Bioavailability of Two Coated-Tablet Formulations of a Single Dose of Pantoprazole 40 mg: An Open-Label, Randomized, Two-Period Crossover, Comparison in Healthy Mexican Adult Volunteers

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Abstract

Pantoprazole is a proton pump inhibitor indicated for the treatment of acid-related gastrointestinal diseases such as reflux esophagitis, duodenal and gastric ulcers. The aims of this study were to compare the bioavailability and to determine the bioequivalence of a test and reference formulation of oral pantoprazole 40 mg, administered as a coated tablet, and to generate data regarding the oral bioavailability of this drug in the Mexican population. This single-dose, randomized-sequence, open-label, 2-period crossover study was conducted on a total of 34 healthy Mexican adult subjects of both genders, with a 7-days washout period. Study formulations were administered after a 10-hour overnight fast. For pharmacokinetic analysis, blood samples were drawn at 0 (baseline), 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2.0, 2.5, 3, 3.5, 4, 5, 6, 8, and 10 hours after administration. Plasma concentrations of pantoprazole were determined using HPLC coupled to a UV detector. The test and reference formulations were to be considered bioequivalent if the 90% CIs for the geometric mean test/reference ratios were within a predetermined range of 80% to 125%.

The estimated pharmacokinetic parameters of pantoprazole for the reference (Pantozol®) and test (Prazolan®) formulations were C\text{max} (3448 ± 1214 ng/ml, 3610 ± 1344 ng/ml); AUC\text{0–t} (5521± 2454 ng•h /ml, 5720 ± 2527 ng•h /ml) and AUC\text{0–∞} (6997 ± 2548 ng•h /ml, 6292 ± 2548 ng•h /ml), respectively. The 90% CIs for the geometric mean ratios of C\text{max}, AUC\text{0–t}, and AUC\text{0–∞} were 90.13% to 117.04%, 92.45% to 113.18%, and 94.50% to 108.16%, respectively. In this study a single dose of the test formulation met the regulatory requirements to assume bioequivalence, based on the rate and extent of absorption.

Keywords: Pantoprazole; Bioequivalence; Bioavailability; Pharmacokinetics; HPLC

Introduction

Pantoprazole, (R,S)-6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-1H-benzo[d]imidazole, is a proton pump inhibitor [1,5] indicated for the treatment of acid-related gastrointestinal diseases such as reflux esophagitis, duodenal and gastric ulcers [8,2]. Its absolute bioavailability is 77% and it is not affected by multiple dosing. Pantoprazole shows linear pharmacokinetics after both i.v. and oral administration. It is extensively metabolized in the liver, has a total serum clearance of 0.1 l/h/kg, an elimination half life of approximately 1.1 hour, and an apparent volume of distribution of 0.15 kg/l. Almost 80% of an oral or intravenous dose is excreted as metabolites in urine; the remainder is found in feces and originates from biliary secretion. The bioavailability of pantoprazole is not affected by the concomitant intake of food [7].

The test (Prazolan®, Laboratorios Liomont, S. A. de C. V., Mexico City, Mexico) and reference [9] coated-tablet formulations are marketed in Mexico. Pantozol® was selected as the reference formulation [9] because it is included in the list of Drug Reference Medications issued by the Mexican Federal Commission for the Protection Against Sanitary Risks [4]. It is important to point out that the reference medications (formulations) indicated in this list are mandatory for the bioequivalence studies performed in Mexico. The test formulation was selected because the sponsor of the present study wanted to obtain the renewal of its marketing authorization in Mexico.

A search of PubMed, MEDLINE and Google data bases for literature published up to may of 2011, using the combination terms pantoprazole, bioequivalence, bioavailability, pharmacokinetics, Mexico, Mexican and population, did not identify any published data concerning the bioavailability of oral pantoprazole in the Mexican population. Therefore, the aims of this study were to compare the bioavailability and to determine the bioequivalence of a test and reference formulation of oral pantoprazole 40 mg and to generate data regarding the oral bioavailability of pantoprazole 40 mg in the Mexican population for the purpose of renewing marketing authorization of the test formulation in Mexico.

Subjects, materials and methods

The study protocol (P3275034/V004) and the informed-consent form were approved by an independent ethics and research committee of Medica Sur Hospital (Mexico City, Mexico) on August 11 of 2010. The study was conducted in accordance with the principles of Helsinki and its amendments and the International Conference on Harmonisation Guideline for Good Clinical Practice. The principal...
investigator informed the subjects of all procedures, duration of the study, anticipated risks and discomfort it could entail, and an individual written informed-consent was obtained prior to the initiation of the study. The study was conducted from August to October of 2010.

Inclusion/exclusion criteria

Healthy Mexican adults aged 18 to 55 years and of either gender were eligible for inclusion. Subjects were recruited from the out-patient records retrieval database within the Pharmacological Research Unit (clinical unit) at Medica Sur Hospital, Mexico City, Mexico.

A physical examination was conducted in each participant. Subject’s health was based on unremarkable findings on a clinical health evaluation, which consisted on the following: a personal interview; complete physical examination (blood pressure [BP], heart rate, weight height, temperature and respiratory rate); and diagnostic testing that included 12-lead ECG, chest radiography, and laboratory testing (complete blood cell count, metabolic and liver function tests [alanine and aspartate amino transferase], biochemistry [glucose, blood urea, nitrogen and creatinine, and serological tests for hepatitis B and C and HIV antibodies], urinalysis, and a pregnancy test in women. Systolic and diastolic BP was measured with a sphygmomanometer (Tyco; 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. Candidates were excluded if laboratory values were significantly out of the reference range and/or if all tests had not been completed. Laboratory testing was performed at Medica Sur Hospital, which has been certified by the Mexican government and the College of American Pathologists. The scope of the certifications included the laboratory data were reviewed by investigators at the clinical site. Before the enrollment of the participants, the laboratory data were reviewed by investigators at the clinical unit. Selected candidates were compensated for their participation.

Study design and drug administration

A single-dose randomized-sequence, open label, 2-period crossover design was used. A total of 34 subjects (17 men, 17 women) were admitted to the clinical site on the day before the study was begun and were randomly assigned by the quality assurance personnel at the clinical unit, in a 1:1 ratio using a computer-generated table of random numbers, to 1 of the 2 sequences (test formulation containing pantoprazole [lot 10-VI-46; expiration date; June 30, 2012] followed by the reference formulation or vice versa). Randomization codes were concealed from all the investigators of the study.

To ensure reliable baseline plasma measurements, participants underwent a 10-hour overnight fast with a 7-day washout period, which excludes the 7 half-lives required by COFEPRIS. Blood samples were drawn for baseline plasma determinations in the following way. An 18-G x 1.6 in (1.1 x 30 mm) indwelling angiocatheter (BD-InSyte, Becton, Dickinson and Co., Sao Paulo, Brazil) was inserted in suitable anatomical veins and 7.5-ml blood sample was drawn into heparin- treated vacuum tube (S-Monovette, Sarstedt AG & Co., Nümbrecht, Germany).

Subjects were administered a single 40-mg coated tablet of the test or the reference formulation with 250 ml of water. Additional blood samples were drawn at 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 10 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 event (AEs). Plasma was obtained by centrifugation (1000 g for 15 minutes at 25°C) and stored at -75°C±5°C until analyzed using HPLC. After 7-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 3 hours after study drug administration. Their diet, for each treatment period, consisted of 3 standardized meals (1464 kcal/d) at 3, 7 and 10 hours after study drug administration.

Determination of pantoprazole plasma concentrations

Chemicals: (R/S)-Pantoprazole sodium (lot: 10H266) and (R/S)-omeprazole (IOF081) reference standards were obtained from the USP (Rockville, MD). All solvents were HPLC grade (Avantor Performance Materials, Inc., Phillipsburg, NJ) and all reagents were analytical grade (Mallinkrodt Baker, Inc., Phillipsburg, NJ).

Method and sample preparation: Pantoprazole plasma levels were determined by using a HPLC method developed and validated by personnel of Biokinetics at Mexico City, Mexico. The method included the following: 500 µl of plasma, 10 µl of internal standard (omeprazole, 100 µg/ml) and 500 µl of acetonitrile. These components were vortexed in a 2.0-ml conical tube, (Sarstedt AG & Co.) for 1 minute. The tube was cooled in a freezer (-75°C±5°C) for 1.5 minutes and then centrifuged at 3500 rpm for 15 minutes at room temperature (25°C). The supernatant was separated and injected (volume of injection = 20 µl) into the chromatographic system (HPLC, Agilent Technologies, model 1200, Palo Alto, California).

Chromatographic conditions

Pantoprazole concentrations were determined with a 150 × 4.6- mm internal-diameter column of 5-µm particle size (Zorbax SB-C18, Agilent Technologies) equipped with a pre-column (125 mm × 4.6-mm internal-diameter, 5-µm particle size, Zorbax SB-C18, Agilent Technologies) and eluted with a mobile phase consisting of a mixture (60:40 v/v) of an aqueous buffer solution (ammonium acetate, 10 mM; pH 3.0 ± 0.1) and acetonitrile. The column temperature was 25°C. Flow rate was maintained at 1 ml/minute and the pantoprazole was detected by a UV (Agilent Technologies) detector set at a wavelength of 302 nm. Typical retention times for pantoprazole and the internal standard were 4 and 3 minutes, respectively. The peak area was measured for calculation of the peak area ratio of pantoprazole with respect to the internal standard, and the concentration was calculated.

Method validation

The analytical method was validated according to Mexican [4] and international guidelines [1]. The selectivity of the method was tested by the analysis: of blank human plasma samples from 6 different subjects; blank human (hemolyzed and lipemic) plasma samples, as well as with regard to anticoaugulants (heparin), xanthines (caffeine and theobromine), and another drug substance commonly used as analgesic (acetylsalicylic acid, paracetamol and naproxen). No interferences were observed in the resulting chromatograms. The range of the method was 100 to 6000 ng/ml, with lower limits of quantification and detection of 100 and 50 ng/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 pantoprazole were 1.45% to 3.58% and -2.02% to 0.46%, respectively, while inter-assay %CV and accuracy were 0.01% to 0.07% and -1.45% to -0.04%. The absolute recovery was above 93.3%.
Pantoprazole in plasma was found to be stable after 24 hours at room temperature (25°C), after 3 freeze-thaw cycles and after 10 weeks at -75 ± 5°C. Quality control samples (QC) samples were included in every analytical run to verify its performance. These QC samples were prepared at 3 different concentration levels (designated as low, [500 ng/ml], medium [1500 ng/ml] and high [2500 ng/ml]) of pantoprazole independent of the calibration curve. This method was considered suitable by the study investigators for the bioequivalence study of pantoprazole.

Tolerability

Tolerability was determined using clinical assessment, monitoring of vital signs (BP, heart rate, and armpit body temperature) at baseline, after the drug administration during hospitalization, and at the end of the clinical stage of the study. Laboratory results were also considered.

The subjects were interviewed (using open-ended questions) by the investigators during hospitalization and at the end of the clinical stage of the study concerning the occurrence 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 designed by the principal investigator.

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

Sample size calculation (Chow and Liu, 2000) was based on the within-subject variability of pantoprazole AUC_{0–∞} from a pilot study (n = 7) that had a %CV of 29% (data on file, Laboratorios Liomont, Mexico City, Mexico; study: BK-PE-10-010, completed April 2010). This calculation was performed considering the following values: 1 - β = 0.8, α = 0.05, %CV = 29, and an equivalence range of 80% to 125%; which yielded a sample size of 30 subjects. In this research, a sample size of 34 subjects was used, which included 4 additional subjects (with respect to the required sample size) considered in case of dropouts. In addition, this sample size exceeded the minimum sample size of 24 subjects requested for bioequivalence studies by COFEPRES.

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 according the non-compartmental method using the trapezoidal rule. From the terminal log-decay phase, the elimination rate constant (k_{e}) was estimated using linear regression, and t_{e} was estimated using the following equation [3].

\[ t_{e} = \frac{\ln 2}{k_{e}} \]

where ln was defined as the natural logarithm. Extrapolation of AUC from baseline to infinity (AUC_{∞}) was calculated as follows:

\[ AUC_{∞} = AUC_{0–t} + \left( \frac{C_{i}}{k_{e}} \right) \]

where C_{i} was the last measurable plasma concentration. To assess the bioequivalence between the test and reference formulations, C_{max}, AUC_{0–∞}, and AUC_{0–t} were considered as the primary variables. ANOVA for a 2 × 2 crossover design using log-transformed data for these parameters was carried out at the 5% significance level (α = 0.05).

The 90% CIs of the geometric means ratios (test/reference) of C_{max}, AUC_{0–∞}, and AUC_{0–t} were calculated using log-transformed data. The test and the reference formulations were to be considered bioequivalent if the 90% CIs of these parameters fell within a predetermined range of 80% to 125% and if the probability of exceeding these limits was <0.05. The probability of exceeding the 80% to 125% range was obtained using the two 1-sided test described by Schuirmann [10]. All pharmacokinetic and parametrical-statistical analyses were performed using WinNonlin version 5 (Pharsight, Mountain View, California).

T_{max} was considered as a secondary variable because there is no bioequivalence requirement for this pharmacokinetic parameter in Mexico [4]. It was analyzed using the distribution-free Hodges-Lehmann interval [6] using the untransformed data. For the purpose of this study, the test and the reference formulations were to be considered bioequivalent, if the 90% distribution-free confidence interval for the difference of the median of all possible pairwise differences (test - reference) fell within a predetermined range of ± 30% of the T_{max} of the reference formulation. This statistical analysis was performed using EquivTest/PK, 2006 (Statistical Solutions, Ltd., Saugus Massachusetts).

Results

A total of 34 subjects (17 men, 17 women; mean [SD] age, 31 [10] years [range, 18-52 years]; weight, 62.3 [9.6] kg [range, 44.8-81.7 kg]; height, 162 [9] cm [range, 143-179 cm]; and body mass index [BMI], 23.56 [2.19] kg/m² [range, 19.20-26.00 kg/m²]) were enrolled and completed the clinical stage of the study.

Pharmacokinetic parameters

Mean plasma concentration–time curves of the 2 pantoprazole formulations are shown in Figure 1. The pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0–t}, AUC_{0–∞}, and AUC_{0–t}) and the weight-adjusted parameters (C_{max}, AUC_{0–t}, and AUC_{0–∞}) are shown on Table 1.

On ANOVA of C_{max}, AUC_{0–t}, and AUC_{0–∞}, no significant formulation or period-sequence effects were detected (data not provided). However, when subjects’ weights were used as covariate, a significant weight-related effect was detected for C_{max}. Therefore, the weight-adjusted parameters were also estimated and included in Table 1.

Table 2 shows the bioequivalence statistics (using the log-transformed data of C_{max}, AUC_{0–t}, and AUC_{0–∞}); geometric Mean ratios
### Pharmacokinetic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Cmax, ng/ml</td>
<td>3610 (1344)</td>
<td>3448 (1214)</td>
</tr>
<tr>
<td>Adjusted Cmax, kg·ng/ml/mg</td>
<td>5444 (1711)</td>
<td>5269 (1678)</td>
</tr>
<tr>
<td>AUC0-t, ng·h/ml</td>
<td>5720 (2527)</td>
<td>5521 (2454)</td>
</tr>
<tr>
<td>Adjusted AUC0-t, kg·ng·h/ml/mg</td>
<td>8738 (3643)</td>
<td>8515 (3675)</td>
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<tr>
<td>AUC0-∞, ng·h/ml</td>
<td>6292 (2548)</td>
<td>6097 (2415)</td>
</tr>
<tr>
<td>Adjusted AUC0-∞, kg·ng·h/ml/mg</td>
<td>9625 (3681)</td>
<td>9399 (3694)</td>
</tr>
</tbody>
</table>

### Discussion

Considering that all of the 90% CIs of the geometric mean ratios of the pharmacokinetic parameters (Cmax, AUC0-t, and AUC0-∞) were found to be within the predetermined range of bioequivalence (80%-125%) and that the Schuirmann 1-sided tests (i.e., probability of exceeding limits of acceptance) found all of the probability values to be <0.05, these results satisfied the accepted regulatory requirements to assume bioequivalence. In addition, the 90% distribution-free confidence interval for Tmax was found to be within the range of bioequivalence of ± 30%, supporting the bioequivalence conclusion. Although a significant weight-related effect on Cmax was detected on ANOVA, using the F test, it was found that this effect did not affect the comparison of the bioavailability of the 2 formulations. None of the reported 12 AEs by 8 subjects were considered by the principal investigator to be serious.

### Limitations

As with any clinical trial, and in particular for most bioavailability studies, the current study had some limitations that should be considered. First, this was an open-label 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 [4], within a specific age range, who were administered a single dose; the PK parameters of pantoprazole might differ in target populations. For example, differences in absorption, metabolism and excretion of pantoprazole might exist in patients, with respect to healthy subjects. Thus, the results of this study might not be generalized to this population.

In addition this study was conducted under fasting conditions because the bioavailability of pantoprazole has been reported not to be affected by the concomitant intake of food are [7]. However, further studies would be useful to assess the food effect on the bioavailability of this drug on the target population.

Because of the limited data (small sample size, single dose, healthy subjects, age range, and fasting conditions) in the present study, we are unable to predict the response of the drug at any time following alternative doses and/or administration intervals with the present data set. 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 pantoprazole in Hispanic population.

### Conclusions

In this small study in healthy, fasting, Mexican adult subjects, single doses of oral pantoprazole 40 mg met the Mexican [4] regulatory requirements to assume bioequivalence based on the rate and extent of absorption. Both formulations were well tolerated.

### Acknowledgments

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S.A. de C.V., Mexico City, Mexico. The authors have indicated that they have no other conflicts of interest regarding the content of the article.

References


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