BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **28**: 177–186 (2007)
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/bdd.543

Liquid Chromatographic–Mass Spectrometry Analysis and Pharmacokinetic Studies of a Novel Rabeprazole Formulation, Sterile Powder for Injection, in Dogs and Rats

Feng Shao, Jianguo Sun, Guangji Wang*, Haitang Xie, Xiaoyan Zhu and Jingwei Zhang
Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, 210009, Jiangsu Province, China

ABSTRACT: Rabeprazole is among the most potent proton pump inhibitors (PPI) identified to date and it has been demonstrated that it is effective in such diseases as gastroesophageal reflux disease (GERD), duodenal ulcer and gastric ulcer. There is currently interest in developing a new formulation: rabeprazole sterile powder for injection (RSPI). This investigation was conducted to evaluate the preclinical pharmacokinetics of RSPI in rats and at the same time a comparative study was carried out in dogs between RSPI and Pariet® tablets using liquid chromatographic–mass spectrometry analysis.

The liquid chromatographic–mass spectrometry method was first conducted and validated as being specific, and having accuracy, precision, sensitivity and a satisfactory recovery. After intravenous administration of RSPI (i.v.: 2, 6 and 18 mg/kg) to rats, no significant dose-dependency was found in the *CL* (4.20–5.72 l/h/kg), V^{d}_{area} (0.94–1.32 l/kg), dose-normalized *AUC* (197.20–245.82 μg/l*h based on 1 mg/kg) and $t_{1/2}$ ($p > 0.05$). In the dog, a randomized, open-label, crossover experiment was carried out to show that the mean area under the plasma concentration-time curve ($AUC_{0-\infty}$) after i.v. administration of RSPI was at least four times larger than that following oral administration of Pariet® tablet at an equivalent dose but the elimination half-life of these two formulation was similar ($p > 0.05$). The results showed that the pharmacokinetics of RSPI was linear ($r^2 = 0.98$) in the dose range 2–18 mg/kg and the RSPI had a much higher $AUC_{0-\infty}$ and similar $t_{1/2}$ values compared with the enteric-coated tablet. Copyright © 2007 John Wiley & Sons, Ltd.

Key words: rabeprazole; pharmacokinetics; sterile powder; tissue distribution; enteric-coated tablet; liquid chromatographic/mass spectrometry

Introduction

Rabeprazole [1–4], a new PPI (proton pump inhibitor), is a substituted benzimidazole with both antisecretory and gastroprotective properties. Rabeprazole is a protonatable weak base with a pKa (negative logarithm of the acid-ionization) value of 5 [5–6], so it accumulates selectively in acidic spaces at pH < 5, which are found primarily in the secretory canaliculus of the gastric parietal cell. In an acidic environment, protonation of the pyridine and benzimidazole nitrogens results in the formation of a tetracyclic sulfonamide, which represents the active form of the drug. Because of the higher pKa than other PPIs with values of ~4, rabeprazole has a faster onset of inhibitory action on H^+/K^+-ATPase and acid secretion in comparison with other PPIs such as omeprazole, lansoprazole or pantoprazole [7]. It has a two-fold to ten-fold greater antisecretory activity *in vitro* than the progenitor

*Correspondence to: Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, 210009, Jiangsu Province, China. E-mail: guangjiwang@hotmail.com

Received 14 May 2006
Revised 2 December 2006
Accepted 12 December 2006

PPI, omeprazole [8]. The results of clinical trials have demonstrated that rabeprazole is as effective as omeprazole in promoting ulcer healing and in alleviating gastroesophageal reflux disease (GERD) symptoms [9–10] and that it may provide superior pain relief [11].

Rabeprazole has little interaction with other drugs compared with omeprazole, lansoprazole and pantoprazole [12–15]. Rabeprazole enteric-coated tablets have been used successfully to treat many acid-related diseases such as GERD, duodenal ulcer and gastric ulcer. Rabeprazole is acid-labile, and is therefore administered orally as an enteric-coated (gastro-resistant) tablet formulation. However, it is difficult for infants and severely ill patients to swallow tablets, and injections are an effective administration route and not subject to the influence of food and secretion from the gastrointestinal tract, besides its rapid effect and absorption. Thus rabeprazole sterile powder for injection (RSPI) was developed. Rabeprazole tablets [16–18] exhibited first-pass metabolism following oral administration and PPIs exhibit polymorphism in metabolism and there were great differences between individuals. RSPI was dissolved in 0.9% saline solution for injection and directly injected to blood circulation, escaped first-pass metabolism and rapidly reached higher plasma concentrations, which was beneficial for binding the proton pump and so inhibiting acid secretion.

RSPI is a different dosage form and administration route compared with Pariet® tablets, which have been successfully used in the clinic for many years. It was necessary to study the pharmacokinetics of rabeprazole in RSPI in animals in order to establish its safety in humans. Thus the pharmacokinetic profile of rabeprazole in RSPI was systematically investigated and a randomized, open-label, crossover experiment was carried out in dogs in order to compare the tested (RSPI) and the control (Pariet® tablets) preparation.

Materials and Methods

Materials and equipment

Rabeprazole (MW = 359) and omeprazole (internal standard, IS., MW = 345) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Rabeprazole sterile powder for injection was provided by Xi'an Xintong Drug Research & Development Ltd. Pariet® 10 mg tablets were bought from Janssen Pharmaceutical (Pty) Ltd. HPLC grade methanol was obtained from Fisher Scientific (Toronto, Canada). Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA). Ammonia water and other chemicals and solvents used were analytical grade.

A Shimadzu 2010A liquid chromatographic–mass spectrometry system (Qarray-Octapole-Quadrupole mass analyser) with ESI interface, and Shimadzu LCMS solution workstation software (Ver 2.02) for data processing were utilized to perform all analytical procedures.

Animals

Male and female Sprague-Dawley rats (body weight, 210–240 g) and six beagle dogs (body weight, approximately 10 kg) were obtained from the Laboratory Animal Center of China Pharmaceutical University. The animals were maintained on a 12 h light/dark cycle (light at 8:00) at ambient temperature (22–24°C) and relative humidity of $50 \pm 10\%$. All animals in this experiment were acclimated for 1 week prior to experiment. The animals utilized in the fasting experiment were fasted overnight (approximately 16 h) prior to and 4 h following dosing. Drinking water from the local water supply was readily available *ad libitum*. Each group comprised six animals. The experimental protocol was approved by the Animal Care Committee of China Pharmaceutical University.

Experiments and sample preparation

Rabeprazole solutions for intravenous administration were prepared by dissolving RSPI in 0.9% saline solution for injection. Intravenous doses were administered via the forelimb vein in dogs or the caudal vein in rats. Rabeprazole tablets (Pariet®) were administered manually.

Pharmacokinetic studies. Sprague-Dawley rats were divided into three groups: high, middle and low dosage groups. There were six rats

DOI: 10.1002/bdd

(three males and three females) per group. Each rat was administered rabeprazole solution as an intravenous dose of 2, 6, 18 mg/kg. The total injection volume was 1.0 ml/kg. Blood samples (0.2 ml) were collected via the femoral vein into a polyethylene cannula at 0 (to serve as a control), 2, 4, 8, 12, 20, 30, 45, 60, 90, 120 and 150 min after intravenous administration. The blood volume was replaced with an equal volume of 0.9% saline solution. The blood was collected into a heparinized tube and immediately centrifuged at 8000 rpm for 10 min at 4°C. A 100 µl volume of plasma was finally obtained, and stored at −20°C until analysis.

During the experimental period each dog was placed in the upright position in the stand. A 10 mg dose sterile powder of rabeprazole was given intravenously or 20 mg dose tablet (2 tablets) was given orally in a randomized, open-label, crossover study with a week washout period between doses. The legs were shaven and an opposite forelimb vein was cannulated using an 18-gauge cannula. Blood (0.2 ml) was collected via the cannula. The times of blood sampling were at 0 (to serve as a control) 0.03, 0.08, 0.17, 0.33, 0.5, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 h and 1.0, 1.5, 2, 2.17, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 6.00 h for RSPI and control (Pariet® tablet) preparation, respectively. The blood was placed in heparinized tube and immediately centrifuged at 8000 rpm for 10 min at 4°C. A 100 µl volume of plasma was finally obtained, and frozen at −20°C until analysis.

Tissue distribution. Twenty four rats were divided into four groups (six rats per group) and killed by exsanguination from the abdominal aorta under isoflurane anaesthesia at 0, 4, 20 and 60 min after i.v. administration of RSPI at a dose of 6 mg/kg weight. Selected tissues and plasma were removed, rinsed with ice-cold phosphate-buffered saline (PBS), blotted and then stored in polypropylene tubes at −20°C until analysis.

Sample preparation. The rabeprazole concentrations in plasma and tissue were determined by liquid chromatographic–mass spectrometry. The tissue was weighed, cut with scissors and homogenized in 1 ml phosphate-buffered saline (PBS, pH 7.4). Plasma or tissue homogenate (0.1 ml) and the internal standard solution 10 µl (1 µg/ml) were added to a test tube and mixed. Rabeprazole and omeprazole were extracted with 1 ml of a mixture of ethyl acetate-isopropanol (99:1, v/v). The samples were vortex-mixed for 3 min, and centrifuged at 8000 rpm for 5 min. The organic layer (0.8 ml) was transferred to another tube and evaporated to dryness in a Speed Vac System (Thermo Savant SPD 2010, Thermo Electron Corporation, USA). The residue was reconstituted in 0.1 ml of methanol, and centrifuged at 20000 rpm and 4°C for 10 min. The supernatant (10 µl) was injected into the liquid chromatographic–mass spectrometry system using an autosampler.

Liquid chromatographic–mass spectrometry analysis

The chromatographic separation was carried out on a Zorbax Extend-C_{18} analytical column (2 mm × 50 mm i.d, 5 µm, Agilent Technologies, USA). The mobile phase consisted of methanol (B) and water containing 0.1% ammonia water (A) under a gradient elution program. The operating conditions were programmed with a 3.8 min gradient from 35% B to 80% B followed by reducing to 35% in 4.2 min and maintaining at 35% for 3 min; the mobile phase was delivered at 0.2 ml/min directly into the ESI source; the column was maintained at 40°C. The optimized MS parameters were selected as followed: the curve dissolution line (CDL) temperature of 250°C and block temperature of 200°C, detector voltage of 1.65 kV and a probe voltage of 4.5 kV. Liquid nitrogen (99.995%, from Gas Supplier Center of Nanjing University, China) was used as the nebulizer gas and curtain gas source at 1.5 l/min and 2.0 l/min, respectively. Mass spectra were obtained at a dwell time of 0.2 s in SIM mode and 1 s in scan mode. The analytes were assayed by quantifying the $[M + H]^+$ ion of rabeprazole at m/z 360, and IS at m/z 346.

Method validation

Specificity was ascertained by analysing drug-free plasma or tissue homogenates without adding internal standard to determine the interference with the quantification of analytes. Five sets of calibration curves ranging from 1 to

DOI: 10.1002/bdd

2000 ng/ml or 5 to 250 ng/ml for rabeprazole were constructed by plotting the peak-area ratios of target/internal standard versus concentrations in different matrices (plasma and tissues), respectively, on a single day. The assay precision was determined by intra-day and inter-day relative standard deviation (RSD) at three concentrations (2.0, 500.0, 1000.0 ng/ml). The accuracy was determined by comparing the calculated concentration (obtained from the calibration curve) to the theoretical concentration of each sample and expressed as the percent of the nominal value. The extraction recovery of the rabeprazole and IS was determined by comparing the peak areas of the spiked plasma samples to the peak areas of the standard solution at the same concentration not carried through the extraction procedure.

The stability was assessed at three concentration levels (2.0, 500.0, 1000.0 ng/ml). The freeze and thaw stability study samples at three concentrations were stored at -20°C and subjected to three freeze-thaw (37°C) cycles. The short-term stability of rabeprazole during storage in the autosampler at 4°C, was performed by repeated injection every 4 h for a period of 24 h. The long-term stability of rabeprazole in plasma was assessed at three concentration levels after storage at -20°C for 4 weeks.

Pharmacokinetic analyses

Pharmacokinetic parameters were calculated by noncompartmental analyses [19]. The estimation of the elimination rate constant (k_{el}) was obtained by the log-linear least-squares regression method, using the terminal portion of the plasma concentration-time curve. The corresponding half-life ($t_{1/2}$) was calculated as $\ln 2/k_{el}$. The area under the concentration-time curves from time zero to infinity ($AUC_{0-\infty}$) was calculated by the linear trapezoidal method with extrapolation to infinity. The maximum concentration of drug in plasma (C_{max}) and the time to reach C_{max} (T_{max}) were determined directly from the observed plasma concentration vs time curves. The systemic clearance (CL) was obtained as the ratio of intravenous dose/$AUC_{0-\infty}$, and the volume of distribution (V_d) was estimated as CL/k_{el}. The results are expressed as the mean and the standard deviation.

Statistical analysis

The AUC, $t_{1/2}$ and CL derived from plasma concentrations of rabeprazole were assessed in rats and dogs. Pharmacokinetic variables in dogs were compared between the powder SPI and enteric-coated tablet using analysis of variance; the statistical comparison for the elimination variable was based on $t_{1/2}$, AUC and CL. Statistical analyses of the experimental data were performed using Student's t-test and one-way ANOVA (Statistical Product and Service Solution, SPSS 11.5).

Results

Method validation

Using the acquisition of negative SIM (selective-ion monitoring) mode, blank rat and dog plasma and tissues all yielded good resolution chromatograms without co-eluting interference peaks at the retention time of rabeprazole and IS. Typical chromatograms of the blank and spiked plasma are given in Figure 1 (chromatograms of rabeprazole in tissues are not shown). The representative peaks had the same m/z values as the standard samples. The retention times of rabeprazole and IS were about 3.7 and 3.3 min, respectively. Five sets of calibration curves were constructed in the range 1–2000 ng/ml for rabeprazole in different plasma and tissues, respectively. Non-weighted least-squares linear regression analysis was used. The mean regression equations and their correlation coefficients (r^2) for the curves were $y = 0.00537x + 0.0047$ ($r^2 = 0.9990$) for rat plasma; $y = 0.00531x - 0.007\,5$ ($r^2 = 0.9992$) for dog plasma; and for the tissues which were shown in Table 1. The lower limit of quantitation was 1 ng/ml (RSD < 20%) for rabeprazole and is sufficient to support the pharmacokinetic studies. Recovery, precision and accuracy data are shown in Table 2. Intra-day precision (RSD) ranged from 5.8% to 10.5%, and intra-day accuracy values ranged from 95.0% and 105.1%. The method showed reproducibility with

DOI: 10.1002/bdd

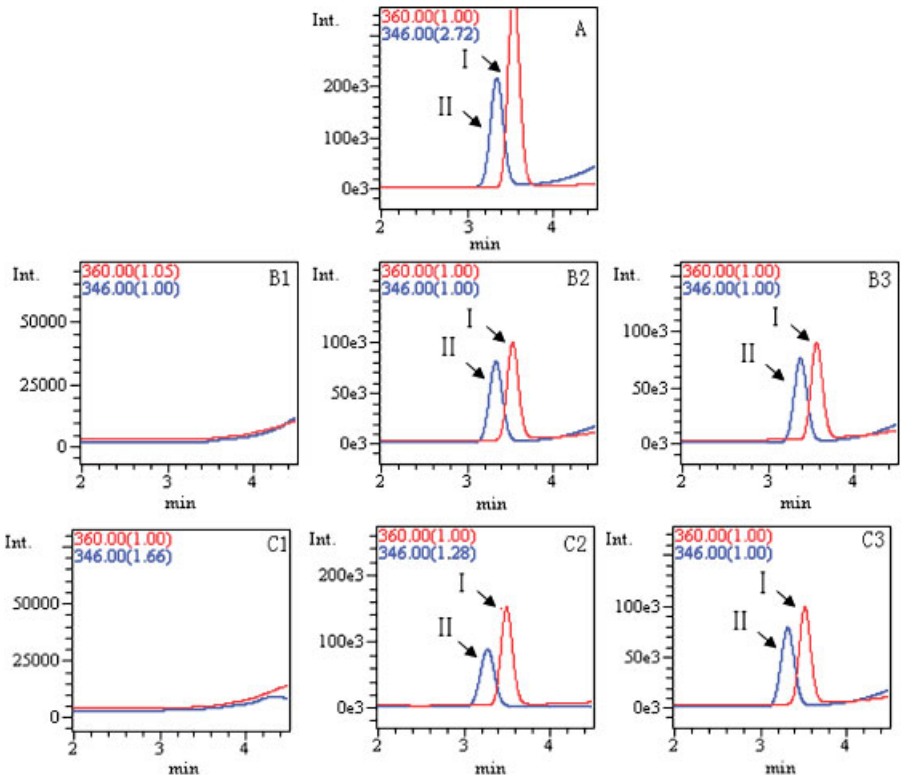

Figure 1. Representative SIM chromatograms of rabeprazole and omeprazole (internal standard, IS). (A) Rabeprazole (1000 ng/ml) and IS in methanol sample; (B) Rat plasma sample; (C) Dog plasma. 1: Blank plasma; 2: Plasma spiked with RA (500 ng/ml) and IS; 3: Plasma obtained from a rat or dog after i.v. injection of rabeprazole. I: sign of peak of rabeprazole (m/z 360); II: sign of peak of omeprazole (m/z 346). The numbers in the parentheses represent ratio of the displayed peak to the original peak

inter-day precision ranging from 6.0% to 10.0%. The inter-day accuracy ranged from 99.1% to 105.0%. These results indicated that the present method has a satisfactory accuracy, precision and reproducibility. The extraction recovery was more than 70%, indicating a satisfactory extraction efficiency had been achieved by using a mixture of ethyl acetate-isopropanol as the extraction solvent.

Table 3 displays the stability of rabeprazole under the following conditions: (1) stability of rabeprazole in rat and dog plasma through at least three freeze–thaw cycles, (2) stability of rabeprazole in rat and dog plasma for at least 24 h at 4°C in the autosampler, (3) stability of rabeprazole stored at −20°C for at least 4 weeks. As a result, rabeprazole showed a very good stability under the three conditions.

Pharmacokinetic phase

The plasma concentration-time profiles of rabeprazole following intravenous administration to rats and dogs are illustrated in Figures 2 and 3, respectively. A significant difference ($p < 0.05$) in elimination rates between the two animal species was shown following intravenous administration, with a mean terminal phase $t_{1/2}$s of 0.21 ± 0.11, 0.21 ± 0.02, 0.20 ± 0.01 h , respectively, at 2, 6, 18 mg/kg dosages in rats, and 0.51 ± 0.01 h in dogs (Table 4). The *CL* values in dogs were 4.97 ± 0.51 l/h/kg and the *CL* values in rats, were 5.52 ± 1.62, 4.20 ± 0.70, and 5.72 ± 1.31 l/h/kg at doses of 2, 6, 18 mg/kg, respectively. The *CL* values in dogs and rats were similar ($p > 0.05$), but it was also shown that the rate of rabeprazole elimination was slower in

DOI: 10.1002/bdd

Table 1. Calibration curves of rabeprazole in various tissues, $n = 5$

Tissue	Spiked concentration (mean ± SD ng/ml)						Calibration curve[a]		
	5	10	25	50	100	250	*a*	*b*	r^2
Brain	0.0271 ± 0.0052	0.0878 ± 0.0087	0.2014 ± 0.0181	0.3493 ± 0.0327	0.6936 ± 0.0976	1.6378 ± 0.2157	0.023	0.0065	0.9991
Duodenum	0.0203 ± 0.0026	0.0603 ± 0.0062	0.1502 ± 0.0173	0.3055 ± 0.0279	0.6331 ± 0.0765	1.4927 ± 0.2194	0.0035	0.006	0.9992
Kidney	0.0294 ± 0.0024	0.0551 ± 0.0074	0.1667 ± 0.0156	0.3504 ± 0.0426	0.6824 ± 0.0988	1.8166 ± 0.2325	−0.0185	0.0073	0.9995
Liver	0.0573 ± 0.0056	0.1032 ± 0.0094	0.2780 ± 0.0322	0.5133 ± 0.0457	0.9061 ± 0.1077	2.2106 ± 0.3300	0.0392	0.0087	0.999
Lung	0.0269 ± 0.0033	0.1215 ± 0.0123	0.2599 ± 0.0278	0.4958 ± 0.0633	1.006 ± 0.1253	2.3911 ± 0.2578	0.016	0.009	0.9993
Muscle	0.0485 ± 0.0064	0.1044 ± 0.0134	0.2721 ± 0.0322	0.5785 ± 0.0746	1.1434 ± 0.1415	2.7453 ± 0.3165	0.0077	0.011	0.9996
Spleen	0.0291 ± 0.0052	0.0599 ± 0.0057	0.2626 ± 0.0245	0.6044 ± 0.0673	1.1578 ± 0.1637	3.2022 ± 0.3218	−0.0652	0.013	0.999
Stomach	0.0396 ± 0.0048	0.1405 ± 0.0188	0.2267 ± 0.0231	0.4522 ± 0.0562	0.9816 ± 0.1208	2.3689 ± 0.3253	0.0075	0.0095	0.9991
Ovary	0.0544 ± 0.0074	0.1093 ± 0.0097	0.2630 ± 0.0321	0.5191 ± 0.0645	0.9558 ± 0.0988	2.3597 ± 0.2245	0.024	0.0094	0.9997
Testis	0.0724 ± 0.0096	0.1747 ± 0.0156	0.4066 ± 0.0435	0.7304 ± 0.0876	1.5086 ± 0.1989	3.5596 ± 0.4346	0.0358	0.0142	0.9994
Heart	0.0153 ± 0.0020	0.0582 ± 0.0068	0.1538 ± 0.0203	0.3116 ± 0.0346	0.6575 ± 0.08526	1.6142 ± 0.2042	−0.0093	0.0065	0.9998

[a]Calibration curve: $y = a + bx$, where x was rabeprazole concentration (ng/ml) and y was rabeprazole peak area/IS peak area.

Table 2. Recovery, precision and accuracy of liquid chromatographic/mass spectrometry method for analysing rabeprazole in different matrices, $n = 5$

Matrix	Spiked concentration (ng/ml)	Recovery		Intra-day			Inter-day		
		Mean ± SD (%)	RSD (%)	Concentration found (mean ± SD) (ng/ml)	Precision[a] (%)	Accuracy[b] (%)	Concentration found (mean ± SD) (ng/ml)	Precision[a] (%)	Accuracy[b] (%)
Rat plasma	2	74.4 ± 3.2	4.3	1.9 ± 0.2	10.5	95.0	2.1 ± 0.2	9.5	105.0
	500	75.2 ± 4.3	5.7	498.4 ± 38.6	7.7	99.7	511.4 ± 30.6	6.0	102.3
	1000	73.5 ± 6.7	9.1	1025.5 ± 65.8	6.4	102.6	1011.5 ± 65.7	6.5	101.2
Dog plasma	2	73.4 ± 3.2	4.4	2.2 ± 0.2	9.1	110.0	2.0 ± 0.2	10.0	100.0
	500	72.5 ± 5.3	7.3	525.4 ± 30.6	5.8	105.1	495.4 ± 25.6	5.2	99.1
	1000	70.2 ± 6.3	9.0	1009.5 ± 60.8	6.0	101.0	1001.5 ± 70.6	7.0	100.2

[a]Expressed as % RSD: (SD/mean) × 100.
[b]Calculated as (mean concentration found/nominal concentration) × 100%.

DOI: 10.1002/bdd

Table 3. Stability of rabeprazole in different matrices under different stored conditions, n=5

Matrix	Spiked concentration (ng/ml)	At 4°C 24 h		At −20°C 4 weeks		Freeze–thaw	
		Concentration found (mean ± SD ng/ml)	Remaining percentage[a] (%)	Concentration found (mean ± SD ng/ml)	Remaining percentage[a] (%)	Concentration found (mean ± SD ng/ml)	Remaining percentage[a] (%)
Rat plasma	2	1.8 ± 0.2	90.0	2.2 ± 0.2	110.0	2.1 ± 0.10	105.0
	500	485.9 ± 20.5	97.4	535.3 ± 24.7	107.1	523.6 ± 28.9	104.7
	1000	1050.5 ± 53.9	105.1	998.6 ± 70.6	99.9	985.5 ± 60.1	98.6
Dog plasma	2	2.1 ± 0.3	105.0	1.9 ± 0.3	95.0	2.0 ± 0.2	100.0
	500	499.9 ± 24.5	100.0	485.7 ± 34.7	97.1	497.8 ± 39.8	99.6
	1000	1005.5 ± 58.9	100.6	984.6 ± 65.8	98.5	1028.5 ± 68.5	102.9

[a]Remaining percentage (%) = Con. found/Con. added × 100. n = 5.

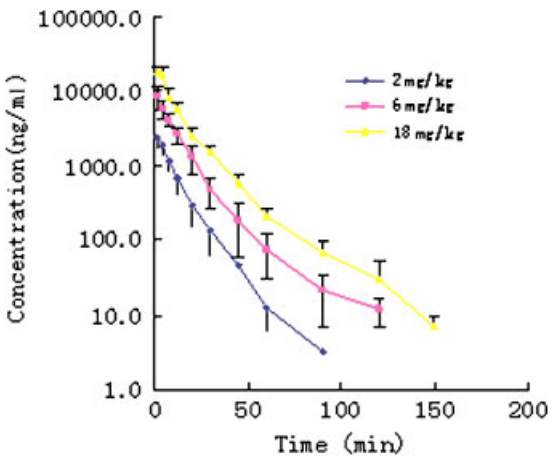

Figure 2. Mean plasma concentration–time profiles for rabeprazole as RSPI formulation in the rat after intravenous administration of 2 mg/kg, 6 mg/kg and 18 mg/kg (data are shown as mean ± SD)

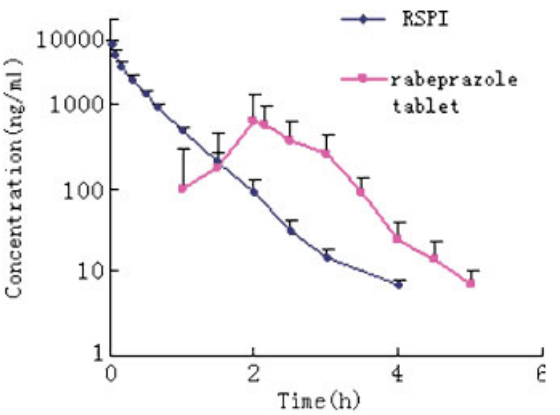

Figure 3. Mean plasma concentration–time profiles for rabeprazole as RSPI formulation and enteric-coated tablet formulation after administration to 6 beagle dogs (data are shown as mean ± SD)

dogs than in rats because there is about 7 l tissue fluid in a dog, much more than 0.1 l in a rat.

After oral dosing of two 10 mg rabeprazole tablets to dogs, plasma rabeprazole concentrations reached a maximum at 2.42 ± 0.51 h, with a mean C_{max} of 909.2 ± 521.3 ng/ml, an MRT of 2.52 ± 0.50 h, and $t_{1/2}$ of 0.51 ± 0.11 h, similar to the $t_{1/2}$ of 0.51 ± 0.01 h following intravenous administration of 10 mg/dog ($p > 0.05$). Comparison of the mean $AUC_{0-\infty}$/dose after intravenous dosing with that following oral administration indicated that the $AUC_{0-\infty}$/dose value after intravenous administration was at least four times larger than that after oral administration.

The mean pharmacokinetic parameters observed in male and female rats following intravenous administration of rabeprazole at three different dosages were not significantly different ($p > 0.05$) between the sexes (Table 4). Similarly, the profiles of the mean concentration of drug in plasma following intravenous administration of rabeprazole were not significantly different ($p > 0.05$) between male and female dogs.

DOI: 10.1002/bdd

Table 4. Pharmacokinetic parameters of rabeprazole following intravenous and oral administration in rats and dogs[a]

Species	Dose	Route[b]	Sex (no. of animals)[c]	T_{max} (h)	C_{max} (ng/ml)	$AUC^{d}_{(0-\infty)}$ (μg/l*h)	MRT^{d} (0-tn)(h)	CL^{d} (l/h/kg)	V^{d}_{area} (l/kg)	$T_{1/2}$ (h)
Rats	2 mg/kg	i.v.	M(3)	—	—	377.51 ± 48.10	0.12 ± 0.01	5.41 ± 0.70	1.30 ± 0.61	0.21 ± 0.11
			F(3)	—	—	411.32 ± 219.31	0.21 ± 0.01	5.70 ± 2.41	1.32 ± 0.80	0.21 ± 0.12
			M and F(6)	—	—	394.40 ± 143.22	0.20 ± 0.02	5.52 ± 1.62	1.31 ± 0.60	0.21 ± 0.11
	6 mg/kg	i.v.	M(3)	—	—	1516.40 ± 240.31	0.19 ± 0.11	4.01 ± 0.60	0.90 ± 0.21	0.22 ± 0.10
			F(3)	—	—	1433.41 ± 362.50	0.21 ± 0.01	4.40 ± 1.01	1.01 ± 0.22	0.22 ± 0.02
			M and F(6)	—	—	1474.90 ± 278.82	0.20 ± 0.02	4.20 ± 0.70	0.94 ± 0.22	0.21 ± 0.02
	18 mg/kg	i.v.	M(3)	—	—	3459.91 ± 721.11	0.32 ± 0.01	5.41 ± 1.32	1.32 ± 0.21	0.21 ± 0.01
			F(3)	—	—	3175.33 ± 967.40	0.20 ± 0.02	6.01 ± 1.60	1.31 ± 0.30	0.20 ± 0.01
			M and F(6)	—	—	3317.64 ± 778.91	0.22 ± 0.01	5.72 ± 1.31	1.32 ± 0.21	0.20 ± 0.01
Dogs	10 mg/dog	i.v.	M(3)	—	—	1951.01 ± 140.23	0.51 ± 0.2	5.13 ± 0.34	7.10 ± 0.90	0.51 ± 0.02
			F(3)	—	—	2066.40 ± 298.10	0.53 ± 0.02	4.84 ± 0.70	7.01 ± 1.01	0.51 ± 0.02
			M and F(6)	—	—	2008.71 ± 217.71	0.51 ± 0.02	4.97 ± 0.51	6.90 ± 0.80	0.52 ± 0.02
	20 mg/dog	p.o.	M(3)	2.73 ± 0.52	1124.82 ± 566.34	994.71 ± 473.01	2.82 ± 0.41	—	—	0.44 ± 0.21
			F(3)	2.02 ± 0.103	1214.64 ± 517.13	953.82 ± 578.21	2.24 ± 0.40	—	—	0.05 ± 0.02
			M and F(6)	2.39 ± 0.51	909.23 ± 521.30	974.34 ± 473.02	2.52 ± 0.50	—	—	0.51 ± 0.11

[a]Values represent mean ± standard deviations. [b]p.o., oral administration; i.v., intravenous administration.
[c]M, male; F, female. [d]*AUC*, area under the plasma concentration time curve; *MRT*, mean residence time; *CL*, clearance; V_{area}, the volume of distribution.

Table 5. Tissue concentration of rabeprazole in rats after i.v. administration of 6 mg/kg (mean ± SD, ng/ml or ng/g, $n = 6$)

Time (min)	Plasma	Brain	Duodenum	Kidney	Liver	Lung	Muscle	Spleen	Stomach	Ovary	Testis	Heart
4	5914.8 ± 1437.1	4.4 ± 3.4	8.3 ± 6.6	70.3 ± 6.6	ND	80.2 ± 7.9	31.2 ± 13.8	8.6 ± 1.1	10.1 ± 8.9	142.3 ± 22.7	162.4 ± 30.5	80.1 ± 1.8
20	1266.9 ± 675.6	ND	ND	11.0 ± 0.9	ND	ND	ND	5.6 ± 0.2	ND	25.8 ± 2.5	20.6 ± 6.9	9.97 ± 1.1
60	38.4 ± 10.3	ND	ND	ND	ND	ND	ND	5.7 ± 1.07	ND	ND	ND	ND

ND, not detected.

DOI: 10.1002/bdd

Tissue distribution

The results are summarized in Table 5. Following a single i.v. dose of 6 mg/kg of RSPI to male and female rats, rabeprazole was rapidly distributed to the major tissues except the liver. Whereas rabeprazole concentrations in these tissues were far lower than that in plasma, the highest concentration in the testis was about 36 times lower than the corresponding plasma concentration. Rabeprazole was rapidly eliminated in tissues; it was not detected in most tissues at 60 min post-dose.

Discussion

A reliable, accurate and precise analytical method should first be established for a pharmacokinetic investigation. The liquid chromatographic–mass spectrometry method for determining the rabeprazole in different matrices (rat and dog plasma, tissues) has been developed and validated as robust and sensitive to satisfy the need of pharmacokinetics in rats and dogs.

The pharmacokinetic phase investigation demonstrated that plasma concentrations of rabeprazole decreased rapidly with a short half-time, i.e. about 0.2 h for rats and 0.5 h for dogs following i.v. administration of rabeprazole sterile powder. In rats and dogs, similar pharmacokinetic parameters were obtained in male and female animals following both intravenous and oral administrations, suggesting no gender difference in pharmacokinetics for rabeprazole both as RSPI formulation and enteric-coated tablet formulation in the two species (Table 4). After a bolus intravenous administration of RSPI to the rat: the *AUC* of rabeprazole increased with increasing doses ($r^2 = 0.98$), indicating linear pharmacokinetics from 2 to 18 mg/kg; The noncompartmental parameters of half-time, clearance and *MRT*, as noted in Table 4, were similar ($p > 0.05$) and showed dose independence.

The randomized, open-label, crossover study in dogs demonstrated that there was no significantly difference in $t_{1/2}$ ($p > 0.05$) between the test (RSPI) and the control (Pariet® tablets) preparation. The mean area under the plasma concentration-time ($AUC_{0-\infty}$) after i.v. administration of rabeprazole was at least four times larger than that following oral tablets under equivalent dose, which ensured that rabeprazole after i.v. administration could bind much faster with the proton pump (H,K-ATP) and disturb the function of the proton pump, thereby resulting in a potent acid inhibition.

The tissue distribution experiment showed that there was a lower concentration of rabeprazole in tissues, which may be due to the 96% of plasma protein binding [20]. Rabeprazole was rapidly eliminated and could not be detected at 60 min post-dose in tissues except the spleen, which may be because the spleen is an important organ serving to store blood. It was interesting to find that rabeprazole was not detected in the liver. It was speculated that rabeprazole entering the liver was rapidly metabolized by the abundant metabolism enzymes because we had ever detected some perhaps metabolites of rabeprazole during sample liquid chromatographic-mass spectometry analysis. In addition, excretion experiments for RSPI were carried out, but rabeprazole was not detected in the urine, feces or bile (data not shown).

The safety of RSPI was always given consideration in clinics. In comparison with the rabeprazole enteric-coated tablets, there was a similar terminal elimination half-time for the sterile powder, which showed that there was no significant difference in the elimination properties of the two rabeprazole formulations—the sterile powder and enteric-coated tablet—in the disposition of rabeprazole in dog.

In conclusion, there was a linear relation in rats at three different dosages. In the dog pharmacokinetic study, there was a similar elimination property, but the *AUC* was over four-fold after i.v administration of RSPI than that following oral tablets after an equivalent dose. Therefore a dose adjustment should be considered in the clinical study of RSPI.

Acknowledgements

This study was supported by National '863' Project (No. 2003AA2Z347A, 2005AA2Z3C70), Jiangsu International Cooperation Fund (BZ2004042) Jiangsu Nature Science Fund (BK2005098). The authors are grateful to Profes-

DOI: 10.1002/bdd

sor Sun Fenzhi for reviewing the primary manuscript.

References

1. Kinoshita Y. Review article: treatment for gastro-esophageal reflux disease—lifestyle advice and medication. *Aliment Pharmacol Ther* 2004: **20**(Suppl 8): 19–23.
2. Robinson M. Review article: pH, healing and symptom relief with rabeprazole treatment in acid-related disorders. *Aliment Pharmacol Ther* 2004; **20**(Suppl 6): 30–37.
3. Robinson M. Review article: the pharmacodynamics and pharmacokinetics of proton pump inhibitors—overview and clinical implications. *Aliment Pharmacol Ther* 2004; **20**(Suppl 6): 1–10.
4. Robinson M, Horn J. Clinical pharmacology of proton pump inhibitors: what the practising physician needs to know. *Drugs* 2003; **63**: 2739–2754.
5. Sachs G. Proton pump inhibitors and acid-related diseases. *Pharmacotherapy* 1997; **17**: 22–37.
6. Sachs G, Shin JM, Bring C, *et al*. The pharmacology of the gastric acid pump: The H^+,K^+ ATPase. *Annu Rev Pharmacol Toxicol* 1995; **35**: 277–305.
7. Williams MP, Poumder RE. The pharmacology of rabeprazole. *Aliment Pharmacol Ther* 1999; **13**(Suppl 3): 3–10.
8. Prakash A, Faulds D. Rabeprazole. *Drugs* 1998; **55**: 261–267.
9. Lim PW, Goh KL. Review article: efficacy and safety of rabeprazole in treating gastroesophageal reflux disease. *J Gastroenterol Hepatol* 2004; **19**(Suppl 3): S61–S68.
10. Thjodleifsson B. Review of rabeprazole in the treatment of gastro-esophageal reflux disease. *Expert Opin Pharmacother* 2004; **5**: 137–149.
11 Dekkers CPM, Beker JA, Thjodlerfsson B, *et al*. Comparison of rabeprazole 20 mg vs. omeprazole 20 mg in the treatment of active gastric ulcer—a European multicentre study. *Aliment Pharmacol Ther* 1998; **12**: 789–795.
12. Humphries TJ, Merritt GJ. Review article: drug interactions with agents used to treat acid-related diseases. *Aliment Pharmacol Ther* 1999; **13**(Suppl 3): 18–26.
13. Furuta T, Shirai N, Sugimoto M. Influence of CYP2C19 pharmacogenetic polymorphism on proton pump inhibitor-based therapies. *Drug Metab Pharmacokinet* 2005; **20**: 153–167.
14. Horn J. Review article: relationship between the metabolism and efficacy of proton pump inhibitors—focus on rabeprazole. *Aliment Pharmacol Ther* 2004; **20**(Suppl 6): 11–19.
15. Klotz U, Schwab M, Treiber G. CYP2C19 polymorphism and proton pump inhibitors. *Basic Clin Pharmacol Toxicol* 2004; **95**: 2–8.
16. Setoyama T, Laurent A, Humphries T. Pharmacokinetics of rabeprazole following single intravenous and oral administration to healthy subjects. *Int J Clin Pharmacol Ther* 2005; **43**: 37–42.
17. Chen J, Jiang WM, Gao XL. Bioequivalence evaluation of two rabeprazole enteric coated formulations in healthy Chinese volunteers. *Eur J Drug Metab Pharmacokinet* 2004; **29**: 103–106.
18. Mondal U, Ganesan M, Pal TK. Bioequivalence study of rabeprazole sodium on healthy human volunteers. *J Indian Med Assoc* 2004; **102**: 26, 28, 30.
19. Wei SL. *Biopharmaceutics and Pharmacokinetics*. Beijing Medical University and Peking Union Medical College United Publishing Company: Beijing, 1997; 120.
20. Li J. Pariet. *Chin J New Drugs* 2001; **10**: 623–624.

DOI: 10.1002/bdd