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Bioequivalence Evaluation of Two Brands of Ketoconazole Tablets (Onofin-K® and Nizoral®) in a Healthy Female Mexican Population

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ABSTRACT: A randomized, crossover study was conducted in 24 healthy female volunteers to compare the bioavailability of two brands of ketoconazole (200 mg) tablets; Onofin-K® (Farmacéuticos Rayere S.A., México) as the test and NIZORAL® (Janssen-Cilag, México) as the reference products. The study was performed at the Clinical Pharmacology Research Center of the Hospital General de México in Mexico City. Two tablets (400 mg) were administered as a single dose with 250 ml of water after a 12 h overnight fast on two treatment days separated by a 1 week washout period. After dosing, serial blood samples were collected for a period of 12 h. Plasma harvested was analysed for ketoconazole by a modified and validated HPLC method with UV detection in the range 400–14000 ng/ml, using 200 µl of plasma in a full-run time of 2.5 min. The pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\alpha}$, C_{max}, T_{max} and $t_{1/2}$ were determined from plasma concentrations of both formulations and the results discussed. AUC_{0-t}, $AUC_{0-\alpha}$ and C_{max} were tested for bioequivalence after log transformation of data, and no significant differences were found either in 90% classic confidence interval or in the Anderson and Hauck test ($p < 0.05$). Based on statistical analysis, it is concluded that Onofin-K® is bioequivalent to Nizoral®. Copyright © 2004 John Wiley & Sons, Ltd.

Key words: ketoconazole; bioequivalence; HPLC; pharmacokinetics

Introduction

Ketoconazole (*cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3,-dioxolan-4-yl]methoxy]phenyl]piperazine is an imidazole derivative with antimycotic properties. Its main effect when it reaches serum levels during systemic administration is the inhibition of sterole 14-α-demethylase in fungi. This enzyme is coupled with the CYP450 complex and such an inhibition allows the accumulation of ergosterol in the cytoplasmic membrane of fungi, modifying the phospholipid arrangement and disrupting the function of ATPases and other membrane electronic transporter systems, resulting in the blocking of fungi proliferation [1].

Ketoconazole has been used clinically in the treatment of blastomycosis, histoplasmosis, coccidiomycosis and some types of candidiasis with a high degree of success [2]. Recently, there has been renewed interest in the clinical use of ketoconazole in certain opportunistic infections in immunocompromised patients [3,4] due to its effect on preventing metastases of certain kinds of cancer [5,6], and for its effect on enhancing the bioavailability of other drugs [7,8].

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The oral absorption of ketoconazole is highly dependent on gastric pH, being well absorbed at lower pH. It is 99% bound to plasma proteins and is widely distributed in the organism. During passage through the liver, it is metabolized into inactive by-products, which are excreted in the faeces and urine.

Ketoconazole presents a clearance of 8.4 ± 1.6 l/kg and a terminal half-life of 3.3 ± 1.0 h [9]. However, it has been reported that oral bioavailability of ketoconazole administered in tablet form showed no considerable intersubject variability [10].

The aim of the present work was to determine the bioequivalence between Onofin-K® (ketoconazole tablet 200 mg) as the test formulation and Nizoral® (ketoconazole tablet 200 mg) as the reference formulation in healthy female Mexican volunteers.

Materials and Methods

Study formulation

The test product was Onofin-K® 200 mg tablets; Batch no: 2652/Expires 11-2004; Manufacturer: Farmacéuticos Rayere, S.A. Mexico City, Mexico. The reference product was Nizoral® 200 mg tablets; Batch no: 1HDX5/Expires 08-2006; Manufacturer: Janssen-Cilag, S.A. Puebla, Mexico.

Clinical design

Volunteers. Twenty four healthy female volunteers participated in the study at the Research Center of Clinical Pharmacology in the Hospital General de Mexico under the following inclusion criteria: age between 18 and 45 years; nonsmokers or having quit smoking 72 h before the beginning the study, with a body mass index (BMI) between 20 and 29, a normal clinical history, thorax radiography and electrocardiogram without abnormalities, ±10% normal values in laboratory tests (haematology, blood biochemistry, hepatic function and urine analysis) and negative results for AIDS, hepatitis types B and C and pregnancy test. Exclusion criteria were a recent allergic process treated with astemizole, any illness 4 weeks prior to the study, a history of drug addiction, or the use of any drug 2 weeks before study initiation. Throughout the study, care was taken to avoid the presence of exclusion criteria such as hypersensitivity toward ketoconazole, loss of two or more samples around C_{max}, or dietetic transgression (eating or drinking something extra or different from that established on the clinical protocol). Signed informed consent forms were obtained from each volunteer and the study protocol was reviewed and approved by the Ethics Board of Hospital General de México.

Drug administration and sample collection. The study was carried out with a single 400 mg (two tablets) dose of ketoconazole; the trial design was two treatments, two periods, two sequences, double-blind, crossover and randomized. Treatment groups were balanced, having the same number of volunteers randomly distributed into product administration sequences. Reference and test products were blinded by an identification code for both clinical and analytical phases of the study. Decoding was carried out prior to the statistical analysis.

Volunteers entered 12 h before phase 1 initiation, having dinner at 8:00 pm and fasting overnight (12 h). The following morning, an indwelling cannula was fixed and a single dose (400 mg ketoconazole) of either tablet (reference or test) was taken with 250 ml of water, orange juice and breakfast. Lunch and dinner were served 6 and 12 h after dose administration. Volunteers left the research center after dinner and re-entered phase 2 after 6 days (the day before the second administration).

Blood samples, approximately 5 ml, were taken through the cannula at 0 h (prior to administration) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 9 and 12 h after dosing. Samples were collected in heparinized glass tubes and centrifuged at 4000 rpm for 10 min at 4°C, while plasma was separated in labelled cryovials and kept frozen at −70°C until chromatographic analysis.

Sample preparation for HPLC analysis. Sample purification and HPLC conditions used with our modifications were previously reported [11]. Briefly, a 200 µl aliquot of plasma samples were measured into a 1.5 ml microcentrifuge tube

and deproteinized by adding 300 µl of cool acetonitrile (4°C). The mixture was vortexed for 30 s at a higher speed in a vortex mixer and then centrifuged at 17500 × g for 5 min. The supernatant was transferred into an HPLC insert and injected into the chromatographic system.

Chromatographic conditions. An analytical method was modified to use UV detection and validated before the study. All solvents used were HPLC grade. Solvents and other chemical reagents were purchased from J.T. Baker (Mallinckrodt Division, Xalostoc, México).

The ketoconazole standard was obtained from Rayere Pharmaceuticals, México. The HPLC system was from Waters Co. (MA, USA) and consisted of a solvent delivery binary pump (mod. 1525), autosampler (mod. 717), UV-VIS detector (mod. 2487) and temperature column heater (Methaterm, Methachem. Tech., Inc.). Integration was done using Millennium® 32 software (Waters Co.).

Chromatographic separation was performed using XTerra® MS C18, 4.6 × 100 mm, 5 µm particle column (Waters). The mobile phase consisted of 0.05 M disodium hydrogen orthophosphate and acetonitrile (50:50 v/v) adjusted to pH = 6.0 with glacial acetic acid. A volume of 50 µl of supernatant was injected into the HPLC system and analysis was run at a flow rate of 1.5 ml/min, the autosampler temperature was maintained at 15°C, the column temperature was controlled at 30°C and the detector was fixed at 240 nm. The samples were quantified using peak height vs concentration in a log-log regression model.

Assay of validation. The analytical method was validated following criteria established in Mexican regulatory guidelines [12]. Standard calibration curves were constructed by spiking drug-free human pool plasma with a known amount of ketoconazole at concentrations of 400, 1000, 4000, 8000, 12000 and 16000 ng/ml.

The ketoconazole stock solution was prepared weekly by dissolving 10 mg of ketoconazole standard in 10 ml of methanol to achieve a concentration of 1000 µg/ml; this solution was maintained at 4°C. A working ketoconazole solution was prepared daily by a 1:10 dilution of stock solution with ultra pure grade water (18 megaohm.cm). Quality control points at low, medium and high levels (2000, 6000 and 10000 ng/ml, respectively) were used to determine absolute recovery and within-day and between-day precision and accuracy. Stability, limit of quantification and selectivity were also evaluated.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated considering plasma data, a single extravascular dose in a non-compartmental model, using WINNONLIN™ version 3.1 software [13]. Elimination half-life ($t_{1/2}$), area under curve to last measurable concentration (AUC_{0-t}), and area under curve extrapolated to infinity ($AUC_{0-\infty}$) were software outputs. The maximum observed concentration (C_{max}) and time of C_{max} (T_{max}) were experimentally obtained by observation.

Statistical analysis. ANOVA for a standard 2 × 2 crossover design was used to evaluate fixed effects such as period, sequence, formulation and carryover. For bioequivalence analysis, AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as primary variables. Decimal logarithm values of these parameters were considered to construct a classic confidence interval at 90% (90% CI) with a significance level (α) of 0.05 and assuming normal distribution of data. Moreover, an interval hypothesis based on Anderson and Hauck procedure was tested, with a significance level (α) of 0.05. Both data analyses were conducted according to FDA recommendations for establishing bioequivalence [14]. All statistical procedures were performed with SAS/STAT® version 8.2 software [15].

Results and Discussion

The original 24 volunteers concluded the study; the demographic and mean health parameters of all participants are summarized in Table 1; as can be observed, volunteers formed an homogeneous population in terms of age, weight, height and BMI. Ketoconazole was generally well tolerated by the volunteers. Five cases of adverse effects

Table 1. Demographic and health parameters of all 24 female healthy volunteers considered in the bioequivalence study

	Age (years)	Weight (kg)	Height (m)	BMI (kg/m^2)	Breath rate (min^{-1})	Heart rate (min^{-1})	Temp. (°C)	Blood pressure (mm Hg)
Mean	22.2	61.16	1.60	23.83	18.38	71.92	36.34	107/64
SD	2.40	8.99	0.05	2.03	1.47	6.50	0.35	9.2/8.0

(slight cephalea) possibly associated with drug administration were reported in phase 1 (three volunteers with Onofin-K® and two with Nizoral®), and three additional cases of adverse affects were reported in phase 2 (two volunteers with Onofin-K® and one with Nizoral®) for different volunteers. Gastrointestinal disorders, the most common adverse effect associated with oral administration of kentoconazole, were not reported. Although it has been reported previously that oral absorption of ketoconazole is highly dependent on gastric pH [9,10], it did not seem that the co-administration of ketoconazole with orange juice and breakfast resulted in a more homogeneous absorptive phase, as can be seen in the pharmacokinetic profiles.

The elimination phase of ketoconazole was biphasic, becoming greater ($t_{1/2}$=10 h) 8–10 h after dosing; thus the washout period of 1 week during clinical trial was sufficient, due to the fact that no sample prior to administration on phase 2 showed ketoconazole levels.

The described analytical method used for measurement of ketoconazole in plasma was shown to be accurate and sensitive. An internal standard was not used in the study, and the log-log model constructed between the peak height and ketoconazole concentration (log [height] = 0.4599 + 1.007 log [conc.]) proved to be linear over a range of concentration measured ($r = 0.996$) and with less % deviation for each point compared with a first-order model.

The run time was 3.0 min and the retention time of ketoconazole was 2.04 min (Figure 1). The peak of ketoconazole was well resolved and diode array scanning demonstrated that it was chromatographically pure and spectroscopically homogeneous. Data of absolute recovery and between-day and within-day precision are summarized in Table 2.

Throughout stability tests, ketoconazole proved stable in biological samples for at least two freeze-and-thaw cycles, with a final mean recovery of 100.30% and coefficient of variation (C.V.) of 1.62%. Moreover, ketoconazole in plasma was stable at room temperature (approximately 20°C) for at least 6 h, with a final recovery of 101.88% and C.V. of 6.14%. During long-term stability, ketoconazole in plasma was stable at −70°C for at least 30 days with a mean recovery of 102.76% and C.V. of 5.74%. Supernatant samples containing ketoconazole after the extraction technique were stable in an autosampler for at least 12 h, with a recovery of 101.83% and C.V. of 1.73%.

Finally, during validation other drugs were tested to establish the selectivity of the assay, evaluating possible interference with ketoconazole quantification. Heparin, sodium citrate, chlorphenamine and acetaminophen showed no interference with ketoconazole measurement.

The adapted method used in our laboratory is as precise and accurate as reported previously [11,16]. The limit of quantification was 400 ng/ml with C.V. of 6.7%; thus, it was sufficiently sensitive for the purpose of the present work and proved to be faster.

The concentration-time value profiles of all volunteers for the two formulations are shown in Figure 2, indicating that the plasma drug concentrations of two brands were similar, Nizoral® having a higher variability in each point. T_{max} was observed 2 h after drug administration and the last samples were sufficient for calculating at least 80% of $AUC_{0-\alpha}$. All calculated pharmacokinetic parameters are summarized in Table 3 and agree with previous reported values [9,10,16] except with those reported by Yuen and Peh [11], who observed the same values with fluorescent detection employing a dose of 200 mg, which was half the dose used in this and other work.

As can be seen in Table 3, 90% CI for all compared pharmacokinetic parameters (ratios of

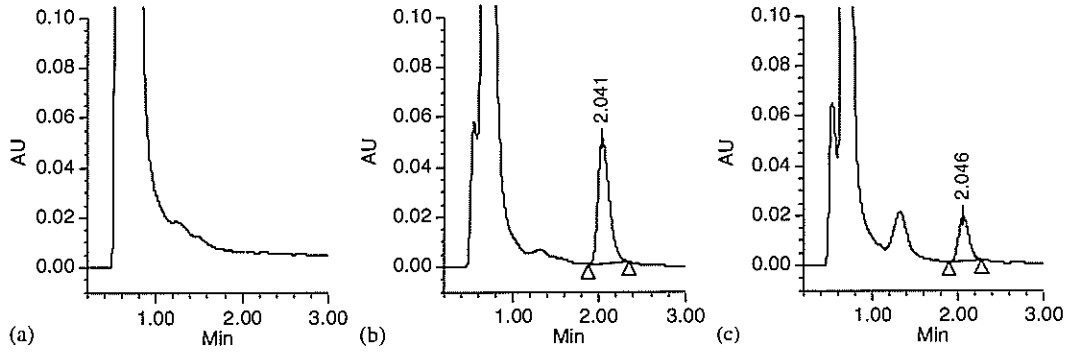

Figure 1. Chromatograms for analysis of ketoconazole in plasma. (a) blank plasma, (b) plasma spiked with 12000 ng/ml of ketoconazole, and (c) a volunteer plasma containing 5500 ng/ml 2 h after single oral administration of 400 mg of ketoconazole

Table 2. Absolute recovery, within-and between-day precision and accuracy of HPLC method used in quantification of ketoconazole in plasma

Concentration	Recovery ($n = 4$)		Within-day ($n = 5$)		Between-day ($n = 6$)	
(ng/ml)	Mean (%)	C.V.%	Accuracy (%)	Precision (C.V.%)	Accuracy (%)	Precision (C.V.%)
2000	108	0.9	104.2	10.42	100.2	8.04
6000	106	0.4	98.3	9.34	95.1	6.58
10000	105	0.2	97.3	5.75	101.0	7.63

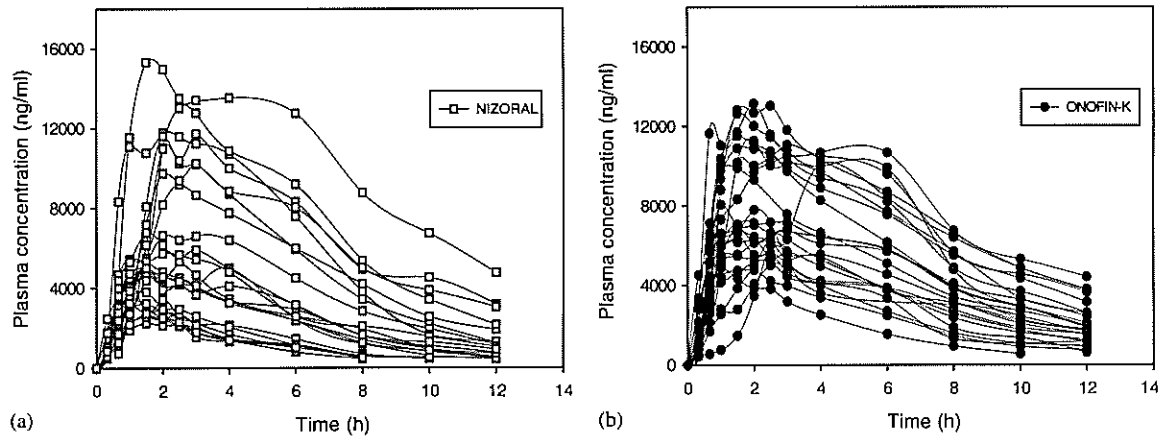

Figure 2. Plasma concentration profiles of ketoconazole tablets after single oral administration of 400 mg of two brands in 24 female healthy volunteers

AUC_{0-t}, $AUC_{0-\alpha}$, C_{max}) were contained in the 80%−125% interval and were very narrow, which showed close similarity between the two formulations. Moreover, the Anderson and Hauck test for all analysed parameters showed no significant differences.

The aim of bioequivalence trials is to assure interchangeability between an innovator formulation and a generic formulation in terms of efficacy and safety. When a pharmacological effect is difficult to measure, then plasma levels of the drug may be used as an indirect indicator of clinical activity. Therefore, the ketoconazole plasma levels obtained in this study with a very fast and accurate method suggest equal clinical efficacy of the two brands of ketoconazole tested

Table 3. Pharmacokinetic parameters of both brands of ketoconazole tablets

Pharmacokinetic Parameter	Onofin-K® (test)	Nizoral® (reference)	90% CI[a] (80%−125%)	Anderson and Hauck test[a] ($p<0.05$)
AUC_{0-t} (ng/mlh)	57809.13 ± 4439.93	39963.46 ± 5772.09	103.06 to 106.05	1.9×10^{-14}
$AUC_{0-\infty}$ (ng/mlh)	71628.41 ± 6163.48	46161.10 ± 7135.89	104.22 to 107.53	3.84×10^{-13}
C_{max} (ng/ml)	8494.64 ± 561.21	6646.71 ± 763.87	102.54 to 105.28	5.78×10^{-19}
T_{max} (h)	2.3 ± 0.24	2.02 ± 0.18		
$t_{1/2}$ (h)	4.25 ± 0.19	3.47 ± 0.40		

Values are given as mean ± standard error.
[a]Statistics were applied on decimal logarithm-transformed data; $n = 24$.

and provide pharmacokinetic data from a Mexican population under a regulatory framework [12] not reported previously.

Conclusions

Statistical analysis of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ between Onofin-K® and Nizoral® (both 200 mg ketoconazole tablets) indicated no significant differences in any compared pharmacokinetic parameters. Therefore, it can be established that Onofin-K®, produced by Farmaceúticos Rayere, S.A., México, is bioequivalent to Nizoral® produced by Janssen-Cilag, México, and that both formulations can be considered equally effective in therapeutics.

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