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A Bioequivalence Study of Two Formulations of Levetiracetam

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Abstract

This study was conducted to compare the bioavailability of 1000 mg levetiracetam film-coated tablet, marketed in Italy as Italept®, with Keppra® coated tablet.

Twenty-four (24) healthy subjects were enrolled in a single-center, randomized, single-dose, laboratory-blinded, 2-period, 2-sequence, crossover study, with a minimum washout period of 7 days. Twenty (20) out of 24 subjects completed the study. Plasma samples were collected up to 36 hours post-dosing. Levetiracetam levels were determined using a validated LC-MS/MS method. The pharmacokinetic parameters used for bioequivalence assessment trough a non-compartmental analysis were: area under the plasma concentration-time curve from time zero to time t (AUC_t), from time zero to infinity (AUC_∞), and maximum observed concentration (C_{max}). The 90% confidence intervals obtained by analysis of variance resulted within the predefined ranges: 95.66 - 113.94% for C_{max} (Test to Reference ratio of geometric least squares means, LS means, 104.4%), 97.83 - 102.58% for AUC_t (Test to Reference ratio of geometric LS means 100.18%) and 97.33 - 101.82% for AUC_∞ (Test to Reference ratio of geometric LS means 99.55%).

Bioequivalence between formulations was achieved considering both rate and extent of absorption.

Keywords: Levetiracetam; Oral Absorption; Pharmacokinetics; Tablet; Urinary Excretion

Introduction

Levetiracetam (LEV) is a novel antiepileptic drug approved in the US in 1999 as an adjunctive therapy for adults with focal epilepsy. Nowadays it is registered, both in the EU and the US, as an adjunctive therapy for primary generalized tonic-clonic seizures, myoclonic seizures of juvenile myoclonic epilepsy and partial onset seizures, with or without secondary generalization.

In 2006 it was approved as monotherapy, but only in the EU, for adults and adolescents above 16 years of age with newly diagnosed focal-onset seizures with or without secondary generalization [1,2]. Its pharmacological mechanism is peculiar, and it differs from those of other antiepileptic drugs. It can bind to the synaptic vesicle protein 2A that can participate in the exocytosis of synaptic vesicles and regulate the release of neurotransmitters, especially excitatory amino acids, and thus depress the epilepsy discharge [3,4].

LEV is almost completely absorbed after oral administration and the absorption is unaffected by food. The bioavailability is nearly 100%. 66% of LEV is excreted unchanged by the kidney. Its major metabolic pathway is independent of hepatic cytochrome system and, for that reason; no clinically meaningful drug interaction with other antiepileptic drugs was found [5]. A published systematic review of LEV suggests that LEV has an equal efficacy compared with conventional antiepileptic drugs, and that it is well tolerated for long-term therapy without significant effects on the immune system [6].

Clinicians and main Regulatory Authorities (FDA and EMA) agree that a bioequivalence limit of 80 and 125% for the geometric means ratio of AUC and C_{max} is acceptable for most generic drugs and may grant the same efficacy and safety outcomes as the brand drug [7-9].

In a study conducted in order to compare the bioavailability of two tablet formulations containing 1000 mg levetiracetam, 18 healthy subjects were enrolled in a single-center, randomized, single-dose, open-label, 2-way crossover study, with a minimum washout period of 7 days. The 90% confidence intervals obtained by analysis of variance were 88.98 - 108.75% for C_{max}, 99.90 - 104.81% for AUC_{last} and 100.11 - 105.23% for AUC_{inf} [10].

The present trial was planned to evaluate and compare the relative bioavailability, and therefore the bioequivalence, of 1000 mg levetiracetam film-coated tablet marketed in Italy as Italept® with Keppra® coated tablet.

Material and Methods

Population

Twenty-four (24) Caucasian volunteers of both genders (10 female and 14 male subjects), aged between 18 and 53 years, were screened for the study. Both male and female volunteers were included in the study sample as, for this drug, no specific pharmacokinetic gender effects are known [5,11]. All volunteers were considered as being eligible to participate in the study and fulfilled all the inclusion and none of the exclusion criteria defined in the study protocol. Since LEV is mainly excreted via the urinary tract, this fact has been considered in inclusion/exclusion criteria. At the time of enrolment, all volunteers showed good health conditions and 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 study showed negative tests for hepatitis B (HBsAg and Anti-HBc IgM), hepatitis C, HIV and urine HCG (pregnancy test only for female subjects).

The protocol and the informed consent were approved by an institutional review board (ETHIPRO) on 2007/09/13. Guidelines as drawn up by the institutional review board were followed with regard to the treatment of human subjects in the study. These guidelines met the requirements of the Declaration of Helsinki; they also met the requirements of the U.S. Code of Federal Regulations (Title 21, part 56), the directive 2001/20/EC (Europe) and the Tri-Council Policy Statement (Canada). This study was conducted in compliance with Good Clinical Practice (GCP).

After explaining the nature and purpose of the study, all volunteers provided their written informed consent for participation.

Since gender effects on pharmacokinetics of LEV are thought to be likely related to differences in body weight and show no differences when weight is normalized [11], no specific gender analyses were foresaw.

Study Treatments

The following treatment was administered under fasting conditions: Test, one LEV Helm 1000 mg film-coated tablet (Helm is the owner of LEV that is marketed in Italy with the brand name Italept®) and Reference, one Keppra® 1000 mg coated tablet.

The products were administered to the 24 healthy volunteers according to the following design: Sequence 1 (n=12) Period 1/Test-Period 2/Reference; Sequence 2 (n=12) Period 1/Reference-Period 2/Test.

Test Batch Treatment No was: 26401; expiry date 01/2008; manufacturer Bluepharma Ind. Pharm S.A. Portugal.

Reference Batch Treatment No was: 33189; expiry date 11/2009; manufacturer UCB S.A. France.

Study Design

The study was a single center, randomized, single dose, laboratory-blinded, 2-period, 2-sequence, crossover design in healthy male and female Caucasian subjects. In each period subjects were asked to arrive at the clinical site at least 10 hours before dosing. After a supervised overnight fast, a single oral dose of the assigned formulation was administered with 240 mL of water at ambient temperature, starting at 8:00 a.m., to one subject per minute. Meals were provided no less than 4 hours after drug administration. Water was allowed *ad libitum* until 2 hours pre-dose and 2 hours after drug administration. Subjects were allowed to leave the clinical site after the 24-hour post-dose blood draw and were asked to return to the clinical site for the remaining blood sample collection. The wash-out period was of minimum 7 days; the duration of this study was expected to be approximately 10 days. Study participants were aware that they were receiving different formulations of a same drug although they were not aware of which product (Test or Reference) was administered. Enzyme-modifying drugs were not allowed for 28 days, subjects were instructed not to take any prescription medications for the 14 days prior to dosing and during the study, except for systemic contraceptives and hormone replacement therapy and any over-the-counter products for the 7 days prior to dosing and during the study (including cold preparations, ASA, vitamins and natural products used for therapeutic benefits and antacid preparations). Blood samples for pharmacokinetic measurements were collected prior to and up to 36 hours (serial sampling) after each drug administration. The first sample of each period, i.e. the blank plasma sample, was collected in two tubes of 4 mL (K_2 EDTA Vacutainers), while the others were collected 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24 and 36 hours after drug administration in a tube of 4 mL. As soon as possible following blood collection, samples were centrifuged at a temperature of 4 °C nominal and at 1500 g for 10 minutes. The plasma obtained was separated into duplicate polypropylene culture tubes, when feasible. The tubes were labelled with a code number that did not reveal formulation identity. The subject samples were collected between 2007/11/14 and 2007/11/22, including re-assays. The samples were frozen in an upright position and retained in the clinic's freezers at a temperature of -20 °C nominal for 39 days until sent on dry ice to the laboratory for assay. Clinical, analytical, and statistical stages of the study were conducted by Algorithme Pharmac Inc., located in the city of Laval, Quebec, Canada.

Method of Measurement

Plasma samples were received frozen by Algorithme Pharma's analytical facility. The experimental samples were assayed for LEV at the analytical facility of Algorithme Pharma using a validated LC-MS/MS method. The date of first sample collection through the last date of sample analysis spanned a period of 39 days. The long-term stability of LEV in human plasma covers 92 days at a temperature of -20 °C nominal [12].

Applied Biosystems API 3000 quadrupole mass spectrometer using a Turbo ion spray source and operating in positive ion mode was used for the detection of LEV; LEV concentrations were determined by a validated LC method using MS/MS detection according to internal SOPs. The lower limit of quantitation (LOQ) and upper limit of quantitation (ULQ) were 0.250 and 50.000 µg/mL, respectively.

The sample analysis of this study was conducted in compliance with Good Laboratory Practices (GLPs) as described in FDA Title 21 CFR part 58 in compliance with OECD Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17 (as revised in 1997), and under EMA regulation as stated in Research Protocol N. LVA-P7-234.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters of interest for this study were C_{max}, AUC_t and AUC_∞. Other parameters such as $AUC_{t/\infty}$, K_{el}, T_{max} and $T_{1/2el}$ were calculated for information purposes only. The natural logarithmic transformation of C_{max}, AUC_t and AUC_∞, as well as the rank-transformation of T_{max}, was used for all statistical inference. The main absorption and disposition parameters were estimated using a non-compartmental approach with a log-linear terminal phase assumption. The trapezoidal rule was used to estimate the AUC and the terminal phase was estimated by maximizing the coefficient of determination. However, they were not to be estimated for individual concentration-time profiles where the terminal log-linear phase could not be reliably characterized. All un-transformed and ln-transformed pharmacokinetic parameters were statistically analysed using a random Analysis for Variance (ANOVA) model. The fixed factors included in this model were the treatment received, the period at which it was given as well as the sequence in which each treatment was received. A random factor was also added for the subject effect (nested within sequence). The sequence, the period and the treatment effects were assessed at the two-sided 5% significance level. Furthermore, the 90% confidence interval for the exponential of the difference in Least Squares (LS) means between the Test and the Reference product (Test to Reference ratio of geometric LS means) was calculated for the ln-transformed parameters; C_{max}, AUC_t and AUC_∞ had to be within the 80 and 125% bioequivalence range. The formula used to estimate the intra-subject coefficient of variation was: $\sqrt{e^{MSE}-1}$, where MSE is the Mean Square Error obtained from the ANOVA model of the ln-transformed parameters. If a pharmacokinetic parameter could not be determined for one period in a subject, the corresponding subject was excluded from the particular statistical comparison. Statistical and Pharmacokinetic analyses were generated using Kinetic, version 8.00, an application developed at Algorithme Pharma and SAS® version 9.1 (mixed procedure).

Determination of Sample Size

Pharmacokinetic studies showed that LEV has a low intra-subject variation [11]. The intra-subject variation following a single dose of LEV appeared to be as high as 18% for C_{max} and around 5% for AUC_t. This Hypothesis done in 2008 seems to be reliable considering recent published data [13-15]. Statistically, given that the expected Test to Reference ratio of geometric LS means should fall within 92.2 and 105.7%, it was estimated that the lowest number of subjects to meet the 80 and 125% bioequivalence range with a statistical *a priori* power of at least 90%, was 20. Therefore, the inclusion of 24 subjects was estimated to be sufficient to also take into account the possibility of drop-outs.

Results

Out of 24 healthy participant volunteers 20 completed the study, 3 subjects withdrew their consent for personal reasons, 1 subject withdrew due to an adverse event (transient ischemic attack of moderate intensity). The volunteers had mean age of 37 years, ranging from 19 to 53 years; mean weight of 76.1 kg (60 to 91.1 kg); mean height of 1.69 m (1.48 to 1.82 m) and mean BMI of 26.5 kg/m² (20.3 to 29.2 kg/m²). LEV was well tolerated at the administered dose. No serious adverse event was seen or reported, and no pregnancy was detected. The mean curves for plasma concentration vs time obtained for test and reference drugs are shown in Figure 1. The curves are overlapped, showing a similar pharmacokinetic profile between the two drugs.
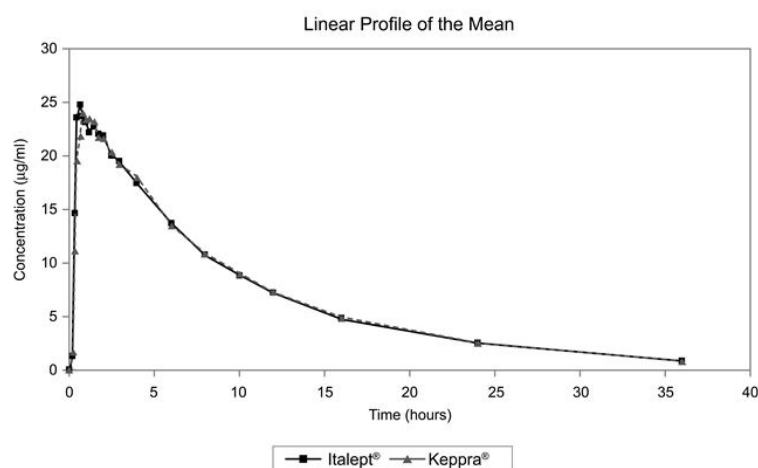

Figure 1: Mean Curve for Plasma concentration vs Time obtained for Italept® and Keppra®

Table 1 shows the main study results and Table 2 shows the comparison of results with standards for bioequivalence.

The mean elimination half-life ($T_{1/2el}$) was 7.43 (CV 12.5%) for Test and 7.64 (CV 13.7%) for Reference p=NS.

The $AUC_{t/\infty}$ was 96.57 (CV 1.3%) for Test and 95.57 (CV 2.3%) for Reference; p < 0.10.

PARAMETER	TEST		REFERENCE		F (treatment)	P*
	MEAN	CV (%)	MEAN	CV (%)		
C_{max} (µg/mL)	31.893	27.8	30.510	21.6	1.22	N.S.
ln (C_{max})	3.426	7.9	3.396	6.3	0.73	N.S
T_{max} (hours) §	0.75	55.5	0.83	52.1	1.43	N.S
AUC_t (µg h/mL)	241.691	16.9	240.767	15.3	0.09	N.S
ln (AUC_t)	5.474	3.1	5.472	2.8	0.02	N.S
AUC_∞ (µg h/mL)	250.351	17.1	250.850	15.0	0.01	N.S
ln (AUC_∞)	5.509	3.1	5.5142	2.7	0.12	N.S
$AUC_{T/\infty}$ (%)	96.57	1.3	95.97	2.3	3.85	<0.10
K_{el} (hour $^{-1}$)	0.094	13.2	0.092	12.4	2.69	N.S
$T_{1/2el}$ (hours)	7.43	12.5	7.64	13.7	2.08	N.S

*N.S.: Not Significant whenever *p*-value <0.05
§ For T_{max}, the median is presented and the statistical analysis is based on a rank-transformation
Table 1: Pharmacokinetic Parameters. Summary of Main Study Results

PARAMETER	INTRASUBJECT CV (%)	GEOMETRIC LS MEANS *		RATIO (%)	90% CONFIDENCE LIMITS (%)	
		TEST	REFERENCE			
C_{max}	16.0	30.967	29.662	104.40	95.66	113.94
AUC_t	4.3	237.893	237.467	100.18	97.83	102.58
AUC_∞	4.1	246.462	247.574	99.55	97.33	101.82

*Units are µg/mL for C_{max} and µg h/mL for AUC_t and AUC_∞
Table 2: Statistical Analysis. Comparison of Results with Standards for Bioequivalence

Discussion

A two-period, two-sequence crossover design was considered as the design of choice for the two formulation comparison, taking into account the European bioequivalence guideline and the pharmacokinetic profile of LEV [5,9,11,16,17]. LEV is approved in the EU in the 250, 500, 750 and 1000 mg formulations as film-coated tablets. The 1000 mg formulation was selected to test for bioequivalence. Both male and female volunteers were included in the study sample as, for this drug, no specific pharmacokinetic gender effects are known [5,11].

Healthy subjects were used, minimizing variability and thus allowing for a better comparison between the pharmaceutical products [9]. A minimum 7-day washout period was set, based on literature $T_{1/2el}$ of 6-8 hours [5,11,17], allowing proper elimination of the drug administered in the first period. The sampling schedule over 36 hours was considered enough to allow a full characterization of the plasma profiles and was confirmed by study results. The results of mean $AUC_{t/\infty}$ imply that the blood sampling schedule was defined adequately to characterize at least 80% of the AUC of both products.

Results showed in Table 2 clearly demonstrate that the 90% confidence interval of the C_{max}, AUC_t and AUC_∞ geometric LS means of the Test to Reference formulation are within the pre-specified 80 and 125% bioequivalence range [9].

Clinicians and main Regulatory Authorities (FDA and EMA) agree that a bioequivalence limit of 80 and 125% for the geometric means ratio of AUC and C_{max} is acceptable for most generic drugs and may grant the same efficacy and safety outcomes as the brand drug [7-9]. However, this could not be true in some specific therapeutic areas such as for the antiepileptics.

Antiepileptic therapy is frequently linked with particular risks in many of the drugs generally used, especially in older compounds, with a narrow therapeutic range, where changes in the rate and extent of absorption may have clinical relevance such as with carbamazepine, phenytoin, valproic acid and divalproex [18,19].

These latter drugs probably should need a narrow 90% confidence interval (90-110%).

Levetiracetam is a new generation AED with a wide therapeutic range that does not require such narrow interval [20,21].

One possible limitation of this study is that dissolution profiles of the formulations used in studies were not presented; this could be helpful to better correlate with the kinetics of the two formulations.

LEV pharmacokinetic data seem to be in line with published data. Furthermore, this study provides detailed information on the pharmacokinetics of a 1000 mg dose, for which little information can be found in the literature [5,10,11,17].

Conclusion

This bioequivalence study was well designed, demonstrating bioequivalence between 1000 mg levetiracetam film-coated tablet, marketed in Italy as Italept®, with Keppra® coated tablet (European reference formulation), in terms of both rate and extent of absorption. 90% confidence intervals of the main pharmacokinetic parameters, C_{max}, AUC_t and AUC_∞ comply with the 80 and 125% acceptance interval.

Acknowledgment

References

1. Verrotti A, Giulia L, Giangennaro C, Alberto S, Angelika M, et al. (2011) Pharmacotherapy for children and adolescents with epilepsy. Expert Opin Pharmacother 12: 175-94.

2. Bassel AK (2008) Levetiracetam in the treatment of epilepsy. Neuropsychiatr Dis Treat 4: 507-23.

3. Matagne A, Margineanu DG, Kenda B, Michel P, Klitgaard H (2008) Anti-convulsive and anti-epileptic properties of brivaracetam (ucb 34714), a high-affinity ligand for the synaptic vesicle protein, SV2A. Br J Pharmacol. 154: 1662-71.

4. Rigo JM, Hans G, Nguyen L, Rocher V, Belachew S, et al. (2002) The anti-epileptic drug levetiracetam reverses the inhibition by negative allosteric modulators of neuronal GABA- and glycine-gated currents. Br J Pharmacol 136: 659-72.

5. Patsalos PN (2003) The pharmacokinetic characteristics of levetiracetam. Methods Find Exp Clin Pharmacol 25: 123-9.

6. French J, Edrich P, Cramer JA (2001) A systematic review of the safety profile of levetiracetam: a new antiepileptic drug. Epilepsy Res 47: 77-90.

7. Bialer M (2007) Generic product of antiepileptic drugs (AEDs): Is it an issue? Epilepsia 48: 1825-32.

8. Chen ML, Shah V, Patnaik R, Adams W, Hussain A, et al. (2001) Bioavailability and bioequivalence: an FDA regulatory overview. Pharm Res 18: 1645-50.

9. EMEA (2001) Note for guidance on the investigation of bioavailability and bioequivalence. CPMP, London 2001: 1-18.

10. Franco Spínola AC , Almeida S, Filipe A, Neves RI, Tanguay M, et al. (2008) Bioequivalence of two formulations of levetiracetam. Int J Clin Pharmacol Ther 46: 591-6.

11. Radtke RA (2001) Pharmacokinetics of levetiracetam. Epilepsia 42: 24-7.

12. EMA/CHMP/323600/2011Committee for Medicinal Products for Human Use (CHMP) Assessment report, Matever. International nonproprietary name: levetiracetam Procedure No. EMEA/H/C/2024.

13. Clinical Pharmacology/Biopharmaceutics Review UCB Inc.

14. London, 19 May 2011 EMA/457042/2011 Committee for Medicinal Products for Human Use (CHMP) Assessment report Levetiracetam Teva International non proprietary name: levetiracetam Procedure No. EMEA/H/C/002316.

15. Boudriau1 S, Hanzel C, Massicotte J, Sayegh L, Wang J, et al. (2016) Randomized Comparative Bioavailability of a Novel Three-Dimensional Printed Fast-Melt Formulation of Levetiracetam Following the Administration of a Single 1000-mg Dose to Healthy Human Volunteers Under Fasting and Fed Conditions. Drugs R D 16: 229-38.

16. Stefan H, Feuerstein TJ (2007) Novel anticonvulsant drugs. Pharmacol Ther 113: 165-83.

17. Zhao Q, Jiang J, Li X, Lu Z, Hu P (2006) Single-dose pharmacokinetics of levetiracetam in healthy Chinese male subjects. Br J Clin Pharmacol 63: 614-7.

18. Ritchens A (1997) Impact of generic substitution of anticonvulsants on the treatment of epilepsy. CNS Drugs 8: 124-33.

19. Berg MJ (2007) What's the problem with generic antiepileptic drugs? A call to action. Neurology 68: 1245-6.

20. Jacob S, Nair AB (2016) An Updated Overview on Therapeutic Drug Monitoring of Recent Antiepileptic Drugs. Drugs R D 16: 303-16.

21. Shaw SJ, Hartman AL (2010) The Controversy over Generic Antiepileptic Drugs. J Pediatr Pharmacol Ther 15: 81-93.