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Short communication

HPLC method for the pharmacokinetics and tissue distribution of taspine solution and taspine liposome after intravenous administrations to mice

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Abstract

Taspine is a bioactive aporphine alkaloid, which has many potent pharmacological effects. A simple, rapid HPLC method to quantify taspine in mouse plasma and tissue homogenates containing either taspine solution or liposome was developed and validated. Sample preparation was achieved by liquid–liquid extraction with acetoacetate. Taspine was separated on a C_{18} reversed phase HPLC column, and quantified by its absorbance at 245 nm. The pharmacokinetics and tissue distribution after intravenous administrations of taspine liposome (L-Ta) and taspine solution (Ta) to ICR mice were then compared. The area under the plasma concentration–time curve (AUC) was higher for L-Ta than for Ta. In contrast, the total body clearance (CL), apparent volume of distribution V_c and plasma half-life for the distribution ($t_{1/2\alpha}$) and elimination phase ($t_{1/2\beta}$) were lower for L-Ta, in comparison to the respective parameter of Ta. The AUC values were higher in the lung than in other organs for both L-Ta and Ta. The AUC in the spleen, kidney and liver of L-Ta were higher than those of Ta. However, the heart and brain AUC of Ta was higher than that of L-Ta. It can thus be concluded that incorporation into liposomes prolonged taspine retention within the systemic circulation, increased its distribution to the spleen and liver but reduced its distribution to the heart and brain.

Keywords: Taspine; Liposome; Pharmacokinetics; HPLC

1. Introduction

Taspine (Fig. 1), an alkaloid, can be extracted from many plants, such as *Leontice eversmanii* Bge. (Berberidaceas) of Central Asia, Iran and Afghanistan [1], *Croton lechleri* (*sangre de dreago*) of South American as well as Blue Cohosh Rhizomes [2–5]. On the one hand, taspine is a bioactive aporphine alkaloid, which has many potent pharmacological effects. For instance, taspine hydrochloride is a highly effective anti-inflammatory agent [1]. Taspine has also been shown to enhance wound healing largely through stimulating the chemotaxis of fibroblasts that migrate into the wound from the local tissues thereby increasing extracellular matrix synthesis [6–8]. On the other hand, taspine is a cytotoxic substance [9]. It exhibited a high embryotoxicity in the rat embryo culture [5].

We previously demonstrated that taspine may have anti-angiogenesis and anti-tumor activity since it significantly inhibited rat thoracic aorta ring angiogenesis, vascular endothelial cell (VEC) proliferation and migration in vitro and chicken chorioallantoic membrane (CAM) angiogenesis in vivo [10]. With the growing anti-tumor potential of taspine, there is an increasing demand for investigating on its pharmacokinetics. However, due to its hydrophobic nature, taspine is poorly soluble in any pharmaceutical solvent. The only pharmacokinetics of taspine in rat plasma after oral administration with taspine suspension was studied in our previous work [11]. An intensive investigation on the pharmacokinetics and tissues distribution of taspine after intravenous administration is desired.

To surmount taspine solubility obstacle, we prepared the taspine solution which could be administrated via intravenous injection to mice. But the preparation was not completely satisfying; a formulation of encapsulation of taspine into liposomes was developed. Since the drug is incorporated into one or more lipid bilayers, its pharmacokinetics profiles may be completely altered [12–14]. Many methods have been published describing procedures for the determination of liposomal pharmacokinetics, for example, HPLC-fluorescence method [15], LC–MS/MS method [16,17] and assay of radioactivity [18]. HPLC method

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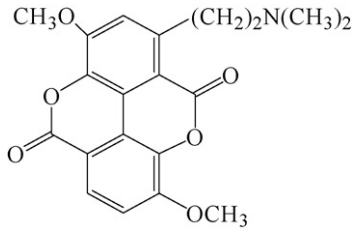

Fig. 1. Chemical structure of taspine ($C_{20}H_{19}NO_6$).

using reverse-phase columns is the most common means, along with ultraviolet or visible absorbance as the method of detection [12,13,19].

In the present study, a simple, sensitive, and rapid HPLC method was developed to quantify taspine in mouse plasma and tissues homogenates. Furthermore, the pharmacokinetic parameters of taspine solution (Ta) and taspine liposome (L-Ta) in ICR mice following intravenous administration were evaluated.

2. Experimental

2.1. Materials and animals

Taspine, a white amorphous powder with a melting point of 370 °C, was extracted from Racix Rhicoma Leonticis and then chemically identified using UV, IR, NMR and MS [2]. Its purity was measured using high-performance liquid chromatography and it was found to be above 97%. The compound was stable and practically insoluble in water but soluble in chloroform or methanol.

Egg phosphatidylcholine (EPC) was kindly supplied by Xi'an Libang Pharmaceutical Technology Company (Shannxi, China). Cholesterol (CHOL) was obtained from Beijing Aoboxing Biotechnology Co. HPLC grade solvents were from Fisher (Hampton, NH, USA), and all other analytical grade chemicals were obtained from Xi'an Chemical Regent Co.

Male ICR mice, weighing 18–22 g, were supplied from the Experimental Animal Centre of Xi'an Jiaotong University (Xi'an, China). All experimental protocols involving animals were reviewed and approved by the Institutional Animal Experimentation Committee of Xi'an Jiaotong University.

2.2. Preparation of taspine liposome

Taspine liposome was prepared by thin film evaporation–extrusion technique, using the procedures described previously [14]. The lipid composition was EPC/CHOL at a molar ratio of 50:50. The lipid mixture (10 g) was dissolved in chloroform/methanol (4:1 v/v). 20 mL of taspine chloroform solution (taspine concentration: 3.0 mg/mL) was added to the lipid mixture and mixed for 5 min using a vortex agitator (SK-1, China). The mixture was transferred to a 250 mL round bottom flask and allowed to dry under vacuum on a rotary evaporator (RE-52, China) at 40 °C for 60 min. The dried, lipid film was re-hydrated with 100 mL of 5% glucose aqueous solution by homogenizing at room temperature for 30 min. The concentration of sucrose as a lyoprotectant was 13% (w/v). The liposome suspension thus obtained was extruded for 4 extrusion cycles (the Extruder, Lipex, Northern Lipids Inc.) through a polycarbonate membrane with 0.4, 0.2 and 0.1 μm pores (Nuclepore, Whatman). The extruded suspension was transferred into 2.0 mL glass bottles, frozen by submerging the glass bottles in liquid nitrogen and lyophilized on a laboratory freeze drier (EZ-DRY, FTS System Company, USA) for 48 h. The lyophilized preparation was flushed with nitrogen, stored at 4 °C and re-hydrated with 2.0 mL of 5% glucose for use in subsequent experiments.

2.3. HPLC determination

2.3.1. Chromatographic equipment and conditions

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10ATvp pump, an SPD-M10Avp UV detector, a Diamonsil C_{18} column (150 mm × 4.6 mm, 5 μm particle size) (Dikma Technologies, Beijing, China), a guard column (C_{18}, 10 mm × 4.6 mm) installed ahead of the analytical column, and a column compartment set up at room temperature. The isocratic mobile phase was composed of 20 mmol/L aqueous biphosphat sodium–methanol–triethylamine (55:45:0.2 v/v/v), adjusted to pH 5.0 with glacial acetic acid, running at a flow rate of 1.0 mL/min. Taspine was detected using a UV detector at 245 nm. Data collection and processing were performed using CLASS-VP software (Shimadzu Corporation).

2.3.2. Assay method

Mouse plasma samples were collected from ICR mice into vials containing sodium heparin and stored at −20 °C until use. Thawed blank plasma was spiked with taspine stock solutions (0.9 mg/mL) to prepare quality control samples. Predetermined volumes of the taspine stock solution were added separately to 200 μL blank plasma to obtain taspine concentrations of 0.009, 0.09, 0.9, 4.5, 9.0 and 45 μg/mL.

Tissues (heart, liver, spleen, lungs, kidneys and brain) from ICR mice were weighed and homogenized in 1.0 mL normal saline (0.9% NaCl). Homogenates were aliquoted (0.8 mL) and spiked with taspine stock solution to prepare tissue homogenate quality control samples with taspine concentrations of 0.009, 0.09, 0.45, 0.9, 4.5, 9.0, 45 and 90 μg/g. The actual concentration ranges may differ slightly in various tissues.

Taspine was extracted from the plasma or the homogenates using the liquid phase extraction method described in Section 2.4. The HPLC method was evaluated for selectivity, linearity, precision, extraction efficiency and freeze–thaw stability.

2.4. Pharmacokinetic and tissue distribution study

Pharmacokinetics studies were performed as described elsewhere [20]. Mice were randomly divided into two groups (40 per group). Group 1 was treated with taspine solution whilst group 2 was treated with the taspine liposome. Taspine was dissolved in the mixture of Tween 80:dimethyl sulphoxide:5% glucose aqueous solution (0.1:55:45 v/v/v) by ultrasonic processing in ultrasonic cleaner (SB5200DTD, China) to obtain taspine solution with taspine concentration of 0.5 mg/mL, whilst the lyophilized taspine liposome was re-hydrated in 5% glucose

aqueous solution to obtain the same concentration. Each preparation was injected through the tail vein at the taspine dose of 12 mg/kg mouse.

Blood samples were taken from the terminal retro-orbital bleeding at various times (0.05, 0.08, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h) into micro-tubes containing sodium heparin as an anticoagulant, and centrifuged immediately (10 min, 12,000 × *g*). An aliquot (0.2 mL) of each plasma sample was accurately transferred to a centrifuge tube and buffered with 0.2 mL NH_3–NH_4Cl solution (pH 9.0). Two millilitres of acetoacetate was added to the buffered mixture and mixed for 2 min by vortex to extract taspine. Following centrifugation at 3000 × *g* for 10 min, the organic phase was transferred to a glass tube and the solvent was evaporated under nitrogen stream at 40 °C. The dry sample was then dissolved in 100 μL methanol and 10 μL of the solution was injected into the HPLC column to measure taspine peak area and calculate its concentration by standard curve method.

The heart, liver, spleen, lungs, kidneys and brain of each mouse was rapidly excised following blood collections, and immediately washed twice with normal saline (0.9% NaCl), wiped with filter paper, weighed and homogenized with 1.0 mL normal saline (0.9% NaCl). 0.8 mL of each homogenate was mixed with 0.8 mL NH_3–NH_4Cl buffer (pH 9.0) and taspine was extracted twice, each with 1.6 mL acetoacetate. After vortexing for 3 min, the sample was centrifuged for approximately 10 min at 3000 × *g*. The supernatant was collected and the solvent was evaporated under nitrogen gas at 40 °C. The dry sample was reconstituted in 100 μL methanol for measurement of taspine by HPLC.

2.5. Data analysis

The pharmacokinetic parameters of taspine in mice were calculated by the 3p97 software supplied by the Pharmacological Society of China (Beijing, China). Elimination distribution and disposition were assessed using following parameters: area under the curve (AUC); total body clearance (CL_s); apparent volume of distribution (V_c); plasma half-life for the distribution and elimination phase ($t_{1/2\alpha}$ and $t_{1/2\beta}$).

3. Results and discussion

3.1. Preparation and characterization of taspine liposome

Pre-lyophilized taspine liposomes showed a mildly translucent liposome dispersion whilst lyophilized taspine liposome was a white and amorphous powder. Taspine was poorly soluble in water but taspine liposome was found to be readily soluble in water.

3.2. Assay method

Chromatograms obtained from blank plasma and blank tissue homogenate of the heart (as a representative sample) are shown in Figs. 2A and 3A, respectively. No significant peak was observed at or near the retention time of taspine, suggesting that the extraction procedure was capable of obtaining highly purified samples which in turn ensured a high selectivity of the HPLC method.

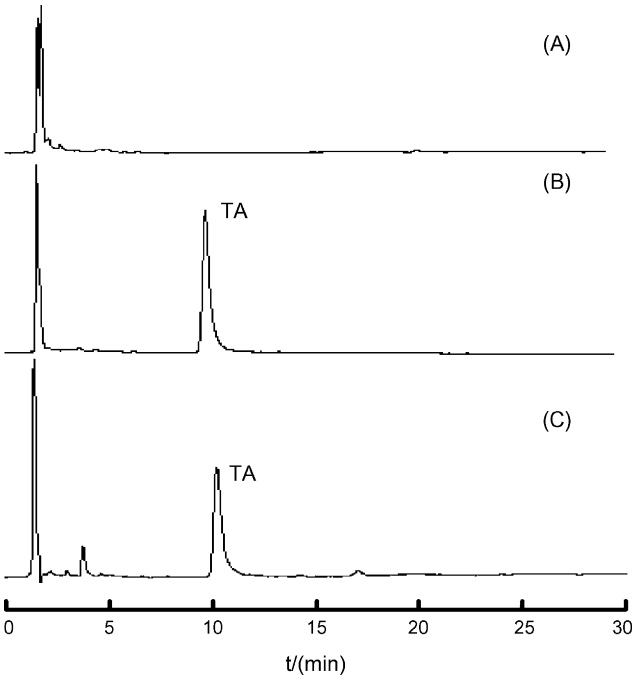

Fig. 2. Representative chromatograms of taspine (TA): (A) blank plasma; (B) blank plasma spiked with taspine; (C) plasma sample after intravenous administration of taspine solution.

Plasma quality control samples and tissue homogenate quality control samples were determined by HPLC. The standard curves between the peak area of taspine in quality control samples and taspine concentration were evaluated by

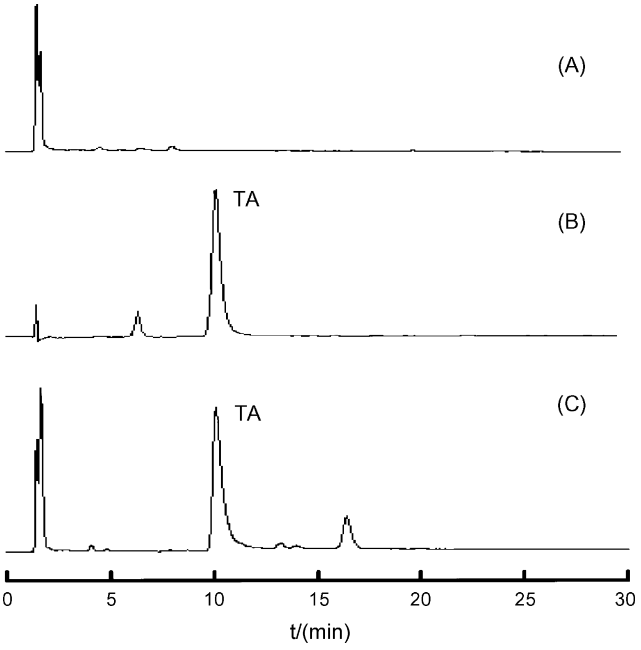

Fig. 3. Representative chromatograms of taspine (TA): (A) blank heart homogenates; (B) blank heart homogenates spiked with taspine; (C) heart homogenates sample after intravenous administration of taspine solution.

Table 1
Between-run and within-run precision and extraction recovery of taspine in mice plasma the quality control samples ($n = 5$)

Nominal concentration (μg/mL)	Mean measured concentration (μg/mL)	Precision (%)	Mean extraction recovery (%)
Within-day			
0.009	0.0073	13.12	80.58
0.9	0.85	9.95	93.94
45.00	43.34	4.75	96.31
Between-day			
0.009	0.0074	14.29	82.58
0.9	0.75	12.41	84.11
45.00	39.74	7.25	88.30

least squares regression analysis. Standard curves were linear ($r = 0.9915$–0.9995) over the ranges 0.009–45 μg/mL in plasma and 0.009–90 μg/g in tissue. The LOQ (limit of quantitation) was 5.0 ng/mL in plasma with precision, expressed as coefficient of variation (%CV), of 15.3% and accuracy (expressed as recovery) of 98.7%. In tissue samples, the LOQ was 8.0 ng/g with precision ranging from 7.65 to 17.4% and accuracy from 94.9 to 105.5%, respectively.

The precision was assessed by analyzing quality control samples at three concentrations in five duplicates. Table 1 shows the between-run, within-run precision and extraction efficiency for plasma samples. Table 2 shows the between-run, within-run precision and extraction efficiency of samples in tissue homogenates. These data demonstrate that both precision and extraction efficiency meet the requirements of the current Chinese Pharmacopoeia (2005 edition, part II) [21].

Taspine was shown to remain stable in plasma after treatment by three freeze–thaw cycles. It was also found to be stable in mice plasma after storage for at least 4 h at room temperature, and at least 4 weeks at −20 °C. The relative recoveries were detailed in Table 3.

3.3. Pharmacokinetics and tissue distribution study

Plasma pharmacokinetic parameters of taspine liposomal formulations were compared to those of taspine solution formulation in ICR mice. The mean plasma concentration–time profiles are given in Fig. 4. The pharmacokinetic parameters and the compartment model were analyzed by software program 3p97. The results showed that both taspine liposome and solution plasma concentration–time curves can be fitted into the open two-compartment model. The relevant pharmacokinetic parameters are listed in Table 4.

From Fig. 4 it can be seen that taspine plasma concentration was 8.97 μg/mL at 0.05 h after intravenous administration of L-Ta, which was ca. 10 times the value (0.84 μg/mL) obtained after administration of taspine solution. The plasma level of taspine was detectable up to approximately 24 or 12 h after administration of L-Ta or Ta, respectively.

As is shown in Table 4, the distribution half-life of L-Ta (ca. 0.02 h) was shorter than that of Ta (ca. 0.19 h), suggesting that L-Ta was taken up by other tissues more rapidly than Ta. The volume of distribution of taspine liposome was 0.41 (mg/kg)/(μg mL) and this was considerably smaller than that (12.42 (mg/kg)/(μg mL)) of the taspine solution. In addition, L-Ta gave a much greater area under curve (4.56 mg/mL × h) than Ta (1.94 mg/mL × h). Conversely, Ta had a much larger CL

Table 2
Between-run and within-run precision and extraction recovery of taspine in mice tissue homogenates quality control samples ($n = 5$)

Organs	Nominal concentration (μg/g)	Within-run precision		Between-run precision		Mean within-run extraction recovery (%)
		Mean measured concentration (μg/g)	Precision (%CV)	Mean measured concentration (μg/g)	Precision (%CV)	
Heart	0.09	0.074	13.56	0.073	17.12	82.34
	9.00	4.24	13.23	7.72	8.24	80.44
	90.00	79.56	10.21	79.39	11.56	84.40
Liver	0.009	0.0066	16.14	0.0070	10.68	73.60
	0.45	0.32	13.56	0.34	9.33	71.58
	9.00	7.05	11.73	7.40	5.48	78.34
Spleen	0.009	0.0073	14.18	0.0076	16.80	80.74
	0.9	0.76	10.24	0.72	14.26	84.15
	45.00	38.80	4.14	39.70	7.10	86.23
Lung	0.09	0.072	15.46	0.075	16.09	80.08
	4.5	3.93	5.85	3.91	11.58	87.29
	90.00	80.94	6.39	78.67	10.39	89.93
Kidney	0.09	0.074	14.30	0.073	13.57	82.78
	4.5	3.91	7.29	4.02	10.54	86.92
	45.00	39.82	5.46	39.63	6.27	88.49
Brain	0.009	0.0073	18.45	0.0072	12.60	81.15
	0.45	0.37	4.76	0.36	12.35	81.68
	9.00	7.89	15.42	7.73	10.03	87.62

Table 3
Stabilities of taspine in mice plasma ($n = 5$)

	Nominal concentration (μg/mL)	Mean measured concentration (μg/mL)	Precision (%CV)	Mean extraction recovery (%)
Room temperature storage for 4 h	0.009	0.0076	15.74	84.64
	0.9	0.80	13.21	89.42
	45.00	41.56	9.63	92.36
−20 °C for 4 weeks	0.009	0.077	12.66	85.25
	0.9	0.79	10.96	87.92
	45.00	40.83	7.84	90.74
−20 °C for 4 weeks (three freeze–thaw cycles)	0.009	0.0074	18.52	82.86
	0.9	0.80	14.85	88.91
	45.00	38.36	10.24	85.24

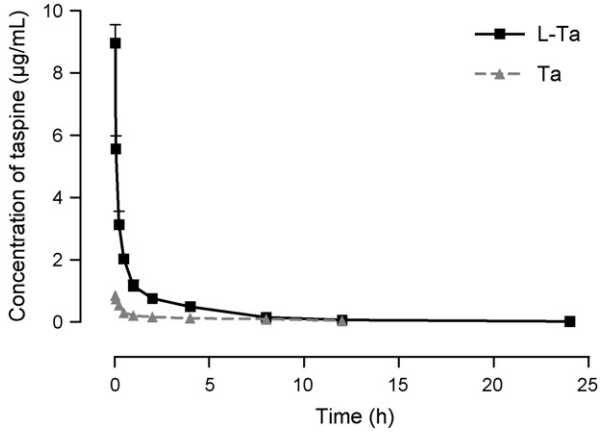

Fig. 4. Mean plasma concentration–time curve of taspine in mice after intravenous administration of taspine liposome (L-Ta) or taspine solution (Ta) at a dose equivalent to 12 mg/kg of taspine ($n = 4$). (■) Taspine liposome and (▲) taspine solution.

as compared to L-Ta, possibly due to the fact that taspine solution had a larger volume of distribution than taspine liposome.

Both L-Ta and Ta were taken up by the heart, liver, spleen, lungs, kidneys and brain of ICR mice. Fig. 5 shows the taspine levels in the liver and spleen at different time points after intravenous administration of either L-Ta or Ta. These were the raw data from which the pharmacokinetic parameters listed in Table 5 were calculated. It is clear that the concentration–time curves of both L-Ta and Ta can be fitted into a two-compartment model in the samples collected from the heart, liver, spleen, lungs, kidneys and brain. However, the concentration–time curve obtained from the liver samples after administration of taspine solution appeared to follow a non-linear dynamics model.

Similar values of CL_s in the heart were obtained for taspine solution and liposome. The heart AUC of Ta was higher than that of L-Ta, indicating that incorporation of taspine within liposomes decreases heart uptake of the molecule. Furthermore, the $t_{1/2\beta}$ (16.57 h) in the heart for Ta was substantially longer than that for L-Ta (3.79 h), suggesting that taspine liposme was cleared from the heart more rapidly than taspine solution. Encapsulation of taspine within liposome may therefore significantly reduce accumulative action of taspine to the heart.

The simulated pharmacokinetic process of taspine solution in the liver followed a non-linear dynamics at a dose of 12 mg/kg. Main pharmacokinetic parameters for Ta included V_{max} (46.74 μg/(g h), K_m (1.21 μg/g) and V (0.28 mg/kg/μg/g). However, the distribution and elimination of Ta in the liver at other doses is a subject of further investigations. The AUC in the spleen, which is rich in reticuloendothelial cells, was much higher for L-Ta than for Ta. The comparison of other parameters within the spleen shows that L-Ta had a higher $t_{1/2\alpha}$ and $t_{1/2\beta}$ but a lower $K_{10V(c)}$ and CL_s than the respective value for taspine solution. These data indicated that encapsulation of taspine in conventional liposomes might promote the phagocytosis of the

Table 4
Pharmacokinetic parameters of taspine liposome and solution in mice plasma following intravenous administration ($n = 4$)

Parameters	Definitions	Units	Taspine liposome	Taspine solution
A	Hybrid parameter	mg/mL	25.60	0.75
α	distribution rate constant	h^{-1}	30.98	3.62
B	Hybrid parameter	mg/mL	3.71	0.21
β	elimination rate constant	h^{-1}	0.99	0.12
$t_{1/2\alpha}$	Plasma half-life for the distribution phase	h	0.02	0.19
$t_{1/2\beta}$	Plasma half-life for the elimination phase	h	0.70	5.63
K_{21}	Transport rate constant from periphery compartment to central compartment	h^{-1}	4.77	0.89
K_{10}	First-order elimination rate constant	h^{-1}	6.43	0.50
K_{12}	Transport rate constant from central compartment to periphery compartment	h^{-1}	20.76	2.35
AUC	Area under the curve	mg/mL × h	4.56	1.94
CL_s	Total body clearance	mg/kg/h (μg/mL)	2.63	6.19
V_c	Apparent volume of distribution	(mg/kg)/(μg mL)	0.41	12.42

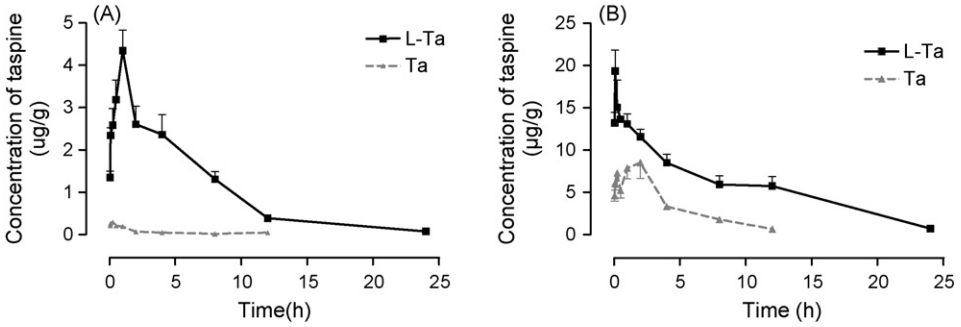

Fig. 5. Taspine levels over time in liver (A) and spleen (B) after i.v. injection of taspine liposome (■) and taspine solution (▲) in mice at a dose of 12 mg/kg ($n = 4$).

Table 5
Pharmacokinetic parameters of taspine liposome (L-Ta) and taspine solution (Ta) in various organs of ICR mice after intravenous administration ($n = 4$)

Organs	Taspine formulations	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	K_{10} (h^{-1})	AUC (mg/g × h)	V_c ((mg/kg)/(μg g))	CL_s (mg/kg/h (μg/g))
Heart	L-Ta	1.22	3.79	0.42	87.45	0.31	0.13
	Ta	1.65	16.57	0.33	93.10	0.42	0.14
Liver	L-Ta	2.82	4.51	0.16	13.67	5.63	0.88
	Ta[a]						
Spleen	L-Ta	0.02	5.67	0.31	124.28	0.31	0.10
	Ta	0.01	3.61	0.43	36.07	0.78	0.33
Lung	L-Ta	0.09	2.13	0.12	233.30	0.44	0.05
	Ta	2.72	5.26	0.21	228.54	0.25	0.05
Kidney	L-Ta	1.79	11.57	0.12	150.55	0.66	0.08
	Ta	0.39	12.10	0.08	47.85	3.27	0.25
Brain	L-Ta	0.12	4.21	1.93	3.66	1.70	3.28
	Ta	0.22	10.69	0.21	4.23	13.32	2.84

[a] Ta in the liver displayed a non-linear dynamics model.

molecule by mononuclear phagocytic cells. The AUC for L-Ta was slightly greater than that for Ta in the lung, but the CL values for both L-Ta and Ta were equivalent, suggesting that liposomes encapsulation of taspine did not alter taspine pharmacokinetics in the lung. The pharmacokinetic parameters in the kidney indicated that both L-Ta and Ta were primarily cleared from the kidney. In this tissue, the CL for Ta was 3.13 times higher than that for L-Ta whilst the V value for Ta was 4.95 times higher than that for L-Ta. Thus, taspine solution is more rapidly eliminated from the kidney than taspine liposome, the latter having markedly longer the residence time in the kidney than the former.

Taspine in the brain could be detected by the HPLC after intravenous injection of both L-Ta and Ta, suggesting that taspine could penetrate through the blood brain barrier. L-Ta gave a slightly lower brain AUC but markedly lower brain V, in comparison to Ta. Hence, liposomal encapsulation of taspine may not be able to prevent its penetration into the brain.

4. Conclusion

An HPLC method was developed and validated for quantitative measurement of taspine in ICR mouse plasma and tissue homogenates. This simple, rapid and sensitive method meets the current requirements for bioanalytical methods as specified by The Pharmacopoeia Committee of China.

Taspine in biological samples could be quantified with the HPLC method after intravenous administration of L-Ta and Ta. Liposomal encapsulation was found to change in vivo disposition of taspine after intravenous administration into ICR mice. Taspine liposome gave a markedly longer residence time in the systemic circulation than taspine solution. After administration of L-Ta, taspine level in the liver was higher than that of Ta. In addition, the accumulation of taspine in the heart was substantially reduced by liposomal encapsulation.

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