Bioequivalence Evaluation of Two Oral Formulations of Acetaminophen in Healthy Subjects: Results From a Randomized, Single-Blind, Crossover Study

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Abstract

Despite widespread availability of acetaminophen in Mexico, data on its pharmacokinetic properties in Mexican populations are limited. This single-center, single-blind, randomized, 2-period, 2-treatment, crossover, single-dose-per-period, 2-sequence study evaluated the bioequivalence of a test acetaminophen product available in Mexico compared with a reference 500-mg acetaminophen product in 28 healthy adults under fasting conditions. Blood samples were collected predose and at specified intervals across a 16-hour period following administration and were analyzed for acetaminophen using a validated reverse-phase high-performance liquid chromatography method. Drug products were considered to be bioequivalent if confidence intervals of natural log-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ data were within the range of 80% to 125%. Results were inconclusive for C_{max} due to high levels of intrasubject variability with this parameter. However, criteria for bioequivalence were met for AUC_{0-t} and $AUC_{0-\infty}$. All measured acetaminophen concentrations in this study were within a safe therapeutic range, and no adverse events were reported. The level of C_{max} intrasubject variability observed in this study does not have any apparent clinical implications that could affect either safety or efficacy.

Keywords

acetaminophen, bioequivalence, pain management, paracetamol, pharmacology

Acetaminophen (paracetamol) is a widely used nonprescription drug with analgesic and antipyretic properties that is effective for the symptomatic management of mild to moderate acute and chronic pain of diverse etiology.[1] Many international guidelines recommend acetaminophen for pain management because of its well-established efficacy and safety profiles across diverse patient populations.[1–4] For example, the American Geriatrics Society guidelines for the management of persistent pain in older adults state that acetaminophen is effective for the management of osteoarthritis pain and that the safety profile of acetaminophen at recommended doses is generally more favorable than that of nonsteroidal anti-inflammatory drugs in older patients.[3]

The pharmacokinetic properties of acetaminophen have been studied extensively in various populations. Acetaminophen has a reported oral bioavailability of 79% for tablets and 87% for an elixir formulation, and has been shown to have a fast onset of action, with a time to peak plasma concentration (T_{max}) of about 30 to 45 minutes.[5,6] Acetaminophen is eliminated predominantly through hepatic metabolism, with an elimination half-life in adults of between 1.5 and 3.5 hours.[1,2,5,7–10]

In Mexico, there are currently more than 50 marketed products that contain acetaminophen, either

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alone or in combination with other drugs, and in different dosages and formulations.[11] However, despite such widespread availability of acetaminophen in Mexico, data on the pharmacokinetic properties of acetaminophen in Mexican populations are quite limited.[11,12]

The objective of the current study was to evaluate the bioequivalence of a test acetaminophen product available in Mexico (Mejoral® 500-mg tablets; GlaxoSmithKline Consumer Healthcare México, S.A. de C.V., Mexico City, Mexico) compared with a reference 500-mg acetaminophen product (Tylenol® caplets; Janssen-Cilag México S.A., de R.L. de C.V., Mexico City, Mexico) in healthy adults under fasting conditions.

Methods

The study protocol was approved by the Research Committee plus the Research Ethics Committee of Arete Proyectos y Administración S.C. and COFEPRIS (Federal Commission for the Protection against Sanitary Risks). All study participants provided written informed consent. This single-center, single-blind, randomized, 2-period, 2-treatment, crossover, single-dose-per-period, 2-sequence study was designed to test the bioequivalence of test and reference acetaminophen products based on comparisons of pharmacokinetic parameters in healthy subjects. The study was conducted in accordance with Declaration of Helsinki, International Conference for Harmonisation, and Good Clinical Practice guidelines, and followed Official Mexican Regulation NOM-177-SSA1-2013 for establishing tests and procedures to prove drug products are interchangeable.

Subjects

Healthy male and female volunteers between 18 and 55 years of age were eligible for study enrollment if they had a body mass index of 18.0 to 27.0 kg/m², were in a good state of health based on clinical history and safety laboratory parameters, and were able and willing to comply with all study procedures and restrictions as evidenced by voluntary written informed consent. Study exclusion criteria included variations in vital sign measurements outside predefined limits or clinically significant abnormalities in the electrocardiogram at the screening visit; history of cardiovascular, renal, hepatic, muscular, metabolic, gastrointestinal (including constipation), neurologic, or endocrine disorders; history of dyspepsia, gastritis, esophagitis, or duodenal or gastric ulcers; history of hematopoietic disorders or any other type of anemia, asthma, mental illness, or any other organic abnormalities; or muscle trauma within 21 days before the study. Subjects were also excluded if they had taken any drug product, in-cluding vitamins or herbal remedies, within 30 days (or 7 half-lives) prior to the beginning of the study or required any drug product during the course of the study, apart from the drug product being studied; had exposure to hepatic enzyme inducers or inhibitors, or drugs that alter urinary pH, within 30 days before the study start; had used any other investigational product within 90 days before the study; had donated or lost ≥450 mL of blood within 60 days before the study; had been hospitalized within 7 months before the study; had drug, food, or substance allergies or required a special diet; were unable to comply with study requirements; or had a history of or tested positive for drug abuse or alcoholism. Women were excluded from the study if they were lactating, had a positive pregnancy test, or intended to become pregnant during the study. Employees of the sponsor or the study site or members of their immediate families were also excluded.

To participate in the study, subjects were required to avoid alcohol, carbonated beverages, beverages containing xanthines (eg, coffee, tea, cocoa, chocolate, cola), coal-roasted food, grapefruit and orange juice, and smoking for 24 hours before the beginning of both study periods. In addition, from the time of the study selection visit until the end of the study, subjects were to avoid strenuous exercise, and subjects and their spouses were to avoid pregnancy from the time of study selection to 60 days after the last dose of study drug.

Study Design

This was a randomized, single-blind, 2-period, 2-treatment, crossover, single-dose-per-period, 2-sequence study in healthy subjects under fasting conditions. The study was conducted at a single research center, Investigación Farmacológica y Biofarmacéutica (IFaB), in Mexico City, Mexico, in August 2015 (ClinicalTrials.gov identifier: NCT02504775).

Eligible subjects were admitted to the study center for each of two 34-hour (±2 hours) periods that spanned from approximately 12 hours before to 22 hours after study drug administration. Subjects were admitted to the study center the day before administration of the study drug between 5:00 PM and 9:00 PM. Shortly after admission to the study center, subjects underwent screening and physical examinations, received a light dinner, and were instructed to fast for a minimum of 10 hours prior to study drug administration at approximately 8:00 AM the next day.

Subjects were randomized to 1 of 2 possible treatment sequences (AB or BA), in which treatment A consisted of a single 500-mg dose of acetaminophen reference product, and treatment B consisted of a single 500-mg dose of acetaminophen test product. Treatments were administered to subjects in a fasted state with 250 mL of room-temperature water.

There was a 96-hour washout period between administration of the first and second study drugs to ensure elimination of the first study drug.

Blood samples (6 mL) for pharmacokinetic analyses were collected over a 16-hour period based on the reported elimination half-life of acetaminophen[13] and variability in drug absorption after oral administration. By taking samples over approximately 4 half-lives, it was expected that more than 90% of the administered drug would have been eliminated. In each study period, blood samples were taken predose and at 0.25, 0.33, 0.50, 0.67, 0.83, 1, 1.25, 1.50, 1.75, 2, 4, 6, 8, 12, and 16 hours after administration of the study drug. At the final study evaluation, additional blood samples were taken for safety laboratory testing (10 mL) and liver function monitoring (6 mL).

Vital sign measurements (blood pressure, heart rate, respiratory rate, and body temperature) were taken at 2, 6, 12, and 22 hours after administration of the study drug.

Study Assessments

Blood samples (with a solution of citrate phosphate dextrose adenine added as an anticoagulant) were analyzed for acetaminophen using a validated chromatographic method.[14,15] Plasma acetaminophen quantification was established by a liquid-liquid extraction and separation technique by liquid chromatography through a reverse-phase column with ultraviolet detection using diprophylline as an internal standard. The relationship between the chromatographic response with respect to concentration in each calibration curve was fitted by linear least-squares regression to the equation $y = mx + b$, with arrangement $1/x^2$, where the "y" variable was the ratio of the areas of acetaminophen/diprophylline obtained for the respective nominal concentration "x" of acetaminophen.

Because of the sensitivity required for the characterization of the pharmacokinetic profile of acetaminophen, the analytical method was validated in the range of 0.1 to 40.0 μg/mL. The performance of the analytical run was evaluated with values obtained from the calibration and results of quality control samples. Each volunteer sample was integrated and quantified with the calibration curve on the day of the analysis; these curves met the linearity and accuracy parameters established during validation. Acceptance criteria for assay accuracy required intraday, interday, and reproducibility of reinjection percentage of absorption deviation to be ≤15%, except for the lower limit of quantification, which was required to be ≤20%. Acceptance criteria for reproducibility required the interday coefficient of variation (CV) to be ≤15%, except for the lower limit of quantification, which was required to be ≤20%. The acceptance criteria for baseline selectivity required that the analytical response of interferences close to the retention time be ≤20% for the analyte of interest and ≤5% for the internal standard.

Analytical samples were stored in cryotubes at −70°C. Acceptance criteria for sample stability required that the percentage of absolute deviation was ≤15% relative to the value of recently prepared samples. Data showed that samples were stable for 4 freeze-thaw cycles at −70 ± 10°C; samples were also stable at room temperature (15–30°C) and under refrigeration (2–8°C) for 60.7 hours. The main acetaminophen standard solution was stable under refrigeration and frozen for 55.8 days.

Pharmacokinetic parameters determined based on plasma acetaminophen concentration-time data included maximum plasma drug concentration (C_{max}), T_{max}, area under the plasma drug concentration-time curve (AUC) from time zero up to the last sampling time (AUC_{0-t}) and extrapolated to infinity ($AUC_{0-\infty}$), and elimination half-life ($t_{1/2}$).

Safety was assessed by evaluating reported adverse events, abnormal clinical laboratory values, and changes in electrocardiogram parameters or vital signs during both study periods.

Statistical Analyses

Sample size calculations were based on an acetaminophen intrasubject CV of 24.23%, which was observed in previous pilot studies in Mexican populations and is consistent with a CV of 20.92% estimated on the basis of the relative bioequivalence interval and sample size in the study conducted by Dominguez and colleagues.[12] Using a CV of 24.23% for the main pharmacokinetic parameters of acetaminophen (AUC_{0-t} and C_{max}), the Shein-Chung Chow equation for crossover design[16] was used to determine that a total sample size of 22 subjects was sufficient to demonstrate the bioequivalence of the test and reference acetaminophen products. To ensure that at least 22 subjects completed the study, the goal was to randomize 28 subjects.

Pharmacokinetic parameters were determined using noncompartmental methods and were summarized by treatment using descriptive statistics, including arithmetic mean, geometric mean, standard deviation, standard error, median, minimum, maximum, and CV for each variable. Natural log comparisons of the pharmacokinetic parameters of C_{max} and AUC were performed for the test versus the reference drug using an analysis of variance model that included sequence, period, and formulation as fixed effects based on linear mixed effect models. Analysis of variance comparisons were performed using Phoenix/WinNonlin® version 6.4 (Certara, LP, Princeton, New Jersey) considering the type III sum of squares.

Table 1. Subject Demographic and Baseline Characteristics

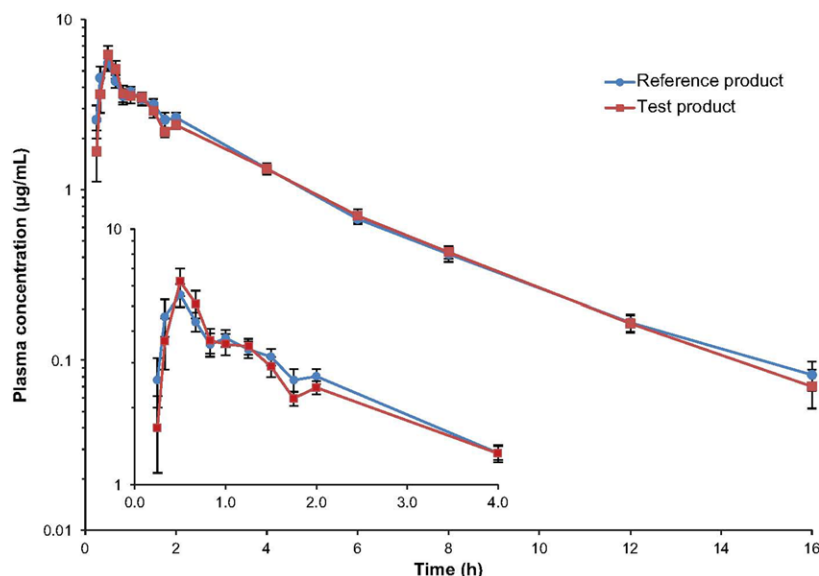| Variable	Men (n = 18)	Women (n = 10)	Total (N = 28)
Age, mean (SD), y	28.1 (9.5)	35.4 (10.6)	30.7 (10.4)
Weight, mean (SD), kg	70.3 (10.4)	59.6 (6.9)	66.5 (10.5)
Height, mean (SD), m	1.7 (0.07)	1.6 (0.07)	1.7 (0.1)
Body mass index, mean (SD), kg/m^2	23.4 (2.4)	24.3 (2.6)	23.7 (2.5)

SD, standard deviation.

Figure 1. Mean ($\pm$SE) plasma acetaminophen concentration-versus-time profiles for test and reference drug products (semi-log plot). SE, standard error. Values below the limit of quantification were entered as zero in the calculation of mean acetaminophen concentration.

Bioequivalence of the test and reference drug product was determined based on logarithmic comparisons of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. Drug products were considered to be bioequivalent if confidence intervals of natural log-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ data were within the range of 80% to 125%, with a confidence level of 90% and statistical power >0.8. The null hypothesis that the test and reference products were not bioequivalent was to be rejected on the basis of results of applied limit tests using Schuirmann's two 1-sided t-tests and the Anderson-Hauck test on the quotient of the averages of the test to the reference drug products for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ if classic confidence intervals were not within the range of 80% to 125%, with 90% confidence and alpha significance levels of 0.05.

Results

Subjects
Twenty-eight subjects were randomly assigned to a treatment sequence, and all 28 subjects completed the study. There were no major deviations from the study protocol.

Subject demographic and baseline characteristics are presented in Table 1. The study population included 18 (64%) men and 10 (36%) women. In the overall study population, mean age was 31 years, mean body weight was 66.5 kg, mean height was 1.67 meters, and mean body mass index was 23.7 kg/m^2. On average, men were younger (28 vs 35 years of age), taller (1.73 vs 1.57 m), and heavier (70.3 vs 59.6 kg) than women.

Pharmacokinetics
As shown in Figure 1, mean plasma acetaminophen concentration-vs-time curves were similar for the test and reference products.

Pharmacokinetic parameters of the test and reference products are summarized in Table 2. On average, differences between the test and reference products for the pharmacokinetic parameters of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were less than 20%.

Table 3 shows the results of the statistical tests applied to log-transformed data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ to determine bioequivalence of the test and reference products at a confidence level of 90%. The 90% confidence interval of the ratio of geometric means

Table 2. Plasma Pharmacokinetic Parameters of Acetaminophen Reference and Test Drug Products

Parameter	Reference Acetaminophen Product (n = 27)[a]	Test Acetaminophen Product (n = 27)[a]
C_{max}, μg/mL		
Geometric mean	6.7	7.1
Arithmetic mean	7.2	8.1
SD	2.8	3.9
CV, %	39.6	48.5
AUC_{0-t}, μg • h/mL		
Geometric mean	14.7	13.9
Arithmetic mean	15.2	14.7
SD	4.1	4.5
CV, %	27.2	30.8
$AUC_{0-\infty}$, μg • h/mL		
Geometric mean	15.4	14.9
Arithmetic mean	16.0	15.6
SD	4.2	4.7
CV, %	26.5	30.0
T_{max}, h		
Median (min, max)	0.5 (0.25, 2.00)	0.5 (0.25, 2.00)
CV, %	66.0	67.9
$t_{1/2}$, h		
Geometric mean	3.0	3.2
Arithmetic mean	3.3	3.6
SD	1.5	2.0
CV, %	46.1	55.2

AUC_{0-t}, area under the plasma drug concentration-versus-time curve from time zero to the last measurable drug concentration; $AUC_{0-\infty}$, area under the plasma drug concentration-versus-time curve from time zero extrapolated to infinity; C_{max}, maximum observed plasma drug concentration; CV, coefficient of variation; max, maximum; min, minimum; SD, standard deviation; T_{max}, time to C_{max}; $t_{1/2}$, elimination half-life.
[a]Data from 1 subject were excluded from pharmacokinetic and bioequivalence analyses because this subject had a predose blood sample that tested positive for acetaminophen (at a level equal to 7.74% of the subject's C_{max}); therefore, n = 27. Reanalysis of the predose sample from this subject confirmed an acetaminophen level above the limit of quantification. The subject did not declare any predose ingestion of acetaminophen.

for test to reference products (85.2%–132.3%) was not within the predefined equivalence range of 80% to 125% for C_{max} but was within this range for AUC_{0-t} (88.3%–101.5%) and $AUC_{0-\infty}$ (89.8%–102.6%).

It is noteworthy that the intrasubject variability for log-transformed C_{max} was 50.1%. This value was higher than the CV of 24.23% used for study sample size calculations.

Safety
No adverse events were reported during this study in either treatment group.

Discussion
Findings from this study showed that there were no significant differences between the test and reference acetaminophen products based on pharmacokinetic parameters associated with degree of drug absorption (ie, AUC_{0-t} and $AUC_{0-\infty}$). In addition, the results showed that the difference in the ratio of geometric means between the test and reference products for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ was consistently <20%. However, a limitation of this study is that, due to high intrasubject variability (>50%) for the log-transformed C_{max} parameter and small sample size, bioequivalence cannot be inferred in terms of rate of absorption for the test and reference products.

A previous pharmacokinetic study[12] performed in Mexico with the same acetaminophen active ingredient used in the current study found that C_{max} intrasubject CV was considerably lower than reported here (~20% vs ~50%). The reason for the level of C_{max} intrasubject variability observed in the current study is not clear.

The overall pharmacokinetic profiles of both the test and reference acetaminophen products exhibited in the current study conducted in a healthy adult population in Mexico were similar to those reported in patient populations from other geographic regions.[17,18]

Table 3. Bioequivalence of Acetaminophen Test vs Reference Drug Products[a]

Parameter	Average reference (A)[b]	Average test (B)[b]	Ratio of B/A (%)	Classic CI Lower	Classic CI Upper	Schuirmann's two 1-sided t-tests $P < 80$	Schuirmann's two 1-sided t-tests $P > 125$	Anderson-Hauck test	Power
Ln C_{max}	6.7	7.1	106.1	85.2	132.3	0.02	0.1	0.09	0.5
Ln AUC_{0-t}	14.7	13.9	94.7	88.3	101.5	0.0002	0.0000	0.0002	1.0
Ln $AUC_{0-\infty}$	15.5	14.8	96.0	89.8	102.6	0.0000	0.0000	0.0000	1.0
Bioequivalence criterion				>80	<125	<0.05	<0.05	<0.05	>0.8

AUC_{0-t}, area under the plasma drug concentration-versus-time curve from time zero to the last measurable drug concentration; $AUC_{0-\infty}$, area under the plasma drug concentration-versus-time curve from time zero extrapolated to infinity; CI, confidence interval; C_{max}, maximum observed plasma drug concentration; Ln, log-transformed.
[a]Data from one subject were excluded from pharmacokinetic and bioequivalence analyses because this subject had a predose blood sample that tested positive for acetaminophen (at a level equal to 7.74% of the subject's C_{max}); therefore, n = 27.
[b]Geometric mean.

Conclusions

Results of the current study evaluating the bioequivalence of two 500-mg oral formulations of acetaminophen marketed in Mexico were considered inconclusive for C_{max} due to high levels of intrasubject variability with this parameter. However, the criteria for bioequivalence of the 2 products were met for the other pharmacokinetic parameters assessed (AUC_{0-t} and $AUC_{0-\infty}$). Importantly, all measured acetaminophen concentrations in this study were within a safe therapeutic range. The level of C_{max} intrasubject variability observed in this study does not have any apparent clinical implications that could affect either safety (because the plasma concentrations of acetaminophen 500 mg were $<70\ \mu g/mL$ [toxic concentration]) or efficacy (therapeutic range of 2.5–25 $\mu g/mL$),[19] and both the test and reference products are currently approved for use in Mexico.

Declaration of Conflicting Interests

C.B.N. and L.D. are employees of GlaxoSmithKline Consumer Healthcare. I.R.O., A.G.M.N., and L.G.A. are employees of Investigación Farmacológica y Biofarmacéutica.

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