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Bioequivalence Assessment of Azomycin® (Julphar, UAE) as Compared with Zithromax® (Pfizer, USA)—Two Brands of Azithromycin—in Healthy Human Volunteers

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ABSTRACT: Two studies have been performed to assess the relative bioavailability of Azomycin® (Julphar, UAE) as compared with Zithromax® (Pfizer, USA) at the International Pharmaceutical Research Center (IPRC), Amman, Jordan. One study involved Azomycin® capsules and the other Azomycin® suspension. Each study enrolled 24 volunteers and in both studies, after an overnight fasting, the two brands of azithromycin were administered as single dose on two treatment days separated by a 2 weeks washout period. After dosing, serial blood samples were collected for a period of 192 h. Plasma harvested from blood, was analysed for azithromycin by HPLC coupled with electrochemical detection. Various pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$ and K_{elm} were determined from plasma concentrations for both formulations and found to be in good agreement with the 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 intervals for the test/reference ratios of these parameters were found within the bioequivalence acceptance range of 80–125%. Based on these statistical inferences it was concluded that Azomycin® capsule is bioequivalent to Zithromax® capsule and Azomycin® suspension is bioequivalent to Zithromax® suspension. Copyright © 2001 John Wiley & Sons, Ltd.

Key words: azithromycin; bioequivalence study; HPLC; pharmacokinetics

Introduction

Bioequivalence of the two formulations of the same drug comprises equivalence with respect to the rate (C_{max}) and extent of absorption (AUC) especially in conventional drug formulations [1]. In the present work the bioequivalence of two brands of azithromycin capsules and two brands of azithromycin suspension was evaluated in two separate studies by comparing these pharmacokinetic parameters.

Azithromycin is the prototype of a subclass of macrolide antibiotics known as the azalides [2]. This agent differs structurally from erythromycin by insertion of a methyl-substituted nitrogen at position 9a in the lactone ring, creating a 15-membered macrolide [3]. Azithromycin reportedly possesses superior pharmacokinetic properties as compared to erythromycin, including greater tissue penetration and a significantly longer elimination half-life, enabling once-daily dosing [4–6]. Azithromycin has greater acid stability than erythromycin, which may enable superior absorption [5,7]. Azithromycin inhibits protein synthesis in bacterial cells by binding to the 50 S subunit of bacterial ribosomes but not to

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the 80 S mammalian ribosome and this accounts for its selective toxicity [8]. It possesses antibacterial activity against Gram-positive organisms, such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *S. pyogenes* and *S. pneumoniae*; and Gram-negative *Haemophilus influenzae* and *Moraxella catarrhalis*. *Chlamydia trachomatis* is also susceptible to azithromycin [9].

Azithromycin has several unique pharmacokinetic characteristics. Oral absorption of azithromycin is rapid but inhibited by food; reported oral bioavailability is 37% [5,10–12]. When administered with food bioavailability decreased with capsules and increased with suspension [9,11,13]. Peak plasma concentrations occur at 2–4 h [5,14–16] after oral administration. Azithromycin exhibits significant intracellular penetration and concentrates within fibroblasts and phagocytes. As a result, tissue levels are significantly higher than plasma concentrations. Total protein binding is reported as being 7–50% [5]. The exact biodisposition of azithromycin is still being elucidated. The drug undergoes some hepatic metabolism to inactive metabolites, but biliary excretion is the major route of elimination. Only 6.5% of the drug is excreted unchanged in the urine [17]. The elimination half-life was reported to be 68 h and is prolonged because of extensive tissue sequestration and binding [17]. The elimination of azithromycin from serum is biphasic, exhibiting a short tissue distribution phase followed by a longer elimination phase [12,18]. The terminal elimination half-life is determined by the movement of azithromycin from distribution sites [12]. A half-life in the range of 11–14 h was reported after 8–24 h of dose administration; however, the half-life increases with time after the dose. For example, after 24–72 h of dose administration, the half-life increased to 35–40 h. After multiple-doses (1 g followed by 500 mg once daily), the apparent half-life was 57 h [5,14].

Objectives of the Study

The purpose of this study was to compare the bioavailability (rate and extent of absorption) of generic formulations of azithromycin (Azomycin® capsule/suspension, Gulf Pharmaceutical Industries, Julphar, United Arab Emirates) relative to the reference formulation (Zithromax® capsule/suspension, Pfizer, USA). The bioequivalence of the test formulation was assessed by statistical analysis of the pharmacokinetic parameters as recommended by the FDA [19].

Material and Methods

Study Products

Study I: Azithromycin Capsules. Test product: Azomycin® 250 mg capsules (dose 2 capsules = 500 mg). Batch No.: 0013, expiry 01/2001. Gulf Pharmaceutical Industries, Julphar, UAE.

Reference product: Zithromax® 250 mg capsules (dose 2 capsules = 500 mg). Batch No.: 71036070, expiry 12/2001. Pfizer, USA.

Study II: Azithromycin Suspension. Test product: Azomycin® 200 mg/5 mL suspension (dose 12.5 mL = 500 mg). Batch No.: 0014, expiry 01/2001. Gulf Pharmaceutical Industries, Julphar, UAE.

Reference product: Zithromax® 200 mg/5 mL suspension (dose 12.5 mL = 500 mg). Batch No.: 80764107, expiry 05/2000. Pfizer, USA.

Study Subjects

Twenty-four healthy adult male volunteers were enrolled in each study at Ibn-Al-Nafis Hospital, Irbid, Jordan. The mean age was 23.8 ± 5.6 years with a range of 18.0–39.0 years and mean body weight was 73.1 ± 10.9 kg with a range of 53–92 kg in the capsule study. Similar figures were calculated for the suspension study, namely a mean age of 23.5 ± 4.2 years with a range of 18.0–34.0 years and mean body weight of 71.2 ± 10.2 kg with a range of 55–88 kg. On the basis of medical history, clinical examination and laboratory investigation (haematology, blood biochemistry and urine analysis), no subject had a history or evidence of hepatic, renal, gastrointestinal or haematologic deviations or any acute or chronic diseases or drug allergy. The subjects were instructed to abstain from taking any medication for at least 1 week prior to and during the study period. Informed consent was obtained from the subjects after explaining the

nature and purpose of the study. The study protocols were approved by the Institutional Review Board (IRB) of Ibn-Al-Nafis Hospital, Irbid, Jordan.

Drug Administration and Sample Collection

In both studies after an overnight fasting (10 h) subjects were given single dose of either formulation (reference or test) of azithromycin. In study I, 2×250 mg capsules of either test or reference were given as a single dose with 240 mL of water; in study II, 12.5 mL suspension of either test or reference was given with 240 mL of water. No food was allowed until 5 h after dose administration. Water intake was allowed after 2 h of dose and then water, breakfast, lunch and dinner were given to all volunteers according to a time schedule. Volunteers were ambulatory during the study but prohibited from strenuous activity; they were under direct medical supervision at the study site. Approximately 10-mL blood samples for azithromycin assay were drawn into heparinized tubes through an indwelling cannula before (0 h) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, 24, 48, 72, 96, 120, 144, 168 and 192 h after dosing. The blood samples were centrifuged at 4000 rpm for 10 min, plasma was separated and kept frozen at -20°C in coded polypropylene tubes. After a period of 14 days, the study was repeated in the same manner to complete the crossover design.

Chromatographic Conditions

All solvents used were of HPLC grade, azithromycin and clarithromycin (internal standard) were obtained from Gulf Pharmaceutical Industries, UAE.

The HPLC system (Shimadzu, Japan) used was an iosocratic system consisting of a solvent delivery pump (LC-10AD), an electrochemical detector (L-ECD-6A), a communication bus module (CBM-10A) and a manual injector (Rheodyne). The separation was performed by using a stainless steel C_8 (100×4.6 mm) cartridge column with a particle size of 3.5 μm (Waters, USA). The mobile phase consisted of acetonitrile and phosphate buffer in a 36.5:63.5 (v:v) ratio; pH was adjusted to 7.40. The mobile phase was pumped at a flow rate of 1.2 mL/min; effluent was monitored using an electrochemical detector with an applied potential of 0.85 V at attenuation 5, which represents 32 mV full scale.

Sample Preparation for HPLC Injection

Plasma samples were analysed for azithromycin according to an HPLC method developed at the IPRC laboratory and validated following international guidelines [20]. A 0.5-mL plasma sample was taken in a glass stoppered tube, 100 μL of internal standard (clarithromycin 5.0 μg/mL) was added and shaken on a vortex mixer for 30 s. Basification of the sample was done using 250 μL of 0.2 M sodium carbonate and vortex mixed for 30 s. Six millilitres of *tert*-butyl methyl ether was added, the mixture again shaken for 30 s and centrifuged at 3000 rpm for 5 min. The supernatant organic layer was transferred to a 10-mL test tube and evaporated to dryness at 40°C under a nitrogen stream in a water bath. The residue was reconstituted with 200 μL of mobile phase, followed by vortex mixing for 30 s and then transferred to a disposable polypropylene microcentrifuge tube (1.5 mL Eppendorf) and centrifuged for 2 min at 13000 rpm; 50 μL of aliquot was then injected into the column and peak areas were recorded.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by means of a model independent method. The maximum azithromycin concentration (C_{max}) and the corresponding peak times (T_{max}) were determined by the inspection of the individual drug plasma concentration–time profiles. The elimination rate constant (K_{elm}) was obtained from the least-square fitted terminal log–linear portion of the plasma concentration–time profile. The elimination half-life ($T_{1/2}$) was calculated as $0.693/K_{elm}$. 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 $AUC_{0-t} + C_t/K_{elm}$, where C_t is the last measurable concentration.

Statistical Analysis

For the purpose of bioequivalence analysis AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as primary variables. Bioequivalence was assessed by means of an analysis of variance (ANOVA GLM model) [21] for crossover design and calculating standard 90% confidence intervals (CIs) [19,22] for the ratio of test/reference (T/R) using log-transformed data. Differences between two compared parameters were considered statistically significant for p-values equal to or less than 0.05 with a 95% CI.

Results and Discussion

The most important objective of bioequivalence testing is to guarantee the physicians and patients that generic products are safe and clinically effective within certain boundaries. In the present work, azithromycin was well tolerated by the subjects; unexpected incidents that could have influenced the outcome of the study did not occur. All volunteers who started the study continued to the end and were discharged in good health. Both formulations were readily absorbed from the gastrointestinal tract and azithromycin was measurable at the first sampling time (0.5 h) in almost all volunteers. The mean concentration–time profiles for the two formulations of the two dosage forms are shown in Figures 1 and 2 for azithromycin capsules and suspension, respectively. All calculated pharmacokinetic parameter values were in good agreement with the previously reported values [5,9–18]. According to published data, the oral bioavailability of azithromycin is around 37% [5,10–12]. After an oral dose of 500 mg azithromycin the reported C_{max} was 370–450 ng/mL and the area under the curve was 3700 ng/mL·h [23,24]. Our data, although in the upper limit, are within those ranges. Table 1 shows the pharmacokinetic parameters for two brands of azithromycin capsule/suspension.

On the basis of the mean plasma levels of the 24 subjects completing both studies, the relative bioavailabilities are given in Table 2. For bioequivalence evaluation, various statistical modules were applied to AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} as per current FDA guidelines [19]. Table 3 shows the 90% CI for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for log-transformed data.

The mean and standard deviation (S.D.) of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for the two dosage forms of the two products were found very close, indicating that the plasma profiles generated by Azomycin® are comparable to those produced by Zithromax®. ANOVA for these parameters, after log-transformation of the data,

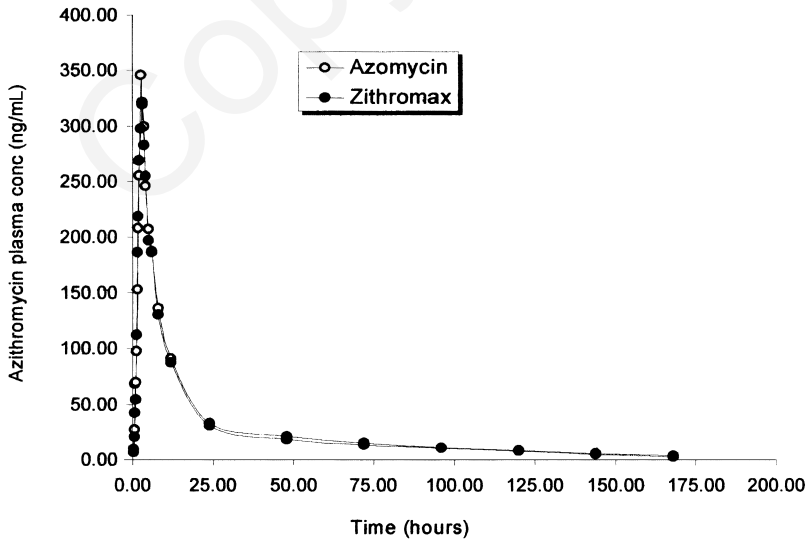

Figure 1. Mean plasma concentration of azithromycin, 2 × 250 mg capsules, after oral administration of a single dose of the two brands to 24 healthy human volunteers

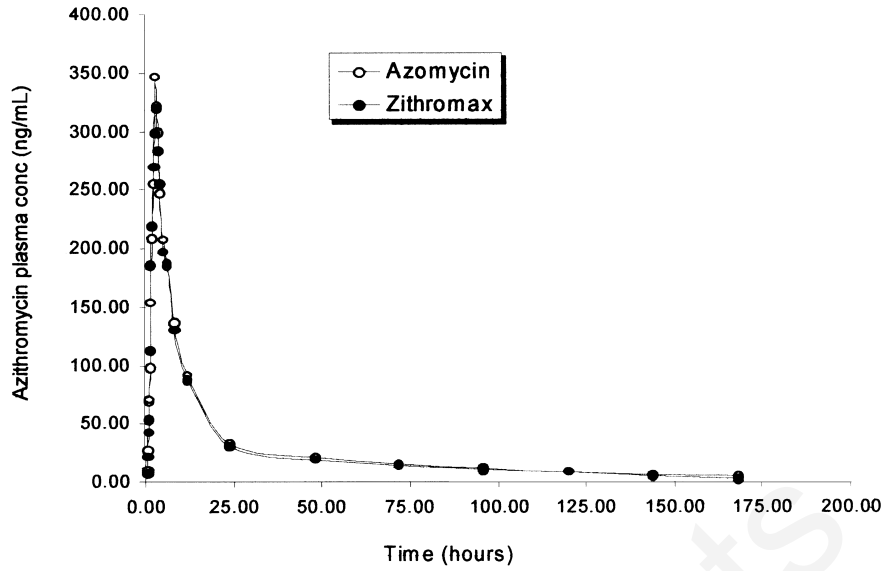

Figure 2. Mean plasma concentration of azithromycin, 200 mg/5 mL suspension, after oral administration of a single dose (12.5 mL) of the two brands to 24 healthy human volunteers

Table 1. Pharmacokinetic parameters of azithromycin formulations (mean ± S.D.; $n = 24$)

Pharmacokinetic parameter	Study I: azithromycin capsules		Study II: azithromycin suspension	
	Azomycin® (test)	Zithromax® (reference)	Azomycin® (test)	Zithromax® (reference)
AUC_{0-t} (ng/mL · h)	3986.92 ± 1295.41	3745.50 ± 1059.01	5761.75 ± 1705.85	5516.04 ± 2188.60
$AUC_{0-\infty}$ (ng/mL · h)	4383.08 ± 1290.60	4099.71 ± 1058.70	6451.54 ± 1757.24	6219.54 ± 2545.46
C_{max} (ng/mL)	323.38 ± 122.85	332.08 ± 90.12	466.38 ± 142.55	447.13 ± 119.44
T_{max} (h)	2.86 ± 0.91	2.82 ± 1.11	2.52 ± 0.82	2.49 ± 0.71
$T_{1/2}$ (h)	49.56 ± 12.81	44.98 ± 12.31	65.04 ± 14.50	70.65 ± 22.88
K_{elim} (/h)	0.01 ± 0.01	0.02 ± 0.005	0.01 ± 0.00	0.01 ± 0.003

showed no statistically significant difference between the two formulations in both studies. ANOVA did not show any significant difference in periods, formulations or sequence, having p-values greater than 0.05. In both studies, 90% CIs also demonstrated that the ratio of these parameters of the two formulations and for the two periods lie within the FDA [19] accepted range of 80–125%.

Table 2. Relative bioavailabilities of reference and test formulations

Pharmacokinetic parameters	Study I: azithromycin capsules (%)	Study II: azithromycin suspension (%)
AUC_{0-t}	106.44	104.27
$AUC_{0-\infty}$	106.91	102.12
C_{max}	97.38	104.30

When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus therapeutically equivalent [25]. To demonstrate bioequivalence certain limits should be set depending on the nature of drug, patient population and clinical end points. It is generally accepted that for basic pharmacokinetic characteristics, such as AUC and C_{max}, the standard equivalence range is 0.8–1.25 [19]. In the present

Table 3. 90% CI of log-transformed data

Pharmacokinetic parameter	Study I: azithromycin capsules (%)		Study II: azithromycin suspension (%)	
	Formulations	Periods	Formulations	Periods
AUC_{0-t}	94.06–119.36	90.25–114.53	97.95–116.61	98.49–117.25
$AUC_{0-\infty}$	95.50–118.94	90.32–112.50	96.37–115.04	97.54–116.44
C_{max}	83.72–106.54	84.84–107.96	94.54–113.71	98.64–118.64

studies the 90% CIs were found within this acceptable range (using log-transformed data).

Conclusion

Based on the pharmacokinetic and statistical results of this study, we can conclude that Azomycin® capsules/suspension manufactured by Gulf Pharmaceutical Industries, United Arab Emirates is bioequivalent to Zithromax® capsules/suspension manufactured by Pfizer, USA and the two products can be considered equally effective in medical practice.

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