

Bioequivalence Study of Two Oral Formulations of Memantine Tablets in Healthy Brazilian Volunteers after a Single Dose Administration

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Abstract

The bioequivalence of a single dose of memantine (Clomenac[®]) 10 mg tablets manufactured by Cobalt Pharmaceuticals, Canada/Arrow Farmacêutica Ltda was compared with a reference memantine 10 mg tablets (Ebix[®], Lundbeck Inc). The single-dose, randomized-sequence, open-label, two period crossover study was conducted on a total of 26 Brazilian healthy volunteers of both genders. Nineteen blood samples were taken during 72 h. Samples were frozen and kept until time of analysis. Plasma concentrations of memantine were determined using a validated UPLC-MS/MS method. Confidence intervals (CI, 90%) for the peak plasma concentration (C_{max}) and area under the concentration-time curve (AUC_{0-t}) were determined by calculating log-transformed data. The test and reference formulations were considered bioequivalent if the 90% CIs for the geometric mean test/reference ratios were within a predetermined range of 80% to 125%. The 90% CI for the geometric mean ratios for C_{max} was 100.1% (92.9-107.9%) and for AUC_{0-t} was 98.8% (93.9-103.9%). In conclusion, the tested 10 mg memantine tablets (Clomenac[®], Arrow Farmacêutica Ltda.) was bioequivalent to Ebix[®] 10 mg tablets, according to the rate and extent of absorption.

Keywords: Memantine; Tablet formulation; UPLC-MS/MS; Bioavailability; Bioequivalence

Introduction

Alzheimer disease is a fatal progressive neurodegenerative illness and the most common form of dementia, affecting as many as 25 million people worldwide [1]. Severe memory loss, confusion, and impaired cognitive abilities characterize Alzheimer disease [2]. The treatments for the disease involve pharmacological and stimulatory therapies, such as, physical exercises, cognitive training and socialization [3].

The first medications to receive FDA approval for symptomatic treatment of patients with Alzheimer disease were the acetylcholinesterase inhibitors, such as tacrine, donepezil, galantamine and rivastigmine [1].

Memantine (Figure 1), which has a different mechanism of action, is a moderate-affinity, noncompetitive antagonist of N-methyl-D-aspartate receptors, approved for the treatment of moderate to severe Alzheimer disease. In addition to its benefits on cognition function and global status, memantine treatment may also help alleviate behavioral symptoms [4,5].

After oral administration, memantine is completely absorbed, with peak plasma concentrations occurring between 3 and 7 hours. Memantine is 45% bound to plasma proteins presenting a distribution volume of approximately 9-11 L/kg, which suggests an extensive distribution into tissues. Memantine is excreted predominantly in

urine as unchanged parent drug, in part via renal tubular secretion, and it has a terminal elimination half-life of 60 to 80 hours [6,7].

Bioequivalence studies are important to compare the systemic exposure profile of a test drug product to that of a reference product. For two orally administered drug products can be considered bioequivalent, the active drug ingredient in the test formulation must exhibit equivalent rate and extent of absorption to that of the reference product. Pharmacokinetic parameters such as C_{max} and AUC are useful tools to evaluate and to compare the systemic exposure of the drug in different products [8]. Some studies describe the pharmacokinetic evaluation of memantine after administration in human volunteers [6,7,9-11] or rats [12,13], and the bioequivalence between oral formulations of memantine tablets [14].

The aim of this study was to evaluate the bioequivalence of two tablet formulations: memantine 10 mg tablets (Clomenac[®]) manufactured by Cobalt Pharmaceuticals, Canada/Arrow Farmacêutica Ltda, relative to reference tablets (Ebix[®], Lundbeck Inc) after single-dose oral administration in Brazilian healthy volunteers.

Subjects and Methods

Subjects

Twenty six adult healthy volunteers of both genders (13 men and

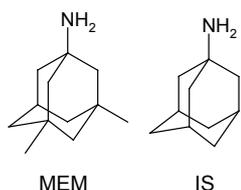


Figure 1: Chemical structures of memantine (MEM) and adamantane, the internal standard (IS).

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Received January 20, 2012; **Accepted** February 18, 2012; **Published** February 20, 2012

Citation: César IC, Duarte Byrro RM, de Santana e Silva FF, Mundim IM, de Souza Teixeira L, et al. (2012) Bioequivalence Study of Two Oral Formulations of Memantine Tablets in Healthy Brazilian Volunteers after a Single Dose Administration. J Bioequiv Availab 4: 014-017. doi:10.4172/jbb.1000104

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13 women) who were between the ages of 18 and 50 and within 15% of their ideal body weight were enrolled in the study. The required sample size was calculated using PASS 2008 software (NCSS, Kaysville, Utah, USA) and considering a minimum of 80% for power of the test. Subjects were judged eligible for enrolment in this study if they were in compliance with all the inclusion and exclusion criteria described in the protocol.

A physical examination was conducted in each participant. Subject's health was based on findings of a clinical health evaluation, which consisted on ECG and the following laboratory tests: blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, Gamma GT, total bilirubin, albumin and total protein, triglycerides, total cholesterol, hemoglobin, hematocrit, total and differential white cell counts, urinalysis and a pregnancy test in women. All subjects were negative for HIV, HBV and HCV.

All subjects were informed with the objectives, drugs, potential risks, dates and activities during the clinical part of the study. A written consent form was signed by each subject. The study protocol was approved by the Ethics Committee of Universidade Estadual de Campinas with the ethical principles described in the Declaration of Helsinki, guidelines for International Conference on Harmonization-Good clinical practices (ICH-GCP).

Drug products

Two products of memantine 10 mg tablets were studied. The test product was memantine 10 mg tablets (Clomenac[®], Arrow Farmacêutica Ltda) and the reference was Ebix[®] (Lundbeck Inc) 10 mg tablets.

Study design

The study was performed to compare the relative bioavailability of two memantine 10 mg tablet formulations under fasting conditions. A single-dose randomized-sequence, open-label, 2-period cross over design was used. A total of 26 subjects of both genders (13 men and 13 women) were admitted to the clinical site on the day before the beginning of the study. Since the evaluated memantine 10 mg tablets are immediate release formulations, a fasting study was performed [8]. Subjects underwent a 10 hour overnight fast and received at 7:00 a.m. either one tablet of Clomenac[®] 10 mg (test product) or Ebix[®] 10 mg (reference product) according to a randomization plan, along with 200 ml of water. All volunteers were then fasted 4 hours following the drug administration, after which a standard lunch was consumed. No other food was permitted during the "in-house" period. Liquid consumption was permitted ad libitum after lunch but xanthine-containing drinks including tea, coffee and cola were avoided. Systolic and diastolic arterial pressure, heart rate and temperature were recorded just before and hourly after drug administration.

For blood samples collection, a cannula was inserted to each subject's forearm vein and remains there until the 24-hour sample. Blood samples (8 mL) were collected into EDTA containing tubes before and 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 24, 36, 48 and 72 hours after administration of each memantine tablet. Plasma was obtained by centrifugation and stored into polypropylene tubes at -70°C until analysis by UPLC-MS/MS.

Determination of memantine plasma concentrations

Chemicals: Memantine reference standard was purchased from Sigma Aldrich Brasil (São Paulo, SP, Brazil) and amantadine (internal standard - IS) reference standard was purchased from the U. S.

Pharmacopeia (Rockville, MD, USA). Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA). Methanol (HPLC grade) was purchased from Tedia (Fairfield, OH, USA) and formic acid, diethyl ether, methylene chloride and sodium hydroxide (analytical grade) were obtained from J.T.Baker (Phillipsburg, NJ, USA).

Sample preparation: A 25 µL aliquot of the internal standard solution (200 ng/mL aqueous amantadine) and a 50 µL aliquot of 0.05 M aqueous sodium hydroxide were added to 200 µL of plasma sample. The sample was vortex mixed for 10 s. A 1 mL aliquot of diethyl ether and methylene chloride (7:3) was added and vortex mixed for 5 min. Then, the samples were centrifuged at 13200 rpm for 5 min, at 4°C. A 0.9 mL of the organic layer was transferred and evaporated to dryness. The dried extract was reconstituted in 200 µL of methanol and vortex mixed for 3 min. A 5 µL aliquot was injected into the chromatographic system (UPLC, Acquity system from Waters, Milford, MA, USA).

Chromatographic and mass spectrometric conditions: Memantine concentrations were determined employing an Acquity BEH C₁₈ column (50 x 2.1 mm i.d.; 1.7 µm particle size, Waters) and eluted with a mobile phase consisted of water and methanol (40:60) containing 0.05% formic acid (v/v), at a flow rate of 0.4 mL/min. The run time was 2.0 min and the injection volume was 5 µL. Mass spectrometric detection was performed using a Quattro Micro triple quadrupole mass spectrometer from Waters (Milford, MA, USA), equipped with an electrospray ion source in positive ionization mode. Nitrogen was used as both the nebulizing and the desolvation gas, and argon was used as the collision gas. The collision energies were optimized at 15 V for memantine and 18 V for IS. The Selected Reaction Monitoring (SRM) fragmentation transitions were m/z 180.3 → m/z 163.1 and m/z 152.0 → m/z 135.2 for memantine and amantadine, respectively.

Method validation: The validation process was carried out according to Guidance for Industry – Bioanalytical Method Validation, recommended by U.S. Food and Drug Administration [15]. Accordingly the method validation was evaluated in terms of selectivity, linearity, sensitivity, inter and intra-run accuracy and precision, recovery and stability under different conditions.

Pharmacokinetic and statistical analyses

Individual plasma concentration–time curves were constructed and pharmacokinetics parameters were calculated from plasma levels applying a non-compartmental statistic using Win Non Lin 6.1 software (Pharsight, USA). C_{max} was the observed maximum plasma concentration of memantine and T_{max} was the sampling time of the maximum plasma concentration. The area under the concentration–time curve (AUC_{0-t}) from time baseline to the last measurable concentration was obtained by the trapezoidal method. Since the estimated $t_{1/2}$ of memantine is around 60 hours, a truncated $AUC_{(0-72h)}$ was obtained, according to the recommendations of FDA Guidance [8]. The total area under the curve (AUC_{0-inf}) was calculated up to the last measured concentration and the terminal elimination rate constant (Ke). Ke was estimated from the slope of the terminal exponential phase of the memantine curve, by means of linear regression, applied to the semi-log plot of the concentration-time data. The elimination half-life, $t_{1/2}$, was then calculated as $\ln(2)/Ke$.

Analysis of variance (ANOVA) using log-transformed data was performed at the 5% significance level to evaluate period, formulation and sequence effects. The 90% CIs of the geometric means ratios (test/reference products) of C_{max} , AUC_{0-t} and AUC_{0-inf} were determined after log transformation of the data. Statistical analyses were performed

using Bioequivalence Wizard of Win Non Lin 6.1 software (Pharsight, USA). Bioequivalence was considered when the ratio of averages of AUC_{0-t} and C_{max} fell within a predetermined range of 80-125%.

Results

Method validation

Selectivity study showed that there are no significant interferences from endogenous compounds of plasma at the retention time of neither memantine nor IS. The calibration curve showed good linearity over the range of 0.1-50 ng/mL, with correlation coefficient higher than 0.99 and a weighted factor $1/x^2$. Under the described conditions, the obtained LLOQ was 0.1 ng/mL.

Intra-run precision ($n=5$) of the method ranged from 4.4 to 7.8%, while accuracy values were from 92.7 to 107.0%. Inter-run precision ($n=15$) ranged from 0.2 to 2.9% and inter-day accuracy were from 94.2 to 107.1%. According to these results the assay is accurate and precise for memantine in the studied concentration range.

The mean recovery rate of memantine ($n=15$), determined at three concentrations, was 54.4%. The mean recovery of the IS was shown to be 61.6% ($n=15$).

In the stability study, memantine proved to be stable in plasma samples for at least 6 h at room temperature and for 60 days at -70°C . In the post-preparative stability study, memantine was stable for 10 h after sample preparation, at 20°C , and after three complete freeze/thaw cycles on consecutive days. In addition, memantine and IS were found to be stable in working solutions for at least 5 days at 5°C and for 6 h at room temperature, as the mean changes in analyte content were within $\pm 15\%$ of initial concentration.

Tolerability analysis

Memantine was well tolerated at the administered dose. All biochemical parameters did not have any clinical relevant alterations. No serious adverse effects were either reported or observed.

Pharmacokinetic and statistical analysis

Two subjects drop out the period 2 for personal reasons, so that a total of 24 volunteers completed the clinical stage of the study. The mean plasma concentration-time curves of memantine obtained for the reference (R) and test (T) formulations are shown in Figure 2. The main pharmacokinetic parameters for both formulations were calculated (Table 1). On ANOVA of C_{max} and $AUC_{(0-t)}$ (using log-transformed data), no significant period, formulation or sequence effects were detected (Table 2).

Table 3 presents the ratios and the respective confidence intervals for bioequivalence analysis. All 90% CIs of the geometric mean ratios of C_{max} and $AUC_{(0-t)}$ fell within the predetermined range of 80% to 125%. These results suggested that the bioequivalence criteria were met.

Discussion

Pharmacokinetic and bioequivalence studies require sensitive and selective methods for the quantitation of drugs and active metabolites in biological matrices without interference of endogenous compounds [16]. Quantitation of memantine in biological matrices represents a challenge in the bioanalytical field, since the molecule does not possess chromophores, so that it is undetectable in the ultraviolet-visible range. In order to make possible the quantitation of memantine using fluorescence detection, pre-column derivatization procedures

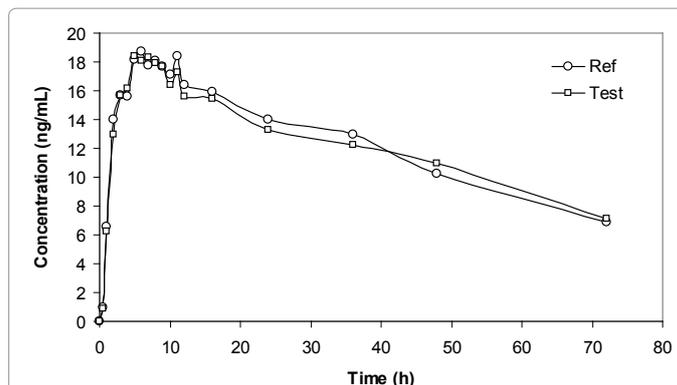


Figure 2: Plasma concentration – time curves of memantine after the oral administration of 10 mg tablets of reference and test formulations in 24 healthy volunteers.

Pharmacokinetic parameters	Reference	Test
C_{max} (ng/mL)	21.6 ± 5.1	21.8 ± 6.0
T_{max} (h)	7.8 ± 3.2	6.4 ± 2.2
AUC_{0-t} (ng.h/mL)	875.6 ± 167.3	865.6 ± 176.3
AUC_{0-inf} (ng.h/mL)	1313.2 ± 274.4	1422.4 ± 388.5
$t_{1/2}$ (h)	42.7 ± 12.1	52.2 ± 19.8

Table 1: Pharmacokinetic parameters (mean \pm standard deviation) of memantine after the oral administration of single dose of reference and test formulations ($n = 24$).

	P-values obtained for the effects of ANOVA test (Sequential)	
	C_{max}	AUC_{0-t}
Sequence	0.1382	0.2781
Formulation	0.4470	0.5353
Period	0.6021	0.3105
	P-values obtained for the effects of ANOVA test (Partial)	
	C_{max}	AUC_{0-t}
Sequence	0.1382	0.2781
Formulation	0.4805	0.6063
Period	0.6021	0.3105

Table 2: P-values obtained for the effects of ANOVA test.

involving the reaction of memantine with fluorescent compounds have been developed [10-13]. However, these methods are complex and time-consuming, besides present poor specificity, since other sample components could also be derivatized. In the last years, liquid chromatography coupled to mass spectrometric detection has almost completely replaced ultraviolet, electrochemical or fluorescence detection in the bioanalytical field, due to its unmatched sensitivity and extraordinary selectivity [17]. In the present work, we developed a rapid and high selectivity method, using ultra performance liquid chromatography coupled to tandem mass spectrometry, which allowed the direct quantitation of memantine in human plasma.

Chromatographic conditions were optimized to achieve good resolution and to increase the signal of analytes, as well as to minimize run times. Different mobile phases were evaluated and water and methanol (40:60) containing 0.05% formic acid (v/v) provided the most efficient chromatography. The use of smaller particles of stationary phase allows UPLC to increase the speed of analysis and

Parameters	C _{max}	AUC _{0-t}
Ratio T/R (%)	100.1	98.8
Lower limit (%)	92.9	93.9
Upper limit (%)	107.9	103.9
Within-subject coefficient of variation (%)	11.59	5.53
Between-subjects coefficient of variation (%)	26.61	20.88

Table 3: Statistical results of memantine pharmacokinetic parameters.

the chromatographic performance. The developed method offers the advantage over those previously reported using LC-MS/MS [14] showing a faster chromatographic total run time. Since a high number of samples are needed for pharmacokinetics and bioequivalence studies, the very fast UPLC separation combined with a low LLOQ (0.1 ng/mL) described in this work allows the application of the method for high throughput analysis.

In this study two formulations of memantine had been evaluated. Washout period was adequate, since there were no quantifiable concentrations of memantine before drug administration in the second period of the study, indicating that there was no carryover effect from the first to second period. The results of this bioequivalence study showed the equivalence of the two studied products in terms of the rate of absorption as indicated by C_{max} and in terms of the extent of absorption as indicated by AUC_(0-t). The mean plasma curves of both products are almost superimposable suggesting that not only C_{max} and AUC_(0-t) but also the time course of plasma levels over the whole sampling period are identical.

Conclusion

In this study in healthy, fasting, Brazilian, adult subjects, single doses of oral tablets of memantine 10 mg met the regulatory requirements to assume bioequivalence based on the rate and extent of absorption. Thus, the tested 10 mg memantine tablets (Clomenac[®], Arrow Farmacêutica Ltda) can be considered interchangeable in medical practice to reference formulation (Ebix[®], Lundbeck Inc).

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