suspension

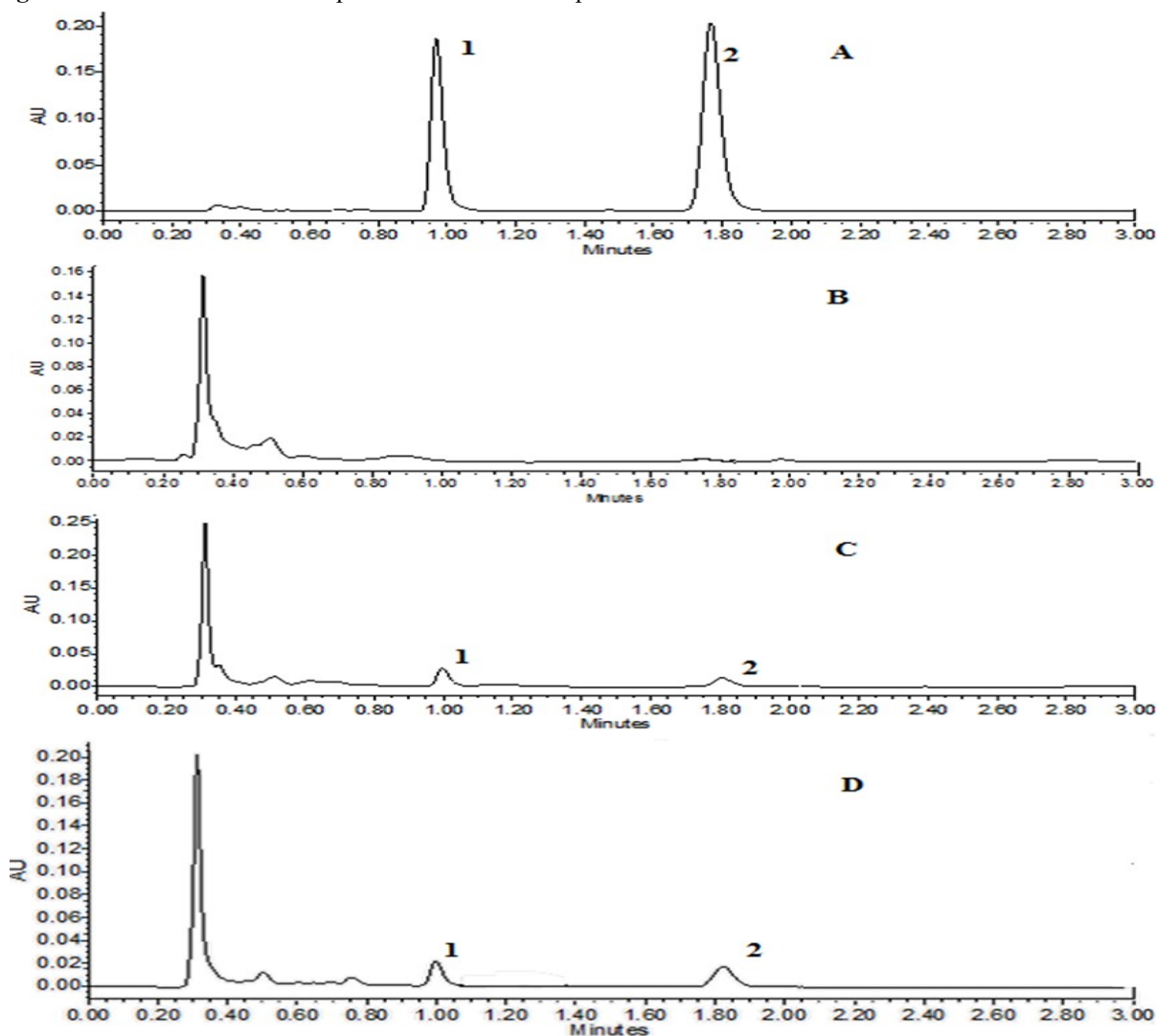


Fig. 4: Representative MRM chromatograms of T-OA and internal standard A: the mixture solution of T-OA and T-GA; B: blank lymph; C: the mixture solution of blank lymph, T-OA and T-GA; D: the mixture solution of T-GA and a lymph sample obtained after the intragastric. 1: T-GA (internal standard); 2: T-OA.

Data analysis

Lymphatic data processing

The analysis of lymphatic absorption is to calculate the accumulation of drug in the lymph fluid over time (Yang 2011). The formula is as follow:

$$X_i = X_{i-1} + C_i * V_i$$

(X_i , the amount of drug accumulated in the lymph from 0 to i hours after administration; X_{i-1} , the amount of drug accumulated in the lymph from 0 to $i-1$ hours after administration; C_i , the concentration of the drug at i -hour; V_i , the volume of lymph fluid collected at i -hour).

STATISTICAL ANALYSIS

All values are expressed as mean \pm standard deviations (S.D.s). To determine the statistical significance of calculated results among experimental groups, the two-tailed nonparametric Mann–Whitney U-test was used for two-group comparison, or the non-parametric Kruskal–Wallis test for multiple comparisons. A p value of less than 0.05 was termed significant (Dahan and Hoffman 2005).

Mathematical processing

In this paper, we try to use matlab spline interpolation method to deal with the experimental data of animal experiments. The experimental result was analysed from different views, so as to more accurately evaluate the experiment.

RESULTS

Partition coefficient of T-OA

Since the concentration in aqueous phase, the C_w , was lower than the detection limit, the partition coefficient was detected by LDA (isocratic chromatography system) and the result showed T-OA's $\log P$ is out of 1.5-5.0. Refer the result of shake-flask method and LDA, characteristic that T-OA rarely dissolves in water, according to the formula of partition coefficient; it can infer that $\log P$ of T-OA is greater than 5.0.

Characteristics of T-OA liposomes

Average particle and zeta potential of T-OA liposomes were (184.05 \pm 10.93) nm and (-21 \pm 0.85) mV, respectively. The entrapment efficiency of T-OA liposomes was estimated to be (93.24 \pm 2.25) %.

In vitro release study

The *in vitro* release curve of T-OA liposomes and T-OA suspension was plotted with which the cumulative release percentage is the ordinate and the sampling time is the abscissa as in fig. 3 (Wang, *et al.*, 2018). The total releasing rate of T-OA liposomes in 36 h was 10.4%, while the suspension's was 4.4%, which means the releasing rate of T-OA liposomes is 1.4 times higher than suspension's. It's inferred that liposome could enhance T-OA's solubility and promote its release.

Method validation

The resulting chromatograms were essentially free from endogenous interferences. Representative chromatograms were shown in fig. 4, including the mixture solution of T-OA and T-GA (internal standard), blank lymph, the mixture solution of blank lymph, T-OA and T-GA, and the mixture solution of T-GA and a lymph sample obtained after the intragastric. The retention time for T-OA and internal standard was 1.051 min and 1.825 min, respectively, and the peak shapes were satisfactory and suitable for quantitative analysis.

The calibration curves were of good linearity within a range of 4.176-1024 μ g/mL in lymph, and the linear regression equations were $Y = 0.029 X + 0.3939$ with linear correlation coefficients (R^2) of 0.9990. X was the concentration of T-OA in lymph; and Y referred to ratio of T-OA peak area over the IS area.

Bioavailability study

Statistical analysis result

The cumulative amount in mesenteric lymph of T-OA after administration is presented in fig. 4. The cumulative amount in mesenteric lymph of T-OA liposomes and T-OA suspension within 12 h were (921.39 \pm 19.73) μ g and (332.31 \pm 21.39) μ g ($n=6$), respectively. The statistical results showed that liposomes promoted the absorption of T-OA significantly ($P < 0.05$).

As showed in fig. 5, the cumulative amount in lymph increased within 7 h after administration liposomes. On the other hand, the drug was hardly detected within 4 h after administrated with T-OA suspension. The result showed the importance of lymphatic transport on the enhanced oral bioavailability of T-OA.

Mathematical processing result

As the fig. 6 shows, the fitting curves of T-OA suspension group's results are different from each other, which means that the data within this group is quite different. The results indicated that T-OA suspensions' absorption didn't have a unified regular pattern and couldn't be standardized quantitative study. But the result still showed that the absorption of the original drug was comparatively slow.

As shown in fig. 7, the fitting curves of T-OA liposome group's results are very similar, which indicated that the rules of T-OA liposomes' absorption could be standardized.

In addition, the fitting curves of liposomes showed that drugs entered into the body and reached to the peak concentration quickly, the decreased rapidly. These indicated that the absorption efficiency of the liposomes group was quite high.

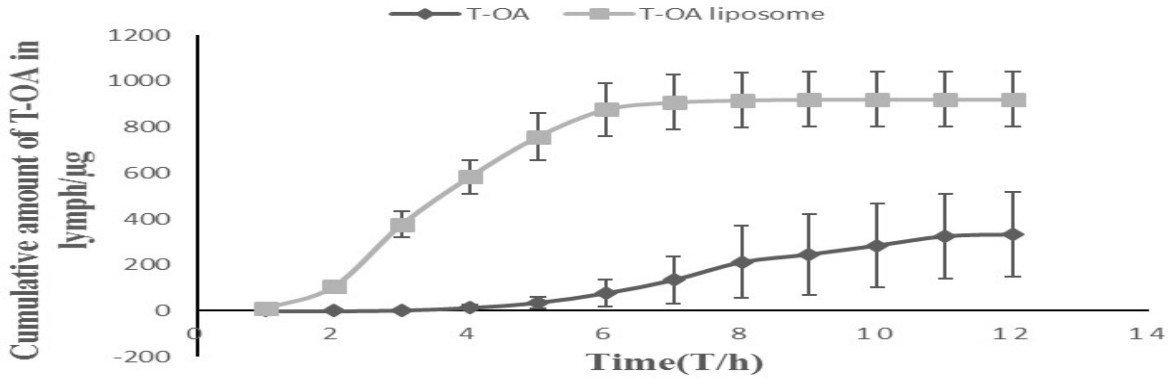


Fig. 5: The cumulative appearance of T-OA (mean \pm S.D.; n=6) in mesenteric lymph as a function of time after oral administration

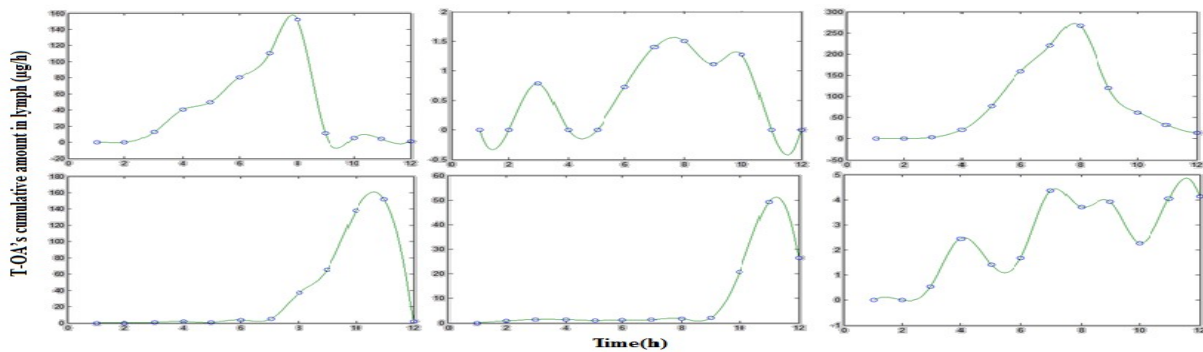


Fig. 6: The fitting curve of the T-OA suspension group (n=6)

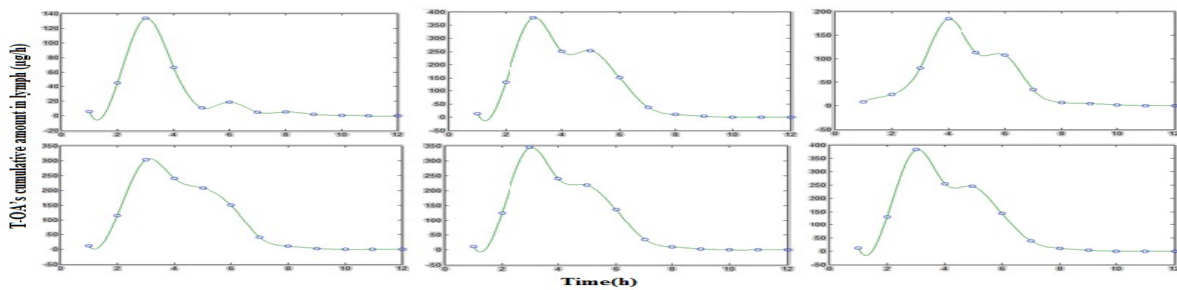


Fig. 7: The fitting curve of the T-OA liposomes group (n=6)

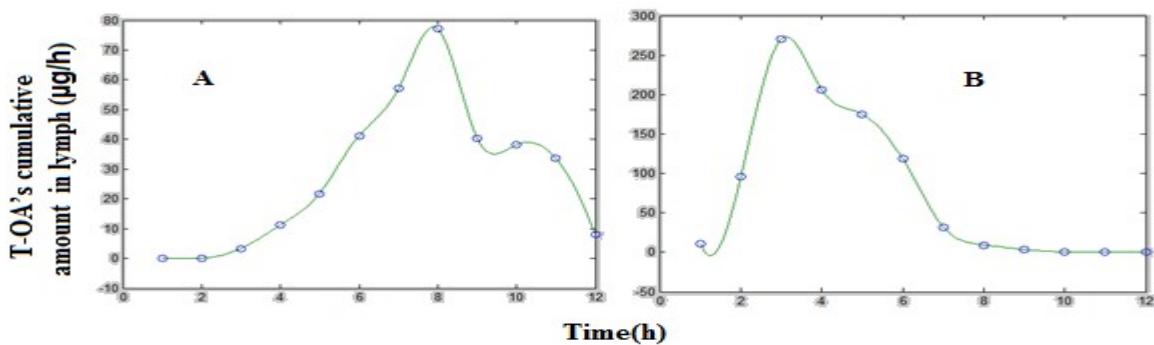


Fig. 8: The fitting curve of liposomes group and the suspension group mean value (A: drug suspension group; B: liposomes group)

The peak of liposomes group appeared earlier and the peak height of liposomes group was higher compared to the original drug group (fig. 7). The liposomes group demonstrated its superiority which could significantly promote the absorption of drugs.

DISCUSSION

In recent years, preparation technology has been greatly developed to improve oral bioavailability of poor solubility drug. Preparing these drugs in lipid formulation has gained increasing attention, because of its improving of drug's solubility and drug bioavailability, besides the enhancement in drug's transport via intestinal lymphatic (Porter 2007). Lymphatic system is another body fluid circulation system, along with blood circulation, plays an important role in substances' transport. Porter (2007) indicated that the fluid flow rate of portal blood is approximately 500-fold more than intestinal lymphatics, and the lipid content of the lymph is only of the order of 1–2% during peak lipid transport. However, in some cases lymph may be the main route of drug absorption, drug recovery in the lymph accounts for 60% to 80% of the dose (Charman *et al.* 2000, Porter 1997, McLennan *et al.* 2005). Drugs through the intestinal lymphatic transport can effectively avoid first-pass effect, increase the concentration of drugs in the lymph and lymph nodes, target the treatment of certain diseases related to lymph such as autoimmune, immune deficiency, allergies, lymphoma (Trevaskis *et al.* 2011). Therefore, this study attempts to use T-OA liposomes to promote T-OA's intestinal lymphatic transport and enhance its oral bioavailability.

There are many animal models are available to investigate the contribution of lymphatic transport to oral bioavailability, such as dog, pig, mice and rat (Chai and Tao 2008, Cai and Li 2013). Rat model is widely used in the study of intestinal lymphatic transport because it's easy procurement, smaller feeding space, relatively simple model and convenient operation. Many studies utilize unconscious rat models to study intestinal lymphatic transport. Whilst these models are simple and do not have problems associated with animals' movement during lymph collection, they have the attendant disadvantage of requiring long periods of anaesthesia to obtain a complete pharmacokinetic profile. But conscious restrained rat models can overcome this disadvantage. In addition, rats of conscious restrained models are closer to the real physiological state, allowing for the continuous collection of lymph over 12 h and no need for the intraduodenal cannula used in the unconscious model (Gong *et al.* 2011, Zhang 2012). Consequently, we choose conscious restrained rat model as animal model for lymphatic drug delivery studies.

There are many factors influencing intestinal lymphatic transport of drugs, such as physical and chemical

properties of drugs, lipid and type of formulations (Chai and Tao 2008, Cai and Li 2013). Studies have shown that lipid can enhance intestinal lymphatic transport, and that formulations of self-microemulsion, liposomes and mixed micelles can enhance intestinal lymphatic transport, too. T-OA is almost insoluble in water. Its low oral bioavailability is mainly attributed to its poor solubility (Trevaskis *et al.* 2011). T-OA liposomes can enhance the solubility of T-OA. T-OA liposomes were prepared by freeze-drying method which can improve liposomes stability. *In vitro* release study, the cumulative release of liposomes was 2.4-fold of the original drug. As T-OA was almost insoluble in water, liposomes could increase its solubility, promoting its release. This phenomenon maybe because that T-OA is almost insoluble in water, liposomes can increase the solubility of T-OA, and the size of T-OA liposomes is small which can more easily enter into dialysis bag to enhance the release, which needs to be further studied.

The data of animals' experiment was analyzed with different data processing methods and interpreted from different perspectives. The results of two methods are consistent. T-OA was not detected in plasma samples of liposomes group and original drug group, which might because the concentration of T-OA in plasma was lower than limit of detection. But the cumulative amount in lymph of T-OA liposomes group is 2.8-fold of the original drug group. Drug through the intestinal lymphatic transport can avoid hepatic first-pass effect and enter into the systemic circulation directly. Experimental results indicate that the liposomes group could significantly promote the absorption of T-OA which could enhance the intestinal lymphatic transport of T-OA.

CONCLUSION

In summary, the liposomes could promote T-OA's intestinal lymphatic transport and had a major contribution to enhance its oral bioavailability. In conscious restrained rat model, where drug was administered by oral, the experimental results indicated that lymphatic transport could be improved by formulation methods using lipid systems. The intestinal lymphatic transport is an exciting area for future research. Future work will assess the impact of the role of lymphatic transport in enhancing the oral bioavailability of lipophilic drugs and strong hepatic first-pass metabolism drugs by lipid systems and thereby define the important features of a lymphotropic lipid delivery system.

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