Bioequivalence of Two Formulations of a Single Oral Dose of 500-mg Azithromycin Granules: A Randomized, Open-Label, Two-Period Crossover Study in Healthy Han Chinese Volunteers

Jing Ren, PhD; Xue-Hua Jiang, PhD; Kejia Li, MS; Chuanchuan Zhang, MS; Chenrui Li, MS; and Ling Wang, PhD

West China School of Pharmacy, Sichuan University, Chengdu, China

ABSTRACT

Background: In recent years, the use of generic drugs has been increasing due to their effectiveness and to the increasing variety of drugs that are now available in generic formulations. Although several generic oral formulations of azithromycin are available in China, information concerning the bioavailability of these formulations in the Chinese population is unavailable.

Objective: The purpose of this study was to compare the bioequivalence and tolerability of a single oral dose of 2 commercial brands of 500-mg azithromycin granules in healthy Han Chinese volunteers.

Methods: In a randomized, open-label, 2-period crossover study, the bioequivalence and tolerability of 2 commercial formulations of azithromycin granules (test: Dayin Ocean Biochemical Company Ltd., Shandong, China; reference: Taiyang Drug Company Ltd., Beijing, China) were compared in healthy adult Han Chinese volunteers. Both the test and the reference formulations were administered to each subject. The 2 treatment phases were separated by a 3-week washout period. Liquid chromatography–tandem mass spectrometry was used to determine plasma drug concentrations. The formulations were considered bioequivalent if the natural log–transformed ratios of \( C_{max} \) and \( AUC \) were within the predetermined equivalence range of 70% to 143% and 80% to 125%, respectively, and if \( P < 0.05 \) for the 90% CIs.

Results: Twenty-four male Han Chinese volunteers (mean [SD] age, 21.0 [2.0] years [range, 18–25 years]; mean [SD] weight, 67.6 [5.6] kg [range, 56–81 kg]; mean [SD] height, 176.0 [5.0] cm [range, 165–188 cm]) were enrolled. Twenty-two subjects completed the study, with 2 withdrawing for personal reasons. No period or sequence effect was observed. The 90% CIs for the corresponding ratios of \( C_{max} \), \( AUC \) from hour 0 to time \( t \), and \( AUC \) from hour 0 to any time point were 85.9 to 103.9, 83.6 to 106.0, and 84.7 to 105.9 (in the 2 one-sided \( t \) tests; all, \( P < 0.05 \)), respectively. Similar results were found in data without a logarithmic transformation. There were no significant differences.
in the plasma concentration–time curves of the test and reference formulations. No adverse events were reported by the subjects or revealed by clinical or laboratory tests.

Conclusions: Single oral doses of 2 commercial brands of azithromycin granules (500 mg) were equivalent with regard to the rate and extent of absorption among these healthy Han Chinese volunteers. Both formulations were well tolerated. (Curr Ther Res Clin Exp. 2007;68:369–377) Copyright © 2007 Excerpta Medica, Inc.

Key words: bioequivalence, pharmacokinetic parameters, azithromycin, Han Chinese volunteers, mass spectrometry.

INTRODUCTION
Azithromycin is a semisynthetic azalide, a subclass of the macrolide antibiotics, that is similar to erythromycin but is composed of a 15-member lactone ring. Compared with erythromycin, azithromycin exhibits an extensive spectrum of activity, improved acid stability, more favorable pharmacokinetic parameters, and a lower prevalence of adverse events (AEs) and drug interactions. In clinical practice, the drug plays an important role in the treatment of skin infections and sexually transmitted and respiratory diseases. For these reasons, azithromycin has been widely used in China since 1993. Azithromycin's share of the macrolide antibiotic market in China increased from 38% in 2000 to 63% in 2005.

In recent years, the use of generic drugs has increased due to their effectiveness and the increasing variety of drugs that are now available in generic formulations. However, their use in clinical practice depends not only on their essential similarity (in formulation and composition, as determined by regulatory agencies), but also on their bioequivalence with their reference counterparts. Therefore, study of the comparative bioavailabilities of test and reference formulations is important for appropriate assessment by the scientific community. When 2 formulations of the same drug present similar bioavailabilities to the extent that they are considered bioequivalent by certain criteria, it is assumed that pharmaceutical equivalents are those whose rate and extent of absorption are not statistically different when administered to patients or subjects at the same molar dose under similar experimental conditions.

Azithromycin is administered orally. Several studies have reported the bioavailability of oral azithromycin in different populations (eg, Mexican, Italian, Iranian, and Dutch). Although several generic oral formulations of azithromycin are available in China and azithromycin was ranked the 19th most frequently used drug in a sample of Chinese hospitals in 2006, no information concerning the bioavailability of these formulations in the Chinese population is available.

The aim of the present study was to compare the bioequivalence and tolerability of 2 commercial brands of azithromycin (500 mg) in healthy Han Chinese volunteers.
SUBJECTS AND METHODS

Subjects

Eligible subjects were Han Chinese volunteers aged 18 to 27 years who were in good health and free of acute or chronic diseases, as documented by pre-study medical records, vital signs (temperature, heart rate [HR], blood pressure [BP], respiratory rate [RR], electrocardiography [ECG]), and routine clinical laboratory tests (hematology, serum biochemistry, urinalysis). Subjects were excluded if they had a history of hepatic, renal, cardiovascular, or gastrointestinal disease or known allergy to any drugs. Subjects were instructed to abstain from administering any medication for ≥2 weeks before the study initiation.

The volunteers were recruited in the Clinical Pharmacology Studies Unit (West China Second University Hospital, Sichuan University, Chengdu, China). Each subject provided written informed consent for participation and was compensated financially.

Study Design

The investigations were approved by the ethics and research committee at Sichuan University and carried out in accordance with the Declaration of Helsinki and the guidelines for Good Clinical Practice. A randomized, open-label, 2-period crossover design was used. After a 12-hour (overnight) fast, each participant randomly received a single dose of the test or reference formulation based on a computer-generated random number table. The test formulation was 500 mg of azithromycin granules (batch no. 05100, Dayin Ocean Biochemical Company Ltd., Shandong, China); the reference formulation was also 500 mg of azithromycin granules (batch no. 060108, Taiyang Drug Company Ltd., Beijing, China). Participants completed a 3-week washout period between the 2 treatments. Both the test and reference products were compared in each subject so that intersubject variables were balanced.

Blood samples were collected in heparinized tubes (Haimen Instrument, Jiangsu, China) predose (hour 0) and at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 12, 36, 60, 84, 108, and 156 hour(s) postadministration. Plasma was immediately separated by centrifugation at 4000 rpm for 10 minutes and stored at −20°C until analysis.

Determination of Plasma Azithromycin Concentration

A sensitive, rapid liquid chromatography–tandem mass spectrometry method for determining azithromycin concentration in human plasma was developed and validated in previous studies. First, 0.5 mL of plasma plus 50 μL of internal standard (finasteride 2 μg/mL), 200 μL of sodium hydroxide 0.5 M, and 3 mL of ethyl acetate were mixed in a test tube and vortexed for 5 minutes (vortex WH-3, Anting Scientific Instrument, Shanghai, China). The tube was centrifuged at 14,000 rpm for 5 minutes (Anke TGL-16C centrifuge, Anting Scientific Instrument). The organic phase was transferred to another tube and evaporated until dry at 40°C under a nitrogen stream. The dry residue was redissolved in 100 μL of mobile phase. After centrifugation at 14,000 rpm
for 5 minutes, 20 µL of the supernatant was injected into the chromatographic system. The compounds were separated in a reversed-phase Diamonsil C₁₈ column (150 × 4.6 mm, inner diameter, 5 µm; Dikma Technologies, Beijing, China) and eluted with a mobile phase consisting of 10 mM ammonium acetate (pH 3.5):methanol (20:80, v/v), which was pumped at a flow rate of 1.0 mL/min with a split ratio of 20:80. The column temperature was 40°C. Detection was carried out by multiple-reaction monitoring using the respective [M+H]+ ions, m/z 375.4/591.5 for azithromycin and m/z 373.4/305.4 for the internal standard. The analytical equipment used included a high-performance liquid chromatography device (model 1100, Agilent, Palo Alto, California) coupled with a mass detector (API 3000 triple quadruple instrument, Applied Biosystems, Foster City, California). Under these conditions, the method was linear over the range of 5 to 1500 ng/mL, accuracy was between 90% and 100%, and the relative SD of the method was <13%. According to the validation report, this modified method was considered suitable for pharmacokinetic study of azithromycin.

**Tolerability Assessment**

To assess tolerability, vital signs (temperature, HR, BP, and RR) were measured and subjects were questioned about AEs after drug administration. Laboratory analyses (hematology, serum biochemistry, and urinalysis) and ECG were also performed prestudy and poststudy. Tolerability of the 2 formulations was the secondary assessment end point; therefore, only descriptive statistics were reported.

**Pharmacokinetic and Statistical Analyses**

Assuming a β of 0.2, it was determined that to achieve a power of 80% (analysis of variance [ANOVA]) with a 90% CI, a total of 22 subjects would be needed for the purposes of this study.

All pharmacokinetic (PK) and statistical analyses were performed using Drug And Statistics (DAS) software, Version 2.0 (DAS, Anhui, China). Cₘₐₓ and Tₘₐₓ were obtained directly from observed plasma concentration–time curves. AUC from hour 0 to time t (AUC₀⁻ᵗ) was calculated using the linear trapezoidal method, whereas AUC from hour 0 to any time point (AUC₀⁻₉) was calculated as the sum of AUC₀⁻₉ and the ratio of time of the last measurable concentration over the elimination rate constant (Cₙ/Kₑ). Kₑ was obtained from the slope of the terminal log-linear phase of the semilog plot of concentration versus time. The t₁/₂ was calculated as natural log (ln)2/Kₑ.

To demonstrate the bioequivalence of the formulations tested, ANOVA for a 2-period crossover design for logarithmically (e-base) transformed Cₘₐₓ, AUC₀⁻₉, and AUC₀⁻₉₉ was carried out. Ratios of ln Cₘₐₓ, ln AUC₀⁻₉, and ln AUC₀⁻₉₉ for both formulations were calculated, and 90% CIs were obtained. ANOVA was performed using the F test. The probability of exceeding the limits of acceptance (80%–125%, US Food and Drug Administration [FDA] guidelines) was obtained using 2 one-sided t tests. The formulations were considered
bioequivalent if the ln-transformed ratios of $C_{\text{max}}$ and AUC were within the predetermined equivalence range of 70% to 143% and 80% to 125%, respectively, and if $P < 0.05$ for the 90% CIs.  

**RESULTS**

Twenty-four healthy Han Chinese male volunteers (mean [SD] age, 21.0 [2.0] years [range, 18–25 years]; mean [SD] weight, 67.6 [5.6] kg [range, 56–81 kg]; mean [SD] height, 176.0 [5.0] cm [range, 165–188 cm]) were included in the study. Two subjects withdrew during the study for personal reasons. The results of 22 subjects were included in the PK analysis. Mean plasma azithromycin concentrations of both the test and reference formulations are presented in the figure. There were no significant differences in the plasma concentration–time curves of the test and reference formulations.

**Pharmacokinetic Parameters**

For the test formulation, the mean (SD) $C_{\text{max}}$ was 426.7 (133.8) ng/mL and the mean (SD) $T_{\text{max}}$ was 2.0 (0.7) hours; for the reference formulation, the $C_{\text{max}}$ was 482.7 (149.7) ng/mL and the $T_{\text{max}}$ was 2.1 (0.8) hours. Mean (SD) $t_{1/2}$ values of the test and reference formulations were 53.9 (23.4) and 57.3 (29.0) hours, respectively. PK properties are shown in Table I. No significant between-group differences were found. No period or sequence effects were observed for the PK properties in the ANOVA.

Table II shows the 90% CIs of the ratios (test/reference) for the ln-transformed values of $C_{\text{max}}$ (as an index of rate of absorption), $AUC_{0-\infty}$, and $AUC_{0-\infty}$ (as an index of extent of absorption); the probability of exceeding the limits of acceptance$^{18}$; and the power of the test.$^{9,11,19}$ The 90% CIs for the corresponding ratios of $C_{\text{max}}$, $AUC_{0-\infty}$, and $AUC_{0-\infty}$ were 85.9 to 103.9, 83.6 to 106.0, and 84.7 to 105.9 (in the 2 one-sided t tests; all, $P < 0.05$), respectively. Similar results were found for data without a logarithmic transformation.

**Tolerability**

No AEs were reported by the subjects or revealed by physical examination, vital sign measurements (body temperature, HR, BP, and RR), ECG, or laboratory analyses (hematology, serum biochemistry, and urinalysis) after a single dose of either of the azithromycin formulations.

**DISCUSSION**

The test and reference formulations of azithromycin exhibited overlapping plasma profiles, especially in the elimination phase, which indicated that the 2 formulations were similar in both absorption and elimination.

To ensure a reliable estimate of the extent of absorption, a collection period of $\geq 3 t_{1/2}$s is recommended by the US FDA$^{17}$ and the Chinese State FDA$^{7}$ guide-
Figure: Plasma drug concentrations of a test formulation (Dayin Ocean Biochemical Company Ltd., Shandong, China) and a reference formulation (Taiyang Drug Company Ltd., Beijing, China) of PO azithromycin 500 mg in healthy male Han Chinese volunteers (N = 22).
Table I. Pharmacokinetic properties of 2 oral formulations of a single 500-mg dose of azithromycin granules in healthy subjects (N = 22). Values are mean (SD).*

<table>
<thead>
<tr>
<th>Property</th>
<th>Test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$, ng/mL</td>
<td>426.7 (133.8)</td>
<td>482.7 (149.7)</td>
</tr>
<tr>
<td>$T_{\text{max}}$, h</td>
<td>2.0 (0.7)</td>
<td>2.1 (0.8)</td>
</tr>
<tr>
<td>$t_{1/2}$, h</td>
<td>53.9 (23.4)</td>
<td>57.3 (29.0)</td>
</tr>
<tr>
<td>$AUC_{0-\text{t}}$, ng/mL $\cdot$ h</td>
<td>4171.9 (932.9)</td>
<td>4466.6 (1198.4)</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$, ng/mL $\cdot$ h</td>
<td>4730.2 (918.2)</td>
<td>5069.3 (1374.4)</td>
</tr>
<tr>
<td>MRT, h</td>
<td>31.9 (8.6)</td>
<td>29.2 (7.2)</td>
</tr>
</tbody>
</table>

$AUC_{0-\text{t}} = \text{AUC from hour 0 to time } t$; $AUC_{0-\infty} = \text{AUC from hour 0 to any time point}$; MRT = mean residence time.

*No significant between-group differences were found.

Table II. Comparison of 90% CIs of natural log (In)-transformed $C_{\text{max}}$, $AUC_{0-\text{t}}$, and $AUC_{0-\infty}$, the probability of exceeding limits of acceptance, and power test.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ratio, %</th>
<th>90% CI</th>
<th>Probability of Exceeding Limits of Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>In $C_{\text{max}}$</td>
<td>88.8</td>
<td>85.9–103.9</td>
<td>$&lt;0.008$</td>
</tr>
<tr>
<td>In $AUC_{0-\text{t}}$</td>
<td>94.1</td>
<td>83.6–106.0</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>In $AUC_{0-\infty}$</td>
<td>94.7</td>
<td>84.7–105.9</td>
<td>$&lt;0.008$</td>
</tr>
</tbody>
</table>

$AUC_{0-\text{t}} = \text{AUC from hour 0 to time } t$; $AUC_{0-\infty} = \text{AUC from hour 0 to any time point}$.

lines. This requirement was fulfilled, and the mean extrapolated area was well below 20% for both formulations, indicating that the extraction period was adequate to fully characterize the PK properties of azithromycin.6

The AUC values obtained with the test and reference formulations were not significantly different, which reflects the similar PK characteristics of the 2 formulations, particularly during the elimination phase. The 90% CIs constructed around the ratio of expected geometric means for AUC after administration of each formulation was 0.836 to 1.060. Because this was well within the bioequivalence range of 0.8 to 1.25, the 2 formulations can be considered bioequivalent according to the US FDA's definition of bioequivalence with respect to the extent of absorption.8 In addition, no significant difference was observed in $C_{\text{max}}$ and $T_{\text{max}}$ values of the test and reference formulations. In this study, the PK values for both formulations were in accordance with findings reported in Mexican subjects by Pineyro-Lopez et al.9
Estimated $t_{1/2}$ values for both formulations were also consistent with those reported in Mexican subjects (~50 hours). However, $t_{1/2}$ values of Iranian subjects were shorter (~10 hours) and $t_{1/2}$ values of Dutch subjects were longer (~96 hours) compared with Han Chinese subjects, indicating that race might be an influential factor. In another clinical trial, the $t_{1/2}$ in Italian subjects was not reported because the duration of sampling was not sufficient to determine it with adequate precision.

This study was limited by the small sample size of healthy volunteers and by using only a single dose of azithromycin. Larger studies in both healthy volunteers and clinical patients are needed to obtain more definitive results and to compare the PK characteristics of different races comprehensively.

CONCLUSIONS

In this study, a single 500-mg dose of 2 commercial brands (test and reference formulations) of azithromycin were compared in these healthy Han Chinese subjects. The rate and extent of drug absorption of both formulations were not significantly different. Elimination was also similar between the 2 formulations, indicating they were bioequivalent according to the FDA definition. Both formulations were well tolerated.

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**Address correspondence to:** Xue-Hua Jiang, PhD, West China School of Pharmacy, Sichuan University, No. 17, Section 3, Southern Renmin Road, Chengdu 610041, China. E-mail: jxh1013@vip.163.com