

Bioequivalence Evaluation of Two Brands of Ketoprofen 50 Mg Capsules (Flogofin® & Profenid®) In Healthy Latin American Volunteers

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Abstract

The study was performed to compare the bioavailability of two ketoprofen capsule (50 mg) formulations: Flogofin®, as test formulation, and Profenid®, as reference formulation. The study was conducted open with randomized two period crossover design and 1 week washout period in 24 fasting, healthy latino-american male volunteers and performed at the Biopharmaceutical Research Center Dominguez Lab. After dosing, serial blood samples were collected for a period of 24 h and plasma was separated and analyzed for ketoprofen, using a sensitive, reproducible, and accurate method by liquid chromatography – tandem mass spectrometry (LC-MS/MS). Pharmacokinetic parameters: AUC₀₋₂₄, AUC_{0-∞}, C_{max}, T_{max}, T_{1/2} and Ke, were analyzed from plasma concentrations of both formulations. The means AUC₀₋₂₄ for test and reference formulation were 50.21 ([μg h]/mL) - 50.28 ([μg h]/mL), 52.38 ([μg h]/mL) - 50.84 ([μg h]/mL) for AUC_{0-∞}, and 21.58 μg/mL - 21.65 μg/mL for C_{max}, respectively. Statistical modules (ANOVA and 90% confidence intervals) were applied to AUC₀₋₂₄, AUC_{0-∞}, and C_{max} to assess the bioequivalence of the two brands which revealed no significant difference between them, and 90% CI fell within the accepted bioequivalence range of 80%-125%. Based on these statistical inferences, both formulations were found to be bioequivalent.

Keywords: Ketoprofen; Bioavailability; Pharmacokinetics; Mass spectrometry

Introduction

The drug 2-(3-Benzoylphenyl)-propionic acid, commonly known as ketoprofen (KPF), belongs to the non-steroidal anti-inflammatory drugs (NSAIDs) which not only produce good analgesia but also exerts antipyretic effects [1]. KPF also exhibits anti-inflammatory properties via inhibition of cyclooxygenase 1 and 2 (COX 1 and COX 2) enzymes reversibly, which decreases production of pro-inflammatory prostaglandin and thromboxane precursors [2].

This drug not only is available for oral administration but also as gel and delivery system patch (TDS), for its topical application. Topical NSAIDs offer the advantage of local, enhanced drug delivery to affected tissues with a reduced incidence of systemic adverse events [3]. KPF has been shown to be well absorbed orally with peak plasma concentrations occurring within 1 hour and it has a short half-life of approximately 2 h. It is 99% bound plasma proteins, and 85-99% of KPF and its metabolites are excreted in the urine rather than the feces [4].

Although the oral bioavailability and pharmacokinetic characteristics of KPF have been well described in clinical studies previously [5,6], they have not reported the bioequivalence of a newly developed generic product, in Latin American population. Here, we compared the bioavailability between the test and its reference product in healthy adult human male subjects using high-pressure liquid chromatography - tandem mass spectrometry (HPLC-MS/MS). The method was developed and validated in the Biopharmaceutical Research Center DominguezLab.

Material and Methods

Formulations and participants

The present study was designed to evaluate Flogofin® (from

Laboratorio Chile S. A., Chile, as test, lot no. 12093261; expiration date 09/2015) and Profenid® (from Sanofi-Aventis de Venezuela S.A., Venezuela, as reference, lot no. 1VE0243; expiration date 10/2015) of KPF 50 mg capsule formulations.

Twenty four healthy Latin Americans male volunteers participated in this study, which was conducted at the Biopharmaceutical Research Center DominguezLab. The ages of subjects were between 18-46 years old (25 ± 7 years), the body weights of subjects were between 54-87 kg (71 ± 9 kg) and the heights of the subjects were between 166-183 cm (174 ± 5 cm). Subjects were selected after screened by physical examination and clinical laboratory tests including renal and liver functions, routine blood (Hb, Ht, RBC, platelet, WBC, BUN, total bilirubin, glucose fasting, total protein albumin, alkaline phosphatase, sGPT, sGOT), and urine analysis (specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC, cast). Subjects were excluded if they were smoker, have a history of any illness of renal and liver, history of alcohol or other medications for long period of time. The consumption of alcohol or beverages and food, containing xanthines was not permitted for the volunteers, 48 h prior to the study and after drug administration, until the last blood sample was collected in the respective study phase.

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Subjects were instructed to abstain from taking any medication for at least 2 weeks prior to and during the study period.

Informed consent was approved by the Institutional Ethical Committee and it was obtained from the subjects after explaining the nature and purpose of the study. The study protocols were approved by the Public Health Institute of Chile (ISP of República de Chile). The present study was also conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and the guiding principles of the 2008 Declaration of Helsinki.

The selection and inclusion of patients according to eligibility criteria were performed following DominguezLab Standard Operative Procedure PG-005-CLI-002.

Study design

This was a single-dose, randomized-sequence, open label, 2-way crossover bioequivalence study. After an overnight fast for 10 h and signing the informed-consent form, the volunteers received a single 200 mg dose (4 capsules × 50 mg) of the test or reference formulation with 240 mL of water in random order, with the 2 study periods separated by a 1 week washout period. The standardized lunch and dinner (8 kcal/kg body weight; 55% carbohydrate, 15% protein, and 30% fat) were provided at 4 and 9 h after administration, respectively. Water intake was allowed 2 h after the dose; water, lunch and dinner were given to all volunteers according to a time schedule.

The washout period was determined based on 5 to 7 times the $T_{1/2}$ of KPF (2 h). The volunteers were confined to the center 12 h before drug administration and for 24 h after administration. The volunteers were under continuously monitored by medical supervision throughout the confinement period of the study. Approximately 8 mL of blood for KPF assay was drawn into heparinized tubes through an indwelling cannula before (0 h) and at 0.17 h, 0.33 h, 0.50 h, 0.75 h, 1 h, 1.17 h, 1.50 h, 2 h, 2.5 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, 16 h and 24 hours after drug administration. The plasma was separated by centrifugation at 3000 ×g for 10 minutes at room temperature (20°C), followed by direct transfer into 2 mL polypropylene tubes and storage frozen at -20°C until analysis. After a 1 week washout period, the study was repeated in the same manner to complete the cross-over design.

Tolerability

In this study, all the volunteers were continuously and carefully monitored. Tolerability was assessed by monitoring vital signs (temperature, blood pressure, heart rate, and respiratory rate) at baseline before dosing and during the study by a qualified nurse. Laboratory tests (hematology, urinalysis, and blood biochemistry), physical examinations, and ECGs were also performed at baseline and at completion of the study.

Adverse events (AEs) were assessed at the time of each blood draw using direct observation, spontaneous reporting, and nonspecific questioning. Any undesirable sign, symptom, or medical condition occurring after the start of the study was recorded regardless of the suspected relationship to the study drug. AEs were graded as mild, moderate, or severe, and their relationship to the study drug was determined by the study physicians as not related, probably not related, uncertain, possibly related, probably related, or definitely related. The physicians who were responsible for determining the clinical significance of AEs were blinded to the treatment.

Chemicals and reagents

KPF (USP, LOT H1H247, 99.8% w/w) and KPF-d3 (TLC, Canada

LOT 1038-047 A1, 99.9% w/w) were supplied by the pharmaceutical industry. Water and Acetonitrile (ACN) were purchased from Carlo Erba, France and formic acid was purchased from Sigma-Aldrich, Germany. All reagents were analytic grade or above.

Sample collection

Samples were obtained from healthy volunteers of Dominguez Lab, according to Standard Operative Procedure of Dominguez Lab P-004-PTG-009: "Samples Identification and Preparation". Briefly, for sampling a catheter system (BD Saf-T-Intima™, BD Vacutainer™) was used and syringes of 5 mL. The blood sample was collected into heparinized polypropylene 4 mL tubes (NAHEP PLH 13X75 4.0 PLBL GN, BD Vacutainer™, Broken Bow NE 68822 US) and centrifuged at 3000×g for plasma separation. Aliquots of 1 mL were preserved in polypropylene 2 mL cryovials and frozen at -20°C ± 5°C until analysis.

Bioanalysis of plasma samples

Plasma KPF samples were analyzed by using a validated HPLC-MS/MS system: Quantitation was achieved by measurement of the peak area ratio of the drug to the KPF-d3 as internal standard (IS). Stock solutions (1 mg/mL) were prepared by dissolving an appropriate amount of each compound in ACN. Working solutions were prepared daily by dilutions of the stock solutions with ACN. Calibration curves of KPF were prepared by spiking blank human plasma in a concentration range of 48-19294 ng/mL.

Samples and Quality Controls (QC's) were thawed at room temperature on the day of analysis: Aliquots of 300 µL sample or QC's were mixed with 200 µL of IS working solution (at 7 µg/mL). A 700 µL aliquot of ACN was added in order to protein precipitation and vortex mixed for 30 s. After samples were centrifuged at 3000 ×g for 5 min., a volume of 500 µL of the supernatant was diluted by adding 1000 µL of water. An aliquot (30 µL) was injected into a Hypersil GOLD C18 analytical column (150 x 2.1 mm i.d., 3 µm). A mobile phase of 0.1% formic acid in water/ACN (40:60, v/v) was pumped isocratically at a flow rate of 0.30 mL/min. Under these conditions, typical retention times were 2.3 min for KPF and IS and the runtime was 4.0 min. Detection and quantification were achieved by using high performance liquid chromatography (HPLC) coupled to a triple-quadrupole mass spectrometer (VARIAN 1200L) with electrospray ionization interface in positive mode. The mass spectrometer was used in the multiple reaction monitoring (MRM) mode and the m/z transition for quantification collected in positive mode were 254.6 → 208.9 and 257.6 → 211.8 for KPF and KPF-d3 respectively.

The proposed analytical method was evaluated in terms of linearity, specificity, accuracy and precision intra-day and inter-day, LOQ, recovery, and stability. To assess stability, QC's plasma samples (104, 8104 and 16208 ng/mL) were subjected to short-term room stability (6 h), post processing stability (12 h remain at the auto-sampler temperature), freeze-and-thaw stability (after 3 cycles of freezing (-20°C) and thawing (room temperature), and long-term stability (60 days at -20°C).

Pharmacokinetics and statistical analysis

To compare the bioavailability (in accordance with the criteria for bioequivalence [the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action] established by the US Food and Drug Administration, in vivo bioequivalence guidelines) [7] of the formulations tested, the following pharmacokinetic (PK) parameters were calculated using

a validated PK software, Win Nonlin version 6.02 (Pharsight Corp. Mountain View, CA): area under the curve from time zero to the last measurable KPF concentration in plasma (AUC_{0-24}), using the linear trapezoidal rule; ($AUC_{0-\infty}$), calculated as the sum of AUC_{0-24} plus C_{last}/K_e (where C_{last} is the last measurable plasma concentration, and K_e is the terminal rate constant of elimination); maximum measured concentration of KPF (C_{max}); time to maximum plasma concentration (T_{max}); and terminal elimination half-life ($T_{1/2}$). After logarithmic transformation, $AUC_{0-\infty}$, AUC_{0-24} and C_{max} values were subjected to analysis of variance (ANOVA). The bioequivalence between the two formulations was evaluated based on the 90% CI transformed back for the geometric mean ratios of $AUC_{0-\infty}$, AUC_{0-24} and C_{max} , which were within acceptance range of 80-125% according to the local and international guidelines [7-11].

Results and Discussion

Analytical performance and method validation

Calibration curves showed a satisfactory linearity within the concentration range of: 48-19294 ng/mL ($r = 0.999$). The LOQ was 48 ng/mL and was defined as the lowest concentration in the calibration curve that can be measured with acceptable accuracy and precision [12-14]. The LOQ was considerably low compared with other works [15,16] and probably this was attributed to the LC-MS/MS technology which allowed more reliable measurements.

Specificity was assessed in six different batches of plasma samples by analyzing blanks and spiked samples at LOQ levels. No significant chromatographic signals of endogenous KPF were observed for any plasma batches at the target analytes retention times (KPF and IS). The intra-day and inter-day precision (RSD%) was less than 3.0 and 7.8%, respectively. Accuracy, evaluated as relative error (RE), was within 12% for the intra-day and 7% for the inter-day. The extraction recoveries of KPF were 87%, 90% and 100% in the concentrations of 116, 8333 and 18333 ng/mL respectively. Post-processing, freeze-and-thaw, short-term and long-term stability tests proved that KPF, did not show any significant degradation at the temperatures and time periods specified. The analytical methods to determine KPF concentrations in human plasma herein described were in agreement with the international guideline [11-13].

Tolerability

All the subjects successfully completed the study according to the protocol, and both KPF formulations were well tolerated at the administered doses. Only one volunteer refereed mild acid sensitive syndrome, probably related to the drug, and it was resolved with ranitidine. All biochemical parameters did not present any clinical relevant alterations. No other adverse effects were reported or observed.

Pharmacokinetic parameters mean

The mean concentration-time profiles for KPF for the test and reference formulations are shown in Figure 1, and they were closely similar and superimposable. The pharmacokinetic parameters (AUC_{0-24} h and $AUC_{0-\infty}$, C_{max} , T_{max} , $T_{1/2}$ and K_e) for the both KPF formulations are shown on Table 1. Peak concentrations of 21.58 ± 6.69 ng/mL and 21.65 ± 8.48 ng/mL for KPF were attained at 1.10 and 1.49 h after drug administration for the test (Flogofin®) and reference (Profenid®) products, respectively, and then declined rapidly and remained detectable until 24 h. The mean and standard deviation of AUC_{0-24} , $AUC_{0-\infty}$ and C_{max} of the two products did not differ significantly, suggesting that the plasma profiles generated by

Flogofin® are comparable to those produced by Profenid®. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, formulations or sequence, having P values greater than 0.05. Table 2 shows the 90% CIs of the ratios (test/reference) for the log-transformed values of C_{max} (as an index of rate of absorption), AUC_{0-24} and $AUC_{0-\infty}$ (as an index of the extent of absorption) and the probability of exceeding the limits of acceptance (Schuirmann's two 1-sided t tests) for KPF capsules. The 90% CIs for the corresponding ratios of C_{max} , AUC_{0-24} and $AUC_{0-\infty}$ were within the 80% to 125% range. All P values were <0.05.

Conclusion

These results suggested that reference and test formulations (KPF capsules) were not statistically different in terms of their PK parameters (C_{max} , AUC_{0-t} and $AUC_{0-\infty}$). In addition, T_{max} and $T_{1/2}$ values had not clinically important differences between them (based on means and standard deviations). Considering that all 90% CIs of the ratios of the

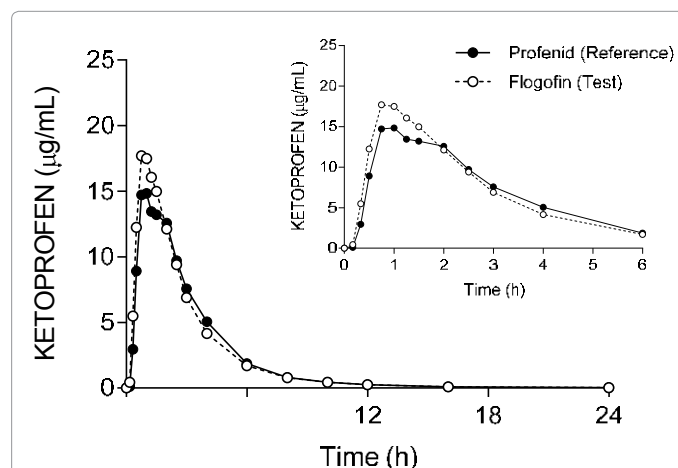


Figure 1: Plasma mean concentrations of 200 mg KPF versus time profile obtained after oral administration of single dose (4 capsules × 50 mg) of two brands (Flogofin® or Profenid®) to 24 volunteers.

Pharmacokinetic parameter	Flogofin® (Test)	Profenid® (Reference)
AUC_{0-24} (µg h/mL)	52.21 ± 1.15	50.68 ± 10.52
$AUC_{0-\infty}$ (µg h/mL)	52.38 ± 11.51	50.84 ± 10.57
C_{max} (µg/mL)	21.58 ± 6.69	21.65 ± 8.48
T_{max} (h)	1.10 ± 0.53	1.49 ± 1.00
$T_{1/2}$ (h)	3.62 ± 0.75	3.54 ± 0.59
K_e (h ⁻¹)	0.20 ± 0.05	0.20 ± 0.05

Table 1: Pharmacokinetic parameters obtained from 24 volunteers after oral administration of 200 mg ketoprofen (4 capsules × 50 mg) formulation (Mean ± Standard deviation).

Parameter	Ratio, %Reference	90%CI	Probability of Exceeding Limits of Acceptance	
			<80%	>125%
Ln C_{max} % ratio	102.4	89.3-117.5	<0.00268	<0.01039
Ln AUC_{0-24} % ratio	103.1	98.1-108.4	<0.00000	<0.00000
Ln $AUC_{0-\infty}$ % ratio	103.2	98.2-108.4	<0.00000	<0.00000

Table 2: Comparison of 90% CIs of natural log-transformed ratios of C_{max} , AUC_{0-24} and $AUC_{0-\infty}$, the probability of exceeding the limits of acceptance, and power test in KPF capsules (4 capsules × 50 mg).

pharmacokinetic parameters (C_{max} , AUC_{0-24h} and $AUC_{0-\infty}$) were within the predetermined range of bioequivalence (80%-125%) with $P < 0.05$, results of both studies satisfied the accepted regulatory requirements to assume bioequivalence.

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Conflict of Interest

No potential conflicts of interest relevant to this article were reported.

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