Bioavailability of Two Oral-Suspension Formulations of a Single Dose of Nitazoxanide 500 mg: An Open-Label, Randomized-Sequence, Two-Period Crossover, Comparison in Healthy Fasted Mexican Adult Volunteers

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Abstract

Nitazoxanide is an oral broad-spectrum parasiticidal agent. In Mexico, the oral powder for suspension of nitazoxanide is indicated for the treatment of antiprotozoal and anthelmintic infections in patients of 1 year or older. The aims of this study were to compare the bioavailability and to determine the bioequivalence of a test and reference formulation of oral nitazoxanide 500 mg, administered as a suspension, and to generate data regarding the oral bioavailability of this drug on the Mexican population.

This single-dose, randomized-sequence, open-label, 2-period crossover study was conducted on a total of 26 Mexican adult subjects of both genders, with a 1-week washout period. Study formulations were administered after a 10-hour overnight fast. For pharmacokinetic analysis, blood samples were drawn at 0 (baseline), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10 and 12 hours after administration. Plasma concentrations of tizoxanide (active metabolite of nitazoxanide) were determined using HPLC coupled to a mass spectrometry (MS/MS). 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 tizoxanide for the reference (Daxon®) and test (Paramix®) formulations were: Cmax (10.40±2.99 μg/ml, 10.73 ± 3.45 μg/ml); AUC 0–t (39.57 ± 15.89 μg•h/ml, 43.31 ± 19.13 μg•h/ml); and AUC 0–∞ (40.93 ± 16.05 μg•h/ml, 45.00 ± 19.63 μg•h/ml), respectively. The 90% CIs for the geometric mean ratios of Cmax, AUC 0–t, and AUC 0–∞ were: 94.58%-110.21%, 100.43%-116.22%, and 101.00%-116.43%, respectively, the within-subject %CV values were 16.23, 15.49 and 15.05, respectively. All the power values were 100%. 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: Nitazoxanide; Tizoxanide; Bioequivalence; Bioavailability; Pharmacokinetics; HPLC; Mexican population

Introduction

Nitazoxanide (2-(acetolxyo)-N-(5-nitro-2-thiazolyl)benzamide) is an oral broad-spectrum parasiticidal agent [10]. In Mexico, the oral powder for suspension of nitazoxanide is indicated for the treatment of antiprotozoal (Entamoeba histolytica, Giardia lamblia and Cryptosporidium parvum) and anthelmintic (Ascaris lumbricoides, Taenia solium, Taenia saginata; Trichuris trichuria Hymenoleptis nana and Fasciola hepatica) infections in patients aged one year or older [9].

Nitazoxanide is a prodrug which is rapidly metabolized (half-life of about 6 minutes in plasma) to its major active metabolites tizoxanide and tizoxanide glucuronide [1]. Therefore, the parent nitazoxanide is not detected in plasma; thus the quantification of tizoxanide in plasma is not detected in plasma and the majority of the reported adverse events (AEs) have been regarded as mild in severity, transient and often associated with the gastrointestinal system [5,7]. The most frequent AEs reported in adults are abdominal pain (6.6%), diarrhea (4.2%), headache (3.1%) and nausea (3.0%) [6].

The test (Paramix®, Laboratorios Liomont, S. A. de C.V., Mexico City, Mexico) and reference [9] powder for suspension formulations marketed in Mexico. Daxon® 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 (COFEPRIS). 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

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published up to 2010, using the combination of the terms nitazoxanide, tizoxanide, bioequivalence, bioavailability, pharmacokinetics, Mexico, Mexican and population, did not identify any published data concerning the bioavailability of the two formulations 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 nitazoxanide 500 mg, administered as a suspension, and to generate data regarding the oral bioavailability of nitazoxanide 500 mg in the Mexican population for the purpose of renewing marketing authorization of the test formulation in Mexico.

Subjects, Materials and Methods

Ethical considerations

The study protocol (P3305026V004) and the informed-consent form were approved by an independent ethics and research committee of Medica Sur Hospital (Mexico City, Mexico) in October 4, 2010, prior to the study initiation. 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 October to December 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 and respiratory rate); and diagnostic testing that include 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 (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. Candidates were excluded if laboratory values were significantly out of the reference range and/or if all tests had not been competed. 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 tests relevant to this study. Before the enrollment of the subjects, 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 cross over design was used. A total of 26 subjects of both genders (13 men, 13 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 [lot 10-VI-43; expiration date; June 23, 2012] followed by the reference formulation [lot 911493; expiration date October 31, 2011]) or viceversa. Randomization codes were concealed from all the investigators of the study.

To ensure reliable baseline plasma measurements, subjects underwent a 10-hour overnight fast with a 1-week washout period which exceeds the 7 half-lives of tizoxanide 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 forearm vein 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 dose of 25 ml of suspension (after constitution of the powder for suspension) containing 500 mg of nitazoxanide of the test or the reference formulation with 250 ml of water. Additional blood samples were drawn at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10 and 12 hours after administration.

During hospitalization, the subjects were under medical surveillance, and during the washout period, subjects maintained contact with the investigators to report any AEs. Plasma was obtained by centrifugation (3000 rpm [1000 g] for 15 minutes at 25°C) and stored at -75°C±5°C until analyzed using HPLC. After 1-week washout period, subjects 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 (1380 kcal/d) at 3, 8.5 and 12 hours after study drug administration.

Determination of tizoxanide plasma concentrations

Chemicals: Tizoxanide was provided by the School of Chemistry of the Universidad Nacional Autonoma de Mexico (National Autonomous University of Mexico, Mexico City, Mexico). All solvents (including water) were HPLC-class spectrometric grade (Avantor Performance Materials, Inc., Phillipsburg, NJ) and all reagents were analytical grade (Mallinckrodt Baker, Inc., Phillipsburg, NJ).

Sample preparation: Tizoxanide plasma levels were determined by using a HPLC method coupled with mass spectrometry (MS/MS) developed and validated by personnel of Biokinetics at Mexico City, Mexico. The sample preparation included the following: 500 µl of plasma and 500 µl of acetonitrile were vortexed in a 2.0-ml conical tube, (Sarstedt AG & Co., Nümbrecht, Germany) for 1 minute. The tube was centrifuged at 5000 rpm for 12 minutes at room temperature (25°C). The supernatant was separated and injected (volume of injection = 5 µl) into the chromatographic system (HPLC, Agilent Technologies, model 1200, Palo Alto, California).

Chromatographic and mass spectrometric conditions: Tizoxanide concentrations were determined with a 50 mm x 4.6-mm column (particle size, 1.8 µm; Zorbax® SB-C18, Agilent Technologies) equipped with a pre-column 12.5 mm x 4.6 mm (particle size, 5 µm; Zorbax® SB-C18, Agilent Technologies) and eluted with a mobile phase consisting of a mixture of (40:60 v/v) of an aqueous buffer solution (ammonium acetate, 10 mM; pH 7.0) and acetonitrile-water (95:5 v/v). The column temperature was 25°C. Flow rate was maintained at 0.4 ml/minute and tizoxanide was detected by a triple-quadrupole mass spectrometer (Agilent Technologies, model G6410B). The spectrometric (MS/MS) analysis was performed by monitoring the transition m/z 264+ m/z 217: The spectrometric conditions were: negative-ionization mode, fragmentor energy (70 V), collision energy

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 [3] was based on the within-subject variability of tizoxanide C\text{max} from a pilot study (n = 8) that had a %CV of 17 (data on file, Laboratorios Liomont, Mexico City, Mexico, study: BK-PE-10-019, completed September 2010). This calculation was performed considering the following values: 1 - β = 0.8, α = 0.05, %CV = 17, and an equivalence range of 80% to 125%; which yielded a sample size of 12 subjects. However, Mexican regulatory requirements [4] call for a minimum sample size of 24 for bioequivalence studies. In this research a sample size of 26 subjects was used, which included 2 additional subjects (with respect to the required sample size) considered in case of dropouts.

Individual plasma concentration–time curves were constructed; C\text{max} and T\text{max} were directly obtained from these curves, the area under the plasma concentration-time curve from time baseline to the last measurable concentration (AUC\text{0–t}) was calculated according the non-compartmental method using the trapezoidal rule. From the terminal log-decay phase, the elimination rate constant (k\text{e}) was estimated using linear regression, and t\text{1/2} was estimated using the following equation [3].

\[ t_{1/2} = \ln 2 / k_e \]

where ln was defined as the natural logarithm. Extrapolation of AUC from baseline to infinity (AUC\text{0–∞}) was calculated as follows:

\[ AUC_{0–∞} = AUC_{0–t} + (C_t / k_e) \]

To assess the bioequivalence between the test and reference formulations, C\text{max}, AUC\text{0–t}, and AUC\text{0–∞} 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\text{max}, AUC\text{0–t}, and AUC\text{0–∞} 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 [8]. All pharmacokinetic and parametrical-statistical analyses were performed using WinNonlin version 5 (Pharsight, Mountain View, California).

Results

A total of 26 subjects (13 men, 13 women; mean [SD] age, 35 [10] years [range, 18-53 years]; weight, 64.4 [7.9] kg [range, 51.6-81.6 kg]; height, 164 [8] cm [range, 146-178 cm]; and body mass index [BMI], 23.76 [1.83] kg/m\textsuperscript{2} [range, 19.70-25.90 kg/m\textsuperscript{2}] were enrolled and completed the clinical stage of the study.

Pharmacokinetic parameters

Mean plasma concentration–time curves of the 2 tizoxanide formulations are shown in the Figure 1. The pharmacokinetic parameters (C\text{max}, T\text{max}, T\text{1/2}, AUC\text{0–t}, and AUC\text{0–∞}) are shown on Table 1. On ANOVA of C\text{max}, AUC\text{0–t}, and AUC\text{0–∞}(using log-transformed data), no significant formulation or sequence effects were detected (data not provided). However, the ANOVA for AUC\text{0–t} and AUC\text{0–∞} detected significant period effects (P = 0.0035, P = 0.0029, respectively). Table 2 shows the bioequivalence statistics (using the log-transformed data of C\text{max}, AUC\text{0–t}, and AUC\text{0–∞}); geometric mean ratios (test/reference) (90% CI); the probabilities of exceeding the limits of acceptance for bioequivalence; and the power values of the test.

Tolerability

Eleven of the 26 subjects reported a total of 22 AEs (several subjects reported more than one AE). The most commonly AEs reported were abdominal pain (6 events reported by 5 subjects [19.2%]), two after administration of reference formulation, 4 after the administration

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of test formulation, and nausea (5 events reported by 4 subjects [15.4%]) two after administration of reference formulation, 3 after the administration of test formulation.

Other AEs were diarrhea (3 events reported by 3 subjects [11.5%]) after the administration of the test formulation; dizziness (3 events reported by 3 subjects [11.5%]) after the administration of test formulation; headache (2 events reported by 2 subjects [7.7%]) after the administration of test formulation; diaphoresis (2 reported by 2 subjects [7.7%]), one after the administration of the reference formulation and the other one after the administration of the test formulation; and an insect bite reported by one subject, which was regarded as unclassifiable. All of the AEs were considered as possibly related to the study drug, except for the insect bite. None of the AEs were considered serious in severity. They were regarded as mild. In addition, all of the AEs spontaneously resolved under medical surveillance during the clinical stage of the study, without the administration of any medication.

Discussion

Considering that all of the 90% CIs of the pharmacometric ratios (Cmax, AUC0–t, and AUC0–∞) were found to be within the predetermined range of bioequivalence (80%-125%) and that the Schuirmann 1-sided tests (ie, probability of exceeding limits of acceptance) found all of the probability values to be <0.05, these results satisfied the accepted Mexican regulatory requirements to assume bioequivalence. Although significant period effects were detected in the ANOVA for AUC0–t and AUC0–∞, we believe that they did not affect the comparison of the bioavailability of the 2 formulations, because no carry-over (sequence effect) was found and the regulatory requirements for bioequivalence were met. In addition the Tmax and t1/2 values suggested that the difference would not be clinically relevant (based on means and SDs), although they were not statistically tested for bioequivalence. None of the 22 reported AEs by 11 subjects were considered by the principal investigator to be serious. All of them, except for the insect bite, were regarded as possibly related to the study drug.

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 (COFEPRIS), within a specific age range, who were administered a single dose; the PK parameters of tizoxanide might differ in target populations. For example, although nitazoxanide suspension is also indicated for children, the results of this study cannot be generalized to this population.

In addition this study was conducted under fasting conditions because these formulations are considered immediate release dosage forms and in Mexico there is no requirement for fed-bioequivalence studies for this type of formulations [4]. However, it has been reported that the bioavailability of tizoxanide is altered, when nitazoxanide was administered as a suspension, the food increased the AUC by 45% to 50% and the Cmax by ≤10% [6]. Hence further studies would be useful to assess the effects of food on the bioavailability of this drug in the Mexican 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 nitazoxanide in Hispanic populations.

Conclusions

In this small study in healthy, fasting, Mexican adult subjects, single doses of oral-suspension of nitazoxanide 500 mg met the Mexican regulatory requirements (COFEPRIS), within a specific age range, and the power test results from a single-dose administration of a test and a reference oral-suspension formulation of nitazoxanide 500 mg in healthy Mexican adult subjects.
(COFEPRIS) regulatory requirements to assume bioequivalence based on the rate and extent of absorption. Both formulations were well tolerated.

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References


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