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Bioequivalence Evaluation of Two Capsule Formulations of Amoxicillin in Healthy Adult Male Bangladeshi Volunteers: A Single-Dose, Randomized, Open-Label, Two-Period Crossover Study

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ABSTRACT

BACKGROUND: Amoxicillin, a semisynthetic penicillin antibiotic, is widely prescribed in Bangladesh due to its extended spectrum and its rapid and extensive oral absorption with good tolerability. Although a number of generic oral formulations of amoxicillin are available in Bangladesh, a study of the bioequivalence and pharmacokinetic properties of these formulations has not yet been conducted in a Bangladeshi population.

OBJECTIVE: The aim of this study was to assess the pharmacokinetic properties and bioequivalence of 2 formulations of amoxicillin 500-mg capsules (test, SK-mox®; reference, Amoxil-Bencard®) using serum data.

METHODS: This single-dose, randomized, open-label, 2-period crossover study was conducted in healthy male subjects in compliance with the Declaration of Helsinki and International Conference on Harmonisation guidelines. Subjects were assigned to receive the test or the reference drug as a single-dose, 500-mg capsule under fasting conditions after a 1-week washout period. After oral administration, blood samples were collected and analyzed for amoxicillin concentration using a validated high-performance liquid chromatography method. The pharmacokinetic parameters were determined using a noncompartmental method. The formulations were considered bioequivalent if the natural log-transformed ratios of pharmacokinetic parameters were within the predetermined equivalence range of 80% to 125%, according to the US Food and Drug Administration (FDA) requirement.

RESULTS: Twenty-four healthy adult male Bangladeshi volunteers (mean [SD] age, 26.92 [3.37] years; age range, 23–34 years; mean [SD] body mass index, 23.09 [1.58] kg/m^2) participated in the study. Using serum data, the values obtained for the test and reference formulations, respectively, were as follows: C_{max}, 9.85 (2.73) and 10.63 (2.12) μg/mL; T_{max}, 1.29 (0.58) and 1.33 (0.49) hours; and AUC_{0-12},

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27.09 (7.62) and 28.56 (6.30) μg/mL · h^{-1}. No period, sequence, or formulation effects were observed; however, significant variation was found among subjects with regard to AUC_{0-12} ($P < 0.001$), $AUC_{0-\infty}$ ($P = 0.002$), area under the moment curve (AUMC) from 0 to 12 hours ($P < 0.001$), and $AUMC_{0-\infty}$ ($P = 0.017$). All CIs for the parameters measured were within the FDA-accepted limits of 80% to 125%.

CONCLUSION: The present study suggests that the test 500-mg amoxicillin capsule was bioequivalent to the reference 500-mg capsule according to the FDA regulatory definition, in this population of healthy adult male Bangladeshi volunteers. (*Curr Ther Res Clin Exp.* 2008;69:504–513) © 2008 Excerpta Medica Inc.

KEY WORDS: amoxicillin, pharmacokinetic, bioequivalence, analysis of variance, confidence interval, Bangladeshi population.

INTRODUCTION

The use of generic drugs is of increasing importance, in terms of efficacy, in the selection of therapeutic alternatives. However, their use in clinical practice depends not only on their similarity (in terms of formulation, composition, and bioequivalence, as considered by regulatory agencies), but also their interchangeability with their reference drugs. Amoxicillin is a widely prescribed antimicrobial agent because of its extended spectrum, rapid and extensive oral absorption, and good tolerability.[1] Several in vitro studies have reported that the bactericidal activity of amoxicillin is significantly better than that of other β-lactam antibiotics (eg, penicillin G, cefpodoxime, cefprozil, cefaclor) against penicillin-susceptible and -resistant pneumococci.[2,3] Oral absorption of amoxicillin is ~90%, and a peak serum concentration of 10 mg/L is attained within 1 to 2 hours with a 500-mg oral dose.[4] The $t_{1/2}$ of amoxicillin in healthy volunteers has been reported to be ~60 minutes.[5] Approximately 10% to 20% of the absorbed dose is hydrolyzed to the corresponding penicilloic acid, and 75% of the unchanged drug is excreted through urine.[6]

The pharmacokinetics of amoxicillin have been reported in various populations including Germans,[7] Americans,[8–10] Brazilians,[11,12] and Belgians,[13] but not in the Bangladeshi population. The aim of the study was to compare the oral absorption and disposition kinetics of a test* and reference† formulation of amoxicillin 500-mg capsules in healthy Bangladeshi volunteers using serum data.

SUBJECTS AND METHODS

MATERIALS

Amoxicillin and cefaclor (internal standard) provided by Eskayef Bangladesh Ltd., (Dhaka, Bangladesh) were used for the study. High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Fisher Scientific (Loughborough, United Kingdom). Deionized water was prepared using a water

*Trademark: SK-mox® (Eskayef Bangladesh Ltd., Dhaka, Bangladesh; Batch #7003).

†Trademark: Amoxil-Bencard® (SmithKline Beecham plc, Middlesex, United Kingdom; Batch #B.526003).

purification system (Arium® 611, Sartorius AG, Goettingen, Germany). All other chemicals were of analytical grade and were used without further purification.

SUBJECTS

Nonsmoking healthy adult male Bangladeshi volunteers were enrolled in the study. Subjects were eligible for the study if they had an unremarkable prestudy medical history, physical examination (height, weight, and temperature), and normal cardiac (heart rate, blood pressure, echocardiogram [ECG]), hepatic (enzyme function studies), renal (creatinine clearance), pulmonary (respiratory rate), neurologic, gastrointestinal, and hematologic profiles. All subjects had to report abstinence from other medications, alcohol, tobacco, and caffeinated products throughout the study. Exclusion criteria were a previous history of hypersensitivity to any of the penicillins, having donated blood within 30 days prior to dosing, and vomiting or any other adverse events (AEs) during the 12-hour study period.

STUDY DESIGN AND CLINICAL CONDUCT

The study was conducted in the Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh, in accordance with the International Conference on Harmonisation Guideline for Good Clinical Practice[14] and in compliance with the Declaration of Helsinki and its amendments.[15] The Bangladesh Medical Research Council (Dhaka, Bangladesh) provided ethical permission for approval of the protocol and consent form for the clinical investigation. Each volunteer signed an informed consent document before entering the study and was free to withdraw from the study at any time without any obligation.

The study was a single-dose, randomized, open-label, 2-period crossover design with a 1-week washout period. A single 500-mg capsule of either formulation (SK-mox or Amoxil-Bencard) was administered with 250 mL of water after an overnight fast. A standardized breakfast and lunch were given at 4 and 8 hours after administration, respectively. Patients were followed up for 12 hours after study drug administration. None of the volunteers vomited and no AEs were identified or reported.

Venous blood samples were obtained prior to dosing (hour 0) and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hours after dosing in both periods. Serum was separated by centrifugation and stored at –40°C until analysis.

BIOANALYSIS

Amoxicillin and cefaclor (internal standard) were extracted from human plasma samples by protein precipitation with slight modification of the method described by Pires de Abreu et al.[12] A 200-µL aliquot of each plasma sample was transferred to a polypropylene tube (Eppendorf, Hamburg, Germany), and 60 µL of cefaclor solution (100 µg/mL) and 340 µL of methanol were added. After brief vortex mixing, the tubes were centrifuged at 10,000 rpm for 5 minutes. Then the supernatants were collected and 20 µL was injected into the chromatographic system.

An HPLC system consisting of an SCL–10Avp system controller and 2 LC–8A pumps (Shimadzu Corp., Columbia, Maryland) was used to quantify amoxicillin in

serum samples. The drug analysis data were acquired and processed using LC Solution version 1.03 SP3 software (Shimadzu Corp.). Ultraviolet (UV) detection was achieved using an SPD–10Avp UV-Visible detector (Shimadzu Corp.). The mobile phase involved a mixture of monobasic potassium phosphate buffer (pH 5.0) and acetonitrile (95:5) pumped at a flow rate of 1.5 mL/min through the column (Nucleosil C_{18}, 5 μ, 4.6 × 250 mm, Varian Inc., Palo Alto, California) at room temperature. Peaks were monitored using UV absorbance at 230 nm at a sensitivity of 0.0005 absorbance units full scale. Quantification of amoxicillin in serum samples was obtained by plotting the amoxicillin to internal standard peak area ratio as a function of amoxicillin concentration. The analytical method was validated in terms of specificity, linearity, precision, accuracy, extraction efficiency, and stability.

TOLERABILITY

Tolerability was determined by clinical assessment and monitoring vital signs (blood pressure, heart rate, body temperature) at baseline, every 4 hours during the study, and at the end of both periods. Subjects were asked if they experienced any AEs during the study. Laboratory analyses (ECG, serum biochemistry, and urinalysis) were also performed before and after the study. Only descriptive statistics were reported for the assessment.

PHARMACOKINETIC ANALYSIS

A total of 24 subjects were used in this study, and the sample size was determined to achieve 80% power with a 90% CI.[16]

The pharmacokinetic parameters of amoxicillin were calculated using a noncompartmental method with Kinetica version 4.4.1 software (Thermo Electron Corporation, Basingstoke, United Kingdom). The C_{max} of amoxicillin was determined by visual inspection of the serum concentration–time profile and the $t_{1/2}$ was calculated using the following equation[17]:

$$t_{1/2} = 0.693/k_{el},$$

where k_{el} was the terminal elimination rate constant calculated using linear least squares regression of the last 3 to 4 time points in the log concentration–time profile. The AUC from time zero to the last sampling time ($AUC_{0-tlast}$) was calculated using the linear trapezoidal rule.[17] $AUC_{0-\infty}$ was calculated as follows:

$$AUC_{0-\infty} = AUC_{0-tlast} + C_{last}/k_{el}.$$

The mean residence time (MRT) was calculated as follows:

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}},$$

where *AUMC* was area under the moment curve.

STATISTICAL ANALYSIS

The analytical method used to measure the 2 formulations of amoxicillin was validated for accuracy, precision, linearity, and sensitivity. Pharmacokinetic parameters were calculated for both the test and reference products using SPSS version 12.0 (SPSS Inc., Chicago, Illinois). $P < 0.05$ was considered statistically significant.

The within-group and between-group differences were examined using analysis of variance, with $P < 0.05$ being considered statistically significant. The effects of formulation, period, sequence, and subject were analyzed according to the statistical model of Jones and Kenward.[18] Log-transformed values of the pharmacokinetic parameters were used in the model and the formulation, period, sequence, and subject effects were evaluated.

Large sample-based 90% CIs for the pharmacokinetic parameters (obtained for both formulations) from log-transformed data were evaluated to assess bioequivalence. The formulations were considered bioequivalent if the natural log (ln)-transformed ratios of pharmacokinetic parameters were within the predetermined equivalence range of 80% to 125%, according to the US Food and Drug Administration (FDA) requirement.[19]

RESULTS

SUBJECTS

Twenty-four healthy adult male Bangladeshi volunteers (mean [SD] age, 26.92 [3.37] years; age range, 23–34 years; mean [SD] body mass index, 23.09 [1.58] kg/m^2) participated in the study. No volunteers were withdrawn from the study.

METHOD VALIDATION

The HPLC method with UV detection used for drug quantification provided the appropriate sensitivity, specificity, and high sample throughput required for pharmacokinetic studies.[19] The retention times for amoxicillin and cefaclor (internal standard) were found to be 3.88 and 15 minutes, respectively. The relationship between concentration and peak area ratio was found to be linear within the range of 0.1 to 10 μg/mL for amoxicillin ($r^2 > 0.997$). The limit of quantification was 0.1 μg/mL. The intraday accuracy of the method used to measure serum amoxicillin concentration ranged from 101.5% to 108.3%, and the intraday precision ranged from 5.7% to 14.0%. The interday accuracy ranged from 101.5% to 108.4%, and the interday precision was 5.9% to 14.4% for serum standards. The absolute recovery of amoxicillin from serum was 97.2% and that of cefaclor was 96.5%. No significant degradation of amoxicillin in serum was observed during this period.

SERUM PHARMACOKINETIC ANALYSIS

The plot of mean serum concentration (N = 24) versus time over a period of 12 hours for all volunteers is shown in the **figure**.

Paired *t* tests were done for all the parameters at the 5% level of significance. There were no significant between-group differences for any of the pharmacokinetic parameters (**Table I**). Amoxicillin was absorbed rapidly, with a mean (SD) T_{max} of 1.29 (0.58) and

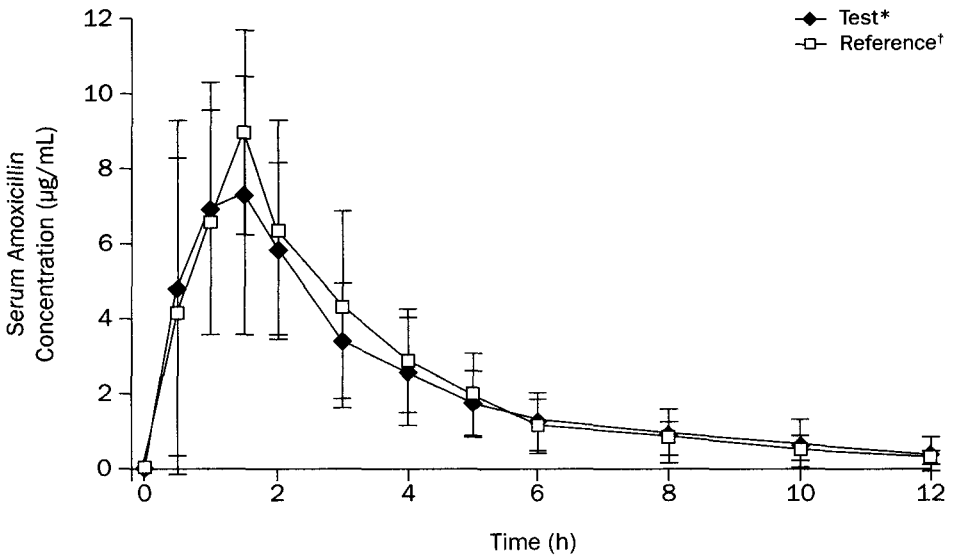

Figure. Mean (SD) amoxicillin concentration-versus-time curve over 12 hours in healthy adult male Bangladeshis (N = 24). *SK-mox® (Eskayef Bangladesh Ltd., Dhaka, Bangladesh); †Amoxil-Bencard® (SmithKline Beecham plc, Middlesex, United Kingdom).

Table I. Mean (SD) serum pharmacokinetic parameters for test* and reference† formulations of amoxicillin 500-mg capsules in healthy adult male Bangladeshis.

Pharmacokinetic Parameter	Test (n = 24)	Reference (n = 24)	*P*
C_{max}, µg/mL	9.85 (2.73)	10.63 (2.12)	0.26
T_{max}, h	1.29 (0.58)	1.33 (0.49)	0.84
AUC_{0-12}, µg/mL · h^{-1}	27.09 (7.62)	28.56 (6.30)	0.16
$AUC_{0-\infty}$, µg/mL · h^{-1}	29.47 (10.21)	29.32 (6.27)	0.93
$t_{1/2}$, h	3.05 (1.78)	3.24 (1.19)	0.78
k_{el}, h^{-1}	0.29 (0.14)	0.25 (0.13)	0.52
$AUMC_{0-12}$, µg/mL · h^{-1}	90.15 (42.99)	93.41 (29.84)	0.65
$AUMC_{0-\infty}$, µg/mL · h^{-1}	134.84 (107.50)	121.31 (42.04)	0.62
MRT, h	4.17 (2.05)	4.00 (1.03)	0.78

k_{el} = terminal elimination rate constant; AUMC = area under the moment curve; MRT = mean residence time.

*SK-mox® (Eskayef Bangladesh Ltd., Dhaka, Bangladesh).

†Amoxil-Bencard® (SmithKline Beecham plc, Middlesex, United Kingdom).

1.33 (0.49) hours for the test and reference formulations, respectively. C_{max} (9.85 [2.73] vs 10.63 [2.12] μg/mL) and AUC_{0-12} (27.09 [7.62] vs 28.56 [6.30] μg/mL · h^{-1}) for amoxicillin were also similar after administration of the test and reference capsules, respectively.

STATISTICAL ANALYSIS FOR BIOEQUIVALENCE

Analysis of variance for the crossover design[18] was used to assess the formulation, period, sequence, and subject effects on the serum pharmacokinetic parameters. The parameters were not significantly different in terms of variation in formulation, period, or sequence. Significant variation was observed among subjects in AUC_{0-12} ($P < 0.001$), $AUC_{0-\infty}$ ($P = 0.002$), $AUMC_{0-12}$ ($P < 0.001$), and $AUMC_{0-\infty}$ ($P = 0.017$) (**Table II**).

C_{max} and AUC_{0-12} for the serum data were analyzed statistically to determine bioequivalence. Large sample-based 90% CIs on the mean of the difference between the 2 formulations were computed. The estimates of 90% CIs for the ratio of these parameters were found to be within 80% to 125% for ln-transformed data, as required by the FDA for drug bioequivalence (**Table III**).

TOLERABILITY

All 24 volunteers completed the study without any AEs occurring during the 12-hour follow-up, as assessed by monitoring vital signs and laboratory analysis. No vomiting, diarrhea, or other AEs were reported during the study period.

DISCUSSION

Evaluation of the bioequivalence of a test drug and a reference drug is required to exclude any clinically important differences in the rate or extent to which the active entity of the drugs becomes available at the site of action. The FDA considers 2 drugs bioequivalent if they are pharmaceutically equivalent and their bioavailabilities are so similar that they are unlikely to produce clinically relevant differences in regard to tolerability and efficacy.[19]

The pharmacokinetic parameters obtained with the test and reference formulations were not significantly different, which reflects the comparable pharmacokinetic characteristics of the 2 formulations. The 90% CIs for C_{max}, AUC_{0-12}, and $AUC_{0-\infty}$ were within the acceptable range (80%–125%) based on the FDA criteria for bioequivalence. Moreover, both formulations were well tolerated.

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

CONCLUSION

The present study suggests that the test formulation 500-mg amoxicillin capsule was bioequivalent to the reference formulation, according to the FDA regulatory definition, in this population of healthy adult male Bangladeshi volunteers.

Table II. *P* values for variations between test* and reference† amoxicillin formulations in healthy adult male Bangladeshis based on formulation, period, sequence, and subject using analysis of variance.

Source of Variation	C_{max}	T_{max}	AUC_{0-12}	$AUC_{0-\infty}$	k_{el}	$t_{1/2}$	$AUMC_{0-12}$	$AUMC_{0-\infty}$	MRT
Formulation	0.302	0.254	0.659	0.725	0.795	0.794	0.271	0.537	0.390
Period	0.242	0.731	0.159	0.616	0.602	0.603	0.218	0.595	0.884
Sequence	0.94	0.567	0.792	0.696	0.815	0.814	0.915	0.983	0.805
Subject	0.036	0.291	<0.001	0.002	0.798	0.798	<0.001	0.017	0.124

k_{el} = terminal elimination rate constant; AUMC = area under the moment curve; MRT = mean residence time.
*SK-mox® (Eskayef Bangladesh Ltd., Dhaka, Bangladesh).
†Amoxil-Bencard® (SmithKline Beecham plc, Middlesex, United Kingdom).

Table III. Large, sample-based 90% CIs for the pharmacokinetic parameters of healthy adult male Bangladeshis administered amoxicillin 500 mg.

Parameter	Point Estimate	90% CI
C_{max}	91.33	82.58–101.02
AUC_{0-12}	93.12	87.54–99.05
$AUC_{0-\infty}$	94.11	86.50–102.38

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