Bioequivalence Evaluation of Two Formulations of Fluconazole 150 mg Capsule in Healthy Arab Men

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ABSTRACT: A randomized crossover study was conducted on 26 healthy Arab males to compare the bioavailability of two formulations of fluconazole 150 mg capsules, Fluconazole™ (test) and Diflucan® (reference). The formulations were administered after an overnight fast with a washout period of 2 weeks. Twenty blood samples (per period) were collected over 168 h, plasma fluconazole concentrations were determined by locally validated high performance liquid chromatography (HPLC) assay and pharmacokinetic parameters were analysed by the standard non-compartmental method.

The mean ± SD maximum concentration ($C_{\text{max}}$), time to reach maximum concentration ($T_{\text{max}}$), area under the curve ($AUC_{0-\infty}$ and $AUC_{0-\tau}$) and elimination half-life ($t_{1/2}$) were $3.17 ± 0.47$ and $3.24 ± 0.59 \mu g/ml$, $2.62 ± 2.01$ and $2.65 ± 1.63$ h, $149.52 ± 29.49$ and $151.36 ± 25.84 \mu g.h/ml$, $163.57 ± 29.9$ and $164.89 ± 26.46 \mu g.h/ml$, and $36.81 ± 5.72$ and $36.56 ± 5.36$ h for the test and reference drug, respectively. These values are similar to previously reported values in other ethnic groups. The parametric 90% confidence intervals on the mean of the difference (test-reference) between the log-transformed values of the two formulations were 95.484% to 101.035%, 96.382% to 101.245% and 94.621% to 102.074% for $AUC_{0-\infty}$, $AUC_{0-\tau}$ and $C_{\text{max}}$, respectively. The results indicate that the two formulations are equivalent in the rate and extent of absorption. Further, a review of the literature indicates that there is no apparent ethnic variation in the absorption and elimination rates of fluconazole.

Key words: fluconazole; pharmacokinetics; bioavailability; bioequivalence

Introduction

Oral fluconazole, a triazole antifungal, is characterized by a highly variable absorption rate [1], a time to peak plasma concentrations of 0.5 to 1.5 h [2], and a plasma elimination half-life of approximately 30 h [2]. Bioequivalence studies are required by the Ministries of Health of the Gulf Cooperation Council (GCC) countries in order to register and market generic drugs in the GCC countries. The aim of this study was to compare the rate and extent of absorption of two 150 mg capsule formulations of fluconazole, Fluconazole™ (test) and Diflucan® (reference), under fasting conditions.

Subjects and Methods

Twenty-eight healthy (medical history, clinical examination and routine laboratory investigation within 30 days), adult Arab males were enrolled in the study; one withdrew before starting, and another after completing, the first part of the study, both for personal reasons. The mean ± SD age and body mass index of the remaining 26 volunteers were $33.3 ± 6.5$ years and $24.5 ± 3.2$ kg/m². The volunteers were non-smo-
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...ers (except for three who did abstain 48 h prior) and confirmed that they had abstained from taking alcohol or caffeine or related xanthene-containing beverages or food for 48 h, and from taking any drug for 2 weeks prior to, and throughout the study (except for one volunteer who took one Panadol® tablet for headache 1 day before starting part two of the study). Standardized breakfast and dinner were given at 4 and 10 h after drug administration. None of the volunteers vomited and no adverse events were identified except for bouts of asymptomatic sinus tachycardia in one volunteer. They signed a consent form that was approved by the Research Ethics Committee of the King Faisal Specialist Hospital and Research Centre. The study was conducted according to the Declaration of Helsinki, Good Clinical Practice and Good Laboratory Practice guidelines.

A single 150 mg capsule of either formulation (Fluconazole™, manufactured by Jamjoom Pharma, Jeddah, Saudi Arabia, lot number 411123, expiry date 11/2005, and Diflucan®, manufactured by Pfizer Italiana, lot number 300706, expiry date 02/2005) was administered with 250 ml of water after an overnight fast, in a two-way crossover random design with a 2 week washout period. Venous blood samples were collected in heparinized tubes, before and at 0.33, 0.66, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144 and 168 h after drug administration. The plasma was harvested in coded polypropylene tubes and stored at –20 °C for 1–7 days at the clinical study site and then at –80 °C until analysed.

A high performance liquid chromatographic assay was locally developed and validated. Briefly, fluconazole and acetophenetidin (internal standard, IS) were separated (retention times of 5.7 and 11.8 min, respectively) at room temperature on a Nova-Pak C18 cartridge and detected spectrophotometrically at 260 μm. The mobile phase, 0.01 M disodium hydrogen phosphate (pH 7.0) and acetonitrile (75:25 v/v), was delivered at 1.0 ml/min. Prior to injection, 200 μl of 0.02 M phosphate buffer (pH 7) and 1 μg of the IS were added to 500 μl plasma samples and the mixture was passed through an Amicon Centrifree-MS filter (Millipore Corporation, Bedford, MA). The relationship between the fluconazole concentration and peak height ratio (fluconazole/IS) was linear (R² > 0.997) in the range 0.2–12 μg/ml. The intra- and inter-day coefficient of variations were ≤8.62% and ≤9.24%, respectively. The quantification limit was 0.2 μg/ml. The mean extraction recoveries of fluconazole and the IS were 90% and 83%, respectively. Fluconazole in plasma was stable (103%) for at least 10 weeks when stored at −20 °C. All samples were analysed blindly within 1 month from collection, and after a single cycle of freeze and thaw.

Pharmacokinetic calculations were performed according to the standard non-compartmental method. Analysis of variance (ANOVA) for crossover design was used to assess the effect of formulation, period, sequence and subjects nested in sequence on natural log-transformed data of $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{\text{max}}$, $K_{\text{el}}$, $t_{1/2}$ and $C_{\text{max}}/AUC_{0-\infty}$ and on untransformed data for $T_{\text{max}}$. The sequence effect was tested against the mean squares term of subjects nested in sequence. Parametric 90% confidence intervals on the mean of the difference between the two formulations (Fluconazole™-Diflucan®) of log-transformed values of $AUC$ and $C_{\text{max}}$ were computed using the mean residual error obtained from ANOVA. In addition, bioequivalence was assessed by Schuirmann’s two one-sided $t$-tests procedure [3].

Results and Discussion

The mean timed plasma concentrations of fluconazole are shown in Figure 1. Pharmacokinetic parameters are shown in Table 1. ANOVA revealed that none of the effects examined was statistically significant except for subjects’ effect, which was calculated as inter-subject and intra-subject coefficient of variations (Table 1). The absence of significant sequence or period effects suggests that the crossover design was properly conducted. The mean ratio of $AUC_{0-t}/AUC_{0-\infty}$ for test and reference formulations of 92.8% and 91.7%, respectively, indicates that the sampling time was adequate [4]. The 90% confidence limits for $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{\text{max}}$ as well as the results of the Schuirmann’s two one-sided $t$-tests are also shown in Table 1. The 90% CI for $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{\text{max}}$ were within the...
bioequivalence acceptable range of 80% to 125% [5]. Furthermore, the results of the Schuirmann’s t-test indicated that the lower and upper limits of the calculated t-test were greater than the critical t-value. Therefore, the two formulations can be considered bioequivalent with regard to the extent and rate of absorption.

Previous studies from Brazil [1,6] and Thailand [7] reported a $K_{el}$ of 0.021 to 0.023 h$^{-1}$ [1,7], $T_{\text{max}}$ of 1.18 to 1.59 h [7], $C_{\text{max}}$ of 2.61 to 3.84 µg/ml [1,6], $AUC_{0-\infty}$ of 152 to 181 µg.h/ml [1,6] and $C_{\text{max}}/AUC_{0-\infty}$ of 0.108 to 0.023 h$^{-1}$ [1,6]. These values are similar to the corresponding values obtained in the current study, suggesting that
ethnicities may have little or no effect on the elimination process, half-life or extent of absorption of fluconazole. This is not unexpected [9], fluconazole possesses certain properties such as linear pharmacokinetics, minimal metabolism, high bioavailability and a low potential for protein binding, which make it less likely to be sensitive to racial differences.

It is concluded that the two formulations of fluconazole capsules examined are bioequivalent. Further, a review of the literature indicates that there is no apparent ethnic variation in the rates of absorption and elimination of fluconazole.

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References