

Comparative Pharmacokinetics and Bioequivalence of Two 50 mg Atenolol Tablet Formulations in Healthy Korean Male Volunteers

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Key words

- atenolol
- bioequivalence study
- volunteers

Abstract

Atenolol is a selective β_1 receptor antagonist that is available as a racemic mixture. The objective of this study was to compare the pharmacokinetics and evaluate the bioequivalence of 50 mg atenolol test and reference formulations in 24 healthy Korean male volunteers.

This study was a single-dose, randomized, open-label, 2 period crossover study. 24 healthy Korean male volunteers randomly received 50 mg of either test or reference atenolol formulations in a 2×2 crossover study. There was a 1 week wash-out period between doses. The area under the curve (AUC)_{0–24h} and C_{max} of 50 mg atenolol were the primary criteria for evaluation of bioequivalence.

The mean ± standard deviation (SD) values of the C_{max}, T_{max}, AUC_{0–24h}, AUC_{0–∞}, k_e, and t_{1/2} of the test and reference formulations were 268.4 (78.96) and 256.9 (79.34), 2.750 (0.9555) and 3.104 (1.053), 1981 (729.2) and 1872 (604.8), 2228 (697.1) and 2187 (628.5), 0.1332 (0.02748) and 0.1421 (0.04223), 5.419 (1.110) and 5.442 (2.357), respectively. The 90% confidence intervals for AUC_{0–24h} and C_{max} were 0.9037–1.166 and 0.9169–1.1987, respectively. These results were within the accepted bioequivalence range of 0.80–1.25, which satisfied the bioequivalence criteria of the European Committee for Proprietary Medicinal Products and the US Food and Drug Administration guidelines. In conclusion, the findings of this study indicate that the 2 formulations of 50 mg atenolol that were tested are bioequivalent. Therefore, these formulations may be prescribed interchangeably.

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Introduction

Atenolol is a selective β_1 receptor antagonist without intrinsic partial agonist or membrane-stabilizing activities. Atenolol is commercially available as a racemic mixture. The (–)S-form is the active isomer, whereas the R(+)-isomer has no significant pharmacological activity [1,2]. Importantly, it is widely used to treat hypertension, angina acute myocardial infarction, supraventricular tachycardia, ventricular tachycardia, and congestive heart failure [3].

The onset of atenolol starts 2–4 h after oral administration. The absorption of atenolol has been shown to be rapid and consistent but incomplete, with a bioavailability of 50% [4]. Approximately 6–16% of atenolol is bound to plasma protein [3], and very little atenolol is metabolized by the liver. Instead, approximately 40–50% of non-metabolized atenolol is excreted in urine after administration [3,5].

The objective of this study was to compare the pharmacokinetics and bioequivalence of 50 mg atenolol test drug manufactured by Hana Pharmaceuticals (Seoul, Korea; Hana Atenolol[®]) with 50 mg atenolol reference drug manufactured by Hyundai Pharmaceuticals (Seoul, Korea; Tenormin[®]) in 24 healthy Korean male volunteers.

Materials and Methods

Test and reference medications

The test medication (Hana Atenolol, 50 mg, lot No. 6003, Hana Pharmaceuticals) and the reference medication (Hyundai Tenormin, 50 mg, lot No. 66042, Hyundai Pharmaceuticals) were supplied as tablets.

Subjects and methods

This study was a single-dose, randomized, open-label, 2 period crossover study. The study was

conducted at Bestian Hospital in Seoul, Korea. The protocol of this study was approved by the Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University (Seoul, Korea) and performed according to the rules of Good Clinical Practice and in accordance with the Declaration of Helsinki. All participants provided a written informed consent after they had been informed of the nature and details of the study in accordance with Korean Guidelines for Bioequivalence Tests [KGBT 2006].

24 healthy male Korean volunteers, ranging in age from 20 to 28 years (median: 24), weight from 58 to 95 kg (71.0 ± 9.15 kg), and height from 168 to 183 cm (176 ± 4.17 cm) completed the study. Volunteers were selected after passing a clinical screening procedure. The screen consisted of a physical examination and laboratory tests, including a blood analysis of hemoglobin, hematocrit, white blood cell (WBC), platelets, differential counting of WBC, blood urea nitrogen, serum creatinine, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, cholesterol, glucose fasting, and alkaline phosphatase. In addition, a urine analysis was conducted for specific gravity, color, pH, glucose, protein, bilirubin, red blood cells (RBC), and WBC assessment. Volunteers were excluded if they were possibly sensitive to this type of medication, had a history of any illness of hepatic, renal, or cardiovascular systems, or had taken alcohol or other medications that may affect drug metabolism over a long period of time. These exclusion criteria were used to ensure that any illness or other medication would not induce variation in assessment. All subjects avoided using other drugs for at least 1 month before the study and until study completion. Patients were also required to refrain from consuming alcoholic beverages and xanthine-containing foods and beverages 48 h prior to each dosing and until collection of the last blood sample. The volunteers were randomly assigned to 2 groups and received an oral dose of 50 mg of atenolol in a standard 2 × 2 crossover model. A 1 week washout period was included between doses.

Subjects were notified at 8 p.m. on the day prior to the study initiation and fasted 12 h before drug administration and 4 h after. The brachial vein was cannulated with a heparin lock catheter and 1 mL heparinized normal saline solution (20 units/mL) was flushed into the cannula to prevent blood clots from forming prior to administration. At 9:00 a.m. on the day of the study, the subjects were given 50 mg atenolol per oral (p.o.) with 240 mL of tap water. At 4 and 9 h after the administration, all subjects were given standardized meals. The subjects were not allowed to be in a supine position or sleep for any period of time during the entire blood collection period. Approximately 10 mL of blood was collected through the cannula at the following times: predose, 30 min, and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h after administration of atenolol. The heparin lock was flushed with 1 mL heparinized normal saline after each blood sampling. The blood sample was immediately centrifuged after collection and the plasma sample was frozen at -70°C for subsequent high performance liquid chromatography (HPLC) analysis.

HPLC assay of atenolol in plasma

The concentrations of atenolol in plasma were analyzed by HPLC using a slight modification [4]. A 50 μL aliquot of internal standard (7.5 $\mu\text{g}/\text{mL}$ metoprolol), 500 μL aliquot of 0.5 N NaOH, and 5 mL aliquot of ether:dichloromethane (70:30 v/v) were added to a 1 mL aliquot of plasma sample. After 10 min of extraction, the tubes were centrifuged at $5400 \times g$ for 10 min. The organic

layer was then collected and dried under a gentle stream of nitrogen gas at 40°C . A 125 μL aliquot of the mobile phase was added to reconstitute the residue and a 50 μL aliquot was injected directly onto a reverse phase HPLC column. The mobile phase consisted of acetonitrile:methanol:10 mM KH_2PO_4 (pH 3.0) (15:15:70, v/v) and was processed at a flow rate of 1.0 mL/min. The column effluent was monitored using a fluorescence detector with excitation and emission wavelengths set at 222 nm and 300 nm, respectively.

Pharmacokinetic analysis

Standard methods [6] were used to calculate the pharmacokinetic parameters using a non-compartmental analysis (Win-Nonlin[®]; Pharsight Corporation, Mountain View, CA). The C_{max} and T_{max} were compiled from the concentration-time data. The area under the curve ($\text{AUC}_{0-24\text{h}}$) was calculated using a linear-log trapezoidal formula up to the last measured time at 24 h in plasma, and the $\text{AUC}_{0-\infty}$ was calculated by extrapolation [7]. The terminal elimination rate constant, k_e , was defined as the terminal elimination rate constant estimated by log-linear regression analysis on data visually assessed to be a terminal log-linear phase. The apparent terminal elimination $t_{1/2}$ was calculated as follows:

$$t_{1/2} = 0.693/k_e$$

Statistical analysis of data

Analysis of variance (ANOVA) was performed using logarithmically transformed $\text{AUC}_{0-24\text{h}}$ and C_{max} was used to assess group or sequence, period, subjects per group, and drug effects. The Schuirmann's 3-sided t -tests were conducted to test the bioequivalence of the pharmacokinetic characteristics of the 2 formulations [8]. The range of bioequivalence for parametric analysis was set to the commonly accepted 80–125% of the test/reference ratios of $\text{AUC}_{0-24\text{h}}$ and C_{max} according to Korean Guidelines for Bioequivalence Tests [KGBT 2006]. All statistical comparisons were made using the Equiv test and confirmed with the K-BE test program [9].

Results



Both atenolol formulations were well-tolerated by the subjects of this study. Clinically relevant drug-related side effects were not observed in any of the 24 volunteers, and no volunteer withdrew from the study.

A representative chromatogram of blank plasma spiked with internal standard (metoprolol) and the chromatogram of a plasma sample are shown in **Fig. 1**. In this HPLC method, no interference from endogenous substances was observed in human plasma. This method, with slight modification, was able to determine the atenolol plasma concentration within 5 min for each plasma sample. The retention times for atenolol and internal standard were approximately 0.7 and 2.8 min, respectively. The detection limit for atenolol in human plasma was 20 ng/mL based on a signal-to-noise ratio of 10. The standard curve was achieved from the least squared regression equation: $y = 0.0007x + 0.0068$ ($r^2 = 0.9983$). The intra- and inter-day precision coefficients of variation for human plasma were 3.839–9.274% and 7.475–10.19%, respectively, and the intra- and inter-day accuracy coefficients of variation for human plasma were 95.66–105.2% and 97.91–108.8%, respectively, from atenolol plasma concentrations in the range of 20–1 000 ng/mL.

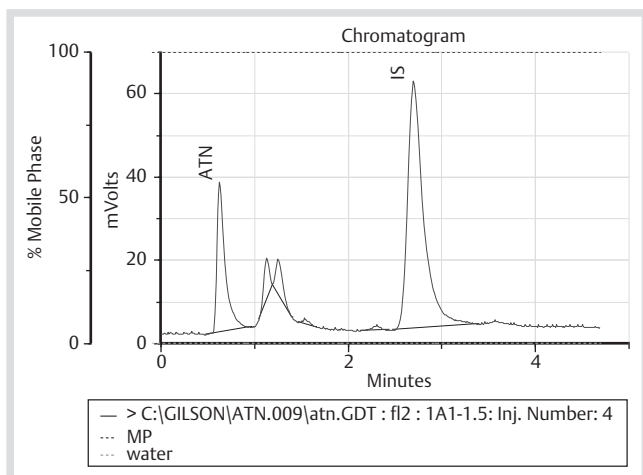


Fig. 1 Chromatogram obtained from plasma of a volunteer following oral administration of atenolol (50 mg): atenolol (370.3 ng/mL) + internal standard (propranolol 7.5 µg/mL).

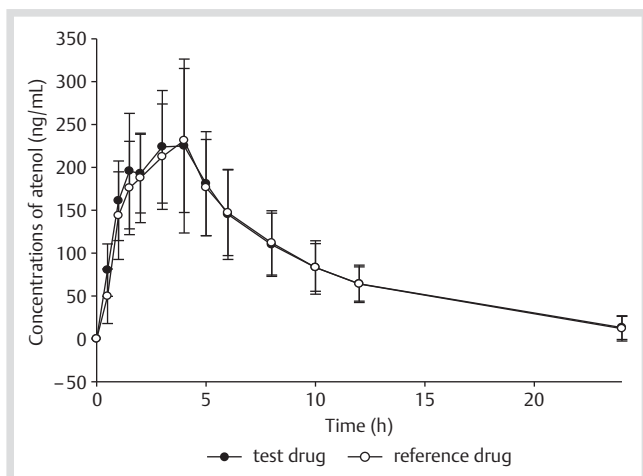


Fig. 2 Plasma concentration-time curve of test drug and reference drug of atenolol 50 mg (n = 24, Bars represent standard deviation).

Table 1 Pharmacokinetic parameters of atenolol 50 mg after a single oral dose of test and reference formulations of atenolol 50 mg tablets in healthy Korean male volunteers.

Parameter	Test drug*	Reference drug**
C_{max} (ng/mL)	268.4 (78.96)	256.9 (79.34)
T_{max} (h)	2.750 (0.9555)	3.104 (1.053)
AUC_{0-24h} (ng/mL×h)	1 981 (729.2)	1 872 (604.8)
$AUC_{0-\infty}$ (ng/mL×h)	2 228 (697.1)	2 187 (628.5)
k_e (h^{-1})	0.1332 (0.02748)	0.1421 (0.04223)
$t_{1/2}$ (h)	5.419 (1.110)	5.442 (2.357)

Data are expressed as the mean(standard deviation(SD))

*atenolol 50 mg test drug manufactured by Hana Pharmaceuticals (Seoul, Korea) (Hana Atenolol®); **atenolol 50 mg reference drug manufactured by Hyundai Pharmaceuticals (Seoul, Korea) (Tenormin®) in 24 healthy Korean male volunteers

The mean plasma atenolol concentration-time profiles are shown in **Fig. 2**. We found that the mean plasma atenolol concentration profiles of the 2 formulations were almost identical (**Fig. 2**). The mean extrapolated section of AUC from the last sampling time to time infinity was 14.78%. The mean pharmacokinetic parameters, such as C_{max} , T_{max} , AUC_{0-24h} , $AUC_{0-\infty}$, k_e ,

Table 2 Standard bioequivalence analysis for log-transformed AUC_{0-24h} (ng/mL×h) and log-transformed C_{max} (ng/mL) based on multiplicative model after a single-dose administration of two 50 mg atenolol tablet formulations in healthy Korean male volunteers. AUC_{0-24h} (ng/mL×h) and C_{max} (ng/mL) were also analyzed based on additive model for completeness.

Multiplicative statistical method	Test/Reference Point estimate	90% Confidence Interval
AUC_{0-24h} (ng/mL×h)	1.026	0.9037–1.166
C_{max} (ng/mL)	1.048	0.9169–1.1987

and $t_{1/2}$ after the administration of test and reference atenolol formulations are listed in **Table 1**. The 90% confidence intervals of AUC_{0-24h} and C_{max} are presented in **Table 2**. The parametric point estimates for the mean ratios of test drug/reference drugs for AUC_{0-24h} and C_{max} were 1.026 and 1.048, respectively (**Table 2**). The 90% confidence intervals for AUC_{0-24h} and C_{max} were 0.9037~1.166 and 0.9169~1.1987, respectively (**Table 2**). The test/reference ratio of T_{max} was 0.8860. The arithmetic mean±standard deviation of T_{max} for the test and reference drugs was 1.794~3.706 and 2.051~4.157 h, respectively (**Table 1**).

Discussion

In this study, we slightly modified the HPLC method described in a previous study to determine plasma atenolol levels in healthy volunteers [4]. The mobile phase, which consisted of acetonitrile:methanol:0.02 M sodium phosphate buffer containing 0.1% sodium dodecyl sulfate (SDS) 35:15:50 (v/v), was changed to acetonitrile:methanol:10mM KH_2PO_4 (pH 3.0) (15:15:70, v/v). Using this modified method, the retention times of atenolol and metoprolol became much shorter from 5.5 and 8 min to 0.7 and 2.8 min, respectively. De Abreu et al. [5] demonstrated that although only 500 µL of plasma per sample was necessary, the retention times remained 3.1 and 6 min for atenolol and metanolol, respectively. However, in this study, the chromatographic run time per sample was only 5 min. In addition, our analysis of atenolol was very precise, effective, and economical, since retention times were shorter and the assay did not require solid-phase extractions.

The pharmacokinetic parameters, including C_{max} , T_{max} , AUC_{0-24h} , $AUC_{0-\infty}$, k_e , and $t_{1/2}$ were comparable between the 2 atenolol formulations and no statistical difference was found. Importantly, our findings in this study were similar to data previously reported [4, 5, 10].

The ANOVA test showed that the F-test values were lower than the F-test table in the group or sequence, period, and drug. These results indicate that the crossover design was properly performed and there were no group or sequence and drug effects. The AUC_{0-24h} , C_{max} , and T_{max} were similar for both test and reference drugs. In addition, the 90% confidence intervals of AUC_{0-24h} and C_{max} were within the accepted bioequivalence range of 0.80–1.25, which satisfied the bioequivalence criteria of the European Committee for Proprietary Medicinal Products and the US Food and Drug Administration guidelines.

In conclusion, the results from this study indicate that the 2 50 mg atenolol formulations are bioequivalent. Therefore, they may be prescribed interchangeably.

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▼
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Conflict of Interest

▼
The authors declare no conflicts of interest with respect to this paper.

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