Original Article

Bioequivalence study of Flunac™ and Diflucan™ in healthy Bangladeshi male volunteers

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Abstract

A bioequivalence study of a local antifungal drug, fluconazole (FlunacTM), was compared with that of innovator product DiflucanTM. The study was conducted on 15 healthy volunteers and single cross-over dose of 150 mg fluconazole was administered orally. Two milliliter of blood was collected at different time intervals for 96 hours. The drug was extracted by liquid-liquid extraction and estimated by high performance liquid chromatography with diode array detector (260 nm). The chromatographic separation was accomplished using C18 analytical column with a mobile phase consisting of water and acetonitril (80:20, v/v). The Cmax of DiflucanTM and FlunacTM was 2.48 ± 0.29 g/mL and 2.23 ± 0.29 g/mL respectively. The Tmax of DiflucanTM and FlunacTM was 2.18 ± 0.98 hours and 2.56 ± 0.81 hours respectively. The area under the curve (0 to 96 hours) of DiflucanTM and FlunacTM was 73.28 ± 13.80 hours/mL and 74.49 ± 16.03 hours/mL. The half-life of both the drugs was 44.59 ± 13.79 hours for DiflucanTM and 42.73 ± 11.71 hour for FlunacTM. This study shows that FlunacTM is comparable to DiflucanTM in pharmacokinetic aspect.

Introduction

Fluconazole is an antifungal antibiotic[1]. Fluconazole is used to treat infections caused by fungus, which can invade any part of the body including the mouth, throat, esophagus, lungs, bladder, genital area, and the blood. It is a triazole antifungal and is used for several years. It is very well absorbed after oral administration. Fluconazole is also used to prevent fungal infection in people with weak immune systems caused by cancer treatment, bone marrow transplant, or diseases such as AIDS. It also achieves good penetration into the cerebrospinal fluid to treat fungal meningitis. Fluconazole is an alternative for Candida albicans infection in clinically stable patients who have not received an azole antifungal recently. Fluconazole decreases ergosterol synthesis by interfering with cytochrome P450 activity, thus inhibiting cell membrane formation of susceptible fungi including B dermatitidis, Epidermophyton spp, *Candida spp, C. immitis, C. neoformans, H. capsulatum, Mycosporum spp, Trichophyton spp, thus leading to cell death.*

The effect of a drug depends on the pharmacokinetic aspect. It is essential to examine whether our local product is comparable to the innovator product in pharmacokinetic aspect. The purpose of this study is to compare the pharmacokinetic aspects of FlunacTM with that of the innovators product DiflucanTM.

Materials and Methods

Chemicals: Fluconazole working standard was supplied by Cadila Healthcare Ltd, Ahmadabad, India. Phenacetin internal standard was supplied by Sigma Chemicals Co. Ltd (USA).

HPLC grade acetonitril and dichloromethane were purchased from E. Merck (Germany). Test Capsule FlunacTM (150 mg; Fluconazole, batch number: 0709)

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was supplied by Drug International Ltd, Bangladesh. Reference capsule DiflucanTM 150 mg (batch number: 814920075) was manufactured by Pfizer Australia PTY Ltd NSW, Australia and imported by Pfizer (Thailand) Ltd, Bangkok, Thailand.

Chromatographic condition: The HPLC-UV diode-array system consisted of Agilent model 1200 series degasser, solvent delivery pump, autosampler, column oven, photo diode array detector. Chromatographic data were collected and analyzed using Chemstation software.

A reverse-phase high performance liquid chromatography (HPLC) was used for the determination of fluconazole in plasma[2,3,4]. The chromatographic analyses were performed on an Agilent 5µm C18 column (150 × 4.6 mm). The mobile phase used for analysis consisted of 20% acetonitril (HPLC grade, E. Merck, Germany) and 80% distilled water and the flow rate was 0.8 ml/ min. Separations were achieved at 400C. The wavelength was set at 260 nm (bandwidth 1 nm). Injection of sample (100 µl) was done using an autosampler. The peak with retention time and area were defined using software.

Volunteers and sampling: Sixteen healthy volunteer was taken for bioequivalence divided into two groups. Each healthy volunteer received each of the treatments as a single dose in accordance with a randomization scheme (according to the protocol) with a washout period of 14 days. After administering fluconazole the blood samples were taken before (2 mL in each time) and at 1, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 96 hours. Drug analysis of fluconazole in plasma was performed by HPLC with a UV-Visible detector. Method validation of fluconazole was performed by preparing 1 mg/ml fluconazole solution which further dilute in 100 µl/ml, 10 µl/ml, 1 µl/ml, 0.1 µl/ml. Twenty micro liter standard solution was inject into the HPLC system. The retention time of fluconazole was 5.8 min. The same concentrated solution was run in different day. Interday and intraday variations were determined. The limit of detection was 0.16 mcg/mL whereas limit of quantification was 0.55 mcg/ml. The plasma assay procedures were validated by taking 100 µl plasma and 400 µl buffer solution extracted three times with dichloromethane after evaporation with nitrogen gas dissolved by mobile phase and injected 100 l into the HPLC system. Blood samples were collected in EDTA tube and centrifuged at 4000 × g for 5 min. The plasma samples were stored at 80°C until analysis. To a 100 µl unknown human plasma sample, 10 µl volume of the I.S. and 400 l of the phosphate buffer (0.1 M, pH 6.6) were added, mixed well and subjected to liquid-liquid extraction using 2 ml dichloromethane as extracting solvent. After vortex mixing for 30 s and centrifugation (2 min at 8000 × g), the organic phase was removed and evaporated to dryness under stream of nitrogen at 50°C. The residue was reconstituted in 400 µl of mobile phase and a volume of 100 µl was injected into the HPLC system.

The extent of absorption was determined by AUCo 96h and AUC0. The rate of absorption was determined by Cmax and Tmax. The half-life of elimination (t1/2) and the rate of elimination (bel) were used to further characterize the pharmacokinetic outcome of this study.

Results and Discussion

One patient was dropped. The age (mean ± SD) of 15 volunteers was 24.18 ± 3.88 years. Height and weight were 168.13 ± 4.62 cm and 57.42 ± 4.13 kg. The C_{max} of DiflucanTM and FlunacTM was 2.48 ± 0.29µg/mL and 2.23 ± 0.29 µg/mL respectively (Table I). The Tmax of DiflucanTM and FlunacTM was 2.18 ± 0.98 hours and 2.56 ± 0.81 hours respectively. The area under the curve (0 to 96 hours) of DiflucanTM and FlunacTM was 73.28 ± 13.80 hours/mL and 74.49 ± 16.03 hours/mL whereas its value from 0 to infinity was 94.43 ± 20.87 hours/mL and 93.80 ± 21.27 hours/mL. The half-life of both the drugs was calculated. It was 44.59 ± 13.79 hours for DiflucanTM and 42.73 ± 11.71 hour for FlunacTM. The elimination rate constant for DiflucanTM and FlunacTM was 0.016 ± 0.004 and 0.017 ± 0.005 respectively. The plasma concentrations of fluconazole following oral administration of DiflucanTM and FlunacTM in 15 healthy male human volunteers are shown in Figure 1,2 and 3.

Single dose of 150 mg of both products were well tolerated by the volunteers

Table I: Comparative study of local and innovator products of fluconazole in pharmacokinetic aspects

Parameters	Diflucan™		Flunac™	
	Mean ± SD	90% CI	Mean ± SD	90% CI
Cmax	2.48 ± 0.29 µg/mL	95.16%-104.83%	2.23 ± 0.29 µg/mL	94.61%-105.38%
Tmax	2.18 ± 0.98 hour	81.65%-118.34%	2.56 ± 0.81 hour	87.10% -112.89%
AUC096h	73.28 ± 13.80	92.24%-109.75%	74.49 ± 16.03	91.45%-108.84%
AUC0'!±	94.43 ± 20.87	90.91%-109.08%	93.80 ± 21.27	90.67%-109.32%
t1/2	44.59 ± 13.79 hour	87.28%-112.71%	42.73 ± 11.71 hour	88.71%-111.28%
Kel	0.016 ± 0.004		0.017 ± 0.005	

AUC0 24h = Area under the plasma concentration-time curve from zero hours to 24 hours; Cmax = maximal plasma concentration; tmax = time for the maximal plasma concentration; t1/2 = half-life; Kel = elimination rate constant.

The 90% confidence intervals for the FlunacTM (test) and DiflucanTM (reference) were found within the acceptance range of 80-125%.

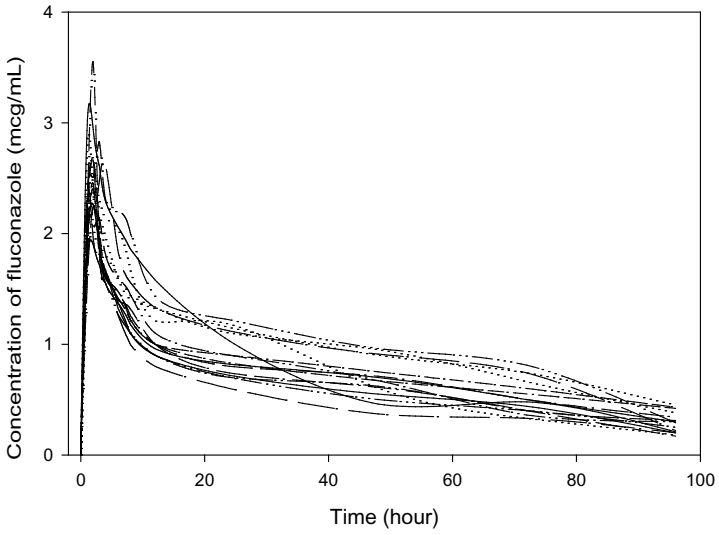

Figure 1: Plasma concentrations of fluconazole following oral administration of single dose (150 mg) of DiflucanTM (Reference) in 15 healthy male human volunteers

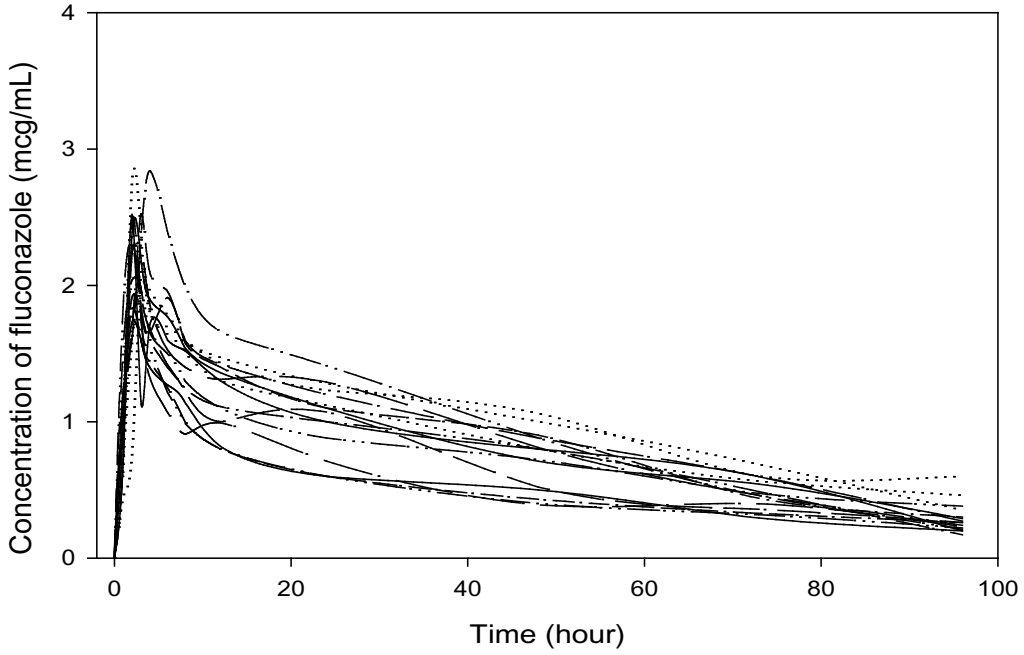

Figure 2 : Plasma concentrations of fluconazole following oral administration of single dose (150 mg) of FlunacTM (Reference) in 15 healthy male human volunteers

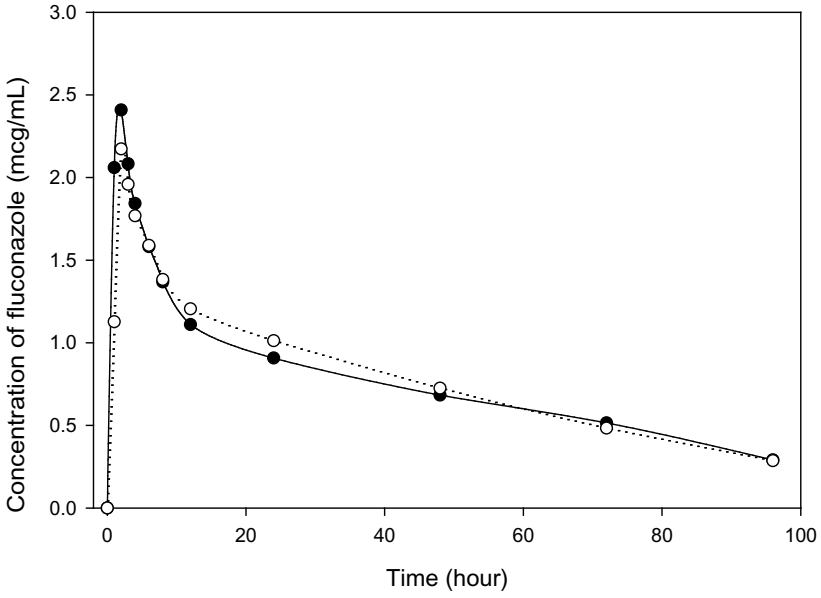

Figure 3: Mean plasma concentrations of fluconazole (DiflucanTM - closed circle; FlunacTM - open circle) in 15 human volunteers

The plasma half-life of fluconazole in Bangladeshi volunteers were lower than the population of other countries[5,6]. It may be due to metabolic effect for this drug. In conclusion, FlunacTM (test) is bioequivalent to Diflucan™ (reference) in terms of absorption and can be used interchangeably in clinical setting.

Acknowledgement

We acknowledge the help of Mr. Mohammed Yusuf, Director of Khwaja Yunus Ali Medical College Hospital.

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