Drug Development and Industrial Pharmacy, 33:335–340, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600868011

informa
healthcare

Pharmacokinetics and Bioequivalence Studies of Galantamine Hydrobromide Dispersible Tablet in Healthy Male Chinese Volunteers

Li-jun Zhang, Xiao-ling Fang, Xue-ning Li, Qing-song Wang, Li-mei Han, Zhi-wen Zhang and Xian-yi Sha
Department of Pharmaceutics, School of Pharmacy, Fudan University, Box 130, 138 Yi Xue Yuan Road, Shanghai, P. R. China

ABSTRACT A randomized, two-way, crossover study was conducted in 18 healthy male Chinese volunteers to compare pharmacokinetics profiles of galantamine hydrobromide dispersible tablet with that of conventional tablet. A single oral dose of 10 mg galantamine was administrated to each volunteer. Plasma concentrations of galantamine were determined by a validated high-performance liquid chromatography (HPLC) method with fluorescence detection, which allowed 1 ng/mL to be assayed as the lowest quantifiable concentration. From plasma concentrations, $AUC_{0\to t}$ (the area under the plasma concentration-time curve from time 0 to the last sampling time, 32 hr), $AUC_{0\to\infty}$ (the area under the plasma concentration-time curve from time 0 to infinity), $t_{1/2}$ (elimination of half-life of the terminal log linear phase), C_{max} (maximum plasma drug concentration) and T_{max} (time to reach C_{max}) were evaluated through noncompartmental pharmacokinetic analysis. $AUC_{0\to t}$ and $AUC_{0\to\infty}$ were calculated by the linear-log trapezoidal rule method. C_{max} and T_{max} were obtained directly from the plasma concentration-time curve. Analysis of variance was carried out using logarithmically transformed $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max}. As far as $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} were concerned, there was no statistically significant difference between the test and reference formulations. Ninety percent confidence intervals (90% CI) for the ratio of $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} values for the test and reference formulations were 100.4–107.8%, 99.0–107.2% and 87.5–111.3%, respectively. As the 90% CIs of $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} were entirely within 80–125%, two formulations were considered bioequivalent.

KEYWORDS Galantamine, High-performance liquid chromatography, Pharmacokinetics, Bioequivalence

Address correspondence to Xian-yi Sha, Department of Pharmaceutics, School of Pharmacy, Fudan University, Box 130, 138 Yi Xue Yuan Road, Shanghai 200032, PR China; E-mail: shaxianyi89@hotmail.com

INTRODUCTION

Galantamine is a tertiary alkaloid that can be isolated from a number of plants (e.g., galanthus, lycoris). As a reversible and selective acetylcholine

esterase (AChE) inhibitor, it has been used for the treatment of mild to moderate Alzheimer's disease (AD). Data from a large number of clinical trials have shown that galantamine significantly improves cognitive function in patients with AD. In addition, galantamine also serves as an allosteric modulator of nicotine ACh receptors to stimulate the release of additional ACh and other transmitters in central synapses, which augments responses to the neurotransmitter Ach (Farlow, 2001; Coyle & Kershaw, 2001; Erkinjuntti, 2002;Huang et al., 2002; Suh et al., 2004).

Alzheimer's disease, a progressive degenerate disorder of the brain, afflicting millions of the elderly people all over the world, has become the most common cause of cognitive impairment in the elderly (Migliaccio-Walls, 2003; Standridge, 2004). Considering most elderly patients with AD may have difficulty swallowing conventional tablet, galantamine hydrobromide dispersible tablet was developed. The dispersible tablet, which is dispersed in water rapidly, forming a stabilized suspension, can facilitate the administration for the elderly people and improve the patients' compliance. Since the new formulation of galantamine was produced by Yixin Pharmaceutical Co., Ltd. (Zhejiang province, China), dispersible tablet (test medication) and Jin kang ling li tablet (reference medication, manufactured by Kang'enbei Pharmaceutical Co., Ltd., Zhejiang province, China) were assessed in 18 healthy male Chinese volunteers in this study. Typical pharmacokinetic parameters such as $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, C_{max}, T_{max} and $t_{1/2}$ of the two galantamine formulations were calculated and compared.

A novel HPLC method was set up and validated to assay plasma concentration of galantamine, reaching the sensitivity of 1 ng/mL as the lowest quantifiable concentration.

Procedures, statistics and results obtained in the pharmacokinetics and bioequivalence studies are comprehensively described in this paper.

MATERIALS AND METHODS

Materials

The test medication was galantamine hydrobromide dispersible tablet (5 mg of galantamine hydrobromide, lot no 050819, Yixin Pharmaceutical Co., Ltd., Zhejiang) and the reference medication, Jin kang ling li tablet (5 mg of galantamine hydrobromide, lot no 050811, Kang'enbei Pharmaceutical Co., Ltd., Zhejiang). Other solvents and reagents were of analytical or HPLC grade. HPLC grade water was produced by the Millipore Direct-Q system. Galantamine hydrobromide and tramadol (used as an internal standard, IS) were supplied from Zhejiang Kang'enbei Pharmaceutical Co., Ltd. and Shanghai Institute for Drug Control, respectively.

Methods

Eighteen healthy Chinese male volunteers ranging in age from 18–30 years (22.22 ± 1.48), weighing 50–80 kg (63.56 ± 4.06 kg), with a height of 160–185 cm (172.94 ± 4.05 cm) were involved in this study. Volunteers were selected after passing a clinical screening procedure including a physical examination and laboratory tests. Volunteers were excluded if they were sensitive to this type of medication, had a history of any illness of hepatic, renal or cardiovascular system, or had taken alcohol of other medications for a long period. This was done to ensure that the existing degree of variation would not be due to the influence of illness or other medications. All volunteers avoided using other drugs for at least two weeks prior to the study and until after its completion. Each volunteer received an oral dose of 10 mg (2 tablets) of galantamine hydrobromide in a standard 2 × 2 crossover model in a randomized order. There was a 2-week period of washout between the doses. The ethical committee of Zhongshan hospital (Shanghai, China) approved the protocol of this study. All participants signed a written consent after they had been informed of the nature and details of the study. There were no dropouts.

The doses were taken with 250 mL of tap water. At 4 hr after oral administration, all subjects were given standardized meals. Approximately 3 mL blood samples were collected via the cannula at 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 5, 8, 12, 24, and 32 hr after the administration of the drug. The heparinized 1 mL normal saline injectable solution was flushed after each blood sample. The blood sample was centrifuged at 6000 rpm for 10 min and the plasma was frozen at –20°C until subjected to the HPLC analysis.

HPLC Assay of Galantamine in Plasma

The concentrations of galantamine were analyzed by HPLC with fluorescence detection. Briefly, calibration standards, validation control samples and volunteer plasma samples were prepared by aliquoting 1mL of plasma into clean glass tubes followed by addition of 50 μL of internal standard solution (1 μg/mL tramadol in methanol) and 100 μL of 2M sodium hydroxide solution. After vortex mixing for 1 min, 5 mL of methyl tertiary butyl ether was added. The mixture was mixed by vortex agitation for 3 min and centrifuged at 8000 rpm for 5 min. The top organic phase (4 mL) was transferred into a 10 mL conical glass tube. The organic phase was evaporated to dryness under nitrogen stream while immersed in a 50°C water bath. The residue was reconstituted with 60 μL mobile phase and vortex mixed. This solution (20 μL) was subsequently injected for HPLC analysis. The analyses were performed on a Shimadzu HPLC system composed of a LC-10ATvp pump and a RF-10A XL fluorescence detector. The analytical column was a Shim Pack CLC-ODS column (150 mm × 4.0 mm i.d. , 5 μm particle size). The mobile phase consisted of acetonitrile, water, triethylamine (20:80:1, v/v/v, pH 7.0). The analyses were conducted at 30°C, flow rate of 1.0 mL/min. Fluorescence detection was performed at an excitation wavelength of 290 nm and an emission wavelength of 320 nm.

Pharmacokinetic Analysis

Noncompartmental PK analysis was employed to analyze plasma drug concentration-time data. The parameters C_{max} and t_{max} were obtained directly from the concentration-time curve. The terminal slope (k) of the concentration-time curve was determined by log-linear regression of at least the last three data points. Elimination of half-life ($t_{1/2}\beta$) of the terminal log linear phase was calculated using the equation $0.693/k$. The $AUC_{0\to t}$ was calculated using the linear trapezoidal rule and was extrapolated to infinity according to the relationship $AUC_{0\to\infty} = AUC_{0\to t} + C_t/\beta$, where $AUC_{0\to\infty}$ is the area under the plasma concentration-time curve from zero to time infinity, C_t is the last quantifiable concentration and β is elimination rate constant at terminal phase.

Statistical Analysis of Data

For the purpose of bioequivalence analysis $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} were considered as primary variables. Bioequivalence between the formulations was determined by calculating 90% confidence intervals (90% CI) for the ratio of $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} values for the test and reference formulations, using logarithmic transformed data. ANOVA was used to assess product, group and period effects. The formulations were considered bioequivalent if the 90% CI of $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} fell within 80–125%.

RESULTS

Validation of HPLC Assay

The representative chromatograms of a blank plasma spiked with tramadol, blank plasma sample spiked with galantamine and tramadol, and plasma sample obtained from a healthy volunteer following an oral 10 mg dose of galantamine are shown in Fig. 1. The analytes were well separated using the present chromatographic conditions. The retention times were 5.5 min for galantamine and 11.6 min for IS. No interfering peaks from the endogenous plasma components were observed at the retention time of galantamine or IS.

The lower limit of quantification was 1 ng/mL based on a signal-to-noise ratio of three. Good linearity was observed within the range of 2.0–160 ng/mL ($y = 69.333x - 0.2527$, $r^2 = 0.9994$). For plasma concentration at 5, 20, and 80 ng/mL, the mean recovery of galantamine were 85.54 ± 5.51%, 84.60 ± 6.20% and 76.75 ± 5.55%, respectively. The method was precise and accurate. Intraassay precision was between 2.74–8.92% and interassay precision was between 6.16–7.53%. Accuracy ranged from 96.95–109.02%. Plasma samples were stable for at least 1 mo at –20°C and after three freeze-thaw cycles.

Safety

Subjects were continuously monitored and periodically questioned for any adverse events throughout the study. The tolerability of both galantamine medications was good. No serious clinical adverse events were observed in all 18 volunteers.

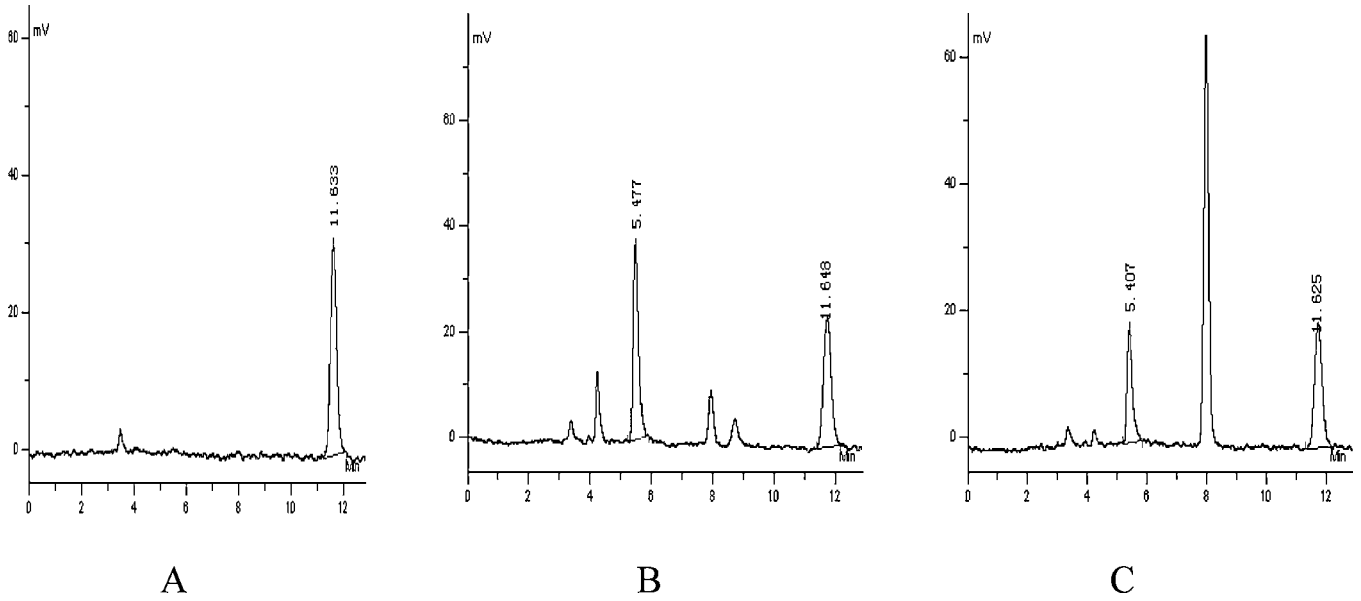

FIGURE 1 **Typical Chromatogram of (A) Blank Human Plasma Spiked with Tramadol, (B) Blank Human Plasma Spiked With Galantamine and Tramadol and (C) Plasma Sample From a Healthy Volunteer.**

TABLE 1 **Pharmacokinetic Parameters of Galantamine After administration of Test and Reference Formulations to 18 Healthy Human Volunteers**

Pharmacokinetic parameter	Test formulation		Reference formulation		Ratio	
	Arithmetic	Geometric	Arithmetic	Geometric	Arithmetic	Geometric
$AUC_{0\to t}$ (ng·h/mL)	485.11 ± 99.12	474.91	468.74 ± 95.36	456.35	1.03	1.04
$AUC_{0\to\infty}$(ng·h/mL)	529.70 ± 111.68	519.25	514.61 ± 105.28	504.11	1.03	1.03
C_{max} (ng/mL)	47.47 ± 10.71	46.35	48.19 ± 10.75	46.97	0.99	0.99
T_{max} (h)	1.01 ± 0.55	0.89	1.23 ± 1.07	0.94	0.82	0.95
$T_{1/2}$ (h)	8.41 ± 1.47	8.29	9.06 ± 1.20	8.98	0.93	0.92

Pharmacokinetic Characteristics

Average values of pharmacokinetic parameters and average galantamine plasma concentration-time profiles after administration of test and reference formulations to 18 healthy human volunteers are shown in Table 1 and Fig. 2, respectively. These parameters show close mean values, with only marginal differences between the test and reference formulations. With both the formulations, galantamine appeared early in plasma, in most cases at 1 hr. The peak was reached on average at 1.01 hr (range 0.5–2.0 hr) with the test and at 1.23 hr (range 0.33–3.0 hr) with the reference, which are consistent with the reported literature values (Zhao et al., 2002). Decrease in concentrations was detectable in most cases by 32 hr. Figure 2 shows that the plasma concentration-time curves of the test and reference formulations are almost overlapping.

Bioequivalence Analysis

The results of analysis of variance for assessments of products, period and group effects and 90% confidence intervals for the ratio of $AUC_{0\to t}$, $AUC_{0\to\infty}$, C_{max} and T_{max} values for the test and reference formulations are shown in Table 2. The results did not show any statistically significant product, period or group effect with $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max}. $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} 90% CI were comprised in the stipulated 80–125% range, but the lower limit of 90% CI of T_{max} was under 80%.

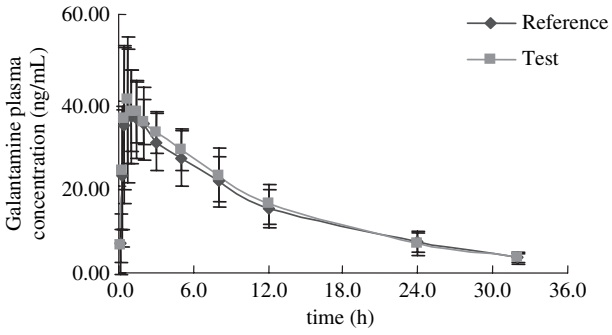

FIGURE 2 **Average Galantamine Plasma Concentration-time Profiles After Administration of Test and Reference Formulations to 18 Healthy Human Volunteers.**

DISCUSSION

The analytical method developed for galantamine hydrobromide quantification in plasma samples showed good specificity, sensitivity, linearity, precision, and accuracy over the entire clinically significant and therapeutically achievable plasma concentrations, thereby enabling its use in bioequivalence and pharmacokinetic trials. The method showed some advantages over other reported methods. In China, some researchers determined the plasma concentration of galantamine using reverse ionic pair HPLC with UV detection without internal standard and the lower limit of quantification was 2.5 ng/mL. In comparison, the method developed in this study uses tramadol as internal standard, which can ensure that the existing degree of variation is not due to the influence of injection volume. Besides, the method developed here also allowed 1 ng/mL to be assayed as the lowest quantifiable concentration. The method proposed earlier (Zhao et al., 2002) uses toluene to extract galantamine from plasma samples twice, which is more toxic and complicated than the method reported herein. The reports (Verhaeghe et al., 2003) show the determination of galantamine using LC-MS-MS detection at high cost, but in developing countries, fluorescence detection is commonly used.

Average plasma decay curve and pharmacokinetic parameters obtained for the test formulation were

TABLE 2 **Analysis of Variance(ANOVA) for the Assessment of the Product, Period and Group Effects and 90% Confidence Intervals (90% CI) for the Ratio of $AUC_{0\to t}$, $AUC_{0\to\infty}$, C_{max} and T_{max} Values for the Test and Reference Formulations, Using Logarithmic Transformed Data, After Administration to 18 Healthy Volunteers ($\alpha = 0.05$)**

	ANOVA (*p*-value) variation source			
Pharmacokinetic parameter	product	period	Group	90% CI
$AUC_{0\to t}$	0.0675	0.8031	0.6538	100.4–107.8%
$AUC_{0\to\infty}$	0.2063	0.7129	0.6993	99.0–107.2%
C_{max}	0.8500	0.4999	0.6471	87.5–111.3%
T_{max}	0.7337	0.1983	0.5975	74.2–124.4%

similar to those of the reference formulation. The 90% confidence intervals of $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} all fell within the range of 80–125%, demonstrating the bioequivalence of the two formulations. However, the 90% CI of T_{max} was out of 80–125%. For the test and reference formulation, the mean values of T_{max} were 1.01 and 1.23 hr, respectively, which indicated that the test formulation could be absorbed more rapidly than the reference one. It may be due to the fact that the test formulation is dispersible tablet, which can disintegrate and dissolute more rapidly than classic tablet.

In conclusion, above results indicate that the two formulations of galantamine hydrobromide are bioequivalent.

REFERENCES

Coyle, J. & Kershaw, P. (2001). Galantamine, a cholinesterase inhibitor that allosterically modulates nicotinic receptors: effects on the course of Alzheimer's disease. *Biol. Psych., 49*, 289–299.

Erkinjuntti, T. (2002). Treatment options: the latest evidence with galantamine (Reminyl®). *J. Neurol. Sci., 203–204*, 125–130.

Farlow, M. R. (2001). Pharmacokinetic profiles of current therapies for Alzheimer's disease: implication for switching to galantamine. *Clinic Therap., 23* suppl A, 13–24.

Huang, F. L., Lasseter, K. C., Janssens, L., Verhaeghe, T., Lau, H., & Zhao, Q. (2002). Pharmacokinetic and safety assessments of galantamine and Risperidone after the two drugs are administrated alone and together. *J. Clin. Pharmacol., 42*, 1341–1351.

Migliaccio-Walle, K., Getsios, D., Caro, J. J., Ishak, K. J., O'Brien, J. A., & Papadopoulos, G. (2003). Economic evaluation of galantamine in the treatment of mild to moderate Alzheimer's disease in the United States. *Clinic. Therap., 25*, 1806–1825.

Standridge, J. B. (2004). Pharmacotherapeutic approaches to the treatment of Alzheimer's disease. *Clinic. Therap., 26*, 615–630.

Suh, G. H., Yeon Jung, H., UK Lee, C., Hoon Oh, B., Nam Bae, J., Jung, H. Y., Ju, Y. S., Kil Yeon, B., Park, J., Hong, I., Choi, S., & Ho Lee, J. (2004). A prospective, double-blind, community controlled comparison of three doses of galantamine in the treatment of mild to moderate Alzheimer's disease in a Korean population. *Clinic. Therap., 26*, 1608–1618.

Verhaeghe, T., Diels, L., Vries de, R., De Mevlder, M., & De Jong, J. (2003). Development and validation of a liquid chromatographic-tandem mass spectrometric method for the determination of galantamine in human heparinised plasma. *J. Chromatogr. B, 789*, 337–346.

Zhao, Q. Y., Brett, M., Van Osselaer, N., Huang, F., Raoult, A., Van Peer, A., Verhaeghe, T., & Hust, R. (2002). Galantamine pharmacokinetics, safety, and tolerability profiles are similar in healthy Caucasian and Japanese subjects. *J. Clin. Pharmacol., 42*, 1002–1010.