Bioequivalence Evaluation of Two Formulations of Lamotrigine Tablets in Healthy Volunteers

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Abstract

Lamotrigine is a phenyltriazine used in the treatment of epilepsy and bipolar disorder type I. The purpose of this study was to compare the bioavailability in healthy Colombian volunteers of two brands of lamotrigine 100 mg tablets: a new generic formulation (test product) developed by Humax Pharmaceuticals S.A (Medellín, Col) and LAMICTAL® (reference product) from Glaxo Operations UK Ltd (Ware, UK). A single-dose, randomized, two-period, two-sequence crossover study, with six weeks washout period, was performed. Blood samples were obtained from 0 to 144 hours after dosing and plasma lamotrigine levels were determined by a validated high performance liquid chromatographic (HPLC) method. The 90% confidence intervals (CIs) for the ratios of the ln $AUC_{0-\infty}$ and ln C_{max} means between the reference and test formulations were constructed under 80/125 rule for bioequivalence limit. Fourteen subjects were enrolled in the study, but only twelve completed both treatment periods. The estimated pharmacokinetic parameters of lamotrigine for the reference and test formulations were C_{max} 2.314 ± 0.414 µg/mL, 2.226 ± 0.355 µg/mL; AUC_{0-120} 70.148 ± 10.824 µg.h/mL, 69.277 ± 13.432 µg.h/mL, and for $AUC_{0-\infty}$ were 78.524 ± 16.000 µg.h/mL, 77.532 ± 15.255 µg.h/mL, respectively. The 90% CIs for the ln-transformed ratio (test/reference) of $AUC_{0-\infty}$ and C_{max} were 88.97 to 110.65 and 87.77 to 106.37, respectively.

Conclusions: In this single dose study it was found that the test and reference products of lamotrigine 100 mg tablets complied with the regulatory criteria for equivalence with respect to rate and extent of absorption according to the guidances of Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA) and FDA.

Introduction

Epilepsy is one of the most common neurological disorders. It is usually controlled, but cannot be cured with medication. Although there are many anticonvulsant medications able to inhibit the development or spread of abnormal spontaneous electrical activity throughout the nervous system, several drugs are characterized by low effectiveness in certain epilepsy syndromes and a relatively high frequency of serious side effects. Newer anticonvulsants offer alternatives to replace or combine with older medications, therefore improving patient safety or enabling greater seizure control.

Lamotrigine was approved for the treatment of epilepsy by Food and Drug Administration (FDA) in 1994. It is currently used and recommended as monotherapy for partial seizures and bipolar disorder type I [1,2]. It may also be an option in add-on therapy for the treatment of partial seizures, generalized seizures of Lennox-Gastaut syndrome [3], and tonic-clonic seizure with primary generalization [4]. It has been proposed that the anticonvulsivant mechanism of action of lamotrigine consists in blocking voltage-gated sodium channels [5], thus inhibiting the release of the excitatory neurotransmitter glutamate [6].

Lamotrigine pharmacokinetics is characterized by complete absorption after oral administration, with an absolute bioavailability of 98% [7,8], reaching peak plasma concentration (C_{max}) between 0.5 to 4.0 hours. The apparent volume of distribution of lamotrigine ranges from 1.0 – 1.4 L/kg [9], its plasma protein binding is approximately 55% [9]. This drug is metabolized by glucuronic acid conjugation into inactive metabolites [10], and 94% of it is recovered in urine [8]. Elimination half-life of lamotrigine varies between 22.8 and 37.4 hours

[11] and the proposed therapeutic range for seizure control is 1 to 4 mg/L [11] although this has not been well-established [12].

In Colombia, the Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA) requires that manufacturers of anticonvulsant generic drugs ensure the quality of these lower cost alternatives through bioequivalence studies, before approving its marketing or renewal license. This strategy is based on the assumption that drugs that have similar plasma concentration profiles over time should produce a similar therapeutic effect.

This study was designed to assess the bioavailability and bioequivalence between a generic formulation of lamotrigine (test product) and the reference product LAMICTAL®, after oral administration of a single-dose.

Subjects and Methods

Study products

Test product, LAMOTRIGINE 100 mg immediate release tablets, was a mixture of the lots LP1607, LP1707 and LP1807 made on an equal number of tablets of each batch. The product was manufactured

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and provided by Humax Pharmaceutical S.A. (Medellín, Colombia). Reference product, LAMICTAL® lamotrigine 100 mg dispersible tablets, lot R306554B, manufactured by Glaxo Operations UK Ltd. (Ware, UK) was purchased from a local pharmacy.

Before the bioequivalence study, the products were tested for potency, dissolution and dosage uniformity through analytical methods used by the manufacturer of the test product in the Laboratorio Especializado de Análisis of the Facultad de Química Farmacéutica, Universidad de Antioquia. The products were judged suitable for the bioequivalence study if the assayed potency of the test product not differ from that of the reference product by more than 5%; the mean percentage dissolved in 30 minutes was not less than 80% and the acceptance value for dosage uniformity of the 10 dosage units is less than or equal to 15%.

Subjects

Healthy Colombian volunteers, nonsmoking, aged 18 to 30 years, and within 15% of ideal body weight for height (Metropolitan Life Insurance Company Statistical Bulletin, 1983) [13] were eligible to be enrolled in the study. Inclusion criteria encompassed no evidence of cardiac, pulmonary, gastrointestinal, hepatic, renal, hematologic, or neurologic disorders, or any acute or chronic disease, no history of drug or alcohol addiction, normal laboratory tests (complete blood counts, urinalysis, liver and kidney function, and fasting blood sugar); and serological negativity HIV, hepatitis B and blood pregnancy test (for females).

Subjects were informed by an investigator about the purposes and risks of the study and written informed consent was obtained from all volunteers. They were asked to abstain from using concomitant medications, including over-the-counter products, dietary supplements and natural products, two weeks prior to dosing and throughout the end of the study. Caffeine and/or xanthine-containing products or alcohol were not allowed 2 days before the first administration of the study medications and throughout the blood sampling periods. Subjects were required to fast for at least 12 hours before each scheduled dosing.

Study design

The protocol and informed consent form were reviewed and approved by the Ethics Committee of the Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia. The study was performed in compliance with the principles of the Declaration of Helsinki [14], the guidelines for Good Clinical Practice [15,16] and the Resolution No. 8430 of Ministerio de la Protección Social, República de Colombia [17].

This study was conducted as a single-dose, two treatment, two-period, two-sequence crossover design with a six weeks washout period. Using a table of random numbers, volunteers were assigned to receive lamotrigine 100 mg test or reference tablet and personnel conducting the study and the analyses were blinded in treatment assignment.

Study drug administration and blood sampling

Subjects were confined to the hospital one-hour prior to dosing of lamotrigine throughout completion of the 24 hour blood sampling period. During the first hour a urine sample was taken from each volunteer to assess illicit drugs or alcohol intake, blood pressure and pulse rate were monitored, and an indwelling intravenous cannula was inserted into an antecubital vein. A dose of the test or reference products was administered with 240 mL of water at room temperature.

Volunteers remained in semi-sitting position three hours after drug administration to ensure adequate gastric emptying and were kept at rest or walking on small areas during the time of confinement. The blood pressure and pulse rate of each subject were monitored at 2, 8 and 24 hours after dosing. During hospitalization, volunteers were under continuous medical surveillance, and for the duration of the additional sampling and washout period subjects maintained contact with the investigators to report any adverse events.

Subjects were allowed to have a fruit juice one hour after drug administration. They had standardized meals at 2.5, 5, 8, 11, and 24 hour after dosing (breakfast, lunch, break, dinner and next day breakfast).

Blood samples were obtained at 0, 0.3, 0.6, 1, 1.3, 1.6, 2, 2.5, 3.5, 6, 12, 24 hours. Subjects later on went to the Pharmacology and Toxicology laboratory of the Facultad de Medicina, Universidad de Antioquia for further sampling at 48, 96, 120, and 144 hours after drug administration. Blood was collected into 10 mL heparinized vacuum tubes (Vacutainer, Becton, Dickinson and Company, Franklin Lakes, New Jersey). Samples were centrifuged at 2000 rpm for 15 minutes, and then the plasma was aliquoted in two containers. One of them was frozen at -20°C until analysis. The other aliquot was frozen at -70°C, as retention sample for a period of five years.

Tolerability

Subjects were monitored for adverse events during both periods of the study and additional sampling visits. Subjects were asked to report any undesirable sign or symptom occurring after the start of the study. Adverse events (AE) were collected based on interview and spontaneous reports and recorded on a case-report form. The study physicians graded the AEs as mild, (it interferes with daily activities), moderate (it interferes with daily activity but it is still able to do it), or severe (it is disabling and requires medical attention).

Determination of plasma concentrations of lamotrigine

Plasma concentrations of lamotrigine were determined by a HPLC method previously reported by Matar et al. [18] with minor modifications included chloroform/ethyl ether (80/20, v/v) as the extraction solvent, carbamazepine as the internal standard and 10 mM potassium phosphate (pH 7.5)-methanol-acetonitrile (60:21:19) at a flow rate of 0.8 mL/min as the mobile phase.

A liquid chromatographic system (model 1100, Agilent Technologies, Palo Alto, California) with photodiode array detector set at 220 nm was used. Chromatographic separation was performed on a 250 x 4.0 mm, 5 μm, C18 column (LiChrospher® RP-Select B, Merck, Darmstadt, Germany). Samples were kept at room temperature in the autosampler, and 100 μL of solution was injected for analysis. Data acquisition and analysis were performed by ChemStation® software (Agilent Technologies, Palo Alto, California).

The Method was validated using established international guidelines [19]. Quality control samples (low 0.25 μg/mL and high 1.00 μg/mL) were included in each analytical run to assess its performance.

Pharmacokinetic and statistical analysis

Pharmacokinetic data were calculated by the non-compartmental method. The maximum plasma concentration (C_{max}) and the time to

reach it (T_{max}) were determined by inspecting each individual plasma level – time curves. The elimination rate constant (k_e) was obtained by ln-linear regression of the terminal decay phase. The area under the plasma level-time curve (AUC_{0-120h}) was obtained by the trapezoidal rule, and the $AUC_{120h-\infty}$ time was determined by dividing the 120 h plasma concentration by k_e, and adding this result to the AUC_{0-120h}.

Analysis of variance (ANOVA) for the cross design (Statistica 6.0, Statsoft Inc, 2001) was performed on ln-transformed data of $AUC_{0-\infty}$ and C_{max} in order to assess the effects of treatment, period, sequence of administration.

The data was also analyzed in relation to the assumptions of the two-formulation, two-period, two-sequence, crossover design to assess: carry over effect, intra-subject variability, intra e inter-subjects residuals normality, residual independence and outliers for t-r, t/r and ln t/r variables untransformed. Differences were considered statistically significant when the *p* value was equal to or less than 0.05.

To conclude bioequivalence the two - one side method suggested by Schuirmann [20] was used, taking into account the standard error of the ANOVA for each parameter. The 90% confidence intervals (CIs) for the ratios of the ln $AUC_{0-\infty}$ and C_{max} means between the reference and test products were constructed under 80/125 rule for bioequivalence limit.

Bioequivalence was accepted if theses CIs were within 80% and 125% of the reference mean.

Results

Study products

Results of potency, dissolution and dosage uniformity both the reference product and the test product are summarized in Table 1. The test product was different from the reference product by 1.7%. Both products met the requirements for the tests.

Subjects

The study was conducted in fourteen (14) Colombian subjects, six (6) men and eight (8) women aged between 19 and 26 years, with an average of 21.9 ± 1.9 years. Their weights ranged from 50.0 to 68.2 kg, with an average of 59.3 ± 5.5, and height ranged between 1.55 and 1.78 m, with an average of 1.65 ± 0.08.

In the second period, two subjects voluntarily withdrew, one belonging to the test product / reference product sequence and the other one to the inverse sequence. Statistical calculations were performed using data obtained from 12 volunteers who completed the test.

Tolerability

In the first period, one volunteer presented dizziness, nausea, vertigo and weakness. During the second period, another volunteer showed erythematous macules, not itchy, with irregular border in the anterior neck and chest.

Bioanalytical method validation

The analysis of six blank samples did not show interference in the chromatograms. The calibration curve of spiked plasma samples showed good linearity in the range of 0.10 - 2.5 µg/mL. Correlation coefficient calculated from curves constructed for interday (n=6) and intraday (n=6) evaluations were 0.9994 and 0.9996, respectively.

The limit of quantification of the assay was 0.10 µg/mL. Intraday accuracy (expressed on the basis of percent recovery) ranged from 95.4% to 100.3%, whereas intraday precision (expressed as coefficients of variation) ranged from 5.7% to 13.7%. Interday accuracy ranged from 96.5% to 107.8%, whereas interday precision ranged from 8.2% to 14.3%. The result obtained from the stability study showed that lamotrigine in plasma was stable kept at -20°C during the analysis period (4 months).

Pharmacokinetic and statistical analysis

The mean concentration-time profiles from the volunteers after oral administration of the products are shown in Figure 1 and 2. The mean (standard deviation) of the parameters $AUC_{0-\infty}$, C_{max} and T_{max} and other associated pharmacokinetic parameters are described in Table 2.

Product	Potency	Dissolution	Dosage Uniformity
Test	99.9	93.5 ± 2.4	7.4
Reference	101.6	91.3 ± 9.2	6.7

Table 1: Results of potency (mean percentage of active ingredient per tablet) dissolution (mean percentage of active ingredient dissolved) and dosage uniformity (acceptance value in percentage) tests to the test and reference products.
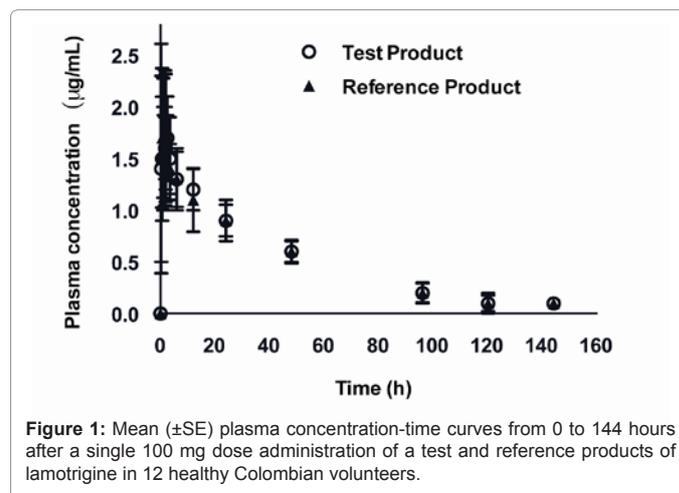
Figure 1: Mean (±SE) plasma concentration-time curves from 0 to 144 hours after a single 100 mg dose administration of a test and reference products of lamotrigine in 12 healthy Colombian volunteers.
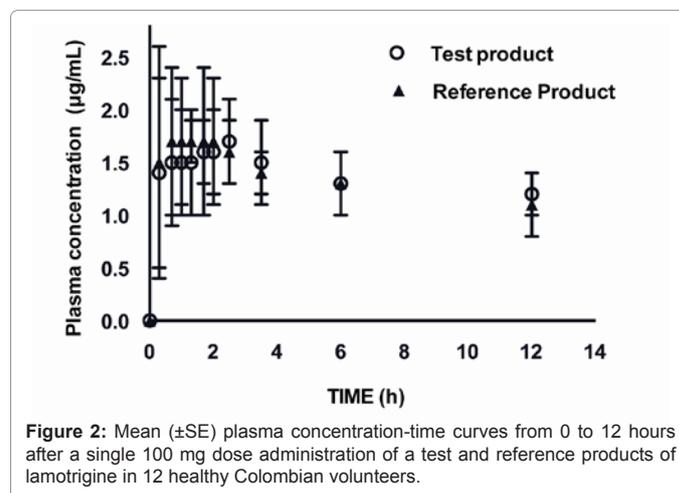
Figure 2: Mean (±SE) plasma concentration-time curves from 0 to 12 hours after a single 100 mg dose administration of a test and reference products of lamotrigine in 12 healthy Colombian volunteers.

None of the effects examined with ANOVA for $AUC_{0-\infty}$ and C_{max} (treatment, period, sequence) were statistically significant. Similarly, carryover effect was no found. Intrasubject variation was similar in both formulations, regarding these two parameters. The intra and inter subject residuals can be assumed normal and independent. No outliers were detected.

The ratios between the test and reference products of ln $AUC_{0-\infty}$ and ln C_{max} means and the 90% CIs obtained are shown in Table 3.

Discussion

The main objective of bioequivalence studies is to assure the efficacy and safety of generic formulations. Therefore, two formulations of the same drug are considered to be bioequivalent and ergo therapeutically equivalent if they exhibit a comparable extent and rate of absorption, when they are administered in the same molar dose and under similar experimental conditions [21].

In this study, the test and reference products were evaluated to assess its "similarity" *in vitro* prior the bioequivalence evaluation to ensure their quality. The results of the tests showed that both products met the requirements (Table 1) and the differences found in the potency and dissolution test were small, indicating that probably the products would have a similar performance *in vivo*.

This study was performed as a pilot study with 12 subjects, to establish values for μ_T/μ_R and σ^2 required to calculate the simple size according to the formula proposed by Julious [22], but once completed, and the parameters calculated, the sample size obtained was a total of six volunteers. In addition the posteriori calculated power of the study gave a value of 94%. These results indicated that a sample size of 12 subjects is adequate to conclude bioequivalence between these two formulations.

No adverse events necessitating subject withdrawal from the study were reported. The adverse reactions reported by two volunteers were resolved spontaneously at the end of each period and were considered mild. In both cases the event was presented with the test product and was consistent with effects reported for lamotrigine.

Lamotrigine plasma concentrations over the quantification limit (0.1 µg/mL) were observed within 0.33 to 120 hours after drug administration. Although blood samples were taken until 144 hours plasma concentrations at this time showed levels of 0.0940 ± 0.0329 µg/mL and 0.0864 ± 0.0442 µg/mL for the test and reference product, respectively. These values were not used to calculate pharmacokinetic parameters.

Pharmacokinetics parameters obtained in this research were similar to those found by Srichaiya et al. [23] in a study with the same drug, at the same dose in a group of healthy Thai male volunteers, using an HPLC method with high sensitivity LOQ (0.05 µg/mL) as shown in Table 4.

The values obtained for 90% CIs for the mean ratio (test/reference) of $AUC_{0-\infty}$ and C_{max} (Table 3) fell within the 80%–125% interval establish as bioequivalence limit [24]. It was concluded that the tested LAMOTRIGINE tablet (100 mg) elaborated by Humax Pharmaceutical S.A. is bioequivalent to the reference formulation, LAMICTAL® 100 mg, manufactured by Glaxo operations UK Ltd. in terms of rate and amount of absorption, according to the guidances of Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA) and FDA.

Acknowledgement

Parameter	Test product	Reference Product
C_{max} (µg/mL)	2.226 (0.355)	2.314 (0.414)
T_{max} (h)	1.380 (1.060)	0.933 (0.729)
k_e (h)	0.020 (0.004)	0.021 (0.005)
$t_{1/2}$ (h)	36.261 (6.801)	35.694 (9.476)
AUC_{0-120} (µg/mL)× h	69.277 (13.432)	70.148 (10.824)
$AUC_{0-\infty}$ (µg/mL)× h	77.532 (15.255)	78.524 (16.000)

Table 2: Pharmacokinetic parameters (mean (SD)) of lamotrigine after a single 100 mg oral dose of the two products in healthy Colombian volunteers (n=12).

Parameter	Ratio Test/Reference	IC 90%
C_{max} (µg/mL)	0.9584	87.77% - 106.37%
$AUC_{0-\infty}$ (µg/mL)×h	0.9982	88.97% - 110.65%

Table 3: Average ratio of test product / reference product and confidence intervals 90% of ln-transformed data for the parameters C_{max} and $AUC_{0-\infty}$.

Pharmacokinetic parameters	Colombian population		Thai population	
	Test	Reference	Test	Reference
$AUC_{0-\infty}$ (µg/mL . h−1)	77.5	78.5	74.9	74.3
C_{max} (µg/mL)	2.2	2.3	1.7	1.7
T_{max} (h)	1.4	0.9	1.2	1.4
$T_{1/2}$ (h)	36.3	35.7	35.0	34.7

Table 4: Comparison of lamotrigine pharmacokinetics obtained by Arunee Srichaiya et al. [23] and those obtained in this study.

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