

Bioequivalence Study of Two Formulations of Atorvastatin Film-Coated Tablets in Healthy Male Subjects Under Fasting Conditions

Mohammed Sultan Alsultan

Department of Clinical Pharmacy, College of Pharmacy,
King Saud University, Riyadh-11451, Saudi Arabia

Abstract: The aim of this study was to demonstrate bioequivalence between a generic atorvastatin calcium film-coated tablet (provided by Saudi Food and Drug Authority, Riyadh, Saudi Arabia) and a reference formulation (Lipitor, Pfizer, Germany), under fasting conditions. The study was a single-centre, open-label, randomized, two-treatment, three-sequence and three-period crossover study. A single oral 20 mg dose of atorvastatin was administered following an overnight-fast in with a 2-week washout period between doses. Forty-one healthy volunteers were enrolled and randomized. After the test formulation and first and second reference formulation dosing, the C_{max} was 10.206 ± 7.572 ng/mL, 9.531 ± 4.744 ng/mL and 10.279 ± 5.960 ng/mL and the AUC_{0-t} was 39.58 ± 23.90 ng.h/mL, 38.20 ± 21.90 ng.h/mL and 37.29 ± 20.18 ng.h/mL, respectively. The geometric mean ratios (90% confidence interval) of test drug/reference drug for atorvastatin were 102.08% (94.22%-110.58%) for AUC_{0-t} , 101.06% (93.81%-108.87%) for AUC_{0-inf} and 91.55% (78.39%-106.92%) for C_{max} . This study showed that the test and reference formulations were within the pre-defined bioequivalence acceptance limits following a 20 mg oral dose, under fasting condition. Both formulations were generally well tolerated in the population studied.

Key words: Atorvastatin • Bioequivalence • Crossover

INTRODUCTION

Atorvastatin is a synthetic lipid-lowering agent and is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. Atorvastatin is indicated as an adjunct to diet for the reduction of elevated total cholesterol, LDL-cholesterol, apolipoprotein B and triglycerides in adults with primary hypercholesterolaemia when the response to diet and other non-pharmacological measures is inadequate [1-4]. The clinical dosage range for atorvastatin is 10-80 mg/day and can be administered at any time of the day [4]. Atorvastatin is rapidly absorbed after oral administration; maximum plasma concentrations occur within 1 to 2 hours [4, 5]. The extent of absorption increases in proportion to atorvastatin dose. The absolute bioavailability of atorvastatin (parent drug) is approximately 14% and the systemic availability of

HMG-CoA reductase inhibitory activity is approximately 30% [4]. Atorvastatin is extensively metabolized by cytochrome P450 3A4 to at least 2 active metabolites, ortho-hydroxy-atorvastatin (which is the dominant metabolite detected in plasma) and para-hydroxy-atorvastatin derivatives (whose plasma concentration is low) and various beta-oxidation products [4, 6]. Atorvastatin and its metabolites are eliminated primarily in bile following hepatic and/or extra-hepatic metabolism [4, 5]; mean plasma elimination half-life of Atorvastatin in humans is approximately 14 hours, but the half-life of inhibitory activity for HMG-CoA reductase is 20 to 30 hours due to the contribution of active metabolites [4, 5].

The aim of this study was to demonstrate bioequivalence between a generic atorvastatin calcium film-coated tablet (provided by Saudi Food and Drug Authority, Riyadh, Saudi Arabia) and a reference formulation (Lipitor, Pfizer, Germany), under fasting conditions.

Corresponding Author: Mohammed Sultan Alsultan, PharmD, PhD, Department of Clinical Pharmacy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451 Saudi Arabia.
Tel: +966-50 7222277, Fax: +966-11-4677480.

MATERIALS AND METHODS

Study Design: This randomized, open label, two treatment, three-sequence and three-period cross-over study involved 41 healthy subjects under a fasting condition, with a 2-weeks washout period. This study was carried out in accordance with the Declaration of Helsinki [7], Good Clinical Practice [8] and Good Laboratory Practice [9]. The protocol, the consent form and the patient information sheet were reviewed and approved by an independent ethics committee of the Saudi Ajal Health Services Co, Saudi Arabia, prior to the study conduct. Written informed consent from every study subject was obtained prior to any trial-related activities and the investigator retained the consent forms.

Subjects: Subjects aged between 18–50 years, with body weight within a normal range (body mass index between 18.5 kg/m² and 30 kg/m²) and who had signed the informed consent, were enrolled in this study. All clinical laboratory tests, such as urinalysis, hematology and blood chemistry, were required to be normal prior to enrollment. Serologic tests (hepatitis B surface antigen, hepatitis C virus antibody and HIV antibody) needed to be negative. Anyone who was already participating in an investigational study, who was using any investigational drugs, or who had donated ≥ 200 mL of blood within 90 days prior to study initiation was excluded. Subjects were excluded if they had taken any prescription medication, or any over-the-counter medication two weeks before or during the study period. Any subjects with a concurrent illness or with a history of hypersensitivity to atorvastatin or related compounds were also excluded. The clinical and analytical parts of the study were conducted at Saudi Ajal Health Services Co (Riyadh, Saudi Arabia). Pharmacokinetic and statistical analyses were performed by ACDIMA BioCenter, (Amman, Jordan).

Study Products: The test formulation (atorvastatin calcium 20 mg film coated tablets, batch number 5NL133; expiration date: 12/2017) was provided by Saudi Food and Drug Authority (Riyadh, Saudi Arabia). The reference formulation (atorvastatin calcium 20 mg film coated tablets, batch number J87935; expiration date: 06/2017) was the innovator product (Lipitor[®]; manufactured by Pfizer, Germany).

Treatment Phase and Blood Sampling: After an overnight fast of ≥ 10 hours, subjects were administered the test or the reference formulation, as per the randomization scheme, as a single oral dose of one film-coated tablet containing 20 mg of study medication, with 240 mL bottled tepid water. Subjects were dosed as specified in the protocol and subsequently fasted for a period of at least 4 hours. Subjects were served a controlled meal not less than 4 hours post-dose and at appropriate times thereafter, in each period. Subjects were served standardized post dose meals similar in composition in each period. With the exception of the volume administered at the time of dosing, fluids were not permitted from 1 hour before dosing to 4 hours after dosing, except for: 120 ml of water 1 hour before dosing, 240 ml of water with the product on dosing and 120 ml of water 2 & 3 hours after dosing. Otherwise, subjects were allowed to drink water as desired. The study had three periods (period 1, period 2 and period 3) and the subjects were randomized to three sequences (test-reference reference [TRR]; reference-reference-test [RRT] and reference-test-reference [RTR]) using SAS 9.3 software. In each study period, subjects were administered the test formulation (Treatment A) or the reference formulation (Treatment B) as per the randomization scheme. The treatment periods were separated by washout periods of 14 days. Decoding of the randomized treatments was done only during kinetic analysis.

From each subject, blood samples were collected in pre-labeled heparinized tubes through an indwelling cannula in the subject forearm immediately before taking the drug (control) and at 0.167, 0.333, 0.500, 0.667, 1.00, 1.33, 1.66, 2.00, 2.33, 2.66, 3.00, 3.50, 4.00, 6.00, 8.00, 10.0, 12.0, 16.0, 24.0, 36.0, 48.0, 60.0 and 72.0 hours post dose in each period. The first few drops were discarded. (0.5) ml, Heparin solution (1.0 IU) was flushed into the cannula to prevent it from clogging. Actual times for blood collection were recorded. Collected blood samples were immediately centrifuged at 4000 rpm for 5 minutes at 10°C. Supernatant plasma was transferred using polypropylene Pasteur pipettes and divided into two pre-labeled Eppendorf tubes. Samples were stored during study conduct in freezers at a nominal temperature of -70°C .

Analysis of Drug Concentration: Samples were analyzed at the bio-analytical site of Saudi Ajal Health Services Co. according to a validated chromatographic method (LC-MS/MS) developed in-house. The analysis was

carried on atorvastatin and its primary active metabolites orthohydroxy atorvastatin in plasma using a linear range of (0.300 - 80.000 ng/ml) for atorvastatin and (0.100 - 100.000 ng/ml) for ortho-hydroxy atorvastatin. The used internal standards were atorvastatin-d5 (200.0 ng/ml) for Atorvastatin and ortho-hydroxy atorvastatin-d5 (100.0 ng/ml) for orthohydroxy atorvastatin.

Tolerability Assessments: Tolerability was determined by monitoring of vital signs (sitting blood pressure, heart rate, breathing rate and oral body temperature), clinical laboratory tests (hematology, blood biochemistry and urinalysis) and physical examinations at baseline and at the end of each study period. Subject interviews were also conducted regarding the potential occurrence of adverse events (AEs) associated with atorvastatin administration.

Pharmacokinetic Evaluation: The non-compartmental pharmacokinetic analysis using Kinetica 5.0.11 program was employed to determine the pharmacokinetic parameters of atorvastatin. The number of observations, mean, standard deviation (SD), within-subject variability (WSV) and geometric mean were calculated for plasma concentrations of atorvastatin for each sampling time and treatment. The terminal elimination rate constant (K_{el} or λ_z) was estimated for each subject and for each treatment via linear regression of the last points (at least three points were used) at the terminal phase of the log-concentration versus time curve of each subject. The elimination half-life $t_{1/2}$ was calculated from $0.693/\lambda_z$. C_{max} (ng/mL) and the time to reach C_{max} (t_{max} hours) were obtained directly from the observed data i.e., concentration versus time curve of each subject. ANOVA was performed on the log-transformed data and the 90% confidence interval was determined. The area under the plasma concentration-time curve (AUC) from time zero to 72 hours (AUC_{0-t}) was calculated by the trapezoidal method. AUC from time zero to infinity (AUC_{0-inf}) was calculated through the following equation: $AUC_{0-t} + (C_t/K_{el})$, where C_t is the drug concentration at time t and K_{el} is the elimination rate constant.

Statistical Analysis: For the purpose of statistical analyses, the pharmacokinetic population included the subjects who completed at least two periods, including one period with test formulation and other with the reference formulation and for whom the pharmacokinetic profile was characterized. Pharmacokinetic parameters

were summarized by treatment. Plasma concentrations were summarized by treatment and time point. Individual and mean plasma concentrations, as well as the plots of the plasma levels for all subjects versus time, were graphically displayed for treatments. All statistical tests were performed at the alpha level of 0.05. The average bioequivalence of the products is concluded if the two-sided 90% confidence interval for the test to reference ratio of the population means is within 80.00% – 125.00 % for each of the logarithmic -transformed data AUC_{0-t} and AUC_{0-inf} . Whereas for C_{max} the reference-scaled average bioequivalence approach was employed to determine the bioequivalence limits. The CI limits are determined based on the reference within-subject variability (WSV). The obtained WSV for the reference product (48.27) allowed the widening of the limits to be (75.00%-133.00%), according to the GCC guidelines for bioequivalence [10]. The assessment of the parent atorvastatin was considered to be primary and essential for the bioequivalence comparison. The evaluation of the active metabolite, ortho-hydroxy and para-hydroxy atorvastatin, would provide supportive evidence for the bioequivalence between the test and reference formulations.

RESULTS

Forty-two subjects from the Saudi populace were randomized to three sequences of treatment (TRR, RTR and RRT) and received at least one dose of the investigational medicinal products under study. The baseline demographic characteristics of the pharmacokinetic population are depicted in Table 1. Nevertheless, as previously stated in the protocol, the subjects used for pharmacokinetic and statistical analysis, the pharmacokinetic population, are those that completed at least two periods, including one test and one administration of the reference product and for whom the pharmacokinetic profile was adequately characterized. Forty-one subjects completed all study procedures. The disposition of subjects is presented in Fig. 1.

Tolerability: The study subjects were monitored throughout the study period for adverse events (AEs). Two subjects (4.9%) experienced headache that was considered as moderate and resolved after concomitant medication administration. In the investigator's opinion, none of the AEs were considered to be associated with

Table 1: Demographic data for the pharmacokinetic population (n=41) - descriptive statistics

Parameter	Age (yr)	Height	Weight (Kg)
Mean \pm SD	32.6 \pm 7.1	1.757 \pm 0.068	81.34 \pm 13.31
Range	21 - 49	1.63 - 1.90	49.0 - 103.9

Table 2: Pharmacokinetic variables for atorvastatin calcium for each treatment/period (N=41)

Parameter	Test Product (Mean \pm SD)	Reference Product (First dose) (Mean \pm SD)	Reference Product (Second dose) (Mean \pm SD)
Atorvastatin			
C _{max} (ng/mL)	10.206 \pm 7.572	9.531 \pm 4.744	10.279 \pm 5.960
AUC _{0-t} (ng.hr/mL)	39.58 \pm 23.90	38.20 \pm 21.90	37.29 \pm 20.18
AUC _{0-inf} (ng.hr/mL)	46.11 \pm 24.95	45.33 \pm 23.69	44.51 \pm 22.03
T _{max} (hour)	1.394 \pm 0.922	0.907 \pm 0.630	0.854 \pm 0.576
T _{1/2} (hour)	11.496 \pm 3.058	11.382 \pm 3.323	11.363 \pm 3.402
K _{elimination} (hr ⁻¹)	0.06578 \pm 0.02192	0.06694 \pm 0.02251	0.06747 \pm 0.02317
Ortho-hydroxy-atorvastatin			
C _{max} (ng/ml)	6.171 \pm 4.091	5.924 \pm 4.034	5.900 \pm 3.784
AUC ₀₋₇₂ (ng.hr/ml)	49.72 \pm 21.94	48.45 \pm 18.90	48.92 \pm 19.65
T _{max} (hour)	1.980 \pm 1.307	1.622 \pm 1.372	1.402 \pm 1.332

AUC_{0-inf}: area under the serum concentration-time curve from time zero to infinity, AUC_{0-t}: area under the serum concentration-time curve from time zero to time of last measurable concentration, C_{max}: maximum serum concentration, N: number of observations, SD: standard deviation, T_{1/2}: elimination half-life, T_{max}: time to C_{max}

Table 3: Statistical comparison i.e., ratios, 90 % geometric confidence intervals (CI) for AUC_{0-t}, AUC_{0-inf} and C_{max} and within-subject variability (WSV) of atorvastatin after oral administration of a 20 mg atorvastatin calcium tablet of the test and the reference drug

Parameter	Ratio ^a (%)	90 % CI ^b (%)	WSV (%)	Accepted CI (%)
C _{max}	91.55	78.39-106.92	48.27	75.00-133.00
AUC _{0-t}	102.08	94.22-110.58	26.49	80.00-125.00
AUC _{0-inf}	101.06	93.81-108.87	25.41	80.00-125.00

^aCalculated using least-squares means

^b90 % geometric confidence interval using logarithmic-transformed data

AUC_{0-inf}: area under the serum concentration-time curve from time zero to infinity AUC_{0-t}: area under the serum concentration-time curve from time zero to time of last measurable concentration, C_{max}: maximum serum concentration

the administration of either study drug. Of the two AEs reported, one subject experienced a headache 2 hours, 45 minutes after administration of the test formulation in period II. Another subject also experienced headache after 45 minutes of administration of reference formulation in period III. Concomitant medication (paracetamol 500 mg, two times) was prescribed for adverse event reported during the study. There were no serious and significant adverse events or deaths reported or observed during the conduct of the study.

Pharmacokinetic Properties of Atorvastatin and Ortho-Hydroxy-Atorvastatin: After oral administration of 20 mg of the test and the reference formulation, the mean plasma concentration versus time curves of atorvastatin and ortho-hydroxy-atorvastatin are displayed in Figure 2 and 3, respectively. The values of the pharmacokinetic parameters (AUC_{0-t}, AUC_{0-inf}, C_{max}, t_{1/2}, t_{max} and K_{elimination}) of atorvastatin that resulted from the test drug as well as

from the reference drug are presented in detail in Table 2. After the test formulation and first and second Lipitor[®] dosing, the C_{max} was 10.206 \pm 7.572 ng/mL, 9.531 \pm 4.744 ng/mL and 10.279 \pm 5.960 ng/mL and the AUC_{0-t} was 39.58 \pm 23.90 ng.h/mL, 38.20 \pm 21.90 ng.h/mL and 37.29 \pm 20.18 ng.h/mL, respectively (Table 2).

Bioequivalence Evaluation: The least-squares means ratios, the 90% geometric confidence intervals and the within-subject variability (WSV) for the reference product are presented in Table 3. The geometric mean ratios (90% CI) of the test drug/reference drug for atorvastatin were 102.08% (94.22%-110.58%) for AUC_{0-t}; 101.06% (93.81% -108.87%) for AUC_{0-inf}; and 91.55 % (78.39%-106.92%) for C_{max}. The 90% CIs (Table 3) of the test/reference AUC ratio (as indices of the extent of absorption) and log-transformed values of C_{max} ratio (as an index of the rate of absorption) of atorvastatin met the predetermined criteria for bioequivalence.

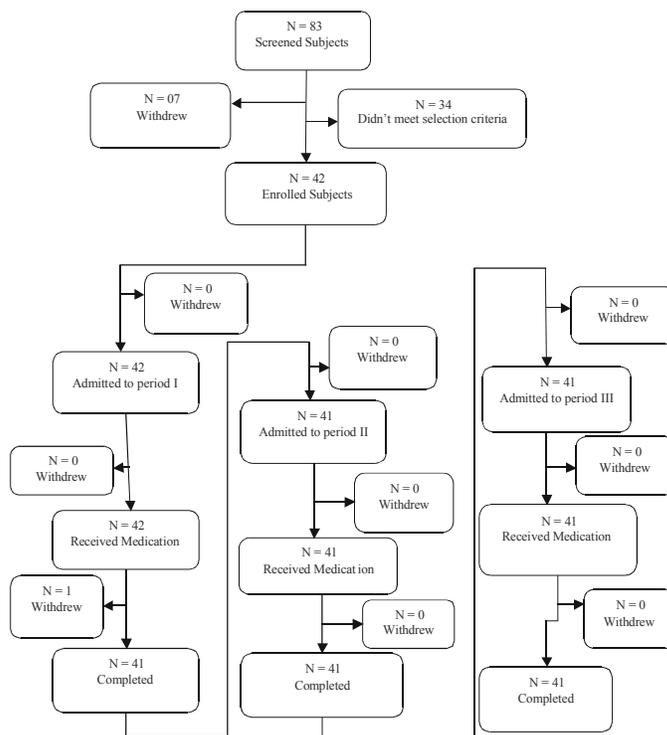


Fig. 1: Subjects Disposition during the Study

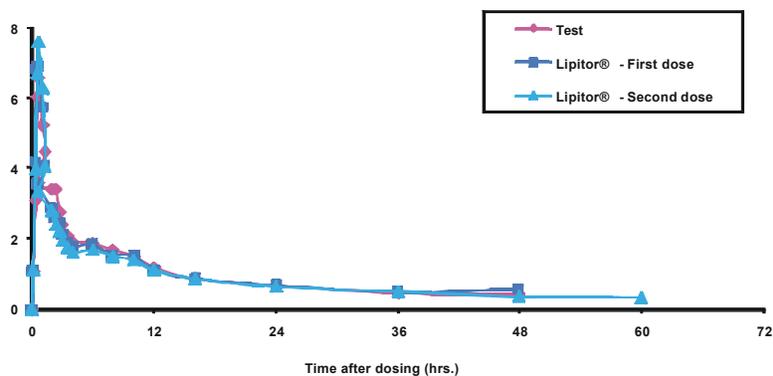


Fig. 2: Mean plasma concentrations versus time profiles of atorvastatin in human subjects (n = 41) after oral administration of 20 mg of atorvastatin calcium tablets of the test drug and the reference drug

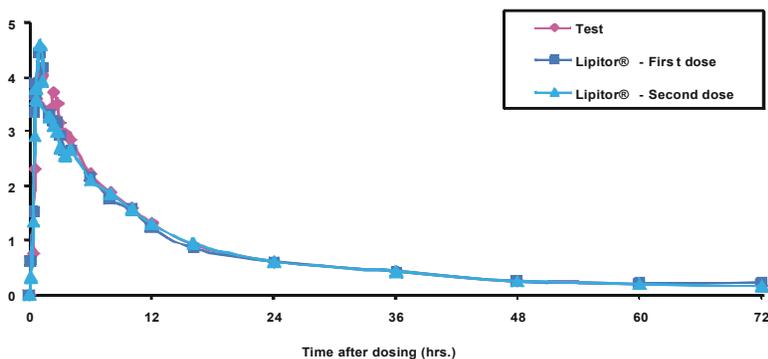


Fig. 3: Mean plasma concentrations versus time profiles of ortho-hydroxy atorvastatin in human subjects (n = 41) after oral administration of 20 mg of atorvastatin calcium tablets of the test drug and the reference drug

DISCUSSION

The aim of the present study was to compare the bioavailability of a commercially available formulation of atorvastatin 20 mg film-coated tablets from a local Saudi pharmaceutical manufacturer to the reference formulation Lipitor. The advantage of providing scientifically sound evidence that the test formulation, or the so-called generic product, is bioequivalent to the reference (which is usually the innovator's product) is that the bioequivalent generic product can be used interchangeably with the reference, yet definitely is available at a more affordable price than the reference. This has made generic products much more accessible to patients in need.

The pharmacokinetic parameters of atorvastatin in the study subjects were found to be in agreement with other studies conducted internationally in foreign populations [11-15]. However, those studies apparently did not evaluate the same preparation that was tested in the present study. The tested preparation in the current study had its own formulation, which was different from those of similar studies. The pharmacokinetic parameters of 20 mg atorvastatin calcium film-coated tablets were assessed based on the plasma concentrations of atorvastatin. In this study, AUC_t , AUC_{inf} and C_{max} of atorvastatin were defined as the main parameters in order to assess possible bioequivalence between both formulations. Based on standard bioequivalence guidelines, the criteria for bioequivalence are the 90% CI of the test/reference geometric means ratio in the range of 80.00% to 125.00% for AUC and 75% to 133% for C_{max} as established by the GCC guidelines for bioequivalence [10]. The results of the present study showed that the geometric mean ratios (90% CIs) of AUC_t , AUC_{inf} and C_{max} of atorvastatin were 102.08% (94.22%-110.58%), 101.06% (93.81%-108.87%) and 91.55% (78.39%-106.92%), respectively. The 90% CIs of the test/reference ratios for AUC_t , AUC_{inf} and C_{max} of atorvastatin were within the acceptable range for bioequivalence. The mean (standard deviation) $t_{1/2}$ of atorvastatin for the test drug was 11.496 (± 3.058) hours and for the reference was 11.382 (± 3.323) hours. These values were within the atorvastatin $t_{1/2}$ based on the literature, which is about 14 hours [4]. Utilizing Student's paired *t*-test; the $t_{1/2}$ values of the test and the reference drug were not significantly different, demonstrating a comparable rate of drug elimination from the body.

Based on the pharmacokinetics and the results of this study, it was concluded that the two formulations of atorvastatin calcium 20 mg film-coated tablets were bioequivalent in terms of the rate and extent of absorption.

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