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Bioequivalence Evaluation of Two Brands of Fluoxetine 20 mg Capsules (Flutin and Prozac) in Healthy Human Volunteers

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ABSTRACT: A bioequivalence study of two oral formulations of 20 mg fluoxetine was carried out in 24 healthy volunteers following a single dose, two-sequence, crossover randomized design at International Pharmaceutical Research Centre (IPRC), Amman, Jordan. The two formulations were Flutin capsules (Julphar, UAE) as test and Prozac capsules (Eli Lilly, UK) as reference product. Test and reference capsules were administered to each subject after an overnight fasting on two treatment days separated by a 28 day washout period. After dosing, serial blood samples were collected for a period of 360 h. Plasma harvested from blood was analysed for fluoxetine by a sensitive, reproducible and accurate LC-MS method. Various pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, and λ_Z were determined from plasma concentrations for both formulations and found to be in good agreement with reported values. AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were tested for bioequivalence after log-transformation of data. No significant difference was found based on ANOVA; 90% confidence interval (94.60%–106.41% for AUC_{0-t}, 94.6%–108.14% for $AUC_{0-\infty}$; 91.88%–103.65% for C_{max}) for test/reference ratio of these parameters were found within FDA acceptance range of 80%–125%. Based on these statistical inferences, it was concluded that Flutin is bioequivalent to Prozac and can be used interchangeably in medical practice. Copyright © 2005 John Wiley & Sons, Ltd.

Key words: fluoxetine; bioequivalence; pharmacokinetics; LC-MS; Julphar

Introduction

Bioequivalence of two formulations of the same drug is concluded based on the lack of difference in the rate (C_{max}) and extent of absorption (AUC) especially in conventional drug formulations [1]. In the present study the bioequivalence of two fluoxetine capsules was evaluated by comparing those pharmacokinetic parameters derived from plasma concentration-time values of fluoxetine.

Fluoxetine is the prototype of selective serotonin reuptake inhibitors (SSRIs) and has the longest half-life of all the SSRIs [2]. It was originally FDA approved for the treatment of major depression and obsessive–compulsive disorder (OCD) [3–7]. Chemically, fluoxetine is unrelated to tricyclic, tetracyclic or other available antidepressant agents and designated (±)-N-methyl-3-phenyl-3-[α,α,α-trifluoro-*p*-tolyl) propylamine hydrochloride. It has the empirical formula of $C_{17}H_{18}F_3NO \cdot HCl$. Its molecular weight is 345.79 [8].

Its most important effect is the enhancement of the actions of serotonin due to highly specific serotonin reuptake blockade at the neuronal

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membrane [9,10]. SSRIs have less sedative, anticholinergic and cardiovascular effects than do the tricyclic antidepressant drugs due to dramatically decreased binding to receptors of histamine, acetylcholine and norepinephrine. Anticholinergic activity is virtually absent [11,12].

Fluoxetine is well absorbed from the GI tract [9,13] and the presence of food can delay the rate of absorption, but not the extent [9]. There may be some first-pass metabolism [13]. Peak plasma concentrations occur in 6–8 h [9,13−15]. Fluoxetine is highly protein-bound (94.5%) predominantly to alpha$_1$-acid glycoprotein [9,13,14]. The drug is well distributed, and readily crosses the blood–brain barrier and presumably the placenta [9,13,14]. Fluoxetine is metabolised primarily via N-demethylation to the active metabolite, norfluoxetine; the half-life of fluoxetine is 2 days [9,13,15,16]. About 60% of an oral dose is excreted in urine within 35 days, and about 12% of the dose is excreted in the feces within 28 days [9,13,14].

Adverse reactions include anxiety, insomnia, dizziness, tremor and headache [13,17]. Nausea/vomiting is the most common ($\sim$20%) adverse reaction of fluoxetine. Diarrhoea, anorexia, xerostomia and dyspepsia are also fairly common ($\sim$10%) and may require medical attention [13,18].

Objectives of the study

The aim of this study was to assess the bioequivalence of two commercial 20 mg capsules of fluoxetine (Flutin from Julphar, UAE and Prozac from Eli Lilly, UK) in healthy volunteers by statistical analysis of the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} as recommended by FDA.

Materials and Methods

Study products

Flutin - Fluoxetine 20 mg capsules
Gulf Pharmaceutical Industries - Julphar, United Arab Emirates
Reference Product: Prozac - Fluoxetine 20 mg capsules
Eli Lilly, UK.

Study subjects

Twenty-four healthy adult male volunteers participated in this comparative study. Mean age was 25.25 $\pm$ 3.49 years (19–32 years) mean body weight 74.33 $\pm$ 9.60 kg (71–94 kg) and mean height was 172.13 $\pm$ 5.38 cm (163–182 cm). The volunteers were instructed to abstain from taking any drug including over-the-counter (OTC) for 2 weeks prior to and during the study period. The study was performed according to the revised Declaration of Helsinki for bio-medical research involving human subjects and the rules of Good Clinical Practices. The study protocol was approved by Institutional Review Board (IRB) of Al-Mowasah Hospital, Amman, Jordan.

Drug administration and sample collection

The volunteers were hospitalised at 6:00 p.m. and had a standard dinner in hospital. After an overnight fast (10 h) subjects were given a single dose of either formulation (reference or test in a randomised fashion) of fluoxetine 2×20 mg capsule with 240 ml of water. Food and drink (other than water, which was allowed after 2 h) were not allowed until 4 h after dosing and then a standard breakfast, lunch and dinner were given to all volunteers according to a time schedule. Beverages and food containing caffeine were not permitted over the entire course of study. Volunteers sat or walked around and were prohibited from strenuous activity until the 4 h blood collection. They were under continual medical supervision at the study site. Approximately 8 ml blood samples for fluoxetine analysis were drawn into evacuated heparinised glass tubes through an indwelling cannula before (0 h) and at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, 24.0, 48.0, 72.0, 96.0, 144.0, 288.0 and 360.0 h after dosing. Blood samples were centrifuged at 3500 rpm for 10 min; plasma was transferred directly into 5 ml plastic tubes and stored frozen at -20°C pending drug analysis. After a period of 28 days, the study was repeated in the same manner to complete the crossover design.

Chromatographic conditions

Plasma samples were analysed for fluoxetine according to a sensitive, selective and accurate

LC-MS method, developed and validated before the study. All solvents used were of HPLC grade; while other chemicals and reagents were of analytical grade; fluoxetine and loratadine (internal standard) were obtained from Julphar, UAE.

The LC-MS Alliance 2690 (Waters, USA) consisted of a system controller (Waters, USA), pump (Waters, USA), autosampler (Waters, USA), degasser and column oven; integration was done using MassLynx software version 3.5 (Micromass, Waters, USA). Chromatographic separation was performed using a X-Terra MS C_{18} (3.9×150 mm, 5 μm) HPLC column. The mobile phase consisted of 59.7% water, 29.85% acetonitrile, 9.95% methanol and 0.5% formic acid, and eluted at a flow rate of 0.3 ml/min; the oven temperature was set at 25°C. Fluoxetine was detected at *m/z* of 310.20, while loratadine was detected at *m/z* of 383.2. The peak area was measured, and the peak area ratio of drug to internal standard and the concentration were calculated by the software. The method was validated following international guidelines [19].

Sample preparation for HPLC injection

100 μl of the internal standard working solution (loratadine 1.0 μg/ml) was added to 0.5 ml plasma sample. The samples were vortexed for 30 s. Seven ml of tert-butylmethylether was added. The samples were then vortexed for 60 s and centrifuged in solvent for 5 min at 32000 rpm. The supernatant (organic layer) was transferred to another 10 ml glass tube and evaporated to dryness at 45°C under nitrogen, then reconstituted with 200 μl of mobile phase and transferred to an eppendorf tube (0.75 ml), and centrifuged for 5 min at 13 000 rpm. A 50 μl aliquot sample was injected to the chromatographic system using an autosampler, where fluoxetine and the internal standard were separated from endogenous substances.

Pharmacokinetic and statistical analysis

Pharmacokinetic analysis was performed by means of a model independent method using a Kinetica™ 2000 computer program [20]. The terminal disposition rate constant (λ_Z) was obtained as the slope of the linear regression of the log-transformed plasma concentration values versus time data in the terminal phase. The elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_Z$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/\lambda_Z$, where C_t is the last measured concentration.

To assess the bioequivalence between two formulations, AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as the primary variables. Two-way analysis of variance (ANOVA GLM procedure; Kinetica™ 2000 Computer program [20]) for a crossover design was used to assess the effect of formulation, period, sequence and subjects on these parameters. Differences between two related parameters were considered statistically significant for *p*-value equal to or less than 0.05. Parametric 90% confidence intervals [21] based on the ANOVA of the mean test/reference (T/R) ratios of *AUC*s and C_{max} were computed.

Results and Discussion

Fluoxetine was well tolerated by the volunteers.

Under the described conditions, the lower limit of quantitation for fluoxetine was 0.50 ng/ml. The relationship between the concentration and peak area ratio was found to be linear within the range 0.50–60.0 ng/ml. The intra-day accuracy of the method ranged from 85.33% to 99.55%, while the intra-day precision ranged from 4.51% to 6.27%. The inter-day accuracy ranged from 92.70% to 106.55%, while the inter-day precision ranged from 8.32% to 11.48%. The absolute recovery was 61.67%, while the relative recovery ranged from 106.11% to 112.74%. Stability studies showed that fluoxetine was stable in plasma for 61 days when stored at −20°C. The method used in this study was found to be reliable, accurate, sensitive and rapid for detecting plasma levels of fluoxetine.

Both formulations appeared to be readily absorbed from the gastrointestinal tract and fluoxetine was measurable at the first sampling time (1.0 h) in all the volunteers. The mean drug concentration-time profile of the two formulations is shown in Figure 1 indicating that the

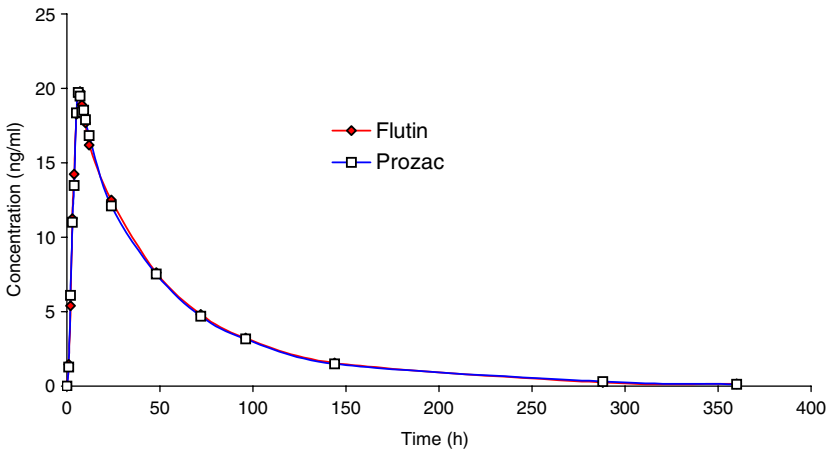

Figure 1. Mean plasma concentrations of fluoxetine after oral administration of the two brands to 24 healthy human volunteers

Table 1. Pharmacokinetic parameters of fluoxetine 20 mg capsules (mean ± standard deviation, $n = 24$)

Pharmacokinetic parameter	Flutin (test)	Prozac (reference)
AUC_{0-t} (ng/ml h)	1008.11 ± 416.98	1020.08 ± 440.87
$AUC_{0-\infty}$ (ng/ml h)	1098.92 ± 439.13	1101.46 ± 463.10
C_{max} (ng/ml)	21.68 ± 5.40	22.56 ± 6.98
T_{max} (h)	6.83 ± 2.06	6.67 ± 2.35
$T_{1/2}$ (h)	46.27 ± 21.56	46.39 ± 22.61
λ_Z (h^{-1})	0.0176 ± 0.01	0.0181 ± 0.01

mean plasma drug concentration profiles of the two brands were superimposable. Peak concentrations were attained at 6.83 and 6.67 h after drug administration and then declined rapidly but were still detectable up to 360 h. All estimated pharmacokinetic parameters were in good agreement with reported values [2,9,13–15].

Table 1 shows the pharmacokinetic parameter values for the two brands of 20 mg fluoxetine capsules. The relative bioavailability of Flutin compared with Prozac was 101.77% for AUC_{0-t}, 102.95% for $AUC_{0-\infty}$ and 99.04% for C_{max}.

Table 2 shows statistical results, specifically analysis of variance (ANOVA) for these parameters, after log-transformation of the data. No statistically significant difference between the two formulations either in period or formulation, had a p value greater than 0.05. Ninety percent confidence intervals also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$ or C_{max} of the two formulations lie within the FDA [21] acceptable range of 80%–125%. For T_{max} the parametric point estimate of difference (test-reference) was 0.16 h, and found to be within the acceptance limits (± 20% of reference mean).

The results of this study suggest equivalent clinical efficacy of the two brands of fluoxetine.

Conclusion

Statistical comparison of the AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} clearly indicated no statistically significant differences exist between Flutin and

Table 2. Statistical analysis of log-transformed data

Statistical analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
ANOVA GLM (p-value)	0.0924 (0.0444)	0.0773 (0.0755)	0.050 (0.0112)
90% CI	94.60%–106.41%	94.60%–108.14%	91.88%–103.65%

Parenthesis values indicate analysis for periods.

Prozac tablets in any of the calculated pharmacokinetic parameters. The confidence intervals for the ratios of mean AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} further demonstrate that these values were within the bioequivalence acceptance range of 80%–125% (using log-transformed data). Based on the above it is concluded that Flutin is bioequivalent to Prozac, and that both products can be considered equally effective in medical practice.

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