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Bioequivalence evaluation of two brands of rivastigmine of different salt forms, an acetylcholinesterase inhibitor for the treatment of Alzheimer's disease, in healthy Beagle dogs

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The bioequivalence of two brands of rivastigmine capsules, of different salt forms, was demonstrated in six healthy beagle dogs after a single oral dose in a randomized cross-over study. Reference (Rivastigmine hydrochloride, Sunve, CN) and test (Rivastigmine tartrate, Novartis, CH) products were administered to fasting beagles on two treatment days separated by a two-day washout period; blood samples were collected at specified time intervals, and the plasma was separated and analyzed for rivastigmine using a validated GC-MS method. The pharmacokinetic parameters $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, C_{max}, T_{max} and $t_{1/2}$ were compared statistically to evaluate bioequivalence between the two brands, using the statistical modules recommended by the State Food and Drug Administration (SFDA) of China. The analysis of variance (ANOVA) did not show any significant difference between the two formulations and 90% confidence intervals fell within the acceptable ranges for bioequivalence. Based on these statistical inferences it was concluded that the two brands exhibited comparable pharmacokinetic profiles and that Sanwei's Rivastigmine hydrochloride was bioequivalent to Rivastigmine tartrate of Novartis, CH.

1. Introduction

Rivastigmine tartrate (Exelon) is an acetylcholinesterase inhibitor for the treatment of Alzheimer's disease (AD), which was approved by the US Food and Drug Administration in 2000 and went on sale in 2007 (Wong 2006). Rivastigmine tartrate is chemically described as (*S*)-*N*-ethyl-3-[(1-dimethylamino) ethyl]-N-methyl-phenyl-carbamate hydrogen tartrate (Bhatt et al. 2007). It may be a well-tolerated treatment option for improving or preventing psychotic and nonpsychotic symptoms associated with AD. Treatment response to Exelon occurs with mild, moderate and moderately severe AD, with the largest effect in patients with advancing severity of disease. It has received approval for use in 60 countries (Bullock and Cameron 2002; Ballmaier et al. 2002; Kennedy et al. 1999; Kurz 2004).

Rivastigmine hydrochloride, a new drug manufactured by Shanghai Sunve Pharmaceutical Co., Ltd (China), has been proved to be effective for the treatment of AD, differing from Exelon regarding the salt forms. Rivastigmine hydrochloride is Waitiny for approval by the State Food and Drug Administration (SFDA) of China. No data was reported about preclinical investigation about rivastigmine hydrochloride.

Bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of their absorption. The area under the concentration time curve (*AUC*) generally serves as the indicator of the extent of absorption while the peak concentration (C_{max}) and the time of its occurrence (T_{max}), reflect the rate of absorption, especially in fast releasing drug formulations (Hauschke et al.1990).

In this paper, the pharmacokinetic parameters of two different salt forms of rivastigmine of were studied in beagle dogs. The aim of the present work was to determine bioequivalence between the two rivastigmine products.

2. Investigations and results

2.1. Pharmacokinetic analysis

Pharmacokinetic analysis was performed by means of model independent method using a 3P97 computer program. The elimination rate constant (k_{el}) was obtained as the slope of the linear regression of the log-trans-formed concentration values versus time data in the terminal phase. The elimination half-life ($t_{1/2}$) was calculated as $0.693/k_{el}$. The area under the curve to the last measurable concentration ($AUC_{0\text{-}t}$) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0\text{-}\infty}$) was calculated as $AUC_{0\text{-}t} + C_t/k_{el}$, where C_t is the last measurable concentration. 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 results are expressed as the mean and the standard deviation.

2.2. Statistical analysis

For the purpose of bioequivalence analysis $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ and C_{max} were considered as primary variables. Analysis of variance (ANOVA) and t-tests were performed to evaluate significant

Table 1: Pharmacokinetic parameters of two brands of rivastigmine of different salt forms 9 mg (3*3 mg) capsules (mean ± standard deviation, n = 6)

Pharmacokinetic Parameter	Rivastigmine hydrochloride (test)	Rivastigmine tartrate (reference)
$AUC_{0\text{-}t}$ (ng/mL h)	88.23 ± 11.93	90.18 ± 13.92
$AUC_{0\text{-}\infty}$ (ng/mL h)	92.44 ± 11.29	94.47 ± 13.36
C_{max} (ng/mL)	44.56 ± 9.89	41.48 ± 4.37
T_{max} (h)	2.00 ± 0.00	2.17 ± 0.25
$t_{1/2}$ (h)	4.05 ± 0.36	4.04 ± 0.35
k_{el} (h^{-1})	0.172 ± 0.016	0.173 ± 0.016

differences between the two formulations. The difference between two related parameters was considered to be statistically significant at values of $p <= 0.05$. Parametric 90% confidence interval based on the ANOVA of the mean test/reference (T/R) ratios of *AUC*s and C_{max} were computed.

2.3. Reaction of animals

Rivastigmine was well tolerated by the beagle dogs. The result of a preliminary test showed that rivastigmine had gastrointestinal irritating effects on fasting dogs which made most of them vomit lightly, dribble or thrill. So, the food has a great effect on absorption of the drug. According to the introduction of Exelon, we fed the dogs with one-third amount of their daily meal before administration. It proved to relieve the side effect of rivastigmine.

2.4. Analytical method validation

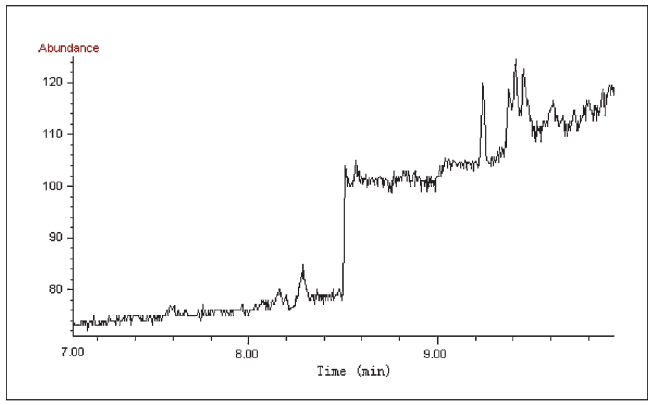
Under the described conditions, the lower limit of quantitation from 0.5 mL plasma was 1.0 ng/mL for rivastigmine. The relationship between the concentration and the peak area ratio was found to be linear within the range 1.0–50.0 ng/mL. The intra-day accuracy of the method for rivastigmine ranged from 94.7% to 119.9%, while the intraday precision ranged from 1.56% to 8.93%. The inter-day accuracy ranged from 100.41% to 104.74%, while the inter-day precision ranged from 7.63% to 11.67%. The absolute recovery was 103.4% while the relative recovery ranged from 71.71% to 79.58%. The stability study showed that rivastigmine was stable in plasma for 2 months when stored at −20 °C. The method used in this study was found to be reliable, accurate, sensitive and rapid for detecting plasma levels of rivastigmine. Figure 1 shows typical chromatograms of this experiment.

2.5. Plasma concentration profiles

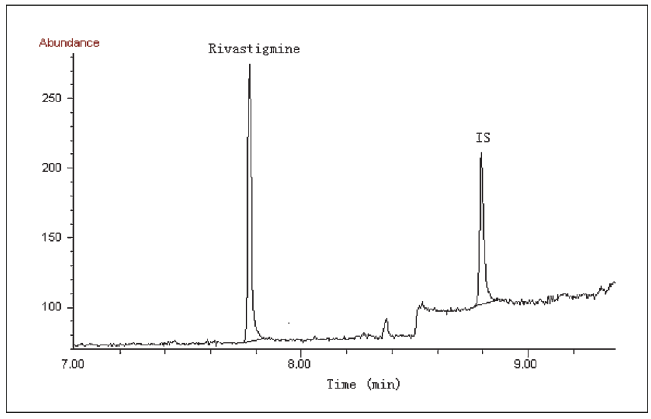
Both brands of rivastigmine were readily absorbed from the gastrointestinal tract in all beagle dogs. The mean concentration-time profile of rivastigmine for the two formulations is shown in Fig. 2. The figure indicates that the mean plasma concentration profiles of the two brands were closely similar and superimposable. Peak concentrations of 41.5 ng/mL and 44.6 ng/mL were attained at 2.2 h and 2.0 h, respectively, after drug administration and then declined rapidly and was detectable up to 8 h only. Table 1 shows the pharmacokinetic parameters of rivastigmine for two brands. The relative bioavailability of test brand was 97.5% for $AUC_{0\text{-}\infty}$.

3. Discussion

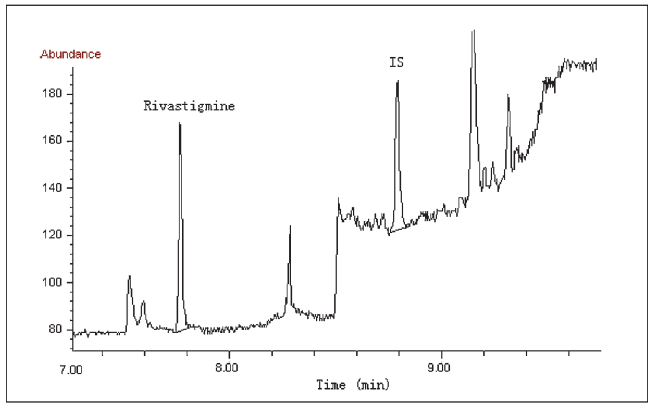
The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When



(A)



(B)



(C)

Fig. 1: Chromatography of (A) blank plasma, (B) blank plasma added with rivastigmine and chlorphenamine (IS), (C) dog plasma sample obtained after single-dose administration of three 3-mg capsules of rivastigmine.

two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent (Schulz and Steinijans 1992). According to the Chinese Pharmacopeia, for basic pharmacokinetic characteristics, the standard equivalence range is 0.8–1.25 for *AUC*, 0.7–1.45 for C_{max}, respectively. The results of statistical analysis are shown in Table 2. The mean and standard deviation of $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ and C_{max} of the two products did not differ significantly, suggesting that the blood profiles generated by rivastigmine hydrochloride are comparable to those produced by rivastigmine tartrate. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in period or formulation, with a value of $p > 0.05$. 90% confidence intervals, also demonstrated that the ratios of $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ of the two formulations lay within the SFDA

Table 2: Statistical evaluation of bioequivalence of two brands of rivastigmine of different salt forms in six beagle dogs

	Individual	Period	Formulation		90% CI
$\ln AUC_{0\text{-}t}$	29.9663	0.6811	0.6811	$AUC_{0\text{-}t}$	101.67%−109.08%
$\ln AUC_{0\text{-}\infty}$	30.1562	0.8517	0.8517	$AUC_{0\text{-}\infty}$	102.57%−109.27%
$\ln C_{max}$	3.3020	2.2526	2.2526	C_{max}	96.60%−118.18%
$F_{0.05}$	$F_{0.05}$ (5,4) = 6.26	$F_{0.05}$ (1,4) = 7.71	$F_{0.05}$ (1,4) = 7.71		

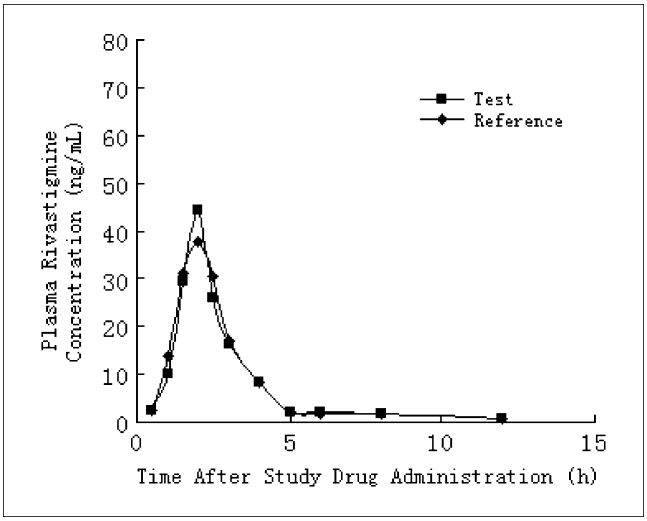


Fig. 2: Mean plasma concentrations of rivastigmine after oral administration of the two brands of different salt forms to 6 beagle dogs.

acceptable range of 80%–125%, and C_{max} lay within range of 70%-145%. For T_{max} the parametric point estimate of difference (test-reference) was 0.08 h, and found to be within the acceptance limits (± 20% of reference mean). Plasma levels may be used as surrogate parameters for clinical activity; therefore the results of this study suggest comparable clinical efficacy of the two brands of rivastigmine of different salt forms. The investigation provided important preclinical pharmaceutical parameters for the further research and clinical test of rivastigmine hydrochloride.

Statistical comparison of the $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ and C_{max} clearly indicated that no statistically significant differences exist between rivastigmine tartrate and rivastigmine hydrochloride capsules in any of the calculated pharmacokinetic parameters in beagle dogs. The confidence intervals for the ratios of mean $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ and C_{max} further demonstrate that these values were within the bioequivalence acceptance ranges. Based on the above it is concluded that rivastigmine hydrochloride is bioequivalent to rivastigmine tartrate (Exelon), and that both products can be considered equally effective in medical practice.

4. Experimental

4.1. Materials

Test product: Rivastigmine hydrochloride - rivastigmine 3 mg capsules, Batch No.: 060918 Manufacturing date: 09/2006; Expiry date: 09/2008, Manufacturer: Shanghai Sunve Pharmaceutical Co., Ltd, CN, Reference product: Rivastigmine tartrate - rivastigmine 3 mg capsules, Batch No.: 200604 Manufacturing date: 04/2006; Expiry date: 04/2008, Manufacturer: Novartis, CH.

4.2. Animals

Six healthy male beagle dogs (body weight, 14–18 kg) participated in the study. They were obtained from the Laboratory Animal Center of School of Pharmacy, Fudan University. The beagle dogs in this experiment were acclimated for 1 week prior to experiment. They were fasted overnight. We fed the dogs with one-third amount of their daily meal before drug administration. Drinking water from the local water supply was readily available.

4.3. Experimental design

The study was open, randomized, cross over design. Six male beagle dogs received each of the two formulations on separate occasions. Three dogs were randomly assigned to each of the two groups. Each dog in the first group received formulation I (three 3-mg capsules) and the other group received formulation II (three 3-mg capsules) at 8:00 a.m., blood samples were collected from jugular vein into heparinized tubes with 10 μL physostigmine (0.1 mol/L) through an indwelling cannula before (0 h) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0 and 8.0 h after dosing. After centrifugation, plasma was separated into amber colored vials and stored at −20 °C until further analysis. After a washout period of 2 days, the study was repeated in the same manner to complete the crossover design.

4.4. Sample preparation for GC/MS injection

Extraction from plasma was accomplished by liquid-liquid phase extraction; 50 μL of internal standard (chlorphenamine, 300 ng/mL) was added to the 0.5 mL plasma. 100 μL of ammonia (1:1, V/V) was added and vortex mixed. To this sample, 3.5 mL of dichloromethane as extraction solvent was added, vortex mixed for 2 min, and centrifuged at 3000 rpm for 10 min. The extraction was repeated twice in similar fashion and the dichlormethane layer was collected on silica cartridge. The total volume of dichloromethane was passed through the cartridge. The cartridge was eluted into clean test tubes and then evaporated to dryness under nitrogen at 40 °C. The residue was reconstituted with 50 μL methanol and 1 μL was injected into the GC-MS, where rivastigmine and internal standard were separated from endogenous plasma substances.

4.5. Chromatographic conditions

The plasma samples were determined for rivastigmine by a validated GC-MS method. All solvents used were of GC grade and were purchased from Merck while other chemicals and reagents were of analytical grade. Rivastigmine were obtained from Sunve, CN; while the internal standard was from Institute for Biochemical Drug Control, CN.

The sample was analysed on a HP 6890 GC system, coupled with an HP MD5980 quadrupole mass spectrometer. Rivastigmine and internal standard were separated on an HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film). Splite injection (50:1) was employed for the plasma samples. The column oven temperature was programmed to rise an initial temperature of 100 °C (1 min) to 280 °C at 20 °C/min and keep 280 °C for 15 min. The injection temperature and ion source temperature were 250 °C and 280 °C, respectively. Helium was used as the carrier gas with a flow rate of 1 mL/min. The volume of injection was 1 μL. The ionizing energy was 70 eV. Rivastigmine was detected at m/z of 250.0 and MS/MS daughter at m/z 235.3, while an internal standard (chlorphenamine) was detected at m/z of 203.2 and MS/MS daughter at m/z of 167.3. The peak area was measured, and the peak area ratio of the drug to the internal standard and the concentration were calculated by the software. The method was validated following international guidelines.

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