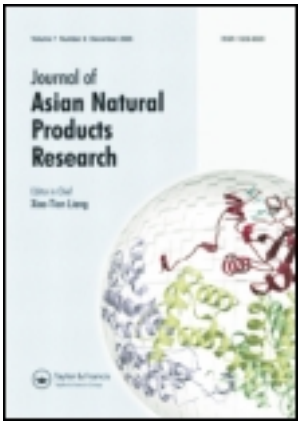

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Pharmacokinetics, tissue distribution, excretion, and metabolism of a new cardioprotective agent 10-*O*-dimethylaminoethylginkgolide B in rats

Dian-Lei Wang[ab], Dai-Yin Peng[a], Xiao-Dong Liu[b]*, Xian Zhang[a], Wei-Dong Chen[a], Yan Liang[b], Xin-Ting Wang[b], Tong Xie[b], Lin Xie[b] and Guang-Ji Wang[b]

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The plasma pharmacokinetics, tissue distribution, excretion, and metabolism of 10-*O*-dimethylaminoethylginkgolide B (XQ-1H), a protective agent against cardiovascular accident for its potential anti-platelet-activating factor activity, were investigated in rats. Plasma profiles were obtained after intravenous administration of 4, 8, 16, and 32 mg/kg of XQ-1H. There was a gender difference in the pharmacokinetics of XQ-1H. The elimination half-life of XQ-1H was 209.55, 200.81, 236.95, and 269.78 min in female rats and was 139.63, 173.83, 191.28, and 228.0 min in male rats at doses of 4, 8, 16, and 32 mg/kg, respectively. At four dose levels, female rats have higher values for area under the curve (AUC) than male rats. XQ-1H had linear pharmacokinetic characteristics in rats within the dose ranges tested. The volume of distribution in rats ranged from 6.05 to 15.09 l/kg. XQ-1H showed an extensive distribution into multiple tissues and reached its maximal concentration in all tissues at 10 min post-dose. About 80% of XQ-1H was mainly converted to its hydrolyzed and demethylated metabolites *in vivo*, and the elimination of unchanged compound was minor ($<20\%$) in rats.

Keywords: 10-*O*-dimethylaminoethylginkgolide B; pharmacokinetics; tissue distribution; excretion; metabolism

1. Introduction

Platelet-activating factor (PAF) is a potent bio-regulator, which appears to play a key role in acute inflammation, asthma, ischemic injury, and tissue rejection through its action at high-affinity receptors [1]. Consequently, the development of PAF antagonists that are suitable for therapeutic use has assumed considerable importance. Among the known types of PAF antagonists, ginkgolides (including ginkgolides A, B, C, J, and M) are especially interesting because of their long history of human use and their notable lack of toxicity [2]. Ginkgolide B, with two hydroxyl groups on C-1 and C-3, is the most powerful antagonist [3]. Pharmacological studies have shown that ginkgolide B possesses many beneficial effects such as anti-inflammatory [4,5], anti-allergic [6–8], ischemia–reperfusion injury protective effects [9,10], and neuroprotective effects [11,12].

Ginkgolide B has therapeutic potential, but has poor water solubility, and poor absorption after being orally administered. Recently, 10-*O*-dimethylaminoethylginkgolide B (XQ-1H, Figure 1A), structurally

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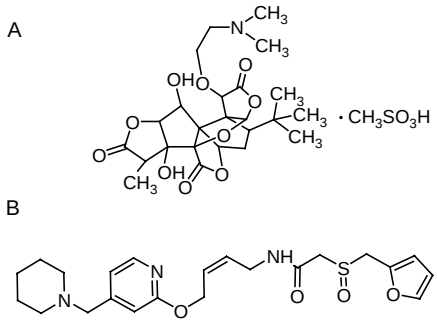

Figure 1. Chemical structure of XQ-1 (A) and IS lafutidine (B).

improved from ginkgolide B, is now being developed as a cardioprotective agent for treating cerebrovascular diseases. XQ-1H mesylate is a white powder and absolutely dissolves in water. Pharmacological research indicated that the C-10 hydroxy derivatives of ginkgolide B showed equivalent or superior activities to ginkgolide B on inhibition of PAF-induced aggregation of rabbit platelets [13–15].

Now, XQ-1H is being developed for treating cerebrovascular accident, but no information is available on systemic and comprehensive pharmacokinetics of XQ-1H. Although the pharmacokinetics of XQ-1H following oral administration has been studied [16], little is known about the pharmacokinetics after intravenous administration to rats. The purpose of the present study was to examine plasma pharmacokinetics, tissue distribution, excretion, and metabolism of XQ-1H in rats by an established liquid chromatography (LC)/ESI-MS method [17] after intravenous administration to rats.

2. Results

The plasma concentration–time curves of XQ-1H in rats following intravenous treatment are depicted in Figure 2. A two-compartment, first-order pharmacokinetic model was best fit to the plasma concentration–time curves obtained from rats. The main pharmacokinetic parameter values are shown in Table 1. There was a gender difference on the pharmacokinetics of XQ-1H. For example, marked differences were found after intravenous administration of 8 mg/kg XQ-1H; the plasma concentrations of XQ-1H in male rats were significantly lower than those in female rats (Figure 3). The clearances of XQ-1H in male and female rats were 0.29 ± 0.06 and 0.14 ± 0.01 l/min/kg, respectively, and the AUC_{0-t} was higher in female (54.77 ± 5.54 μg min/ml) than in male (28.27 ± 5.27 μg min/ml) rats.

The elimination half-lives of XQ-1H were 209.55, 200.81, 236.95, and 269.78 min in female rats and were 139.63, 173.83, 191.28, and 228.0 min in male rats at doses of 4, 8, 16, and 32 mg/kg, respectively. At the four dose levels, female rats have higher AUC values than male rats. The volume of distribution in rats ranged from 6.05 to 15.09 l/kg [10- to 20-fold of the total body water (0.668 l/kg)], suggesting that XQ-1H may be distributed into extravascular compartments. A dose proportionality study indicated that there is good correlation between AUC and dose. In addition, there was no significant difference ($p > 0.05$, one-way ANOVA) in the elimination half-life ($T_{1/2\beta}$) at the four dose levels, suggesting that XQ-1H may have linear pharmacokinetic characteristics in rats within the dose range tested.

The concentration of XQ-1H in various tissues of rats at 10, 45, and 240 min after intravenous administration at a dose of 8 mg/kg is presented in Figure 4. The results indicated that XQ-1H was distributed widely and reached a peak at 10 min in all tissues. As the time proceeded, the concentrations of XQ-1H rapidly decreased from each tissue by several folds in which the drug distribution rate decreased gradually. The large significant differences in the concentration of XQ-1H were found in liver, spleen, and stomach at 10 min between male and female rat tissues. The experimental results demonstrated that XQ-1H showed an extensive

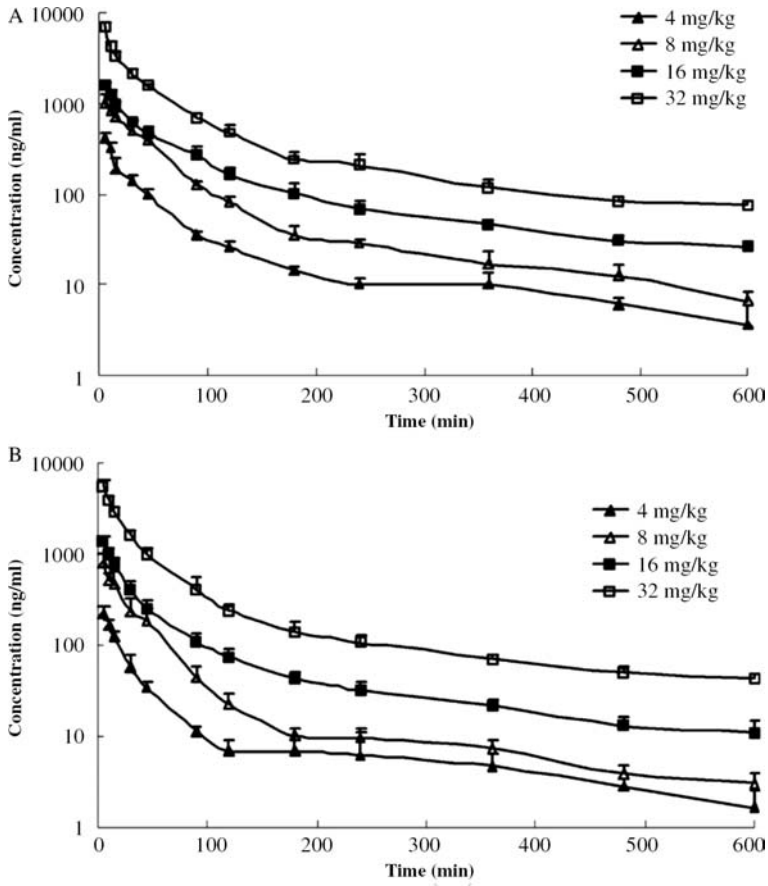

Figure 2. Mean plasma concentration–time profiles of XQ-1H in rats after intravenous administration of XQ-1H mesylate (mean ± SD, $n = 5$; A: female; B: male).

Table 1. Comparison of pharmacokinetic parameters of XQ-1H in rats plasma for single intravenous doses of 4, 8, 16, and 32 mg/kg (mean ± SD, $n = 5$).

Dose (mg/kg)	Gender	V_1 (l/kg)	$T_{1/2\alpha}$ (min)	$T_{1/2\beta}$ (min)	CL (l/min/kg)	AUC_{0-t} (μg min/ml)	$AUC_{0-\infty}$ (μg min/ml)
4	Female	11.18 ± 1.24	19.32 ± 2.42	209.55 ± 89.15	0.20 ± 0.03	18.56 ± 2.25	19.91 ± 2.83
	Male	15.09 ± 2.61	12.28 ± 2.09	139.63 ± 63.90	0.44 ± 0.07	8.38 ± 1.61	9.32 ± 1.30
8	Female	7.87 ± 2.04	25.36 ± 3.32	200.81 ± 47.58	0.14 ± 0.01	54.77 ± 5.54	56.38 ± 5.33
	Male	10.88 ± 2.39	18.03 ± 2.73	173.83 ± 52.55	0.29 ± 0.06	28.27 ± 5.27	28.76 ± 5.56
16	Female	11.11 ± 0.97	26.65 ± 4.02	236.95 ± 48.00	0.16 ± 0.01	91.34 ± 9.44	99.81 ± 8.69
	Male	12.01 ± 2.66	16.96 ± 2.56	191.28 ± 26.61	0.29 ± 0.06	53.38 ± 9.43	56.56 ± 10.52
32	Female	6.10 ± 0.95	24.31 ± 4.33	269.78 ± 109.15	0.10 ± 0.01	291.15 ± 29.96	321.75 ± 33.09
	Male	6.05 ± 0.77	17.25 ± 0.62	228.05 ± 42.16	0.15 ± 0.02	200.56 ± 24.80	215.23 ± 26.64

distribution into multiple tissues followed by rapid elimination from most of the tissues.

The average of excretion of XQ-1H in the bile fluid in female rats was 1.49%, 1.98%, 2.13%, 2.18%, and 2.27% within 2, 4, 8, 12, and 24 h, respectively. The average accumulation of excretion of XQ-1H into the bile fluid in male rats was 1.98%, 2.63%, 2.97%, 3.15%, and 3.36% within 2, 4, 8, 12, and 24 h, respectively. Total excretion of XQ-1H within 24 h was 2.27%

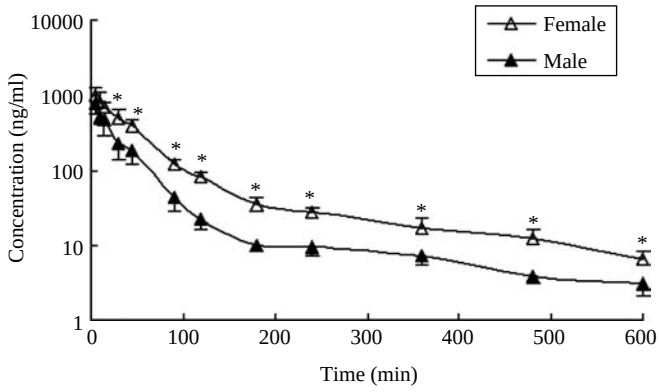

Figure 3. Mean plasma concentration–time profiles of XQ-1H in rats after intravenous administration of 8 mg/kg XQ-1H mesylate (mean ± SD, $n = 5$). $*p < 0.05$ vs. male rats.

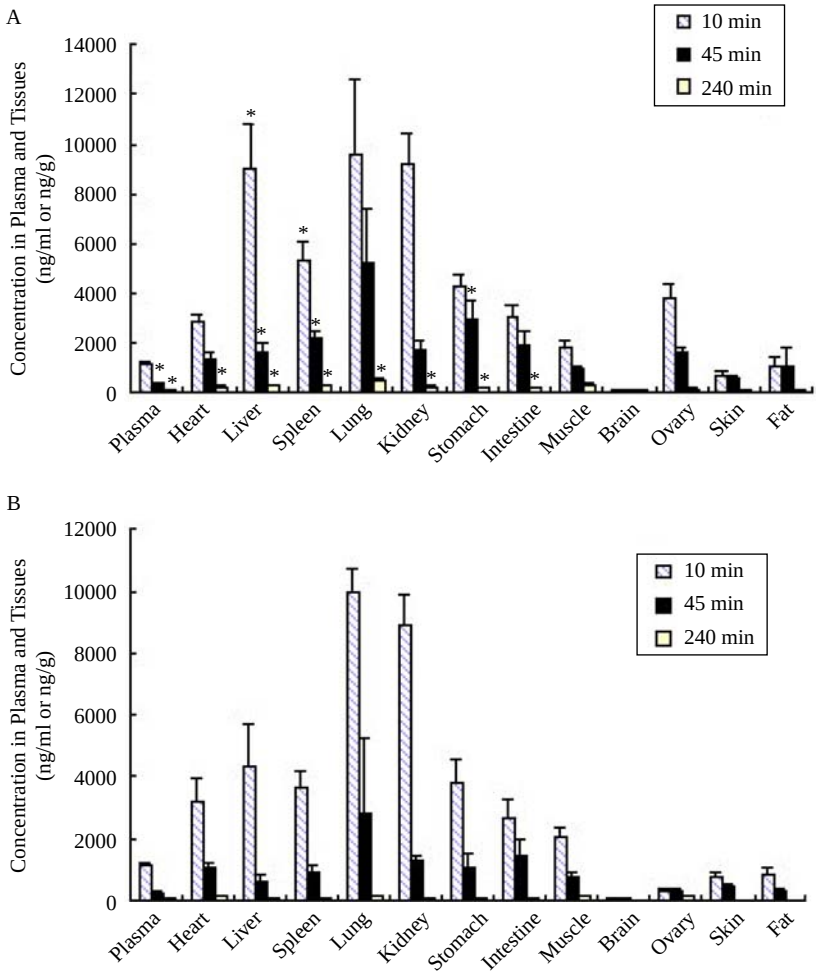

Figure 4. The distribution of XQ-1H in tissues following a single intravenous dose (8 mg/kg) of XQ-1H to rats (mean ± SD, $n = 5$; A: female; B: male). $*p < 0.05$ vs. male rats.

in male rats and 3.36% in female rats (Figure 5A). The average accumulation of excretion of XQ-1H into the urine of male rats was 2.20%, 2.52%, 2.89%, 3.23%, 3.52%, and 3.60% of administered dose within 2, 4, 8, 12, 24, and 48 h, respectively. The average accumulation of excretion of XQ-1H into the urine of female rats was

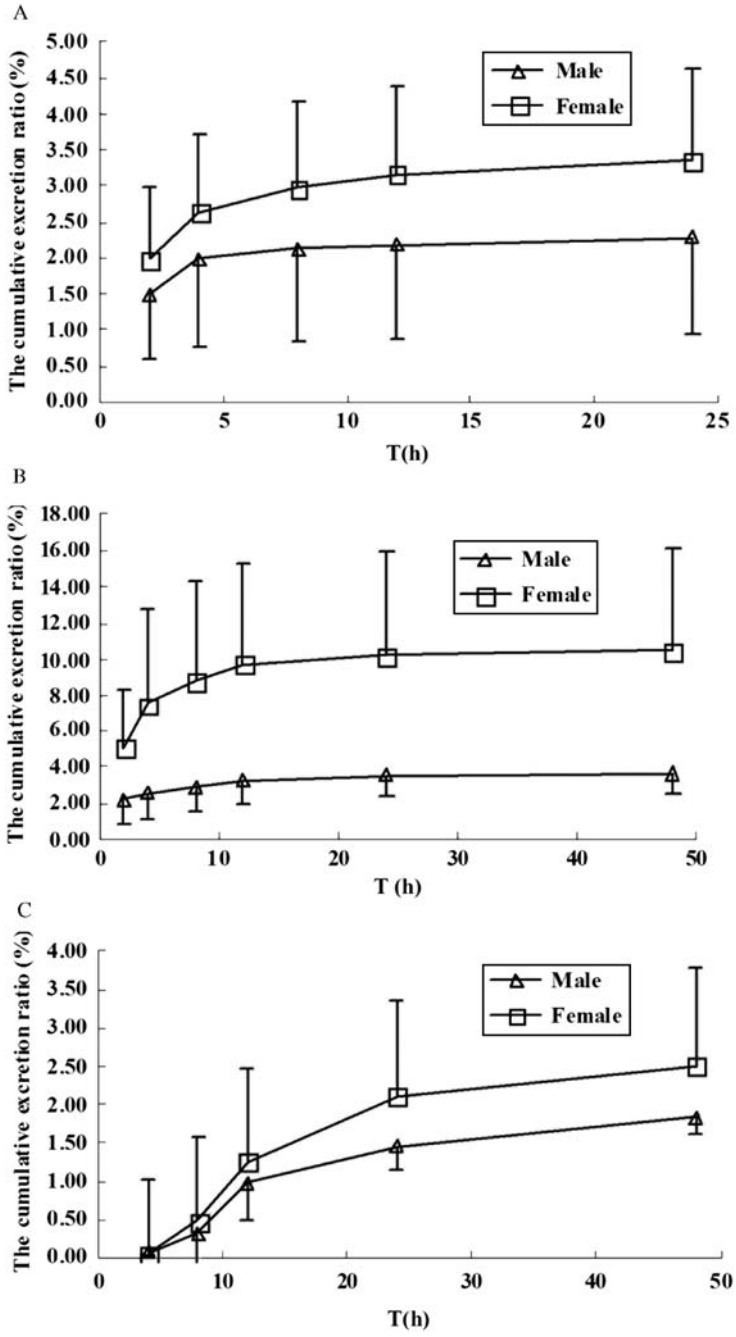

Figure 5. The cumulative excretion ratio of XQ-1H after a single intravenous administration of 8 mg/kg XQ-1H to rats (A: in bile; B: in urine; C: in feces).

5.05%, 7.47%, 8.84%, 9.72%, 10.23%, and 10.52% of administered dose within 2, 4, 8, 12, 24, and 48 h, respectively (Figure 5B). The average accumulation of excretion of XQ-1H into the feces of male rats was 0.07%, 0.32%, 0.97%, 1.45%, and 1.83% of administered dose within 4, 8, 12, 24, and 48 h, respectively. The average accumulation of excretion of XQ-1H into the feces of female rats was 0.05%, 0.48%, 1.25%, 2.12%, and 2.50% of administered dose within 4, 8, 12, 24, and 48 h, respectively (Figure 5(C)).

The excretion of XQ-1H into the bile, the urine, and the feces in female rats was higher than those in male rats but had no difference in statistic analysis. Moreover, the total excretion into the bile, the urine and the feces was 7.7% in male rats and 16.64% in female rats, suggesting that about 80% of XQ-1H may undergo metabolism *in vivo*.

The extracted ion chromatograms were obtained from urine sample after intravenous administration of XQ-1H and compared with those of blank sample (Figure 6) to identify the possible main metabolites in the urine of rat, and their $[M + H]^+$ ions of the possible metabolites were at *m/z* 514 (M_1), 532 (M_2), 482 (M_3), and 500 (M_4), respectively. No significant differences were detected in the formation of metabolites between male and female rats. The accurate mass data and formula of metabolites were obtained using an LC-IT-TOF-MS (Table 2).

The measured accurate masses of the *m/z* 514 metabolite of XQ-1H indicated the charge of $+ H_2O$ moiety from the parent compound. The measured accurate masses of the *m/z* 532 metabolite of XQ-1H indicated the charge of $+2H_2O$ moiety from the parent compound. Relative to XQ-1H, biotransformation to *m/z* 482 resulted in charge of $-CH_3$, and biotransformation to *m/z* 500 indicated the charge of $+ H_2O$ moiety from *m/z* 482. According to the above analysis, the proposed metabolic pathway of XQ-1H in rats could be tentatively presumed as shown in Figure 7.

3. Discussion

The pharmacokinetic study had shown that, after intravenous administration to rats, the linear and dose-independent pharmacokinetics of XQ-1H indicated that the elimination processes of XQ-1H were not saturated. However, there was a gender difference in the clearance of XQ-1H in rats with the elimination of parent drug from plasma occurring faster in male than in female animals. As a result of the gender difference of XQ-1H pharmacokinetics, female rats achieve a higher exposure to the drug than the male rats when administered the same dose.

Sex-related differences in drug pharmacokinetics have been known for more than 60 years, but it was not until recently that the mechanisms for those differences were explored. Recent studies have confirmed that the sexual dimorphism in drug metabolism in rats results from the differential expression of sex-dependent hepatic CYP450. CYP450 is most abundant in the liver, and CYP2C and 3A are major components of the liver microsomes of rat. These enzymes have an important role in detoxification of the liver. It is well known that CYP2C metabolizes tolbutamide, hexobarbital, and warfarin and that CYP3A metabolizes testosterone, cortisol, and aflatoxin B1 [18–20]. The differential expression of *CYP2C* and *CYP3A* genes in rats is responsible for the sex-dependent pharmacokinetics in drugs such as tirilazad [21], ranolazine [22], mephenytoin [23], and indinavir [24]. Thus the sex differences in pharmacokinetics of XQ-1H might be related to the sex-specific expression of rat *CYP2C* and *CYP3A* genes, which needs further studies to confirm. There were no gender differences in XQ-1H pharmacokinetics in beagle dog in our previous study [20].

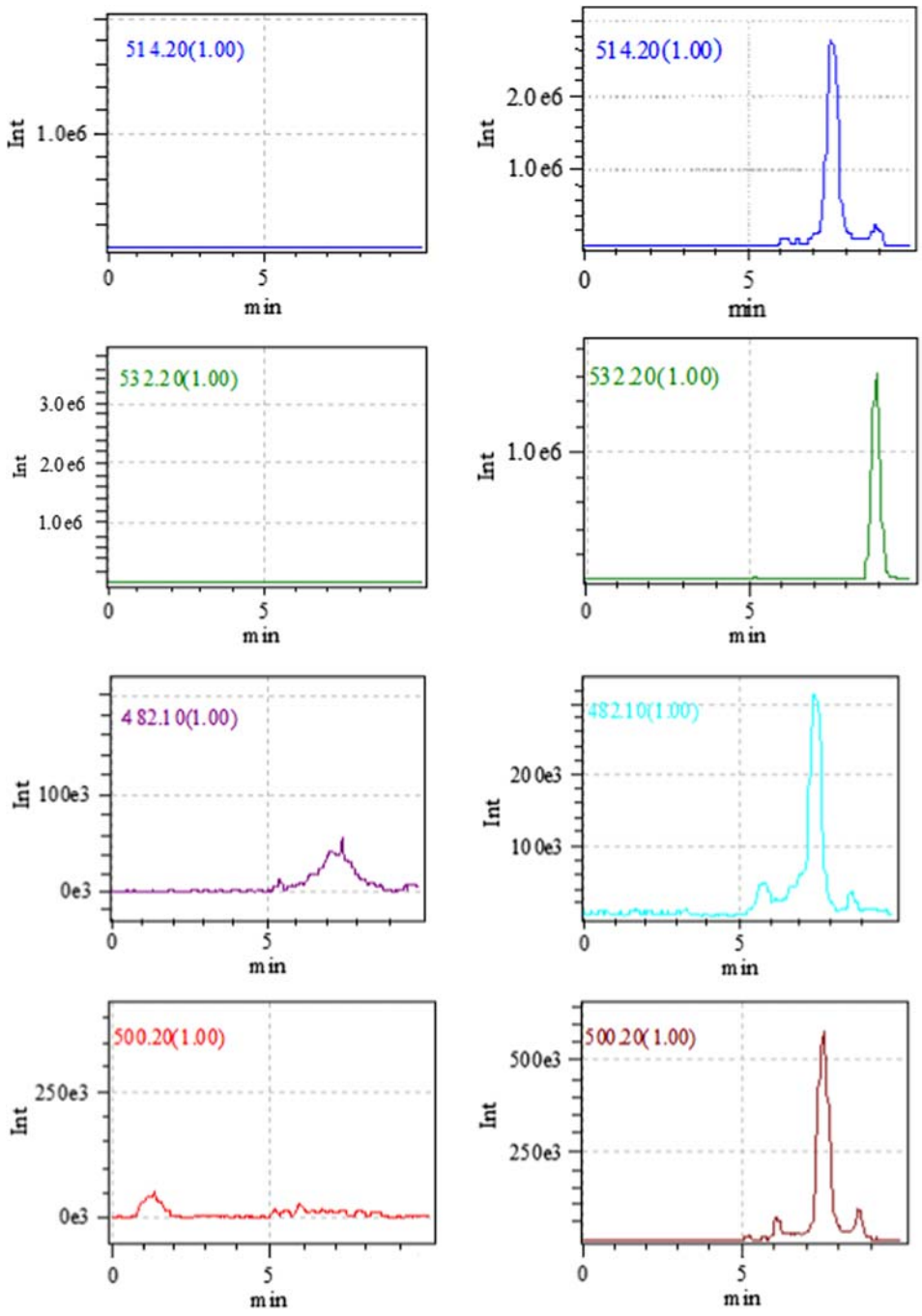

Figure 6. The extracted ion chromatograms of urine before and after intravenous administration of 8 mg/kg XQ-1H to rats: blank urine (left); urine sample collected from 0 to 6 h after intravenous administration of 8 mg/kg XQ-1H to rats (right).

Table 2. Measured accurate mass of the molecular ions $[M + H]^+$ of XQ-1H and its metabolites formed in the urine of rat.

Compound	Measured mass of $[M + H]^+$ (ions/Da)	Predicted mass of $[M + H]^+$ (ions/Da)	Suggested elemental composition	Deviation (ppm)
XQ-1H	496.2169	496.2177	$C_{24}H_{33}NO_{10}$	− 1.16
M_1	514.2268	514.2283	$C_{24}H_{35}NO_{11}$	− 2.92
M_2	532.2380	532.2389	$C_{24}H_{37}NO_{12}$	− 1.19
M_3	482.2016	482.2021	$C_{23}H_{31}NO_{10}$	− 1.04
M_4	500.2093	500.2093	$C_{23}H_{33}NO_{11}$	− 6.6

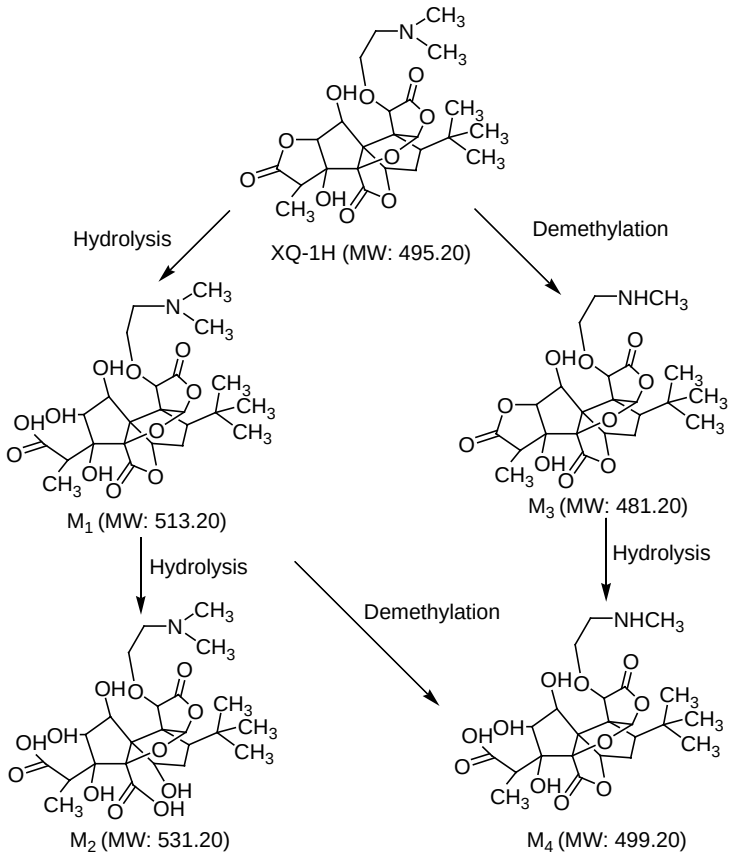

Figure 7. The proposed metabolic pathway of XQ-1H in rats.

Furthermore, XQ-1H appeared to be distributed into extravascular compartments, as predicted from the volume of distribution after intravenous injection (10- to 20-fold volume of total body water in rats). This was further supported by the presence of XQ-1H detected in most tissues. Our findings indicated that about 80% of XQ-1H might undergo metabolism and XQ-1H was mainly hydrolyzed and demethylated *in vivo*.

In summary, XQ-1H would be worthy of further investigation for preventing cardiovascular diseases because of its absolute solubility in water, high bioavailability in dogs [16], superior activities to

ginkgolide B [13–15], and the favorable pharmacokinetic properties in rats.

4. Experimental

4.1 Materials and reagents

XQ-1H (99.0% purity) was obtained from Nanjing Kefeiping Medicine Company (Nanjing, China), and internal standard (IS) lafutidine (Figure 1(B), 99.7% purity) was supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol was of chromatography pure grade and was purchased from Merck (Darmstadt, Germany). Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA). Ethyl acetate and other chemicals and solvents were all of analytical grade.

4.2 LC-MS apparatus and conditions

LC was performed using a Shimadzu LC-10AD (Shimadzu, Kyoto, Japan) HPLC system equipped with an autosampler. The HPLC system was coupled to a Shimadzu LCMS-2010A quadrupole mass spectrometer (Shimadzu) with an ESI interface. Chromatographic separation was achieved on a Shim-pack column (C18, 5 μm, 150 mm × 4.6 mm i.d., Shimadzu) at 40°C. The isocratic mobile phase consisted of a mixture of 1 μmol/l ammonium acetate containing 0.02% formic acid and methanol (55:45, v/v) at a flow rate of 0.3 ml/min. The ESI source was used in positive ion mode. The $[M + H]^+$ ions of XQ-1H (m/z 496.05) and of lafutidine (m/z 432.10) were selected as ions for selected ion monitoring detection. The quantification was performed using peak areas. The MS operating conditions were optimized as follows: drying gas 1.5 l/min, curved desolvation line temperature 250°C, block temperature 200°C, probe voltage + 4.0 kV, peak width 0.7 Th, and dwell time 0.2 s.

Linearity was established for the concentration range from 2 to 1000 ng/ml with a coefficient of determination of 0.9999 and good calculated accuracy and precision. The extraction recoveries ranged from 86.0% to 89.9% in plasma at the concentrations of 5, 50, and 500 ng/ml. The intra- and inter-day precision (relative standard deviation) was lower than 6%, and the accuracy ranged from 97.64% to 104.83%. The lower limit of quantification

Table 3. Standard curves of XQ-1H in plasma, urine, bile fluid, and tissues of rats.

Biological samples	Concentration ranges	Regression equation	Correlation coefficient
Plasma	2–1000 ng/ml	$Y = 0.0075X + 0.0019$	0.9999
Urine	10–5000 ng/ml	$Y = 0.0005X + 0.0014$	0.9997
Bile	10–5000 ng/ml	$Y = 0.0006X + 0.0003$	0.9997
Feces	50–1000 ng/ml	$Y = 0.0202X - 0.1191$	0.9998
Heart	2–1000 ng/0.1 g	$Y = 0.0107X + 0.0090$	0.9999
Liver	2–1000 ng/0.1 g	$Y = 0.0312X + 0.0093$	0.9999
Spleen	2–1000 ng/0.1 g	$Y = 0.0100X + 0.0038$	0.9999
Lung	2–1000 ng/0.1 g	$Y = 0.0105X - 0.0098$	0.9999
Kidney	2–1000 ng/0.1 g	$Y = 0.0229X + 0.0005$	0.9999
Stomach	5–500 ng/0.1 g	$Y = 0.0118X - 0.0024$	0.9991
Intestine	5–500 ng/0.1 g	$Y = 0.0113X + 0.0028$	0.9996
Muscle	2–200 ng/0.1 g	$Y = 0.0132X + 0.0066$	0.9997
Brain	0.5–20 ng/0.1 g	$Y = 0.0105X + 0.0001$	0.9999
Testis	5–500 ng/0.1 g	$Y = 0.0101X + 0.0108$	0.9999
Ovary	5–500 ng/0.1 g	$Y = 0.0122X + 0.0014$	0.9996
Skin	5–500 ng/0.1 g	$Y = 0.0118X + 0.0052$	0.9999
Fat	2–200 ng/0.1 g	$Y = 0.0294X - 0.0182$	0.9998

Note: Y indicates the ratio of analyte to IS and X refers to the concentration of XQ-1H added to blank biological samples.

was reproducible at 2 ng/ml with 100 μl plasma [17]. And the standard curves in other biological samples were established with coefficients >0.999 (Table 3). The extraction recoveries from biological samples were more than 70%.

The accurate mass and formula of metabolites were obtained by using an LC-IT-TOF-MS (Shimadzu) equipped with ESI (+). The probe voltage was −4.5 kV, and both the curved desolvation line temperature and the block heater temperature were 200°C. The ion accumulation time and isolation time were set at 30 and 20 ms, respectively. Full scan quadrupole MS were first acquired for molecular ions of parent compounds and relevant metabolites. Subsequently, the accurate mass and formula of the molecular ions and their fragments were obtained by using time-of-flight (TOF) analyzer.

4.3 Plasma samples preparation

To each tube containing 100 μl plasma, 10 μl of 200 ng/ml solution of IS and 1.0 ml of ethyl acetate were added, and the mixture was then vortexed for 2 min. The samples were then centrifuged for 10 min at 1000*g*. The organic layer was removed and evaporated to dryness in a Thermo Savant SPD 2010 SpeedVac system (Thermo Electron Corporation, Waltham, MA, USA) set at 45°C. The residues were then reconstituted in 200 μl methanol followed by centrifugation at 20,000*g* for 10 min before analysis. An aliquot of 5 μl was injected into the LC/MS system.

4.4 Animals

Sprague–Dawley rats (180–220 g) were provided by Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China; permit no. SCXK Shanghai 2003-0002) and housed five in a cage with unlimited access to food and water. Animals were fasted for 12 h before dosing and afterwards with free access to water. The animals were maintained on a 12-h light–dark cycle (light on from 8:00 to 20:00 h) at ambient temperature (22–24°C) and *ca.* 60% relative humidity. Animal studies were approved by the Animal Ethics Committee of China Pharmaceutical University.

4.5 Pharmacokinetics

Forty rats (20 male and 20 female) were divided into four groups with each group consisted of 10 rats and fasted 12 h before experiments. XQ-1H was dissolved directly in normal saline solution immediately before the pharmacokinetic studies and the injected volume was adjusted to 0.2 ml/100 g. Since doses for pharmacodynamic studies were 4, 8, 16, and 32 mg/kg, the intravenous bolus dose for pharmacokinetic studies was selected as 4, 8, 16, and 32 mg/kg XQ-1H, respectively. Blood samples (250 μl each at 0, 10, 20, 30, 45, 60, 120, 240, 360, and 600 min post-dosing) were collected in heparinized tubes. The blood was then centrifuged at 4000*g* for 10 min, and 100 μl plasma samples were kept at −20°C until analysis.

4.6 Tissue distribution

Thirty rats were randomly divided into three groups with each group consisting of 10 rats: five male and five female. XQ-1H was injected into rats at an intravenous bolus dose of 8 mg/kg following overnight fasting for 12 h. Following intravenous injection, the rats were sacrificed by bleeding at the femoral artery. Selected tissues including the brain, heart, liver, lungs, kidneys, stomach (without contents), muscle, spleen, adipose tissue, skin, duodenum (without contents), testicle, or ovary were collected at 10, 45, and 240 min after intravenous injection.

The rat tissues were accurately weighed and cut into slices and then homogenated after adding the appropriate amount of water (1 ml/0.1 g tissue). To the tissue homogenate, 100 μl of the 200 ng/ml solution of IS and 4.0 ml of

ethyl acetate were added. The samples were then centrifuged at 3000*g* for 10 min after vortexing for 3 min and the supernatant was processed and determined as plasma as described above.

4.7 Excretion into bile fluid

Ten rats (five male and five female) were fasted for 10 h. The rats were anesthetized with ether and secured on the surgery table for surgery in the bile duct. After the rats recovered, 8 mg/kg XQ-1H was injected intravenously. The bile fluid was collected at intervals of 0–2, 2–4, 4–8, 8–12, and 12–24 h.

The amounts of XQ-1H were determined as described above. The percentage of accumulation in the bile was calculated as [(amount of XQ-1H in bile/dose) × 100].

4.8 Excretion into urine and feces

Ten rats (five male and five female) were fasted for 10 h and then 8 mg/kg XQ-1H was injected intravenously into the rats. The rats were placed in the metabolism cages. The urine was collected at intervals of 0–2, 2–4, 4–8, 8–12, 12–24, and 24–48 h, and the feces were collected at intervals of 0–4, 4–8, 8–12, 12–24, and 24–48 h. The volume of urine was accurately measured.

To 100 μl of urine sample, 10 μl of the IS (1 μg/ml) was added. The mixture was processed and determined as plasma as described above. The rat feces were homogenated after adding the appropriate amount of water and the volume of homogenate of feces was accurately measured. The mixture was centrifuged at 1000*g* for 10 min. To 1 ml of supernatant, 10 μl IS (1 μg/ml) and 4.0 ml of ethyl acetate were added. The samples were then centrifuged at 3000*g* for 10 min after vortexing for 3 min and the supernatant was processed and determined as plasma as described above.

The percentage of excretion into urine and feces was calculated as [(amount of XQ-1H in urine or feces/dose) × 100].

4.9 Metabolite profiling

Urine samples (0–12 h) were pooled by the route of administration. Twenty microliters of urine of rat were added to 0.8 ml methanol in a 2-ml centrifuge tube. The sample in the tubes was mixed for 5 min and centrifuged for 5 min at 20,000*g*. Two microliters of the supernatant were injected into the LC-IT-TOF-MS system.

4.10 Pharmacokinetic and statistical analysis

Data fitting and pharmacokinetic parameter calculations were carried out using the DAS 2.0 pharmacokinetic program (Chinese Pharmacology Society, Beijing, China). An appropriate pharmacokinetic model was chosen based on the lowest Akaike's information criterion (AIC) value. Pharmacokinetic parameters of XQ-1H were calculated and presented as mean values. The analysis was performed using one-way ANOVA test and p-values <0.05 were considered significant.

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