Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-190
Volume 106 Issue 2

Comparative Study of the Main Pharmacokinetic Parameters of a Newly Developed Fluconazole Formulationin Healthy Iraqi Volunteers

Jwaid AH[1]*, Mohammed MM[2] and Kharaba HA[3]

[1]Department of Pharmacology and toxicology, University of Baghdad, Iraq
[2]Department of Pharmacy, Al Rasheed University College, Iraq
[3]Department of Pharmaceutics, University of Baghdad, Iraq

Abstract

Objective: Crossover design study was carried out to compare the main pharmacokinetic parameters of a newly developed generic formula of fluconazole as a test product with the standard reference fluconazole product of Pfizer company under the brand name Diflucan®.

Subjects and methods: Twenty-eight volunteers of healthy Iraqi male were involved in this study. The formulations were administered as a single 150 mg dose of the tested and reference fluconazole after an overnight fasting state. Plasma concentration of fluconazole from each volunteer were measured over 24-hourinterval using high performance liquid chromatography (HPLC) assay. From serum concentration versus time, data of each subject,the pharmacokinetic parameters represented by the mean ± SD maximum concentration (C_{max}) of fluconazole, time to reach maximum fluconazole concentration (T_{max}), area under the curve (AUC_{0-t}) were calculated.

Results: The mean±SD (% CV) of the C_{max} (ng/ml), time to reach T max (h), AUC_{0-t} (ng.h/ml) were 2894.52±558.94 (19.4) and 3144.39±564.82 (17.4), 1.94±0.54 (55.3) and 1.82±0.88 (52.9), 88466.1±17632.4 (20.8) and 92274.5±15324.7 (15.9) for the tested and reference produce, respectively. Ratio of C_{max} (ng/ml), time to reach T_{max} (h), AUC_{0-t} (ng.h/ml) for the test verses the reference products were 0.92, 0.95 and 1.06, respectively. Since the 90% confidence intervals for these parameters were within the 80-125% of the interval ratio proposed by FDA, it wasconcluded that the newly developed fluconazole 150 mg tablet was bioequivalent to the reference product produced by Pfizer in term of the rate in addition to the extent of absorption and bioavailability. Consequently, newly developed fluconazole 150 mg tablet is interchangeable with fluconazole 150 mg tablet manufactured by Pfizer and can be prescribed as an alternative in the Iraqi market.

Keywords: Fluconazole; Diflucan; Liquid chromatography

Correspondence to: Jwaid AH, Department of Pharmacology and toxicology, University of Baghdad, Iraq, E-mail:

Citation: Jwaid AH, Mohammed MM, Kharaba HA (2020) Comparative Study of the Main Pharmacokinetic Parameters of a Newly Developed Fluconazole Formulationin Healthy Iraqi Volunteers. Prensa Med Argent, Volume 106:2. 190. DOI: https://doi.org/10.47275/0032-745X-190.

Received: January 07, 2020; **Accepted:** January 31, 2020; **Published:** February 05, 2020

Introduction

Triazole drugs are group of drugs that target the cell membrane of the Candida cells. The do so by depletion of the ergosterol component through inhibiting microsomal CYP (14-a-demethylase) enzyme which catalyze the lanosterol to ergosterol at the final stage of ergosterol's biosynthesis that result in agglomeration of the precursors of sterol. Such pathway of catalyze enzyme inhibition causes cell membrane structure alteration of Candida cells [1], and accumulation of 14-α-methyl sterols which is the key mechanism of fungistatic action of fluconazole [2]. The antifungal Fluconazole that belong totriazole group characterized by their highly variability in their absorption rate and their time to reach peak plasma concentration, which are between 0.5 to 1.5 hr. Furthermore, their plasma elimination half-life could reach to 30 h[3].

As there is exaggerated developing in the rate of invasiveness and superficial fungal infection in the last two decades [4-6]. This increases the need for a powerful antifungal treatment. After the introduction of fluconazole in 1990s, made it the drug of choice to all forms of Candida infections to both immunocompetent furthermore immunocompromised hosts [7,8]. Because of variability in absorption and bioavailability between different product, the study was aimed to compare the rate and extent of absorption of two 150 mg tablet formulations of fluconazole, Fluconazole (test) and Diflucan (reference), under fasting conditions, in healthy male and female volunteers.

Experimental Methods

Reagents and Chemicals

Fluconazole raw material was purchased from SP Farma, India, test tabletswas developed by college of pharmacy, department of pharmaceutics. Carbamazepine standard was purchased from Sigma-Aldrich Co, USA. Diflucan® Pfizer batch no: B289604, Manufactural

Date: 05-2018, expiry date 04-2023 was purchased from local drug store. All solvents (Acetonitrile and dichloromethane) used in extraction and analysis were HPLC grade.

HPLC Analysis

Shimadzu HPLC system with LC pump was the standard for the HPLC method preformation, in addition to SPD-20AVP UV detector, auto sampler and the injection system that fitted with 50 µl loop.

The mobile phase of the HPLC system was a mixture solvent of Acetonitrile and water at a ratio of 60:40 v/v that has isocratic elution mode in which a reversed phase of HPLC was used. On C-18 column (Phenomenix, 4.6 mm × 25 cm, 5 µm packing L1), the flow rate was 0.5 ml/min and 15 min run time at 210 nm using UV detector [9].

Accurately weighted 100 mg of Fluconazole was dissolved in 100 ml of diluents (acetonitrile: water in the ratio of 60:40), sonicated for 2 min, 1ml of the resulting, the final standard concentration was obtained by further dilution to 100 ml with diluent to obtain 100 µg/ml.

Sample preparation

Carbamazepine as internal standard was added to 50 µL tubes for extraction, and the removal of solvent (methanol) was done by evaporation. Two hundred micro litter of plasma was added to driedmicrotubes contain IS, then 50 µl 1.25 M NaOH was added with shaking for 15 seconds. Extraction of fluconazole was done by addition of dichloromethane (3 ml) with continuous shaking for 3 min, then cold centrifugation at 4°C at 4000 rpm for 45 min. The organic phase was separated, and solvent removal was done using rotator evaporator. The dry product was dissolved with 200 µL of mobile phase, 10 µL volumes were injected into the HPLC. The retention times were 9.5 and 13.7 minutes for fluconazole and carbamazepine (IS), respectively.

Sampling times

Twenty-eight volunteers were used in this study by administered 250 ml of tap water after an overnight fasting, 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. Coded polypropylene tubes were used for harvesting of the plasma and stored at -20°C for 1-7 days and then at -80°C till analyzed.

Subjects

Twenty-eight healthy male adults' volunteers with their demographic data shown in the table were accepted to enrollin the study (Table 1). Subjects were got acceptance for enrolment in this study if they have complied with all the inclusion and exclusion criteria stated in the protocol. All the participants were considered healthy depending on physical examination, ECG, and the following laboratory tests which include blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, Gamma GT, total bilirubin, albumin, total protein, triglycerides, total cholesterol, hemoglobin, hematocrit, total and differential white cell counts, routine urine tests, and negative for HIV and HBV. The protocol was carried out according to ethical guideline of Helsinki declaration.

Table 1: Demographic data of 28 subjects participated in the study.

Subjects	Mean	±SD	%CV	Min	Max
Age (Years)	27.4	4.2	20.7	19	44
Weight (Kgs)	73.3	9.3	13.5	65	86
Height (m)	1.69	0.04	3.6	1.54	1.79

Drug analysis

Blood samples (5 ml) from a suitable antecubital vein of each subject were collected into EDTA containing tubes at an interval: 0, 0.33, 0.67, 1,1.33, 1.67,2,2.5, 3,4,6,8,12, and 24 hrs. after administration of each of the test and the reference products. Centrifugation at 3000 rpm for all blood samples for 10 min at 4°C. The separated serum samples were transferred into polypropylene tubes and maintained frozen at -20°C till analysis.

Interassay variation was avoided by analyzing the samples from each single volunteer on the same day.

Concentrations of serum fluconazole weredetermined by HPLC according to the in-house procedure.

Pharmacokinetic and statistical analysis

The maximum observed serum concentration (C_{max}) and the time taken to achieve this concentration (T_{max}), the areas under fluconazole serum concentration were calculated by using the software (Kinetica®, Version 5) applying non-compartment data analysis approach as recommended by international bioequivalence guidance (FDA and EMEA). The bioequivalence between both formulations was determined by calculating individual C_{max}, and the ratio of the mean (test/reference).

Results

Pharmacokinetic and statistical analysis

The mean (±SD) serum concentration time profile of both test and reference formulations were shown in the figure (Figure 1) were similar and superimposable.

The arithmetic meanphar macokinetic parameters of fluconazole capsule after a single 150 mg dose for both formulations and the ratio of pharmacokinetic parameters for the tested versus the reference product are shown in the below tables (Tables 2 and 3).

Discussion

Triazole drugs fluconazole were successfully developed and introduced for the treatment of a wide variety of microbial infection from the time that itis becoming available in the markets because of their effectiveness in the treatment and a wide application in the medical field specially as antifungal therapy [10]. Fluconazole is one of

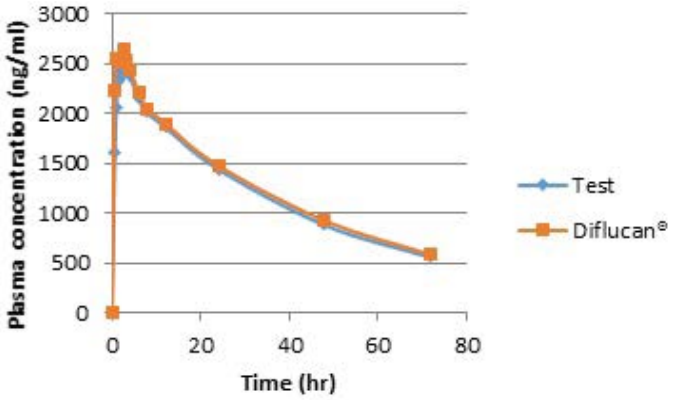

Figure 1: Plasma concentrations *vs.* time profiles of Fluconazole tablet of test product and Diflucan®.

Table 2: Pharmacokinetic parameters of fluconazole capsule after a single 150 mg dose of the test and the reference products.

Pharmacokinetic parameter	Test product Mean±SD (%CV)	Reference product Mean±SD (%CV)
C_{max} (ng/ml)	2894.52±558.94 (19.4)	3144.39±564.82 (17.4)
AUC_{0-t} (ng.hr/ml)	88466.1±17632.4 (20.8)	92274.5±15324.7 (15.9)
T_{max} (hr)	1.94±0.54 (55.3)	1.82±0.88 (52.9)

Table 3: Ratio of pharmacokinetic parameters for the test versus the reference products.

Pharmacokinetic parameter	Difference Test/Reference	Ratio Test/Reference
Cmax (ng/ml)	-249.87	0.92
AUC0-t (ng.hr/ml)	-3808.4	0.95
Tmax (hr)	0.12	1.06

the azoles antifungal drugs (triazole) that contain three nitrogen atoms in a cyclic ring. Fluconazole act by inhibition demethylase conversion of lanosterol to ergosterol. Deficiency in ergosterol effect on cell growth and proliferation. Furthermore, inhibition 14 α-demethylase causes accumulation of methylated sterols as a toxic component that cause membrane stress.

The maximum observed serum concentration (C_{max}), the time taken to achieve this concentration (T_{max}) and the areas under fluconazole serum concentration for the test product were 2894.52±558.94 (19.4), 1.82±0.88 (52.9) and 88466.1±17632.4 (20.8), respectively, and they are within the 80-125% of the interval that have been proposed by the Food and Drug Administration (FDA) in addition to the interval proposed of the European Medicines Evaluation Agency (EMEA).

Conclusion

The test formulation in this study is bioequivalent to the reference formulation obtain from Pfizer company under the brand name Diflucan® for both, the rate and the extent of absorption and such test product could be used as an alternative to the brand product for therapeutic application.

References

1. White TC, Marr KA, Bowden RA(1998) Clinical, cellular, and molecular factors that contributevto antifungal drug resistance. Clin Microbiol Rev 11:382-402.
2. Moraes LA, Lerner FE, Moraes ME, Moraes MO, Corso G, et al. (1999) Fluconazole bioequivalence study: quantification by tandem mass spectrometry. Ther Drug Monit 21: 200-207.
3. Debruyne D, Ryckelynck JP (1993) Clinical pharmacokinetics of fluconazole. Clin Pharmacokinet 24:10-27.
4. Banerjee SN, Emori TG, Culver DH, Gaynes RP, Jarvis WR, et al. (1991) Secular trends in nosocomial primary bloodstream infections in the United States, 1980-1989. National Nosocomial Infections Surveillance System. Am J Med 91:86S-89S.
5. Beck-Sague CM, Jarvis WR (1993) Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990. J InfectDis 167:1247-1251.
6. Diekema DJ, Messer SA, Brueggemann AB, Coffman SL, Doern GV (2002) Epidemiology of candidemia: three-year results from the emerging infections and the epidemiology of Iowa organism's study. JClin Microbiol 40:1298-1302.
7. Rex JH, Walsh TJ, Sobel JD, Filler SG, Pappas PG, et al. (2000) Practice guidelines for the treatment of candidiasis.Clin Infect Dis 30:662-678.
8. Richardson K, Cooper K, Marriott MS, Tarbit MH, Troke F, et al. (1990) Discovery of fluconazole, a novel antifungal agent. Clin Infect Dis12: S267-S271.
9. Santos SR, Campos EV, Sanches C, Gomez DS, Ferreira MC (2010) Fluconazole plasma concentration measurement by liquid chromatography for drug monitoring of burn patients. Clinics 65:237-243.
10. Gadhave PP, Dighe NS, Pattan SR, Deotarse P, Musmade DS, et al. (2010) Current biological and synthetic profile of triazoles: A review. Annals Biol Res 1: 82-89.