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Evaluation of Bioequivalence of Two Formulations Containing 200 mg of Ketoconazole

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The bioequivalence of two oral formulations containing ketoconazole 200 mg was determined in 24 healthy Indian male volunteers. The study was designed as a single dose, two phase, two sequence cross-over study with a wash out period of one week. The content of ketoconazole in plasma was determined by a validated HPLC method using BDS Hypersil C18, (250 × 4.6 mm 5 µ particle size) column. The mobile phase consisted of 0.1 M disodium hydrogen phosphate buffer (pH adjusted to 6.0 with glacial acetic acid) and acetonitrile in the ratio of 60:40 v/v and eluted at a flow rate of 1 mL/min. The eluent was monitored using UV detection at 240 nm. The preparations were compared using the parameters area under the plasma concentration-time curve ($AUC_{0-\infty}$), peak plasma concentration (Cmax) and time to reach peak plasma concentration (Tmax). No statistically significant difference was observed between the logarithmic transformed $AUC_{0-\infty}$ and Cmax values of the two preparations. The 90 % confidence interval for the ratio of the logarithmic transformed $AUC_{0-\infty}$ and Cmax were within the bioequivalence limit of 0.80-1.25. Moreover, the elimination rate constant (Kel) and elimination half life ($t_{½}$) values obtained with the two preparations were comparable and not significantly different statistically.

Key Words: Bioequivalence, Ketoconazole, Pharmacokinetics, HPLC.

INTRODUCTION

Ketoconazole(*cis*-1-acetyl-4-(4-(2-(2,4-dichlorophenyl)-2-(1H-imidazole-1-ylmethyl)-1,3-dioxalon-4-yl)methoxyphenyl 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 Cytochrome P_{450} complex and such an inhibition allows the accumulation of ergosterol in the cytoplastic membrane of fungi, modifying the

phospholipids 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 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 immuno compromised 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]. The oral absorption of ketoconazole is highly dependent on gastric 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. However, it has been reported that bioavailability of ketoconazole administered in tablet form showed no considerable inter subject variability[9].

EXPERIMENTAL

Dermazole 200 mg tablet (Mfd. by Kusum Healthcare, Delhi, India), and the reference product is Nizoral®, (Mfd. by Janssen pharmaceutica, Beerse, Belgium).

Study design: 24 Indian male volunteers aged between 18 and 45 years (23.2 ± 1.5 y) and with body mass index between 20 and 24 (22.5 ± 1.6), were included in a randomized, single dose, two phase, two sequence, cross-over study with a 1 week washout period. Informed consent was obtained from all the volunteers prior to the start of the study. Various physical, biochemical and hematological tests were carried out before enrolling the volunteers for the study. Approval from Drugs Control General of India (DCGI) and Institutional Ethical Committee of Jadavpur University was obtained prior to the start of the study.

Drug administration and sample collection: All the volunteers assembled in Clinical Pharmacology Unit (CPU) ward at 6.00 a.m. on the study day of each session, after overnight fasting of 10 h. Their total pulse rate, blood pressure was recorded. The subjects received either of the study preparations. According to FDA and EMEA regulations, the sampling schedule should be planned to provide a reliable estimate of the extent of absorption. This is generally achieved if AUC_{0-t} is at least 80 % of $AUC_{0-\infty}$. Usually the sampling time should extend to at least three terminal elimination half lives of the active ingredient. Time periods between sampling should not exceed one terminal half life[10]. A total of 15 blood samples were collected at 0 h (before drug administration) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0 12.0, 24.0 h (after drug administration) in the test tubes with EDTA at each time point. Breakfast, lunch and dinner

were provided after 3, 6 and 13 h, respectively after drug ingestion. Collected blood samples were centrifuged immediately, plasma was separated and stored frozen at -20ºC with appropriate labeling of volunteer code number, study date and collection time, till the date of analysis.

Sample preparation: 1.0 mL of plasma was taken in a stopper test tube. To this 0.1 mL of voriconazole (5 mcg/mL) was added and mixed for 1 min. To this mixture 7 mL of dichloromethane was added and vortexed for 10 min manually. It is then centrifuged for 5 min at 5000 rpm. 6 mL of organic layer was removed in a separate tube and evaporated in presence of nitrogen atmosphere at 40-50°C. The residue was reconstituted with 200 µL of mobile phase and was injected into the HPLC system. The use of internal standard, liquid-liquid extraction gives excellent recovery of the drug from plasma, also appearance of peaks after 5th min gives good resolution without interference of plasma peaks which is superior than the other methods for bioequivalence of ketoconazole[11].

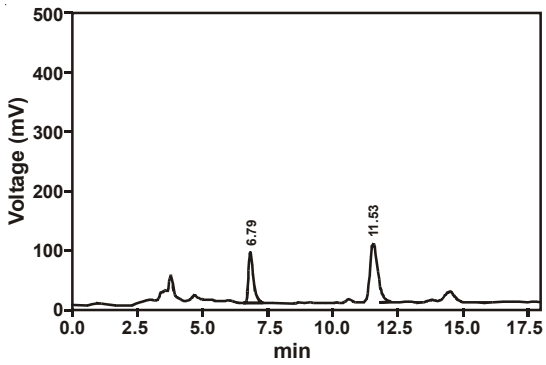
Chromatographic conditions: All the solvents used were of HPLC grade and were purchased from Spectrochem India HPLC grade, while other chemicals and reagents were of analytical grade. Ketoconazole was obtained from Kusum Healthcare, India. The HPLC system was Knauer, Germany and it consisted of a solvent delivery pump (K-501), a Rheodyne injector with 100 µL loop size and an UV-Visible detector (K-2501). Integration was done using Chemitochrom 2000 software. Chromatogram separation was done using a BDS Hypersil C18, (250 × 4.6 mm, 5µ particle size) column. The mobile phase consisted of 0.1 M disodium hydrogen phosphate buffer (pH adjusted to 6.0 with glacial acetic acid) and acetonitrile in the ratio of 60:40 v/v and eluted at a flow rate of 1 mL/min. The effluent was monitored using UV detection at 240 nm. The method was as per the following guidelines[12].

Pharmacokinetic analysis: The following pharmacokinetic parameters were directly determined or calculated by the standard non-compartmental method. Both maximum plasma concentration (Cmax) and time to peak plasma concentration (tmax) were directly obtained from the data. The elimination half-life ($t_{½}$) was calculated as 0.693/Kel, where Kel is the apparent elimination rate constant. Kel was in turn, calculated as the slope of the linear regression line of natural log-transformed plasma concentrations. The last seven quantifiable levels were used to determine Kel. The area under the plasma concentration-time curve (AUC_{0-t}) was calculated from the measured levels, from time zero to the time of last quantifiable level, by the linear trapezoidal rule. ($AUC_{0-\infty}$) was calculated according to the following formula: $AUC_{0-\infty} = AUC_{0-t} + C_{last}/Kel$, where C_{last} is the last quantifiable plasma level.

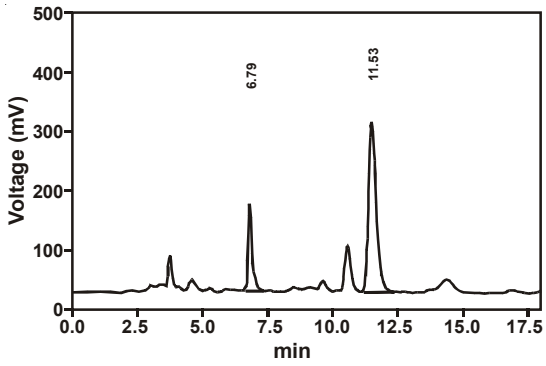
Statistical analysis: Analysis of variance was performed on the pharmacokinetic parameters Cmax, $AUC_{0-\infty}$ using general linear model (GLM) procedures in which sources of variation were subject, formulation and period. The 90 % confidence interval of the test/reference ratios for Cmax, AUC_{0-24} and $AUC_{0-\infty}$ (log transformed) were determined. Bioequivalence between the two formulations can be concluded when 90 % confidence interval for the pharmacokinetic parameters of the two products are found within the acceptable range of 80-125 %.

RESULTS AND DISCUSSION

In HPLC method, no interferences were observed in human plasma. The retention time for ketoconazole was 11 min and voriconazole (internal standard) was 6 min (Fig. 1). The limit of quantification for ketoconazole in plasma was 0.1 µg/mL. Under the described conditions, the lower limit



(A). Plasma spiked with Drug and Internal standard. 6.79 - Retention time of internal standard (Voriconazole); 11.53 - Retention time of Test drug (Ketoconazole)



(B). Plasma sample of volunteers. 6.77 -Retention time of internal standard (Voriconazole). 11.49 - Retention time of Test drug (Ketoconazole)

Fig. 1

of detection was 0.05 μg/mL. The relationship between concentration and peak area was found to be linear within the range of 0.1 to 10 μg/mL quality control points at low, medium and high levels (0.25, 4 and 8 μg/mL) were used to determine absolute recovery and within-day and between-day precision and accuracy. Stability, limit of quantification and selectivity were also evaluated.

Average concentration *vs.* time curves after administration of reference and test products to healthy volunteers are shown in Fig. 2. Average values of pharmacokinetic parameters after administration of reference and test product to healthy volunteers are summarized in Table-1. The limits of the 90 % confidential interval for the ratios of AUC_{0-t}, $AUC_{0-\infty}$ and Cmax for their log transformed data fell within 0.80 to 1.25 (Table-1).

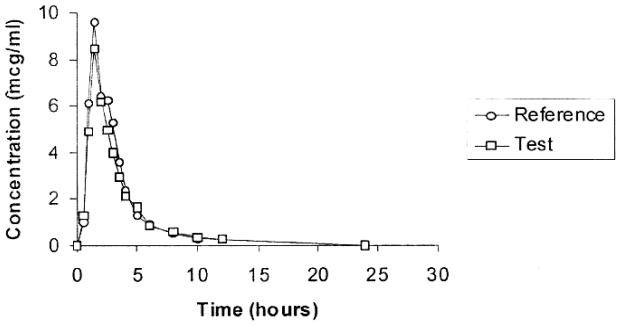


Fig. 2. Plasma concentration *vs.* time profile

TABLE-1
PHARMACOKINETIC PARAMETERS

Parameter	Test	Reference	90 % CI
AUC_{0-t} (μg/mL h)	34.50 ± 2.12	35.20 ± 2.69	91.78 - 100.93
$AUC_{0-\infty}$ (μg/mL h)	38.31 ± 1.83	38.71 ± 2.99	93.88 - 102.28
Cmax (μg/mL)	7.11 ± 0.57	7.10 ± 0.47	91.10 - 101.03
Tmax (h)	2.10 ± 0.36	2.16 ± 0.31	–
$T_{½}$	3.31 ± 0.69	3.07 ± 0.67	–
Kel	0.21 ± 0.03	0.23 ± 0.04	–

AUC_{0-t}, $AUC_{0-\infty}$ = Area under plasma concentration time curve and at infinity time; Cmax = Maximum plasma concentration; Tmax = time to reach maximum plasma concentration; $T_{½}$ = Elimination half-life; Kel = apparent elimination rate constant.

The described analytical method used for measurement of ketoconazole was shown to be accurate and sensitive. An internal standard (voriconazole) was used in the study and the log-log model constructed between peak

height and ketoconazole concentration proved to be linear over a range of concentration measured (r = 0.9987) and with less per cent deviation for each point. The run time was 20 min and the retention time of ketoconazole was 11.0 min. The peak of ketoconazole was well resolved (Fig. 1). The high recovery of extraction and the use of internal standard in the proposed method provide excellent linearity, precision and accuracy.

Throughout the stability tests, ketoconazole proved stable in biological samples for at least two freeze and thaw cycles with a final mean recovery of 98.42 % and C.V. of 4.8 %. Ketoconazole in plasma was stable at room temperature for at least 6 h. The limit of quantification was 0.1 µg/mL with C.V. of 3.2 %.

Ketoconazole was well tolerated by all the volunteers. No adverse events were reported and there were no drop outs. Gastrointestinal disorders, the most common adverse effect associated with the use of anti-mycotic drugs were not observed.

The elimination half life of ketoconazole was in the range 2-3 h. Thus the washout period of one week was sufficient due to the fact that no sample prior to administration in phase 2 showed no ketoconazole levels. Time to reach maximum plasma concentration (Tmax) was observed at 2-10 h after drug administration and the last samples were sufficient for calculating at least 80 % of $AUC_{0-\infty}$. Cmax levels were observed after 2.10 ± 0.36 h (test) and 2.16 ± 0.31 h (reference). The Cmax and $AUC_{0-\infty}$ of test and reference were 7.11 ± 0.57 *vs.* 7.10 ± 0.47 µg/mL and 38.31 ± 1.83 *vs.* 38.71 ± 2.99 µg/mL h. The mean $t_{½}$ for test and reference were 3.31 ± 0.69 h and 3.07 ± 0.67 h, respectively. The relative bioavailability between test and reference was 0.98.

From Table-1, it has been observed that 90 % CI for all the compared pharmacokinetic parameters were obtained within the range of 80-125 %. Moreover the analysis of variance for all the analyzed parameters showed no significant differences.

The aim of the bioequivalence trials is to assure interchangeability between an innovator and a generic formula in terms of efficacy and safety. When a pharmacological effect is difficult to measure, the plasma levels of drug may be used as an indirect indicator of clinical activity. Therefore the ketoconazole plasma levels obtained in this study suggest a equal clinical efficacy of the two brands tested and provide pharmacokinetic data from an Indian population.

Therefore 90 % confidence interval of $AUC_{0-\infty}$ and Cmax ratios of ketoconazole of these two preparations fell in the acceptable range. Both formulations were equal in terms of rate and extent of absorption. Consequently bioequivalence between two formulations can be concluded.

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