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Original Article

Pharmacokinetic study on ginsenoside Rg_1 and Re in rats following intravenous and oral administration of "Shenmai" injection

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Abstract: Rational: Shenmai injection is derived from traditional Chinese medicine Shenmai San (pulse-activating powder) with main active ingredient extracted from Radix ginseng Rubra and Radix Ophiopogonis. Ginsenoside Rg_1 and Re are two major constituents of Radix ginseng Rubra considered responsible for the efficacy of this injection. In the present study, we carried out pharmacokinetics studies on Shenmai injection of ginsenoside Rg_1 and Re in rats with oral and intravenous administration to establish bio-analysis methods of Shenmai injection. Methods: The plasma concentration after giving Shenmai injection in two different routes at different time points was analyzed by HPLC method, and pharmacokinetic parameters calculated with pharmacological software DAS3.0 to estimate the oral bioavailability of two components. Results: Ginsenoside Rg_1 and Re metabolize faster upon intravenous administration, and in the plasma concentration was untraceable after 6 hours after injection, compared with a much slower absorption when administrated orally, in which the plasma concentration peaked at 8 hours after medication. The bioavailabilities of ginsenoside Rg_1 and Re given orally were 7.22% and 7.16%, respectively. Conclusion: The method is specific, simple, sensitive and suitable for the measurement of plasma Rg_1 and Re concentrations. Shenmai injection was absorbed slowly by oral administrating with low bioavailability, while intravenous administration manifests superiority of fast onset.

Keywords: Shenmai injection, ginsenoside Rg_1, ginsenoside Re, pharmacokinetics, bioavailability

Introduction

Traditional Chinese medicine (TCM) included different formulations, and sterile injections of TCM is prepared with modern pharmaceutics techniques that extract effective chemical compounds from TCM and can be directly injected to human body. Intravenous injection provides rapid absorption and rapid onset of efficiency, however, the complex chemical composition makes identification of active compound difficult and potential of adverse event, such as anaphylaxis, when used directly as western medicine [1].

Shengmai San is a widely used formula in modern China, often given as a prepared liquid, a decoction, or as an intravenous drip. The formula is administered to patients who have suffered a serious illness, especially heart attack, congestive heart failure, or severe bronchitis, and to treat a sudden drop in blood pressure associated with cardiogenic or septic shock. Shenmai injection (SMI) is derived from Shenmai San with the main ingredients made from Radix ginseng Rubra and Radix Ophiopogonis. Ginsenoside-Rg_1, as a major constituent of Radix ginseng Rubra, is considered responsible for the efficacy of Shenmai injection, including benefiting vital energy, nourishing Yin and generating body fluid and pulse-activating [2-4].

Ginsenoside Rg_1, ginsenoside Re and ginsenoside Rb_1 are considered as the main active ingredient of SMI [5]. Chromatographic fingerprint analysis has been reported to determine the identity, stability and consistency of Shenmai injection for quality control [6, 7], and biological metabolism of ginsenoside Rg_1, Re, Rd, Rb_1 in rats and rabbits [8-12], as well as the pharmacokinetic study of ginsenoside Rg_1 in human [4]. However, to the best of our knowl-

edge, we have not seen any comparative pharmacokinetic study of ginsenoside Rg_1 and Re in rats following intravenous and oral administration of SMI yet. The aim of this study was to understand the bioavailability of Shenmai injection by comparing the pharmacokinetic parameters of ginsenoside Rg_1 and Re in rats following intravenous and oral administration, which hopeful will provide basis for further pharmacokinetic studies in human and further reference for clinical rational use of this injection.

Methods and materials

Chemicals and reagents

Standard compounds ginsenoside Rg_1 (Lot: 110703-200726, purity > 98.0%), and Re (Lot: 110754-200822, purity > 98.0%) were all purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Shenmai injection was supplied by a Chinese pharmaceutical company (Qingchunbao, Hangzhou, China, Lot: 1001-222). Acetonitrile and methanol were of HPLC grade and both were purchased from Merck (Darmstadt, Germany). Water used throughout the experiments was generated by a Milli-Q academic water purification system (Milford, MA, USA). The experiment animals were supplied by the Animal Center of Zhengzhou University (Zhengzhou, China).

Equipments and chromatographic conditions

HPLC analyses were performed using a WATERS (model Aliance e2695) High Performance Liquid chromatograph (HPLC) system. HPLC separation was achieved using an Agilent Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm particle size; Agilent Technologies, Palo Alto, CA, USA). The mobile phase flow rate was set as 1.0 mL/min. The mobile phase consisted (A) water and (B) acetonitrile (81.5:18.5, v/v). The column temperature was maintained at 30°C, the detection wavelength was set at 203 nm, and the injection volume at 20 μL. Determination was performed using a Waters 2996PDA detector (Waters, Milford, USA). Prior to the analytical column, a C18 guard column (Agilent Technologies, Palo Alto, CA, USA) was placed to prevent column degradation. 4 kinds organic solvents including methanol, methanol-acetonitrile (3:1), n-butanol and acetone, were tested as extraction solvent to select a proper solvent with high recovery for target analytes. Methanol-Acetonitrile (3:1) was applied due to its higher extraction efficiency for all tested saponins.

Preparation of standard solutions and quality samples

Accurately weighed solid portions of standards were dissolved in methanol to prepare stock solutions separately: 23.60 mg/L for ginsenoside Rg_1 and 10.50 mg/L for ginsenoside Re. Then, each stock solution was diluted step by step with methanol.

200 μL of work solution was transferred into a 1.5 mL plastic test tube together with 200 μL of plasma and the mixture was vortex 30 s, followed with adding 800 μL methanol-acetonitrile (3:1) mixture solution. The analytes were extracted from plasma by vortex-mixing for 1 min. Then the sample was centrifuged at 15,000 rpm for 10 min and 600 μL of the supernatant was transferred into another test tube and evaporate to dryness within N_2 current at 40°C. Finally, the residue was reconstituted with 100 μL mobile phase by vortex-mixing for 1 min and centrifuged at 16,000 rpm for 10 min; 20 μL supernatant was injected into chromatographic system for analysis. The effective concentrations in plasma for calibration curve ranged from 2.36 to 23.60 mg/L and 1.05 to 10.50 mg/L for ginsenoside Rg_1 and ginsenoside Re, respectively.

For the validation of the method, plasma samples containing three concentration levels of ginsenoside Rg_1 (9.44, 14.16, 23.60 mg/L), and Re (4.20, 6.30, 10.50 mg/L) were tested as quality control (QC).

Preparation of samples

Transfer 500 μL of plasma sample or blank plasma to a 1.5 mL anticoagulation tube and centrifuged at 15,000 rpm for 15 min. 200 μL of the supernatant was transferred into another test tube and 800 μL methanol-acetonitrile (3:1) mixture solution was added. The analytes were extracted from plasma by vortex-mixing for 1 min. Then the sample was centrifuged at 15,000 rpm for 10 min and 600 μL of the supernatant was transferred into another test tube and evaporate to dryness within N_2 current at 40°C. Finally, the residue was reconstituted with 100 μL mobile phase by vortex-mix-

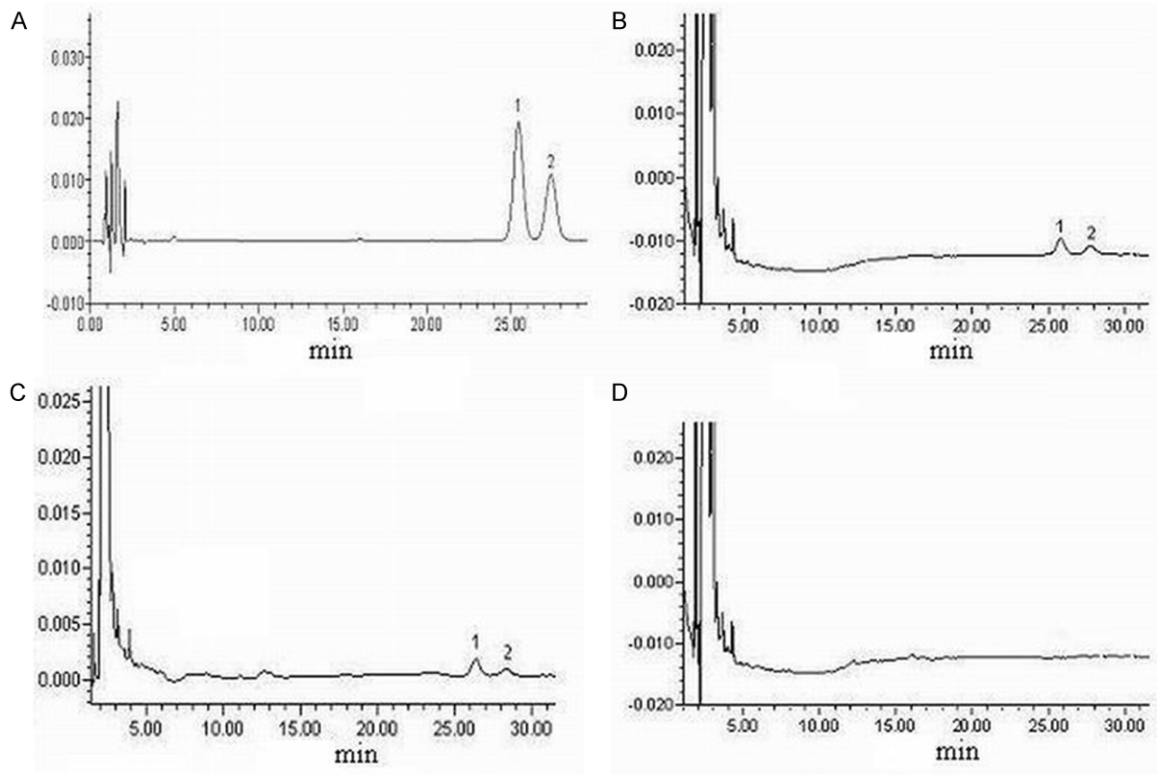

Figure 1. Representative HPLC chromatograms: (A) Standard solutions of ginsenoside (1) Rg_1 (236 mg/L) and (2) ginsenoside Re (105 mg/L); (B) Plasma spiked with (1) ginsenoside Rg_1 (23.6 mg/L), (2) ginsenoside Re (10.5 mg/L), (C) plasma 5 min after intravenous administration of Shenmai injection; (D) Blank plasma.

ing for 1 min and centrifuged at 16,000 rpm for 10 min; 20 μL supernatant was injected into chromatographic system for analysis.

Method validation

The lower limit of quantification (LLOQ) was determined as the lowest concentration point of the standard curve and the lower limit of detection (LLOD) was defined as the minimum amount that could be detected with a signal-to-noise ratio of 3. Plasma samples were quantified using the peak area of analyte as the assay parameter. Standard curves representing peak area versus sample size were described in the form of y = a + bx. To evaluate the linearity of the above formula, plasma calibration curves were prepared and assayed in duplicate on separate 5 days. The intra- and inter-day accuracy and precision were calculated by quantifying QC samples at high, middle, and low concentration levels on three different validation days. The accuracy was expressed by (mean measured concentration)/(spiked concentration) × 100% and the precision by relative standard deviation (RSD%). The extraction recovery of analyte at three QC levels was determined by comparing the peak areas obtained from QC samples with the un-extracted standard working solutions at the same concentration in the same solvent. The QC samples were also used to investigate the stability of the two ginsenosides during the sample storing and processing procedures. The QC samples were stored at ambient temperature for 12 h, and the long-term storage stability was examined by the assay of the QC samples stored at -40°C for a week. The freeze-thaw stability was determined after three freeze-thaw cycles (-40 to 20°C).

Application in rat models

Twelve male Sprague-Dawley (SD) rats (210-260 g) were obtained from the Animal Center of Zhengzhou University (Zhengzhou, China). The rats were kept in an air-conditioned animal center with temperature maintained 22 ± 2°C and relative humidity 50 ± 10%. Animals have free access to water, and were fed with a laboratory rodent chow (Zhengzhou, China) before they

Table 1. The linearity, LLOD and LLOQ of the assay for ginsenoside Rg_1 and Re

Analytes	Calibration curves (y = ax + b)	Linear range (μg)	R	LLOD (mg/L)	LLOQ (mg/L)
Ginsenoside Rg_1	y = 152651.3x-334.4	0.047~0.472	0.9986	1.25	2.36
Ginsenoside Re	y = 235480.4x-2272.8	0.021~0.210	0.9982	0.75	1.05

Table 2. The extraction recoveries of ginsenoside Rg_1 and Re in rat plasma (n = 5)

Analytes	Spiked conc. mg/L	Recovery (%)	RSD (%)
Ginsenoside Rg_1	9.44	84.88	8.56
	14.16	91.64	7.56
	23.60	91.43	11.21
Ginsenoside Re	4.20	82.69	12.37
	6.30	91.02	14.33
	10.50	91.81	11.93

were divided into two groups randomly which with six rats within each group. Animal received jugular vein cannula operation three days after group allocation and were fasted but given free access to water for 12 h prior to experiment.

Animals in Group 1 were administrated a single bolus intravenous injection of Shenmai injection at a dosage of 4 mL/kg (equivalent to ginsenoside Rg_1 0.752 and Re 0.319 mg/kg) though the tail vein. Blood samples (about 500 μL) were drawn in heparinized polythene tubes from jugular cannula immediately after injection and 0.25, 0.5, 1, 2, 4, 6, 8, 12 h after, and 200 μL plasma was separated immediately and stored at -40°C until analysis.

Animals in Group 2 were orally administrated with concentrated solution of Shenmai injection at a dosage of 20 mL/kg (equivalent to ginsenoside Rg_1 37.6 and Re 15.9 mg/kg), blood samples (about 500 μL) were drawn in heparinized polythene tubes from jugular cannula at times 0.5, 1, 2, 4, 6, 8, 10, 12, 24 h and immediately centrifuged to separate 200 μL plasma. The obtained plasma samples were stored at -40°C until analysis.

Results and discussion

Method validation

Specificity: The typical chromatograms of ginsenoside Rg_1 and Re were presented in **Figure 1**. Under the described chromatographic conditions, a good separation was achieved and no obvious interferences from endogenous plasma substances were observed. The mean retention time of ginsenoside Rg_1 and Re were 26.4 min, 28.5 min, respectively.

Linearity and lower limit of detection (LLOD): The linear range and regression equations for quantification of ginsenoside Rg_1 and Re were presented in **Table 1**. The correlation coefficients of these calibration curves were all higher than 0.98. The limits of detection (LLOD) of the analytes were in the range of 0.75-1.25 mg/L, while the lower limits of quantitation (LLOQ) of these analytes were 1.05-2.36 mg/L.

Extraction recovery, precision and accuracy: The extraction recoveries of ginsenoside Rg_1 and Re in rat plasma were shown in **Table 2**. At three concentration levels of these analytes, the extraction recoveries were all higher than 80%. As shown in **Table 3**, the summarized the intra- and inter-day precisions and accuracies of ginsenoside Rg_1 and Re were all ranged 80-120%. The intra- and inter-day precisions (RSD) of these analytes were all no more than 15%. The results demonstrated that the values were all within the acceptable range and the method was accurate and precise.

Stability: The stability of ginsenoside Rg_1 and Re was shown in **Table 4**. The results indicated that these analytes in rat plasma remained stable for 12 h at room temperature, 7 days at -40°C and three cycles of freeze-thaw with RSD values all lower than 15%.

Pharmacokinetic study

The chromatograms method was successfully applied to the pharmacokinetic study of ginsenoside Rg_1 and Re in rats following intravenous of Shenmai injection or oral administration of its concentrated solution. Detailed pharmacokinetic parameters are listed in **Table 5** and the mean plasma concentration-time profiles were illustrated in **Figure 2**. Ginsenoside Rg_1 and Re eliminated quickly in body following intravenous administration and virtually undetectable after 6 h. The pharmacokinetic behaviors of ginsenoside Rg_1 and Re following intravenous and oral

Table 3. The intra- and inter-day accuracies and precisions of ginsenoside Rg_1 and Re in rat plasma at high, middle and low concentration levels (n = 5)

Spiked conc. (mg/L)	Intra-day			Inter-day		
	Measured conc. (mg/L)	Accuracy (%)	Precision (%)	Measured conc. (mg/L)	Accuracy (%)	Precision (%)
Ginsenoside Rg_1						
9.44	10.22	108.24	8.470	9.90	104.84	10.01
14.16	16.35	115.45	7.510	14.55	102.79	3.05
23.60	24.00	101.69	11.150	24.32	103.06	5.88
Ginsenoside Re						
4.20	5.02	119.58	3.77	4.871	116.00	12.88
6.30	7.49	118.89	9.21	7.551	119.89	7.11
10.50	12.60	119.96	8.561	12.42	118.30	4.49

Table 4. The stability of ginsenoside Rg_1 and Re in rat plasma (n = 5)

Components	Spiked conc. (mg/L)	At ambient temperature RSD (%)	At -40°C RSD (%)	Three freeze-thaw cycles RSD (%)
Ginsenoside Rg_1	9.44	4.15	7.75	9.23
	14.16	9.25	6.05	7.88
	23.60	9.95	7.57	8.65
Ginsenoside Re	4.20	5.79	5.95	9.13
	6.30	3.34	12.59	12.12
	10.50	8.32	11.06	5.69

Table 5. Concrete pharmacokinetic parameters of ginsenoside Rg_1 and Re (n = 6)

Parameters	Inject group		Oral group	
	Rg_1	Re	Rg_1	Re
$AUC_{(0-t)}$ (mg/L*h)	25.808	8.322	93.249	29.706
$AUC_{(0-\infty)}$ (mg/L*h)	64.413	20.144	93.995	33.189
$MRT_{(0-t)}$ (h)	2.563	2.582	11.073	11.09
$MRT_{(0-\infty)}$ (h)	11.817	11.531	11.208	13.273
$t_{1/2}z$ (h)	8.425	8.164	2.812	4.474
T_{max} (h)	0.083	0.111	8	8
Vz (L/kg)	0.141	0.182	1.619	2.73
CLz (L/h/kg)	0.013	0.017	0.401	0.5
C_{max} (mg/L)	9.482	2.875	8.195	2.644

administration showed markedly difference. When administrated orally, it could only be detected within 2 h of dosing and the C_{max} in plasma was reached around 8 h and lasted until 24 h. As the pharmacokinetic parameters of ginsenoside Rg_1 and Re were of little difference with reported in the literature [10, 13], the variances may be caused by different administrating routines.

Bioavailability of ginsenoside Rg_1 and Re

According to the following formula, the bioavailability of ginsenoside Rg_1 and Re within Shenmai injection when orally administrated were 7.22% and 7.16%, respectively, which is quite low compared with the intravenous injection.

$$F = \frac{AUCt \times Xiv}{AUCiv \times Xt} \times 100\%$$

Footnote t in the formula represents test formulation, iv represents intravenous administration, and X represents the dose.

Discussions

After intravenous injection of Shenmai injection, the concentration-time curve of ginsenoside Rg_1 and ginsenoside Re in rats plasma is in line with two-compartment open model, while the concentration-time curve of two ginsenosides in rats plasma when fed large doses of concentrated Shenmai injection orally to rats also conformed to two-compartment open model, which were consistent with the literature reported [4] when tested with healthy volunteers.

The oral bioavailability of ginsenoside Rg_1 and Re was calculated based on pharmacokinetic parameters and showed that oral administration has significantly slower absorption distribution and lower bioavailability compared with intravenous injection. The ingredient directly injected into the blood showed fast onset, which is more suitable used for treatment of severe acute in clinical.

The low bioavailability of ginsenoside Rg_1 and Re was consistent with former reports that ginsenoside Re was rapidly cleared from the body with a short half-life (0.2 ± 0.03 h for male and 0.5 ± 0.08 h for female mice after i.v.) and oral absorption was generally poor (0.19-0.28%)

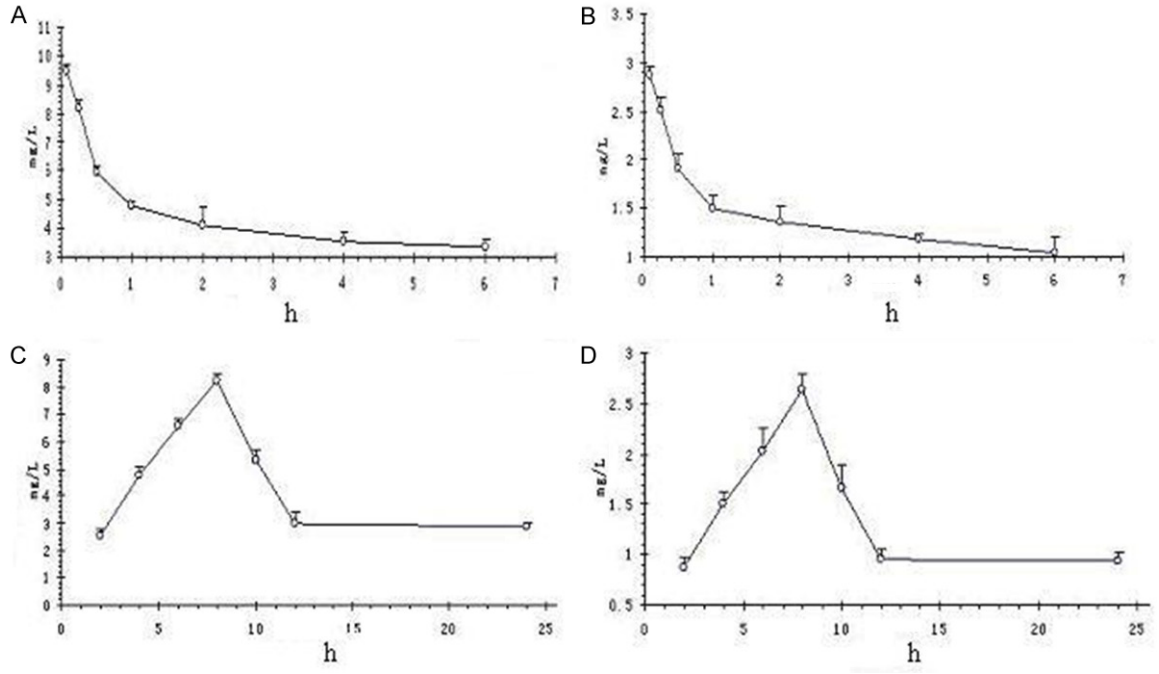

Figure 2. A. Mean plasma concentration-time profiles of ginsenoside Rg_1 in rats after i.v. administration of Shenmai injection, equivalent to Rg_1 at dosage of 0.752 mg/kg (n = 6). B. Mean plasma concentration-time profiles of ginsenoside Re in rats after i.v. administration of Shenmai injection, equivalent to Re at dosage of 0.319 mg/kg (n = 6). C. Mean plasma concentration-time profiles of ginsenoside Rg_1 i.g. administration of Shenmai injection, equivalent to Rg_1 at dosage of 37.6 mg/kg (n = 6). D. Mean plasma concentration-time profiles of ginsenoside Re in rats after i.g. administration of Shenmai injection, equivalent to Re at dosage of 15.9 mg/kg (n = 6). For the experiment, blood was collected via the jugular vein cannula in rat at different time points.

[14]. The administration routine used clinically is intravenous injection, and the injected amount of ginsenoside Rg_1 and Re converted to be equivalent to 4.2 g Panax ginseng or 42 ml of Shenmai injection, which is higher than the treatment dose of shock each time 20 mL one day specified in the Shenmai injection specification. Such result indicated that higher dosage of Shenmai injection may be needed for treatment of shock.

Ginsenosides are triterpene saponins. Due to their bulky molecular structures, the ginsenosides are poorly membrane permeable and prone to degradation. Oral consumption of ginseng preparations exposes ginsenosides to acid hydrolysis accompanied by side-reactions, glycosyl elimination and epimerization of C-20 sugar moiety. Experiments to increase the bioavailability of ginsenosides include co-administration of ginsenosides with adrenaline, emulsification of ginsenosides into lipid-based formulation and suppression of p-glycoprotein efflux system. And the pharmacokinetic characteristics of such modified ginsenoside compounds remain to be further studied.

To sum up, in the present study, the pharmacokinetic behaviors of ginsenoside Rg_1 and Re in rats following intravenous and oral administration of Shenmai injection was compared and the pharmacokinetic parameters were summarized based on a varied HPLC method. The plasma concentration-time curves indicated that there was a significant difference in the elimination speed of ginsenoside Rg_1 and Re in Shenmai injection following intravenous and oral administration and the low bioavailability of ginsenoside Rg_1 and ginsenoside Re when given orally indicated slow absorption and distribution in rat.

Disclosure of conflict of interest

None.

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