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Bioequivalence between Two Tablets of Levetiracetam in Healthy Subjects under Fasting Condition

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Abstract

Bioavailability of two formulations containing levetiracetam 750 mg film-coated tablets was compared in a fasting bioequivalence study. The study was single dose, randomized, open label, and two-period crossover, with Brazilian healthy subjects, males and nonpregnant females. Blood samples were taken for 36 hours after drug administration and plasmatic concentrations were determined using a validated HPLC-MS/MS method. Confidence intervals (CI90%) for the peak plasma concentration (C_{max}) and area under the concentration-time curve (AUC_{0-t}) were determined by calculating LN-transformed data. The ratios and 90% CI for the geometric mean test/reference were 97.41% (91.45- 103.75%) for C_{max} and 99.77% (97.07- 102.54%) for AUC_{0-t}. Test (levetiracetam 750 mg film-coated tablets, Monte Verde S.A.) and reference (Keppra® 750mg film-coated tablets, UCB Biopharm S.A.) formulations were bioequivalent since the 90% CIs for the geometric mean test/reference ratios were within the ANVISA and FDA predetermined range of 80% to 125%.

Keywords: Levetiracetam; Bioequivalence; Pharmacokinetic; Mass spectrometry

Introduction

The active substance levetiracetam is a derivative of pyrrolidone [(S)-α-ethyl-2-oxo-1-pyrrolidine Acetamide] is an antiepileptic drug, indicated as monotherapy for the treatment of partial seizures, with or without secondary generalization in patients from 16 years of age with recent diagnosis of epilepsy [1,2]. It is also indicated as adjuvant therapy in the treatment of partial seizures with or without secondary generalization in adults, adolescents and children with age over 6 years with epilepsy. In Brazil is also indicated in myoclonic seizures in adults, adolescents and children over 12 years of age with juvenile myoclonic epilepsy, generalized primary tonic-clonic seizures in adults, adolescents and children older than 6 years old, with generalized idiopathic epilepsy [2].

The mechanism of action of levetiracetam is not yet fully elucidated but appears to be different from existing antiepileptic drugs. Possibly, the drug binds to a synaptic vesicle protein, SV2A which is believed to impede nerve conduction across synapses. *In vitro* and *in vivo* experiments suggest that levetiracetam does not alter basic cell characteristics or normal neurotransmission. *In vitro* and *in vivo* recordings of epileptiform activity from the hippocampus have shown that levetiracetam inhibits burst firing without affecting normal neuronal excitability, suggesting that levetiracetam may selectively prevent hypersynchronization of epileptiform burst firing and propagation of seizure activity [1-6].

Levetiracetam is rapidly and completely absorbed after oral administration. The pharmacokinetics are linear and time-invariant, with low intra- and inter-subject variability [1,2,6,7]. The extent of bioavailability of levetiracetam is not affected by food. Levetiracetam is not highly protein-bound (<10% bound) and its volume of distribution is close to the volume of intracellular and extracellular water, approximately 0,5 to 0,7 L/kg. Sixty-six percent (66%) of the dose is renally excreted unchanged. The major metabolic pathway of levetiracetam (24% of dose) is an enzymatic hydrolysis of the acetamide group. It is not liver cytochrome P450 dependent. The metabolites have no known pharmacological activity and are renally excreted. Plasma elimination half-life of levetiracetam across studies is approximately 6-8 hours. It is increased in the elderly (primarily due to impaired renal clearance) and in subjects with renal impairment by 40%. Absorption of levetiracetam is rapid, with peak plasma concentrations occurring around one hour following oral administration in fasted conditions. The oral bioavailability of levetiracetam tablets is 100%. The pharmacokinetics of levetiracetam are linear over the dose range of 250 to 5000 mg. Steady state is achieved after 2 days of multiple

twice-daily dosing. Levetiracetam and its major metabolite are less than 10% bound to plasma proteins, clinically significant interactions with other drugs through competition for protein binding sites are therefore unlikely. The major metabolite is inactive in animal seizure models. Two minor metabolites were identified as the product of hydroxylation of the 2-oxo-pyrrolidine ring (2% of dose) and opening of the 2oxo-pyrrolidine ring in position 5 (1% of dose). There is no enantiomeric interconversion of levetiracetam or its major metabolite. The total body clearance is 0.96 mL/min/kg and the renal clearance is 0.6 mL/min/kg. The mechanism of excretion is glomerular filtration with subsequent partial tubular reabsorption [1,2,6,7].

Objective

In this study we compared, in healthy subjects of both genders and in fasting condition, the pharmacokinetic profiles of levetiracetam to assess the bioequivalence between two formulations: 1) levetiracetam 750 mg film-coated tablets, manufacturing by Monte Verde S.A. and imported by Zodiac Produtos Farmacêuticos S.A. and 2) Keppra® 750 mg film-coated tablets, UCB Biopharm S.A (reference drug).

The Study was conducted in compliance with guidelines and standards for researches involving human beings from Resolutions no. 466/12 and 251/97 by the National Health Council - Ministry of Health, Good Clinical Practices according to ICH, and the Document of the Americas and in compliance with the Declaration of Helsinki (adopted by the 18th WMA General Assembly in Helsinki/ Finland, 1964, and with the last amendment by the 64th WMA General Assembly in Fortaleza/ Brazil, 2013). The protocol was submitted and approved before study initiation by the Ethics Committee of Instituto de Ciências Farmacêuticas (Goiânia – BR) accredited by National Research Ethics Commission (CONEP). After explaining the nature and purpose of the study, all subjects provided their written informed consent for participation.

Material and Methods

Population

Thirty-two (32) subjects of both genders (16 female and 16 male subjects) aged 18 to 50 years were screened. All subjects were considered eligible to participate in this study based on the inclusion and exclusion criteria defined in the study protocol. There were no restrictions on ethnic group.

All subjects showed good health conditions or the absence of significant diseases after assessment of medical history, verification of vital signs, physical examination, electrocardiogram, and routine laboratory tests. All subjects enrolled in the studies showed negative tests for hepatitis B (HBsAg and Anti-HBc IgM), hepatitis C and HIV and urine HCG (pregnancy test only for female subjects).

Study Treatments

The test formulation was levetiracetam 750 mg film-coated tablets (batch number: 81522), manufacturing by Monte Verde S.A. and imported by Zodiac Produtos Farmacêuticos S.A. and the reference was Keppra® 750 mg film-coated tablets (batch number: 188330, UCB Biopharm S.A. Before starting the clinical study, test and reference formulations were evaluated *in vitro* to check if they could be considered pharmaceutical equivalents. Tests described in the products specifications/pharmacopeia and dissolution profile was performed. Both products had very fast dissolution (>85% in 15 minutes). Test and reference drugs presented similar performance *in vitro* and were considered pharmaceutical equivalents.

Study Design

The study was conducted using an orally single dose, open-label, randomized, two-period, crossover, balanced design, in fasting conditions with a washout period of 7days between administrations. In each of the study periods, the subjects received a film-coated tablet containing 750 mg of levetiracetam from one of the two formulations mentioned above, with a 200-mL glass of water at room temperature. The drugs were administered after a minimum fasting of 8 hours and the subjects fasted for 4 hours after drug administration. To maintain the standardization, the diet (food and drink) followed the same standard for all subjects and in both periods. The study was conducted with fasting subjects to standardize the condition before receiving investigational drugs.

The intake of alcoholic beverages, food or beverages containing caffeine or xanthine (such as coffee, tea, chocolate and cola- or guarana-based soft drinks) was not permitted. In addition, the use of nicotine was prohibited from 48 hours before hospitalizations until the last blood draw, as well as any regular drugs (for at least 14 days) or occasional drugs (up to 7 days) before the hospitalization.

Blood samples (7.5 mL) were collected in coated tubes, containing Heparine as anticoagulant. The schedule of collects included the pre-dose and 00:10; 00:20; 00:30; 00:40; 00:50; 1:00; 1:10; 1:20; 1:30: 1:45; 2:00; 2:30; 3:00; 4:00; 6:00; 8:00; 10:00; 12:00; 24:00 and 36:00 hours after drug administration of each period. A total of 21 blood samples were collected from each subject in each period.

Immediately after collection, blood samples were centrifuged at 1,646g (3,500 rpm) for 10 minutes at approximately 4 °C. After centrifugation, the plasma was separated and transferred to two previously labeled cryotubes. The cryotubes were stored in freezer at -20 °C and were maintained at this temperature until the analysis.

Quantification of levetiracetam in human plasma

Plasma concentrations of levetiracetam were determined using reversed-phase high-performance liquid chromatography with tandem mass spectrometry (RP-HPLC-MS/MS). The analyte was extracted from plasma using precipitation extraction. Cimetidine Hydrochloride was used as the internal standard. To avoid inter-assay variations, all the samples from the same subject, in both periods, were assessed in the same analytical run.

The detection parameter used was the mass-to-charge ratio (m/z) between precursor ions and product, and the quantification parameter was the ratio of areas under chromatogram peak identified in the retention time between analyte and internal standard. Levetiracetam concentrations in subject samples were calculated using interpolation in the calibration curve.

The analysis was conducted in an HPLC Agilent 1200 Series coupled to the mass spectrometer API 3200 MS/MS with column Inertsil ODS-4 4.6x100mm 5µm, with a flow rate of 0.14 mL/min. The column was maintained at a temperature of 25 °C, while the autoinjector was maintained at 20 °C. The mobile phase used was ammonium acetate with 0.025% formic acid and methanol at a 55:45 ratio (v/v). The injection volume was 5 µL and the total run time set as 2 minutes. The mass spectrometry detection was conducted using electrospray ionization source in positive mode. The multiple reactions monitoring (MRM) method was used and the transitions monitored were *m/z* 171.120 > 126.000 and *m/z* 253.122 > 159.200 for levetiracetam and the internal standard, respectively.

The method was validated in compliance with ANVISA guidance for bioanalytical method validation, RDC Resolution no. 27, dated May 17, 2012 [8]. The validation parameters assessed were selectivity, linearity, intra and inter-run precision, intra- and inter- run accuracy, matrix effect, residual effect, and stability of levetiracetam under different conditions.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters were obtained from the levetiracetam plasma concentration-time curves. These parameters were statistically assessed for bioequivalence determination using software Phoenix WinNonLin version 6.4 and Microsoft Excel. The area under the plasma concentration-time curve was calculated using the linear trapezoidal method, from time zero to the last measurable concentration (AUC_{0-t}). The area under the plasma concentration-time curve was also calculated from time zero to infinity ($AUC_{0-\infty}$), where $AUC_{0-\infty} = AUC_{0-t} + Ct/z$, with Ct being the last drug concentration experimentally defined and z being the terminal phase elimination constant rate. The peak of maximum plasma concentration (C_{max}) of levetiracetam and the time to reach this peak (t_{max}) were obtained directly with no data interpolation. The elimination half-life (t½) was defined using the equation $t½ = \ln(2)/z$.

For the bioequivalence assessment between the formulations, AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were used. The model included a fixed effects term for sequence, period and treatment (ANOVA). Subjects (nested in sequence) were treated as a random effect. A 90% Confidence Interval (CI) was generated for the difference in averages of LN-transformed data from test and reference drugs. The antilog of obtained CI comprised the 90% CI for geometric mean ratio of primary parameters. The drug products are considered as bioequivalent if the bounds of the 90% CI generated for the geometric mean ratio for both primary parameters are equal or higher than 80% and equal or lower than 125%, as established by ANVISA and FDA [9,10].

Results

The validation method used allowed for the selective determination of levetiracetam in a linear range within 200 ng/mL to 40000 ng/ mL. The method developed was robust, selectivity, accurate and precise. There was no carryover effect observed during autosampler carryover experiment. Further, the extent of matrix effect in different lots of plasma was within the acceptable limit. The stability of the levetiracetam and its internal standard in human plasma and stock solutions were evaluated at different storage conditions. Levetiracetam and cimetidine hydrochloride were found to be stable in all tested conditions, including in plasma samples stored below -20 °C and -80 °C for 34 days.

In this study, in fasting condition, all of 32 healthy participants completed the two study periods. These participants had mean age of 33.28 years, ranging from 21 to 50 years; mean weight of 69.29 kg (53.6 to 87.55 kg); mean height of 1.66 m (1.50 to 1.76 m) and mean BMI of 25.06 kg/m² (18.69 to 28.60 kg/m²).

Levetiracetam was well tolerated at the administered dose in the study. No serious adverse events were seen or reported, and no pregnancies were detected. The most common adverse event was leukocyturia, reported by 34,4% of the participants. This event was not related with investigacional drugs and its cause was not investigated in this study.

The mean plasma concentration-time curves for test and reference drugs are shown in Figure 1. The curves were shown to be overlapped, showing a similar pharmacokinetic profile between the drugs. None of the subjects had concentration on the pre-dose collection time in the second period of study, showing adequate washout period.

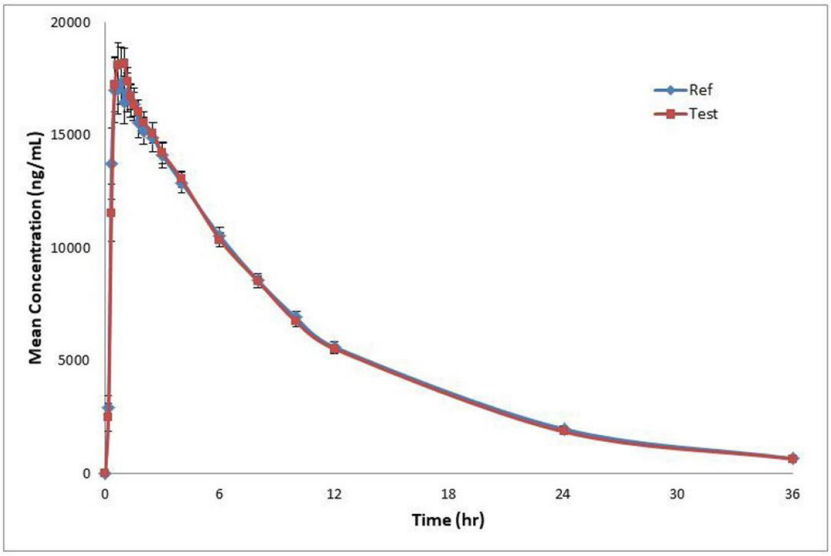

Figure 1: Mean curves of levetiracetam plasma concentration vs. time obtained for test and reference drugs under fasting conditions

The central location and dispersion measures for all pharmacokinetic parameters from both formulations are shown in Table 1.

Parameter	Test Drug	Reference Drug
C_{max} (µg/mL)	21299.3 ± 801.9 (21.3)	21910.5 ± 842.9 (21.8)
t_{max} (h)**	0.81 (0.33-2.0)	1.0 (0.33-6)
AUC_{0-t} (µg*h/mL)	187450.7 ± 5505.0 (16.6)	188845.6 ± 6439.0 (19.3)
$AUC_{0-\infty}$ (µg*h/mL)	194593.8 ± 5906.9 (17.2)	196616.7 ± 6821.5 (19.6)
t½ (h)	7.482 ± 0.156 (11.8)	7.678 ± 0.187 (13.8)

C_{max}: Maximum Plasma Concentration; **t_{max}: Time to Reach the Maximum Plasma Concentration (Median and Range); AUC_{0-t}: Area under the Plasma Concentration vs. Time Curve from Time 0 to t; $AUC_{0-\infty}$: Area under the Curve of Plasma Concentration vs. Time from Time 0 to infinity; t½: Elimination Half-Life

Table 1: Pharmacokinetic parameters [arithmetic mean ± SD (CV%)] of levetiracetam obtained after oral administration under fasting conditions

No significant differences were observed in the parameters C_{max} and AUC after reference and test treatments. Thus, the treatments were considered bioequivalent under study conditions.

Table 2 shows the test/reference geometric mean ratios for pharmacokinetic parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and the respective 90% CIs for the bioequivalence analysis. All 90% CIs were within the range of 80% to 125%.

Parameters	Geometric mean ratio(%)	Confidence interval (90%)	Intra-subject coefficient of variation (%)
Ln(C_{max})	97.41	91.45-103.75	14.95
Ln(AUC_{0-1})	99.77	97.07-102.54	6.46
Ln($AUC_{0-\infty}$)	99.44	96.71-102.25	6.56

Ln: Natural Logarithm; C_{max}: Maximum Plasma Concentration; AUC_{0-t}: Area under the Plasma Concentration vs. Time Curve from Time 0 to t; $AUC_{0-\infty}$: Area under the Curve of Plasma Concentration vs. Time from Time 0 to infinity

Table 2: Test/reference geometric mean ratios for pharmacokinetic parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and the respective 90% CIs for the bioequivalence analysis

Discussion

Two formulations are considered bioequivalent if the rate and extent of drug absorption doesn't show statistically significant differences when administered at the same molar dose of the active ingredient, under the same experimental conditions [9-11]. In this paper, the relative bioavailability of two formulations of levetiracetam was assessed after single-dose administration under fasting conditions. Single dose studies are considered more sensitive to assess bioequivalence when compared to multiple-dose study. The use of multiple-dose study for bioequivalence is recommended in Brazil only when the study is conducted in patients and/or the quantification of the drug in plasma is not possible after single dose.

With the purpose of obtaining a highly sensitive and rapid method for quantification of levetiracetam in plasma, a method by HPLC-MS/MS was developed and validated in this project. In the presented method the lower limit of quantification was 200 ng/mL, which allowed for a sensitive and efficient analysis of levetiracetam plasma concentrations.

The pharmacokinetic results (C_{max}, AUC, t_{max} and t½) found in this study for levetiracetam (Table 1) was very similar to those reported on the literature [7,12-14]. Test drug had a anticipate t_{max}, showing a slightly speeder absorption when compared to reference. As shown in Table 2, 90% CIs obtained for pharmacokinetic parameters defining bioequivalence (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) of formulations of levetiracetam 750mg was shown to be within the bioequivalence limits defined by ANVISA (80%-125%) in RE Resolution no. 1170, dated April 19, 2006 [9].

Antiepileptic therapy is frequently linked with particular risks in many of the drugs generally used, with a narrow therapeutic range, where changes in the rate and extent of absorption may have clinical relevance is written. In this study, the formulations contained levetiracetam showed bioequivalence even considering a narrower therapeutic range (90-110%).

Since the adverse events and laboratory test results were similar for both drugs in the evaluated condition evaluated (fasting), it was also possible to verify that test and reference drugs have similar safety profile and are well tolerated by patients.

Conclusion

Based on the pharmacokinetic and statistical results obtained in bioequivalence study conducted with administration of levetiracetam under fasting conditions, we conclude that the test drug product (levetiracetam 750, Monte Verde S.A.) and reference (Keppra® 750 mg, UCB Biopharm S.A) are bioequivalent. Thus, these drugs may be considered as being interchangeable in medical practice, i.e. having the same efficacy and safety profile.

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