Journal of Chromatography B, 908 (2012) 87–93

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of piperphentonamine and metabolites M1 and M6 in human plasma and urine by LC/MS/MS and its application in a pharmacokinetics study in Chinese healthy volunteers

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ARTICLE INFO

Article history:
Received 26 April 2012
Accepted 18 September 2012
Available online 29 September 2012

Keywords:
Piperphentonamine hydrochloride
LC/MS/MS
Metabolite
Pharmacokinetics
Phase I clinical study

ABSTRACT

Piperphentonamine hydrochloride (PPTA) is a new calcium sensitizer. A liquid chromatography–tandem mass spectrometry (LC/MS/MS) method for determination of piperphentonamine and its metabolites M1 and M6 was developed for the first time and applied to a pharmacokinetics study. Protein precipitation was used for pre-treatment of plasma samples, and solid phase extraction method was used for pre-treatment of urine samples. The chromatographic separation was achieved on a C_{18} column using gradient elution in this study: A: 1% acetic acid aqueous solution, and B: acetonitrile. The whole analysis lasted for 10.5 min and the gradient flow rate was 0.25 mL/min constantly. The detection was performed of a triple quadrupole tandem mass spectrometer by multiple reaction monitoring (MRM) mode via a positive electrospray ionization source. The results were that the m/z ratios of monitored precursor ions and product ions of PPTA, M1 and M6 were 354.0 → 191.8, 356.0 → 148.7 and 358.0 → 148.7, respectively. From the standard curve, the concentration ranges of both PPTA and M1 in blood and urine samples were 0.1–500 ng/mL and 0.1–200 ng/mL, respectively; the concentration ranges of M6 in blood sample and urine sample were 0.2–500 ng/mL and 0.2–200 ng/mL, respectively; and the correlation coefficient of standard curve was $r > 0.99$. A total of 31 healthy Chinese subjects participated in the pharmacokinetic study of single bolus intravenous injection of piperphentonamine hydrochloride. They were divided into three dosage groups and given 0.2, 0.4 and 0.6 mg/kg of PPTA. After drug administration, concentrations of PPTA, M1 and M6 in human plasma and urine samples were determined to evaluation the pharmacokinetic characteristics of PPTA and its metabolites M1 and M6.

1. Introduction

Calcium sensitizers enhance cardiac contractility mainly through promoting the sensitivity of myocardial contraction protein to Ca^{2+}. Therapeutic dosage of calcium sensitizers does not increase intracellular calcium concentration and myocardial consumption of oxygen [1]. Recently, it was found that calcium sensitizers not only have a promising application prospect in congestive heart failure treatment, but also exhibit good effects in treatment of other diseases or conditions, such as anti-shock, regulating peripheral vascular reactivity and improving blood flow in organs and tissues. For example, levosimendan, a commercially available calcium sensitizer, has been used in the clinical treatment of cardiovascular diseases in about 30 countries [2].

Piperphentonamine hydrochloride (PPTA) is a novel synthesized compound. As a new calcium sensitizer, its cardiotonic and vascular dilating functions have been confirmed in multiple experimental settings in vitro and in whole animal models. Pharmacodynamic tests suggested that PPTA exerted cardiotonic functions by enhancing the sensitivity of myocardial contraction protein to Ca^{2+}. It did not augment Ca^{2+} concentration in myocardial cells, but could inhibit the intracellular Ca^{2+} overload induced by anoxia-oxygen recovery, and reduce myocardial consumption of oxygen. These effects lead to decreased cardiac load and maintained cardiac function, and provide protection to cardiac muscle against ischemia-reperfusion-induced injury, all of which make it an ideal drug for the treatment of ischemic heart disease. A preclinical study indicated [3] that PPTA can reverse cognitive deficits induced by cerebral ischemia-reperfusion probably by decreasing the inflammatory responses and cell apoptosis in the brain of SD rats, suggesting its potential as a new therapeutic agent for improving the cognitive function following cerebral ischemia-reperfusion. In addition, another study indicated [4] that the neuroprotective

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http://dx.doi.org/10.1016/j.jchromb.2012.09.031

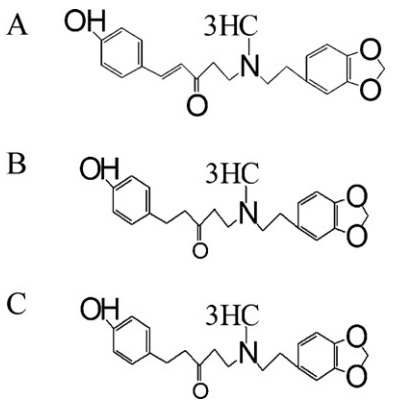

Fig. 1. Structural formulas of PPTA and its metabolites M1 and M6. (a) PPTA, (b) M1 and (c) M6.

effects of PPTA are closely associated with inhibition of lipid peroxidation reaction and scavenging free radicals. Its toxicological tests suggested that this drug has low toxicity, indicating that this compound is a potential new drug with unique action features and minor toxic and side effects.

A HPLC-UV method for determining the concentration of PPTA in plasma of Beagle Dogs has been reported [5]. No reports have focused on determination PPTA in human sample. In order to study the human pharmacokinetics on PPTA for injection use, a rapid and reliable method for determining the concentration of PPTA in plasma and urine samples needs to be established. In this study, we successfully established, for the first time, a liquid chromatography–tandem mass spectrometry (LC/MS/MS) method to determine the concentrations of PPTA and its metabolites M1 and M6 in human plasma and urine samples, and we further applied this method in the pharmacokinetic study of PPTA in healthy Chinese subjects. The structural formulas of PPTA and its metabolites M1 and M6 were shown in Fig. 1.

2. Methods

2.1. Control and chemical reagents

PPTA standard (batch No.: 20080101, purity 100%), metabolite M1 standard (batch No.: 20080912-2, purity 95.6%), and metabolite M6 standard (batch No.: 20080926-1, purity 97.9%) were all provided by Guangzhou Zhongwei Bio-Tech Co., Ltd.; the internal standard (IS), Bepotastine Besilate (batch No.: 06-02, purity 100.1%), was provided by Tianjin Tanabe Seiyaku Co., Ltd. Acetonitrile, methanol and acetic acid (Tedia, USA) were all HPLC grade. Ultrapure water (18.2 MΩ cm, 25 °C) was prepared by Milli-Q® A10 Synthesis purification system (Millipore Corporation, USA). Healthy human blank plasma for methodological study was provided by Beijing Tongzhou Blood Center; blank urine samples were provided by healthy, drug-free volunteers.

2.2. Instruments and experimental conditions

2.2.1. Instruments

Waters 2795 High Performance Liquid Chromatograph – Mricromass Quattro Premier electrospray tandem quadrupole mass spectrometer (LC–ESI-MS–MS) (Waters Corp., Milford, MA, USA); Masslynx ™ 4.0 software (Waters Corp., Milford, MA, USA); Chromatographic column: Phenomenex® Synergi™ Hydro-RP 80A chromatographic column (C_{18}, 150 mm × 2.0 mm, 4 μm); Waters OASIS® HLB 1 cm^3 (30 mg) extraction cartridge.

Table 1
Retention time and mass spectrum parameters of PPTA, M1, M6 and IS.

Compound	R_t (min)	Channel	Cone voltage (V)	Col energy (eV)
PPTA	8.07	354.0 → 191.8	20	15
M1	7.67	356.0 → 148.7	15	28
M6	6.86	358.0 → 148.7	20	25
IS	7.36	388.8 → 201.7	15	20

2.2.2. Liquid chromatographic conditions

Mobile phase: phase A: 1% acetic acid; phase B: acetonitrile. Gradient elution program: during the time period of 0–2 min, A:B ratio was increased from 90:10 to 75:25 at a constant speed; during the time period of 2–8.5 min, a ratio of 75:25 was maintained; during the time period of 8.5–10.5 min, a ratio of 90:10 was maintained; the whole analysis time lasted for 10.5 min and the gradient flow rate was 0.25 mL/min constantly; the column temperature was 30 °C and the sample injection volume was 10 μL.

2.2.3. Mass spectrometric conditions

Electrospray ion source (ESI) with positive ionization mode was adopted and multiple reaction monitoring (MRM) was used for data collection. Capillary voltage was set to 3.2 kV; the flow rates of desolvation gas (N_2) and cone gas (N_2) were 600 L/h and 50 L/h, respectively; the source temperature and desolvation temperatures were set at 105 °C and 400 °C, respectively. With the collision energy off, PPTA, M1, M6 and IS produced precursor ions. Each of the precursor ions was subjected to collision-induced dissociation (CID) to determine the resulting product ions. Argon was used as the collision gas at a flow rate of 0.24 mL/min. The chromatographic retention time, mass spectrum monitoring channels and partial mass spectrum parameters for identifying levels of PPTA, M1, M6 and IS were shown in Table 1.

2.3. Working solution, quality control and standard curve preparation

PPTA standard was added into solution A (acetonitrile:water:acetic acid = 50:48:2), and shaken to dissolve to obtain PPTA working stock solution with a concentration of 400 μg/mL. M1 and M6 stock solutions with the same concentration and internal standard stock solution with a concentration of 100 μg/mL were prepared by the same method. The stock solutions were diluted with solution A to obtain working solutions with corresponding concentrations. All solutions were preserved at 4 °C, and warmed to room temperature prior to use.

2.4. Quality control samples

2.4.1. Plasma quality control samples

200 μL of plasma was added into 20 μL of standard working solutions containing two different concentrations of PPTA (2 and 50 ng/mL) and M1 (1 and 3.75 μg/mL), which generates plasma quality control samples with PPTA of 0.2 and 5 ng/mL, and with M1 of 100 and 375 ng/mL. Similarly, 200 μL of plasma was added into 20 μL of standard working solutions containing M6 with concentrations of 5, 20 ng/mL and 4 μg/mL to prepare plasma quality control samples with M6 concentrations of 5, 20, 400 ng/mL.

2.4.2. Urine quality control samples

1 mL of urine was added into 100 μL of standard working solutions containing three different concentrations of PPTA and M1 (2, 50 ng/mL and 1.6 μg/mL) to generate urine quality control samples with PPTA and M1 of 0.2, 5 and 160 ng/mL, respectively. For M6 quality control samples, 1 mL of urine was respectively added into 100 μL of standard working solutions containing M6 of three

different concentrations: 2, 100 ng/mL and 1.6 μg/mL, which makes final urine quality control samples contain 0.2, 10 and 160 ng/mL of M6.

2.5. Sample pretreatment

2.5.1. Blood samples

A mixture of acetonitrile:water:acetic acid solution (50:48:2) 20 μL and internal standard solution 20 μL were added into 200 μL of human plasma samples, vortexed for 30 s to mix well, followed by addition of 600 μL of acetonitrile containing 2% acetic acid (protein precipitant). Because piperphentonamine is stable under acidic conditions, we finally selected acetonitrile with 2% acetic acid as a protein-precipitating agent. The solution was vortexed once more for 1 min to mix sufficiently, and then centrifuged for 10 min at the speed of 13,000 rpm. 10 μL of supernatant was used to perform sample injection.

2.5.2. Urine samples

Solid phase extraction was used to handle urine samples. Briefly, 1 mL of human urine sample was added into 50 μL of internal standard solution, vortexed for 30 s to mix well, centrifuged for 1 min at the speed of 13,000 rpm. Prior to use, solid phase extraction cartridge was activated by vacuous washing with 1 mL of methanol and 1 mL of water, respectively. Subsequently, 1 mL of sample was loaded onto a HLB cartridge, and then eluted with 1 mL of water, 5% methanol aqueous solution and 10% methanol aqueous solution (containing 2% ammonia), respectively. The sample was finally eluted with 1 mL of 90% acetonitrile aqueous solution (containing 2% acetic acid). 200 μL of collected eluent was taken and mixed with 200 μL of mobile phase (acetonitrile:water:acetic acid = 10:90:1), out of which 10 μL was injected into LC/MS/MS for analysis.

2.6. Method validation

2.6.1. Specificity

Six blank biological samples from different individuals were used to investigate interference. If the responses to blank samples were all less than 20% of lowest limit of quantitation (LLOQ) of test samples, it suggested that endogenous compounds in blank plasma did not interfere the corresponding channels of PPTA, M1 and M6; and there is no interfering peak. The retention time of PPTA and M1 was about 8.05 and 7.65 min, respectively, the retention time of M6 and IS was about 6.90 min and 7.36 min, respectively (the results are shown in Fig. 2).

2.6.2. Linearity

A series of human plasma or urine samples containing corresponding concentrations of PPTA and M1 were prepared and pretreated (refer to Section 2.5), and then the samples were analyzed. The regression equation was obtained by plotting the ratio *R* (the peak area ratio comparing the analytes to the internal standard) and the concentraton of analytes; Calibration curves for M6 plasma and urine samples were prepared by the same method. Each concentration standard needed to the following acceptable criteria:no more than 20% deviation near the LLOQ and no more than 15% deviation for the standards above the LLOQ.

2.6.3. Extraction recovery rates and matrix effects

After QC samples pretreatment (5 samples for each QC concentration), the extraction recovery rates for PPTA, M1 and M6 in plasma samples were analyzed. The extraction recovery was calculated by comparing the peak areas obtained from extracted spiked samples with those of samples spiked post-extraction at corresponding concentrations.

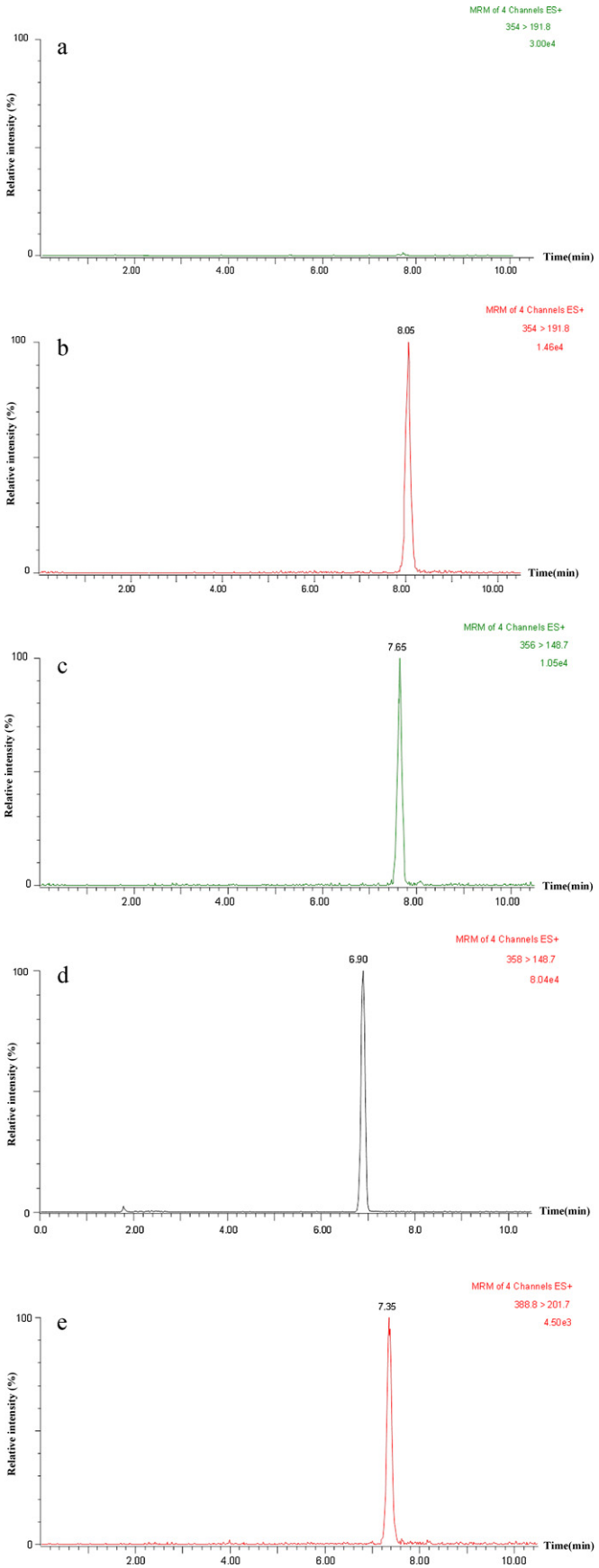

Fig. 2. Blank human plasma (A), blank human plasma + PPTA (B), blank human plasma + M1 (C), blank human plasma + M6 (D), blank human plasma + internal standard (E).

Table 2
Results of regression analysis on calibration curves of PPTA, M1 and M6 in plasma and urine samples ($n = 5$, mean ± SD).

Analyte	Plasma		Urine	
	Regression equation ($y = ax + b$)[a]	Correlation coefficient (r)	Regression equation ($y = ax + b$)[a]	Correlation coefficient (r)
PPTA	$y = (0.15601 \pm 0.00971)x + (0.00885 \pm 0.00475)$	0.9988 ± 0.0005	$y = (0.09523 \pm 0.00890)x + (0.00535 \pm 0.00161)$	0.9978 ± 0.0007
M1	$y = (0.05995 \pm 0.00245)x + (0.00628 \pm 0.00636)$	0.9976 ± 0.0009	$y = (0.10893 \pm 0.01190)x + (0.00300 \pm 0.00179)$	0.9974 ± 0.0014
M6	$y = (0.04925 \pm 0.00468)x + (0.00206 \pm 0.00171)$	0.9969 ± 0.0011	$y = (0.07713 \pm 0.00318)x + (0.00448 \pm 0.00178)$	0.9980 ± 0.0006

a: slope; b: intercept.

The matrix effect was assessed by comparing peak areas of PPTA, M1 and M6 (3 samples at each QC level) obtained from the spiked-after-extraction samples with those from the pure standard solutions at the same concentration level.

2.6.4. Precision and accuracy

To assess the precision and accuracy of the procedure, reproducibility for both intra-day (6 samples at each QC level) and inter-day (3 consecutive days) variations of PPTA, M1, M6 in plasma/urine sample was measured. The precision results are depicted using relative standard deviation (RSD%). Accuracy was assessed by calculating the percentage deviation from the theoretical concentration.

2.6.5. Stability

The purpose of this step is to evaluate the stability of QC samples under different storage conditions, including the stability of PPTA, M1 and M6 working stock solutions preserved at 4 °C, short-term or long-term stability of QC samples containing PPTA, M1 and M6 preserved at room temperature and −70 °C, respectively, stability of QC samples containing PPTA, M1 and M6 preserved at 4 °C after treatment for 24 h, and stability of QC samples subjected to repeated freeze/thaw cycles for 3 times.

2.7. Pharmacokinetics study in Chinese healthy volunteers

2.7.1. Test drug

Freeze-dried PPTA powder for injection (10 mg/bottle, batch No.: 080503) and PPTA placebo for injection (batch No.: 080601) were both provided by Beijing Sihuan Pharmaceuticals Co., Ltd. The drug was added to 30 mL of 5% glucose injection at the time of use, and bolus intravenous injection was performed and finished within 10 min.

2.7.2. Test process

This study is a randomized, single-blind phase I clinical study, approved by Ethics Committee of Beijing Hospital of Ministry of Health. All the subjects signed informed consent forms prior to enrollment in the study. The subjects are from 18 to 40 years old, and their body mass index (BMI) distributed between 19 and 24. Each subject was confirmed to be in healthy status by physical examination, laboratory test and ECG, and met the inclusion criteria. The subjects did not take any prescription drugs or OTC drugs within two weeks prior to the first dose, and did not participate in any other clinical trials within four weeks prior to the administration.

In this human pharmacokinetics study, the subjects were assigned into three dosage groups with single bolus intravenous injection – 0.2, 0.4 and 0.6 mg/kg, respectively. The study was conducted from low dosage to high dosage. A total of 31 subjects were enrolled, of them 19 are males and 12 females. All the 31 subjects completed the study with no subject being withdrawn during the study. Out of the 31 subjects, 27 received test drug and 4 subjects received placebo. A series of time points were chosen to collect the blood samples of the subjects, including before bolus, 5 and 10 min after the initiation of bolus (immediately at the end of bolus), 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 45, 60 min and 2, 3, 4, 6, 8, 10, 12 h after the accomplishment of bolus. Each time for each subject, 3 mL of blood sample was collected. The blood sample was centrifuged for 10 min at 4 °C at the speed of 3000 rpm within 30 min of collection, and subsequently the plasma was divided into 3 parts and transferred to centrifuge tubes, labeled, stored in freezers at −70 °C

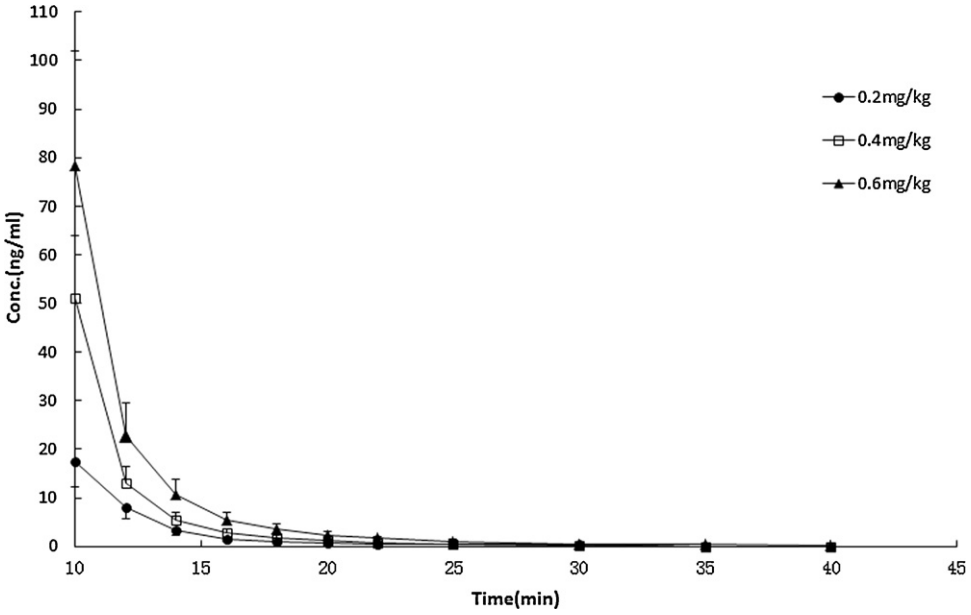

Fig. 3. Mean values of PPTA blood drug concentration–time curve after the subjects received single intravenous injection of 0.2, 0.4 and 0.6 mg/kg of test drug.

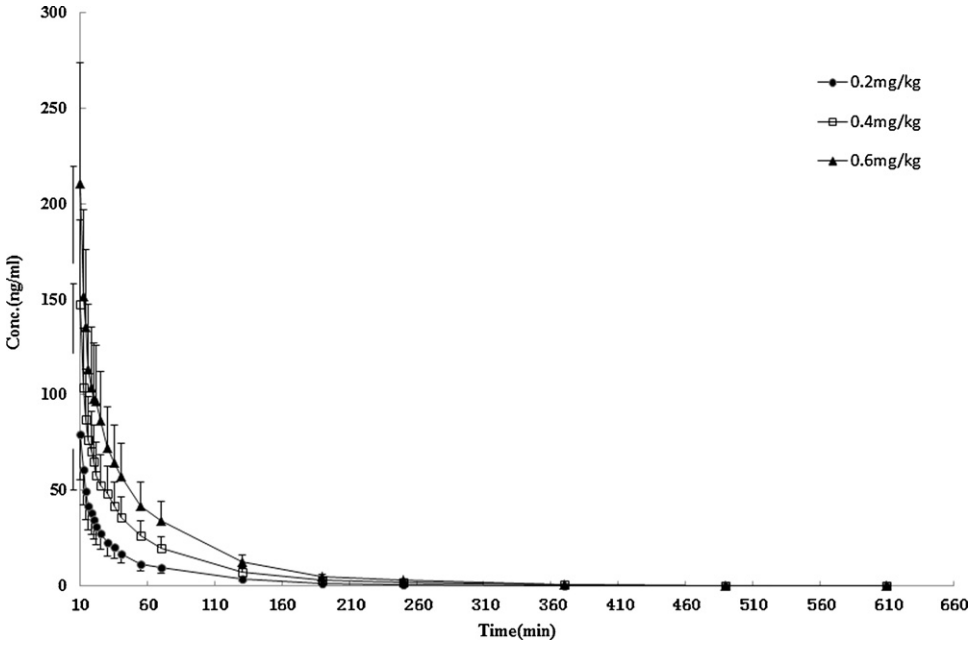

Fig. 4. Mean values of M1 blood drug concentration–time curve after the subjects received single intravenous injection of 0.2, 0.4 and 0.6 mg/kg of test drug.

for later analysis. For urine sample collection: blank urine samples were collected at 0–1, 1–2, 2–4, 4–6, 6–8, 8–12 and 12–24 h after the initiation of bolus. The urine samples were collected at each time point, mixed well, and the volume was accurately measured. 10 mL of each sample was retained in a test tube, divided into 3 parts, sealed immediately and stored in a refrigerator at −70 °C for later analysis.

3. Results and discussion

3.1. Methodological results

3.1.1. Calibration curve and lower limit of quantitation

In this method, the linear ranges for measuring both PPTA and M1 in plasma samples were 0.1–500 ng/mL, and LLOQ for both were 0.1 ng/mL (S/N > 10); the linear ranges for measuring both PPTA and M1 in urine samples were 0.1–200 ng/mL, and LLOQ for both were 0.1 ng/mL (S/N > 10). $1/C^2$ was selected as the weight coefficient for calibration curves of both PPTA and M1. The linear range of M6 in plasma samples was 0.2–500 ng/mL, and LLOQ was 0.2 ng/mL (S/N > 10); in urine samples, the linear range of M6 was 0.2–200 ng/mL, and LLOQ was 0.2 ng/mL (S/N > 10); similar to the above two calibration curves, $1/C^2$ was selected as the weight coefficient for M6 calibration curve preparation. The method exhibited a good linear response over the selected concentration range using linear regression analysis. The results of six representative calibration curves of PPTA, M1 and M6 in plasma or urine samples are listed in Table 2.

3.1.2. Extraction recovery rates and matrix effects

The extraction recovery rates of PPTA, M1 and M6 in plasma were (103.74 ± 2.80)%, (105.16 ± 3.36)% and (101.20 ± 2.45)%, respectively; the extraction recovery rates of PPTA, M1 and M6 in the urine sample were (92.85 ± 5.07)%, (90.09 ± 5.75)% and (96.22 ± 7.19)%, respectively; the extraction recovery rates of internal standard in plasma and urine samples were (101.20 ± 2.45)% and (96.22 ± 7.19)%, respectively. The matrix effect of PPTA was 102.21–114.65% for plasma samples and 96.78–102.15% for urine samples. The matrix effect of M1 was 103.03–114.91% for plasma samples and 96.42–104.73% for urine samples. The matrix effect of M6 was 101.34–116.55% for plasma samples and 92.64–104.70% for urine samples. The results suggested that the recovery of biological samples of each compound was stable, and matrix effect was not observed in this method, which met the requirements of determining biological sample concentration.

3.1.3. Precision and accuracy

The RSD% values for intra-day precision of PPTA in plasma was ≤6.20%, whereas the corresponding inter-day precision was ≤9.76%, the intra-day precision of M1 in plasma was ≤8.90%, and inter-day precision was ≤9.23%. The intra-day precision of M6 in plasma was ≤3.96%, and inter-day precision was ≤7.34%. While the relative recoveries for PPTA, M1 and M6 in the plasma samples were 92.00–111.44%, 86.66–109.36% and 92.04–106.13%, respectively.

The RSD% values for intra-day precision of PPTA in urine sample was ≤10.61%, whereas the corresponding inter-day precision was ≤10.61%, the intra-day precision of M1 in plasma was ≤5.63%, and inter-day precision was ≤8.54%. The intra-day precision of M6 in plasma was ≤8.13%, and inter-day precision was ≤5.34%. While the relative recoveries for PPTA, M1 and M6 in the plasma samples were 91.04–100.00%, 91.86–105.00% and 94.21–108.86%, respectively. They met the requirements for determination of biological sample concentration.

3.1.4. Stability

PPTA and M1 stock solutions were stable at 4 °C for one month. M6 stock solution was stable at 4 °C for two months. While PPTA, M1 and M6 blood samples were stable at room temperature for 1, 2 and 3 h, respectively; they were stable for a longer time at 4 °C, measured as2.5, 3.5 and 4 h, respectively. The urine samples containing PPTA, M1 and M6 were stable at room temperature for 3 h, and stable at 4 °C for 12 h. The plasma and urine samples containing PPTA and M1 were stable at −70 °C for two months, and the plasma sample of M6 was stable at −70 °C for two months. The urine sample of M6 was stable at −70 °C for one month. The plasma and urine samples of M6 were stable for at least 24 h at 4 °C after pretreatment. The plasma and urine samples of M1 can be stable for 12 h and 24 h, respectively, when preserved in a sample room at 4 °C after treatment. Finally, we found that the blood and urine

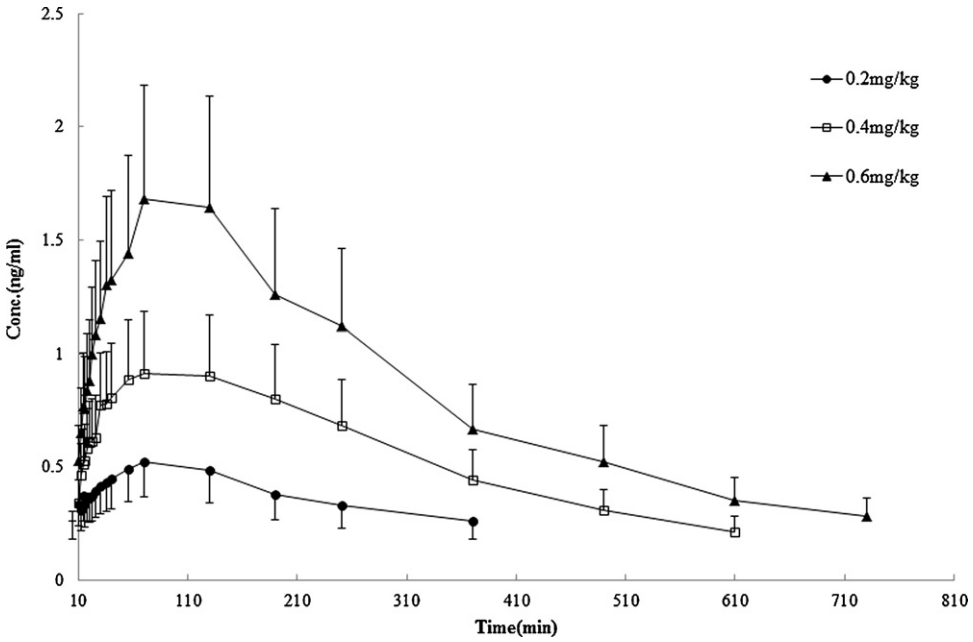

Fig. 5. Mean values of M6 blood drug concentration–time curve after the subjects received single intravenous injection of 0.2, 0.4 and 0.6 mg/kg of test drug.

samples for PPTA, M1 and M6 study were stable even after repeated frozen–thaw cycles for 3 times.

3.2. Results of the pharmacokinetics study

After single bolus intravenous injection of 0.2 and 0.4 mg/kg, PPTA in plasma of studied subjects was undetectable at 35 min; while after single bolus intravenous injection of 0.6 mg/kg, PPTA in plasma was also undetectable at 45 min. The drug levels in urine suggested that, the excretion of PPTA in urine was very low, within (0.20 ± 0.11)%. After single bolus intravenous injection of 0.2, 0.4 and 0.6 mg/kg of PPTA, the mean values of plasma drug concentrations of PPTA, M1 and M6 in subjects and its concentration–time curves were shown in Figs. 3–5.

The mean values of pharmacokinetic parameters of PPTA, M1 and M6 after the subjects received single bolus intravenous injection of 0.2, 0.4 and 0.6 mg/kg of PPTA injection were shown in Table 3.

3.3. Discussion

In the current study, we utilized the HPLC/MS/MS analytic method with high specificity and sensitivity in quantitative analyses of unchanged PPTA and its main metabolites M1 and M6 in the biological samples. We also successfully applied this method to the in vivo pharmacokinetic study of single-dose intravenous injection of PPTA in human subjects.

This study was the first clinical study on human subjects for a novel drug. The results from the pharmacokinetic trial of single-dose intravenous injection in humans showed that: after single-dose intravenous injection of the drug, the pharmacokinetics of PPTA and its metabolite M1 within the used dosage range (0.2, 0.4, 0.6 mg/kg) was in accordance with linear pharmacokinetic characteristics, with the C_{max} of PPTA measured as (17.04 ± 11.67), (47.40 ± 22.10) and (82.83 ± 59.97) ng/mL, respectively; $t_{1/2}$ of PPTA was measured as (4.30 ± 1.08), (5.08 ± 1.76) and (5.27 ± 1.11) min, respectively. These results indicated that the unchanged PPTA was eliminated rapidly from plasma,

Table 3
Pharmacokinetics parameters of PPTA, M1 and M6.

Compound	Parameters	Unit	0.2 mg/kg	0.4 mg/kg	0.6 mg/kg
PPTA	C_{max}	ng/mL	26.94 ± 16.66	59.07 ± 28.91	93.18 ± 63.09
	$t_{1/2}$	min	4.31 ± 1.15	4.64 ± 1.32	5.59 ± 1.12
	CL	L/kg/min	1.32 ± 0.84	1.05 ± 0.61	1.00 ± 0.52
	V	L/kg	8.95 ± 7.51	6.93 ± 4.15	8.20 ± 4.84
	AUC_{0-t}	ng/mL min	209.96 ± 122.96	485.78 ± 217.52	767.36 ± 385.64
M1	C_{max}	ng/mL	91.46 ± 30.08	150.60 ± 44.28	227.57 ± 89.87
	$t_{1/2}$	min	49.80 ± 5.90	59.32 ± 9.55	67.49 ± 12.72
	CL	L/kg/min	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
	V	L/kg	5.74 ± 0.85	7.00 ± 2.01	7.57 ± 1.88
	AUC_{0-t}	ng/mL min	2539.39 ± 493.90	5037.74 ± 979.99	7937.79 ± 1702.53
M6	C_{max}	ng/mL	0.57 ± 0.09	0.98 ± 0.27	1.73 ± 0.44
	$t_{1/2}$	min	305.62 ± 144.02	219.96 ± 58.42	215.88 ± 35.62
	CL	L/kg/min	0.88 ± 0.32	1.06 ± 0.30	0.92 ± 0.20
	V	L/kg	344.75 ± 69.10	335.70 ± 129.66	288.08 ± 72.16
	AUC_{0-t}	ng/mL min	136.80 ± 37.95	314.66 ± 106.80	597.50 ± 150.48
Accumulative urine excretion rate within 24 h of PPTA (%)			0.14 ± 0.07	0.10 ± 0.05	0.20 ± 0.11
Accumulative urine excretion rate within 24 h of PPTA and M1, M6 (%)			0.34 ± 0.25	0.32 ± 0.31	0.50 ± 0.31

and became undetectable 40 min after administration. C_{max} of M1 was measured as (78.69 ± 27.86), (148.66 ± 43.70) and (221.15 ± 89.22) ng/mL, respectively, and its $t_{1/2}$ was recorded as (50.04 ± 5.38), (57.56 ± 9.53) and (60.09 ± 16.06) min, respectively. M1 was still detectable in the plasma 10 h after administration. Although the blood drug concentration of M6 was low in the subjects after intravenous injection of all three concentrations of PPTA (0.2, 0.4 and 0.6 mg/kg), it was stable and still detectable 20 h after administration. In details, its C_{max} was recorded as (0.61 ± 0.14), (0.96 ± 0.27) and (1.83 ± 0.48) ng/mL, respectively, and its $t_{1/2}$ was measured as (271.38 ± 145.26), (198.49 ± 35.61) and (212.82 ± 37.51) min, respectively.

The measured drug concentrations in the urine samples of human subjects showed that, in the dosage group of 0.6 mg/kg, original form of PPTA was undetectable 6 h after administration, indicating that PPTA was a metabolic type compound that can be rapidly transformed to various metabolites in vivo. Further, within 24 h after single-dose intravenous injection of 0.2, 0.4 and 0.6 mg/kg drug, the accumulative urine excretion rates were calculated to be (0.14 ± 0.06)%, (0.10 ± 0.05)% and (0.20 ± 0.11)%, respectively. M1 level was undetectable at 8 h after administration, whereas M6 was still detectable at 24 h after administration, though with a low concentration. Further studies are needed to determine whether M1 and M6 are the ultimate essential metabolites of PPTA.

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