

A Bioequivalence Study of Two Azithromycin Tablet Formulations in Indonesian Healthy Subjects

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Abstract

Aim: To compare the bioavailability of two Azithromycin tablet formulations 500 mg Azivol® tablets as test formulation and 500 mg Zithromax® tablets as reference formulation.

Methods: A single-dosed, open-label randomized two-way crossover design under fasting period with two weeks wash-out period was evaluated in 24 subjects. For the analysis of pharmacokinetic properties, the blood samples were drawn taken up to 120 hours after dosing. Plasma concentration of Azithromycin was determined using liquid chromatography – tandem mass spectrometry method with Turbolon Spray mode. Pharmacokinetic parameters AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were tested for bioequivalence after log-transformation of data and ratios of t_{max} were evaluated non-parametrically.

Results: The point estimates and 90% confidence intervals (CI) for AUC_{0-t} , $AUC_{0-\infty}$, and C_{max} for Azithromycin were 94.63% (86.27-103.81%), 95.35% (87.15-104.31%), 94.16% (80.31-110.41%) respectively.

Conclusion: These results indicated that the two formulations of Azithromycin were bioequivalent and thus may be prescribed interchangeably.

Keywords: Azithromycin; Antibiotic; Bioequivalence and Bioavailability; LC-MS/MS

Introduction

Azithromycin, 9-Deoxy-9a-aza-9a-methyl-9a-homoerythromycin a dihydrate, is a semi-synthetic 15-membered azalide antibiotics derived from erythromycin. Its chemical structure differs from that of erythromycin by the insertion of methyl-substituted nitrogen at position 9a in the lactone ring. This modification results in the improved acid stability associated with more reliable and greater oral bioavailability, more extensive tissue penetration, and significantly longer elimination half-life, which exhibits an extensive spectrum of activity compared with erythromycin. Azithromycin is effective against gram-positive and gram-negative pathogens. Due to its extensive tissue penetration and distribution, Azithromycin appears to be suitable antibiotic for the treatment and prophylaxis of respiratory tract infection, skin and soft-tissue infection, and sexually transmitted diseases [1,2].

Azithromycin given orally is rapidly absorbed from gastrointestinal track but is inhibited by food. Its absolute oral bioavailability is about 37%. Peak plasma concentrations are achieved 2 to 3 hours after a dose, but Azithromycin is extensively distributed to the tissues, and tissue concentrations subsequently remain much higher than those in the blood. Small amounts of Azithromycin are demethylated in the liver, and it is excreted in bile as unchanged drug and metabolites. Azithromycin metabolites are thought to possess no significant antimicrobial activity. About 6% of an oral dose (representing about 20% of the amount in the systemic circulation) is excreted in the urine. The terminal elimination half-life is about 68 hours [2-4].

As for erythromycin, gastrointestinal disturbances are the most frequent adverse effect but are usually mild and less frequent than with erythromycin. The central and peripheral nervous system, predominantly headache and dizziness may occur. Severe hypersensitivity reactions occur rarely but may be prolonged [2].

There are many generic products of Azithromycin in Indonesia and it must also go through the bioequivalence study in order to assure the efficacy, safety, and quality. The present study was conducted to investigate the pharmacokinetics and bioavailability of two Azithromycin tablet formulations in order to prove bioequivalence between both formulations.

Subjects and Methods

The protocol study was reviewed by the Committee of The Medical Research Ethics of the Faculty of Medicine, University of Indonesia (Jakarta, Indonesia) and was approved by the National Agency of Drug and Food Control (Jakarta, Indonesia). This study was conducted in compliance with the ethical principles of the Declaration of Helsinki for biomedical research involving human volunteers and Good Clinical Practice (GCP). All participants signed a written informed consent after they had been informed of the nature and details of the study in accordance with Indonesian Guidelines for Bioequivalence Studies [5,6].

The study was based on single-dose, open-label, randomized two-way crossover design under fasting period with two weeks wash out period. Subjects were randomized to one of the two sequences to receive the formulations according to randomization scheme. The test preparation was 500 mg of Azivol® tablets, manufactured by PT. Novell

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Pharmaceutical Laboratories, Indonesia (Batch no. 11D183) and the reference formulation was 500 mg Zithromax[®] tablets, produced by Pfizer Australia Pty Ltd., (Batch no. B914640151). The sample size $n = 24$ subjects was sufficient to ensure power of 80% for correctly concluding bioequivalence under the following assumption: $\alpha = 0.05$, $0.95 < \mu_T / \mu_R < 1.05$ and an intra-subject variability of 20% [7].

A total of 24 subjects (18 males and 6 females) were selected among Indonesia residents and participated in this study. The demographic data of twenty-four volunteers are shown in Table 1.

Subjects were selected after passing a clinical screening procedure including a physical examination, ECG and clinical laboratory tests (hemoglobin, hematocrit, WBC, platelets, WBC differential, blood urea nitrogen, sGPT, sGOT, alkaline phosphatase, total bilirubin, total protein, fasting glucose, albumin, creatinine, urine analysis, pregnancy test (for female subjects) and negative results of HBsAg, anti HBC and anti HIV. Volunteers were excluded if they had a history of any illness of the hepatic, renal and cardiovascular system, took alcohol or other medications for a long period of time, had hypersensitivity to Azithromycin, had received any investigation drug within four weeks (or suitable longer period for slowly eliminated drugs) of enrollment and donation or loss more than 450 ml of blood within 3 months prior to the screening of the study.

All subjects were avoided using other drugs for at least two weeks prior to the study and after its completion. They were also refrained from ingesting alcohol, caffeine, chocolate, tea or coke containing beverages at least 48 hours before each dosing and until the collection of the last blood sample. Subjects were confined to clinical unit of Clinisindo Laboratories one night before study to assure the fasting condition (10 hours before drug administration). On the study day, subjects were given one tablet of either product with 240 ml of water. No food was allowed until 4 hours after dose administration. Water intake was allowed 2 hours after the dose. Standard meals were served at 4 and 11 hours after drug administration. Snack was served at 8 hours after drug administration. Subjects were remained upright (sitting or standing) for the first 4 hours. Subjects were confined at clinical unit of Clinisindo Laboratories for 24 hours after dosing and were not permitted to take strenuous exercise during the sampling days. Blood pressure, heart rate, body temperature and adverse events were monitored during blood sampling. 5 ml of the venous blood were collected at pre dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours after drug administration in the heparinized tubes. After blood separation, plasma was frozen at -20°C until analysis. After two weeks wash out period, subjects returned to Clinisindo Laboratories and the blood sample analysis was repeated in the second period in the same manner to complete the crossover design.

Safety Evaluation

Analysis of safety-related data was considered using the more common adverse events which occurred after initiation of study treatment and supported by the following more detailed tabulations and analysis (Table 2).

LC-MS/MS assay of Azithromycin in plasma

The concentration of Azithromycin in plasma was determined using LC-MS/MS method with TurboIon Spray mode. Propranolol was used as the internal standard. The method has already been validated in terms of selectivity, sensitivity, linearity, accuracy and precision, recovery, stability, and also has been verified just before being used in

	Mean (± SD)	Value Range
Age (years)	28.6 (4.9)	19-39
Weight (kg)	57.7 (9.3)	41-73
Height (m)	162.4 (7.6)	142.5-174
Body mass index (kg m ⁻²)	21.8 (2.7)	18-25

Table 1: Demographic data for Azithromycin bioequivalence study in 24 volunteers.

Cause relation to study drug	Events	Total
Related	Abdominal discomfort	15
	Dizziness	7
	Somnolence	2
	Nausea	1
Probable	Weakness	2
	Myalgia	1
Total		28

Table 2: Disposition of adverse events.

study. The limit of quantification for Azithromycin was 2.0 ng/mL. The standard calibration curves for Azithromycin were ranged from 2-500 ng/mL. The best linear fit and least-squares residual for the calibration curve were achieved with $1/x^2$ weighing factor. The recoveries of Azithromycin were 84.54-87.91%. The analytical separation was performed on a Synergi 4 μ POLAR- RP-80A, 50 \times 2.00 mm, 4 μ m (Phenomenex[®], USA) and protected by guard column AQ C18, 4 \times 2.0 mm (Phenomenex[®], USA). The mobile phase used gradient of 0.1% formic acid in acetonitrile and 0.1% formic acid in water, pumped 0.7 mL/min for 4.0 min run time. The column temperature was maintained at 40°C. Briefly, a 250 μ L of human plasma in microtube was added with 20 μ L of internal standard (10 ppm). After mixing, 250 μ L of acetonitrile was added and vortex mixed for 30 seconds. The mixture was centrifuged at 3000 rpm for 10 min. A volume of 5 μ L supernatant was injected into LC-MS/MS system. The retention time for Azithromycin and propranolol were 0.95 min and 1.10 min, respectively.

Pharmacokinetic and statistical analysis

The bioequivalence was determined using the primary parameters, AUC_{0-t} , $AUC_{0-\infty}$, C_{max} , C_{min} and t_{max} were obtained directly by inspection of the individual drug plasma concentration time data, and were used as measures of rate of absorption. AUC_{0-t} was calculated using the trapezoidal rule. The elimination rate constant (Kel) was calculated by the technique of least-squares regression from the data of the last 3-5 points of each plasma concentration data curve. The $AUC_{0-\infty}$ values were determined by adding the quotient of Ct and the appropriate Kel to the corresponding AUC_{0-t} , that is:

$$AUC_{0-\infty} = AUC_{0-t} + Ct / Kel$$

The apparent elimination half-life ($t_{1/2}$) of Azithromycin in plasma was calculated by using the following equation:

$$t_{1/2} = (\ln 2) / Kel$$

For the parameters of AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} a multiplicative model was assumed, and analysis of variance (ANOVA) was applied using the respective ln-transformed data. For estimation of bioequivalence the 90% CI of the geometric mean ratio test/reference (T/R) for AUC_{0-t} ,

$AUC_{0-\infty}$ and C_{max} were calculated assuming a multiplicative model. The accepted bioequivalence range for these parameters was 80-125%. All statistical analyses were performed using Equiv Test version 2.0 software (Statistical Solution, Cork, Ireland).

Results and Discussion

Both Azithromycin formulations were well-tolerated at the administered dose and no significant adverse clinical events were observed. All adverse events were of mild intensity and recovered without concomitant medication. There were no serious adverse events. However, all events resolved completely. The disposition of adverse events is shown in Table 2.

The number register of this clinical trial is NCT 01602055. A total of 24 subjects participated in this study and all the subjects were available for pharmacokinetic evaluation. The Azithromycin concentration versus time profiles of twenty four subjects for both formulations are shown in Supplementary Figure 1 and the mean Azithromycin concentration versus time profiles for both formulations are shown in Figure 1. The pharmacokinetic parameters that are used to assess the bioequivalence of the test formulation versus the reference were AUC_{0-t} , $AUC_{0-\infty}$ for the extent of the absorption and C_{max} and t_{max} for the rate of absorption. Descriptive statistics of the pharmacokinetic parameter for Azithromycin test and reference are summarized in Table 3 where the geometric mean values and the range for the AUC_{0-t} , $AUC_{0-\infty}$, C_{max} and $t_{1/2}$ values obtained for each formulation are shown. The pharmacokinetic characteristic t_{max} was presented as mean values. The mean obtained values for test and reference formulations were 586.64 and 623.51 ng/mL for C_{max} ; 4712.31 and 5016.39 ng.h/mL for AUC_{0-t} ; 5370.66 and 5711.19 ng.h/mL for $AUC_{0-\infty}$. The median t_{max} for test and reference formulations were 1.75 h and 2.25 h.

The results of the bioequivalence analysis for Azithromycin are given in Table 4. The intra-subject variability of Azithromycin in the AUC_{0-t} , $AUC_{0-\infty}$, C_{max} , and $t_{1/2}$ estimates from the coefficient of variables as determined by ANOVA were 18.65%, 18.11%, 32.09%, and 11.53%, respectively. As shown in Table 4, 90% confidence intervals (CI) of AUC_p , $AUC_{0-\infty}$, C_{max} , and $t_{1/2}$ log-transformed individual ratios of Azithromycin were included into the range of bioequivalence, i.e. 80-125% when analyzed by parametric statistics. In the same way, individual t_{max} difference was not statistically different between the two formulations. The mean ratio of $AUC_{0-t}/AUC_{0-\infty}$ for all individuals and for both products was around 12%, indicate an adequate sampling time since the extrapolated portion of the total AUC is less than 20%. The

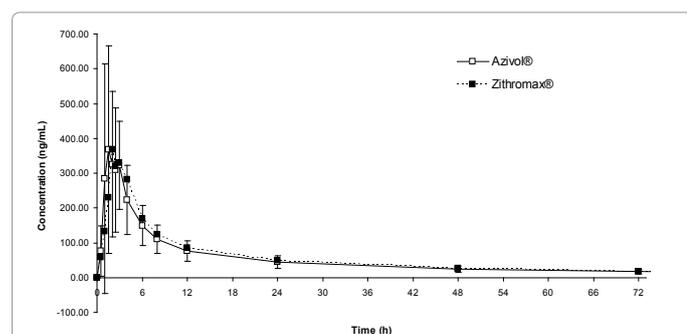


Figure 1: Mean Plasma Concentration Time Profiles of Azithromycin Following the Administration of Each Product.

Parameter	Test Formulation	Reference Formulation
Geometric mean C_{max} (ng/mL) range	534.67 193.87 – 1423.04	567.82 298.80 – 1585.02
Geometric mean AUC_{0-t} (ngxh/ml) Range	4443.48 2178.76 – 8148.69	4695.61 2452.39 – 8203.54
Geometric mean $AUC_{0-\infty}$ (ngxh/ml) Range	5075.61 2615.24 – 9154.81	5323.31 2722.78 – 9340.76
Geometric mean $t_{1/2}$ (h) range	49.99 36.29 – 65.73	47.40 36.63 – 63.81
Median t_{max} (h) range	1.75 1.00 – 6.00	2.25 1.00 – 4.00

Table 3: Mean pharmacokinetic characteristic for Azithromycin after Administration for the two formulations.

Parameter	T/R Point Estimate	Confidence Limits
Geometric mean C_{max} (ng/mL) range	94.16	80.31 – 110.41
Geometric mean AUC_{0-t} (ngxh/ml) Range	94.63	86.27 – 103.81
Geometric mean $AUC_{0-\infty}$ (ngxh/ml) Range	95.35	87.15 – 104.31
Geometric mean $t_{1/2}$ (h) range	105.46	99.59 – 111.67

Table 4: Parametric 90% confidence interval for the mean pharmacokinetic characteristic of Azithromycin formulations.

results for $t_{1/2}$ in the present study (50.50 ± 7.33 h for test product and 47.89 ± 7.23 h for reference product) were consistent with the results reported in the literatures (~ 40-50 h) [4,8,9].

In this research the variability of C_{max} is high but from the previous research in healthy volunteers also showed that the intra-subject variability of C_{max} can be as high as 34.7%. Azithromycin can therefore be considered as a highly variable drug. Highly variable drugs can therefore pose a problem in bioequivalence assessment using standard 0.8 – 1.25 approach. It is therefore justified from that point of view to use wider C_{max} acceptance limits [10].

In conclusion, the application of either parametric or non-parametric statistics reveals the presence of bioequivalence between Azivo[®] FC tablet produced by PT. Novell Pharmaceutical Laboratories and Zithromax[®] FC tablet produced by Pfizer Australia Pty, Ltd for the rate and extent of absorption. Thus, it can be assumed that the two formulations are therapeutically equivalent and therefore interchangeable.

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