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Bioequivalence Study of Two Oral-Capsule Formulations of Pregabalin 300 mg in Healthy Mexican Adult Volunteers

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Abstract

Pregabalin is a ligand for the alpha-2-delta subunit of voltage-gated calcium channels in the central nervous system. It has anticonvulsant, analgesic, and anxiolytic activity.

The bioequivalence of a test formulation was evaluated with respect to its corresponding reference drug formulation of oral pregabalin 300 mg, administered as a capsule.

This randomized-sequence, single-dose, single-blind, two-period crossover design under fasting conditions was done on 25 healthy Mexican adult subjects including both genders. There was a seven-day washout period.

Study formulations were given after an overnight fast (10 hours), and blood samples were collected at baseline was well as 0.16, 0.33, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours after administration.

The pregabalin in plasma was determined using HPLC-MS/MS.

The test and reference formulations were regarded bioequivalent if the 90% CIs for the geometric mean test/reference ratios were within a predetermined range of 80% to 125%.

The 90% CIs for pregabalin C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 94.55% to 107.51%, 95.40% to 102.92%, and 96.16% to 102.36%, respectively. This satisfied the regulatory requirements for assuming bioequivalence, based on the rate and extent of absorption.

Keywords: Pregabalin; Bioavailability; Bioequivalence

Introduction

Pregabalin is a ligand for the alpha-2-delta subunit of voltage-gated calcium channels in the central nervous system, which is structurally related to the neurotransmitter gamma-aminobutyric acid (GABA). It has anticonvulsant, analgesic, and anxiolytic activity [1,2].

Pregabalin is indicated for the management of neuropathic pain associated with diabetic neuropathy and postherpetic neuralgia, fibromyalgia and neuropathic pain associated with spinal cord injury. In addition, pregabalin is indicated as adjunctive therapy of refractory partial seizures and in the treatment of acute pain, generalized anxiety disorder and social phobia [2,3].

Pregabalin oral bioavailability is approximately 90% and has a linear pharmacokinetic profile. Its elimination half-life is approximately 6 hours [4].

Pregabalin is not subject to hepatic metabolism and does not induce or inhibit liver enzymes such as the cytochrome P450 system [1,5].

The sponsor of this study (Laboratorios Liomont, S. A. de C, V.) was interested in obtaining the marketing authorization for pregabalin 300 mg in Mexico (Garbican®, Laboratorios Liomont, S. A. de C. V., Mexico City, Mexico).

Therefore, the aim of this study was to compare the bioavailability and to determine the bioequivalence of a test formulation containing 300 mg of pregabalin, with its corresponding reference drug formulation (Lyrica® 300 mg capsules, Pfizer, S. A. de C. V.).

A literature survey using PubMed, MEDLINE and Google Scholar for the following terms through October 2015 showed no hits regarding the combination of terms: *pregabalin, bioequivalence, bioavailability, pharmacokinetics, capsules, Mexico, Mexican and population.*

Materials and Methods

The study protocol (P418S026V004) as well as the informed-consent documents were reviewed by an independent ethics and research committee (Comité de Ética e Investigación para Estudios en Humanos, Mexico City, Mexico).

This study was approved on July 2, 2012. We also received approval from the Federal Commission for Protection against Sanitary Risks (Comision Federal para la Proteccion contra Riesgos Sanitarios [COFEPRIS]) on September 13, 2012.

The study was performed according to the Declaration of Helsinki (and its subsequent amendments) and the International Conference on Harmonisation for Good Clinical Practice Guideline.

Subjects were informed of procedures, duration of the study, anticipated risks and discomfort it could entail by the principal investigator. All gave written informed consent. The clinical stage of the study was performed in November of 2012.

Inclusion/Exclusion Criteria

Healthy Mexican adults (male and females; 18 to 55 years) were considered eligible for this study.

Subjects were recruited from the volunteer's database at the Center of Pharmacological and Biotechnology Research (clinical unit) in Medica Sur Hospital, Mexico City, Mexico.

The health conditions of all candidates were evaluated. The evaluation included an interview and physical examination (blood pressure [BP], height, weight, temperature, heart rate, and respiratory rate). The diagnostic testing included a 12-lead electrocardiogram as well as chest radiography. The laboratory tests included hematology, blood count and chemistry, urinalysis, alcohol, drug-abuse and pregnancy in women. Serological tests included hepatitis B and C, as well as HIV antibodies. All tests were done at Medica Sur Hospital with certification from the Mexican government and the College of American Pathologists.

Other exclusion criteria included any alcohol consumption or tobacco consumption (48 hours prior to study or during the study).

Systolic and diastolic BP were determined with a sphygmomanometer (Tycos; Welch Allyn, Skaneateles Falls, NY). The BP cuff was applied to the right arm and the reading was taken with the subject in a seated position.

Subjects were to be excluded if laboratory values were considerably out of the reference range and/or if all tests had not been completed.

Prior to enrollment of the subjects, the laboratory data were reviewed and approved by the clinicians.

Study Design and Drug Administration

A single-dose randomized-sequence, single-blind, two-period crossover design under fasting conditions was used. The subjects were admitted to the clinical site on the day before the drug administration, and were randomly assigned by a pharmacist in the presence of quality assurance personnel at the clinical unit in a 1:1 ratio, using a computer-generated table of random numbers to one of the two sequences: test formulation capsules containing pregabalin 300 mg (lot 146B0004-A1; expiration date September 2014) followed by the reference formulation capsules containing pregabalin 300 mg (lot 0944081; expiration date July 2013), or vice versa.

To ensure reliable baseline plasma measurements, participants underwent a 10-hour overnight fast. Based on the reported plasma half-life of approximately 6 hours, a seven-day washout period was considered appropriate because it exceeds the seven half-lives required by COFEPRIS, as well as the number of half-lives required by FDA and EMA [6-8].

Blood samples were drawn for baseline plasma determinations in the following way. An 18-GA x 1.16 in (1.3 x 30 mm) indwelling angiocatheter (BD-InSyte, Becton Dickinson Ind. Cir. Ltda, Minas Gerais, Brazil) was inserted in a suitable forearm vein and a 7.5-mL blood sample was drawn into a lithium heparin-treated vacuum tube (S-Monovette, Sarstedt AG & Co., Nümbrecht, Germany).

Subjects were administered a single capsule of the test or the reference formulation with 250 mL of water. Additional blood samples were drawn at 0.16, 0.33, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours after administration.

During hospitalization, the subjects were under medical surveillance, and during the washout period participants maintained contact with the investigators to report any adverse events (AEs).

Plasma was obtained by centrifugation (3000 rpm for 15 minutes at room temperature) and stored at -70 °C±10 °C (until it was transported to the analytical unit where it was stored at -75 °C±5 °C until it was analyzed). After a seven-day washout period, participants returned to the clinical unit, where the alternative formulation was administered as in the first treatment period.

Subjects were asked to refrain from water and food intake for three hours after the study drug administration. Their diet, for each treatment period, consisted of three standardized meals (2088.6 kcalories/day) at 3.25, 8.25 and 12.75 hours after the study drug administration.

Determination of Pregabalin Plasma Concentrations

Chemicals: Pregabalin (lot: 22004830, purity 98.8%) secondary standard was obtained from Matrix Laboratories Limited (Andhra Pradesh, India). All solvents (including water) were HPLC-mass spectrometric grade (Avantor Performance Materials, Inc., Phillipsburg, NJ) and all reagents were of analytical grade (Mallinckrodt Baker, Inc., Phillipsburg, NJ).

Method and Sample Preparation: Pregabalin plasma levels were determined by using a HPLC method coupled with mass spectrometry (MS/MS) developed and validated by personnel of Biokinetics in Mexico City, Mexico. The method included the following: 250 µL of plasma, 20 µL of internal standard (metformin hydrochloride, 0.1 µg/mL) and 750 µL of acetonitrile. These components were vortexed in a 2.0-mL conical tube (Sarstedt AG & Co.) for one minute. The tube was centrifuged at 8000 rpm for five minutes at 20 °C. The supernatant was separated and injected (volume of injection = 1 µL) into the chromatographic system (HPLC, Agilent Technologies, model 1200, Palo Alto, California).

Chromatographic Conditions

Pregabalin concentrations were determined with a 50 × 4.6-mm internal-diameter column of 1.8-µm particle size (Zorbax® Extend-C18, Agilent Technologies) and eluted with a mobile phase consisting of a mixture (70:30 v/v) of an aqueous buffer solution (ammonium formate, 10 mM; 0.1% formic acid) and acetonitrile. The column temperature was 25 °C. Flow rate was maintained at 0.4 mL/minute and the pregabalin and metformin (internal standard) were detected by a triple-quadrupole mass spectrometer (Agilent Technologies, model G6410B). The spectrometric (MS/MS) analysis was performed by monitoring the transitions 159.9→97.2 m/z for pregabalin and 130.0→60.1 m/z for metformin. The spectrometric conditions were: positive-ionization mode, fragmenter energy (60 and 70 V for pregabalin and IS, respectively), collision energy (10 V), drying gas (nitrogen at 350 °C), gas flow (12 L/minute) and pressure (45 psi). Typical retention times for pregabalin and the internal standard were 1.2 and 1.1 minutes, respectively. Pregabalin peak areas were used for its quantitation.

Method Validation

The analytical method was validated according to Mexican and international guidelines [6,9].

The selectivity of the method was tested by the analysis of blank human plasma samples from six different subjects, blank human (hemolyzed and lipemic) plasma samples, as well as anticoagulants (heparin), xanthines (caffeine and theobromine), and other drug substances commonly used as analgesics (acetylsalicylic acid, ibuprofen, diclofenac, paracetamol and naproxen). No interferences were observed in the resulting chromatograms.

The calibration curve consisted of the following pregabalin concentrations 0.025, 0.10, 0.50, 1, 1.25, 1.75 and 2 µg/mL. Thus, the range of the method was 0.025 to 2 µg/mL, with lower limits of quantification (LLOQ) and of detection (LLOD) of 0.025 and 0.0125 µg/mL, respectively. The method was found to be linear within this range of concentrations with a coefficient of determination of 0.99. The intra-assay %CV and accuracy (relative error) for pregabalin were 1.28% to 3.11% and 0.71% to 8.90%, respectively, while the inter-assay %CV and accuracy were 2.81% to 5.39% and -4.77% to 6.65%. The absolute recovery was above 88%.

Pregabalin in plasma was found to be stable after 24 hours at room temperature (25 °C), after three freeze-thaw cycles and after 16 weeks at –75±5 °C.

Quality control (QC) samples were included in every analytical run to verify its performance. These QC samples were prepared at three different concentration levels (designated as low (0.05 µg/mL), medium (0.75 µg/mL) and high (1.50 µg/mL)) of pregabalin independent of the calibration curve. This method was considered suitable by the investigators for the bioequivalence study of pregabalin.

The acceptance criteria for the approval of the analytical runs and the QC samples, as well as the criteria for performing sample reanalysis, were in accordance with Mexican and international guidelines [6,9].

Tolerability

Tolerability was determined using clinical assessment, monitoring of vital signs at baseline, 2 and 6 hours after the drug administration during hospitalization, and at the end of the clinical stage of the study.

The subjects were interviewed (using open-ended questions) by the investigators during the conduct of the clinical trial and at the end of the clinical stage of the study, concerning the occurrence of AEs. Subjects were asked to spontaneously report any AE to the investigators at any time during the study, including the washout period. Data for all AEs were recorded on a case-report form.

AEs that were life-threatening, led to death, hospitalization, disability, and/or medical intervention to prevent permanent impairment or damage, were considered serious.

Pharmacokinetic and Statistical Analyses

In this study, a sample size of 26 subjects was planned because, at the time the study was conducted, a minimum sample size of 24 subjects was required for bioequivalence studies by COFEPRIS [6].

Individual plasma concentration–time curves were constructed; C_{max} and T_{max} were directly obtained from these curves, the area under the plasma concentration-time curve from time baseline to the last measurable concentration (AUC_{0-t}) was calculated by a non-compartmental method using the linear trapezoidal rule. From the terminal log-decay phase, the elimination rate constant (k_e) was estimated using linear regression, and the t½ was estimated using the following equation [10]: $t_{½} = \ln 2/k_e$, where *ln* was defined as the natural logarithm. Extrapolation of AUC from baseline to infinity ($AUC_{0-\infty}$) was calculated as follows:

$AUC_{0-\infty} = AUC_{0-t} + C_t/k_e$,

where C_t was the last measurable plasma concentration.

To assess the bioequivalence between the test and reference formulations, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were considered as the primary variables. ANOVA for a 2 x 2 crossover design, using log-transformed data for these parameters, was carried out at the 5% significance level ($\alpha = 0.05$).

The 90% CIs of the geometric mean ratios (test/reference) of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were calculated using log-transformed data. The test and the reference formulations were considered bioequivalent if the 90% CIs of these parameters fell within a predetermined range of 80% to 125%. All pharmacokinetic and statistical analyses were performed using WinNonlin Version 5 (Pharsight, Mountain View, California).

Results

Table 1 shows the demographic characteristics for a total of 26 subjects, who were enrolled in the study.

Characteristic	Values
Total No. of subjects (female/male)	26 (9/17)
Age, mean (SD), range, years	34 (11), 18-51
Weight, mean (SD), range, kg	64.82 (6.82), 53.70-79.30
Height, mean (SD), range, m	1.63 (0.09), 1.47-1.76
BMI , mean (SD), range, kg/m^2	24.31 (1.41), 20.24-25.93

BMI = Body mass index

Table 1: Demographic characteristics of subjects

One subject was withdrawn from the study, at the second period, because the subject reported pharyngitis (which was regarded as not related to the study formulations by the principal investigator) and was treated with concomitant medications. Thus, the sample size for the evaluation of bioequivalence was reduced from 26 subjects to 25 subjects.

Pharmacokinetic Parameters

Mean plasma concentration-time curves of the two formulations are shown in Figure 1. suggests comparable mean plasma concentration-time curves.

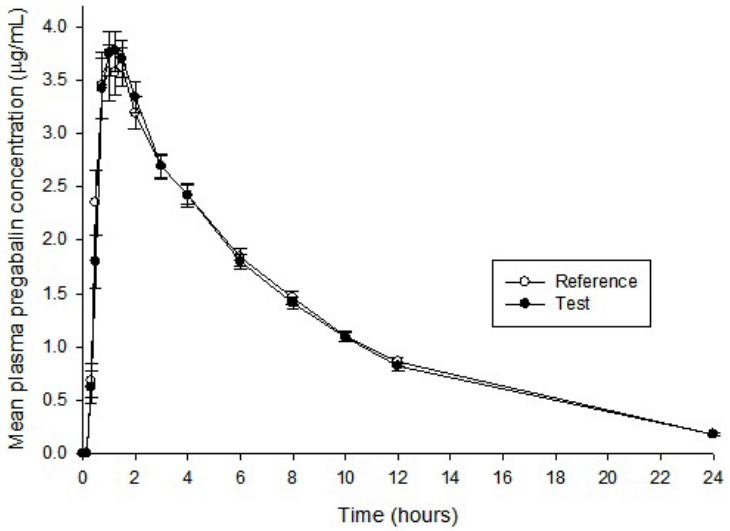

Figure 1: mean plasma concentration-time curves after a single dose of a test (trademark: Garbican®, Laboratorios Liomont, S.A. de C.V.,Mexico) and a reference (trademark: Lyrica®, Pfizer, S.A. de C.V., Mexico) formulation of oral pregabalin 300 mg in healthy Mexican adult subjects (n = 25)

Table 2 shows the pharmacokinetic parameters (C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t} and $AUC_{0-\infty}$) for both formulations.

Parameter	Reference†	Test*
C_{max}, µg/ml	4.29 (1.06)	4.30 (0.94)
AUC_{0-t}, µg•h /ml	28.97 (5.83)	28.61 (4.92)
$AUC_{0-\infty}$, µg•h/ml	30.81 (5.90)	30.48 (4.89)
T_{max}, h	1.43 (0.93)	1.24 (0.57)
$t_{1/2}$, h	5.50 (0.91)	5.59 (0.92)

C_{max} = Maximum plasma drug concentration
AUC_{0-t}= AUC from time 0 (baseline) to the last measurable concentration
$AUC_{0-\infty}$ = AUC from baseline extrapolated to infinity
*Trademark: Garbican® (Laboratorios Liomont, S. A. de C. V., Mexico City, Mexico)
†Trademark: Lyrica® (Pfizer, S. A. de C. V., Mexico City, Mexico)
Table 2: Pharmacokinetic parameters of a reference and a test formulation of oral pregabalin 300 mg after a single-dose administration in healthy Mexican adult subjects (n = 25). Values are mean (SD)

No significant period or sequence effects were detected based on the ANOVA of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ (data not provided).

Table 3 shows the bioequivalence statistics using the log-transformed data of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$: geometric means, geometric mean ratios (test/reference), 90% CI, and the intra-subject %CV.

Parameter	Geometric Means Test/Reference	Geometric Mean Ratio (%)	90% CI	Intra-subject %CV
C_{max}, µg/mL	4.21/4.17	100.82	94.55, 107.51	13.30
AUC_{0-t}, µg•h/mL	28.25/28.51	99.09	95.40, 102.92	7.83
$AUC_{0-\infty}$, µg•h/mL	30.14/30.38	99.21	96.16, 102.36	6.44

C_{max} = Maximum plasma drug concentration
AUC_{0-t} = AUC from time 0 (baseline) to the last measurable concentration
$AUC_{0-\infty}$ = AUC from baseline extrapolated to infinity
Table 3: Geometric means, geometric mean ratios, 90% CIs and the intra-subject %CV of the pharmacokinetic parameters determined for pregabalin after a single-dose administration of 300 mg in healthy Mexican adult subjects

The 90% CIs for pregabalin C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 94.55% to 107.51%, 95.40% to 102.92%, and 96.16% to 102.36%, respectively. The 90% CIs of the geometric mean ratios of the three parameters fell within the predetermined range of 80% to 125%. It is important to mention that although the data obtained from the subject who did not complete the study (only the first period) were not included for the bioequivalence evaluation, a separate statistical analysis, which included data for this subject from the first period, revealed that the bioequivalence conclusions were not affected (data not provided).

Therefore, these results indicate that the bioequivalence criteria were met.

Tolerability

Twenty four of the 26 subjects reported AEs after the administration of both formulations. Most of them correspond to central nervous system and gastrointestinal AEs previously described for the study drug [11,12]: These were dizziness (19 subjects, 73.1%), somnolence (19 subjects, 73.1%), nausea (8 subjects, 30.8%), vomiting (5 subjects, 19.2%) and headache (3 subjects, 11.5%). The frequencies of these AEs were similar for both formulations. However, no statistical analysis was performed to compare the formulations in this regard because we considered that the sample size of 26 subjects to be too small to obtain conclusive results.

Other AEs included diplopia (one subject), fatigue (one subject), tongue paresthesia (one subject) and pharyngitis (one subject).

None of the AEs was considered serious. Instead, all of them were regarded as mild or moderate, and all of the AEs spontaneously resolved under medical surveillance during the clinical stage, except for the subject with pharyngitis who was treated with concomitant medications and withdrawn from the study.

Discussion

All of the 90% CIs of the geometric mean ratios of the pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were found to be within the predetermined range of bioequivalence (80%-125%). These results indicate that the bioequivalence criteria were met.

Both formulations were well tolerated, in view of that fact that none of the reported AEs was considered serious and all had been previously reported or regarded as not related to the study drug.

As with any clinical trial, and in particular with most bioavailability studies, the current study had some limitations that should be considered. First, this was a single-blind study, so it might not objectively address the effectiveness and safety profiles of the formulations tested.

The data were obtained from healthy adult subjects, in accordance with regulatory requirements (COFEPRIS), within a specific age range, who were administered a single dose of the formulation. The pharmacokinetic parameters of pregabalin might differ in target populations. Thus, the results of this study might not be generalizable to a target population.

In addition, this study was conducted under fasting conditions because it has been reported that the administration of pregabalin with food has no clinically relevant effect on its extent of absorption, based on AUC data [1,2].

Further studies are needed to compare the test formulation with the reference formulation in Mexican patient groups. The results of this study might serve as a reference for future controlled studies of pregabalin in a Hispanic population.

Conclusions

In this study of healthy, fasting, Mexican adult subjects, who received a single dose of either the test or reference formulation, it was concluded that the test formulation of pregabalin 300 mg met the Mexican regulatory requirements to assume bioequivalence, based on the rate and extent of absorption. Both formulations were well tolerated.

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