COMPARATIVE STUDIES OF THE BIOEQUIVALENCE OF A GENERIC (TENOLOL®) AND THE INNOVATOR ATENOLOL IN HEALTHY THAI VOLUNTEERS

Maleeya Manorot, Noppamas Rojanasatien, Supanimit Teekachunhatean, Chaichan Sangdee.

Division of Clinical Pharmacology, Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

ABSTRACT

Bioequivalence of 50 mg and 100 mg of the generic atenolol tablets (Tenolol®, Siam Pharmaceutical) were compared to the innovative product (Tenormin®, Zeneca Limited). Each preparation was administered to twelve healthy Thai volunteers according to a randomized balance two-way crossover design with one week washout period. After drug administration, serial blood samples were collected over a period of 24 hr and 30 hr for 50 mg and 100 mg atenolol preparations, respectively. Atenolol plasma concentrations were measured using HPLC technique with fluorometric detection. Pharmacokinetic parameters were analyzed by noncompartmental pharmacokinetic method using TOPFIT. The means and parametric 90% confidence intervals of the ratio (Tenolol®/Tenormin®) of C_{max} and $AUC_{0-\infty}$ were 1.17 (1.00-1.34) and 1.08 (0.96-1.19), as well as 0.94 (0.77-1.12) and 0.98 (0.84-1.14) for 50 mg and 100 mg doses, respectively. These values complied with the acceptable bioequivalence ranges of 0.7-1.43 and 0.8-1.25 for the ratios (Test/Reference) of C_{max} and $AUC_{0-\infty}$, respectively. The mean differences of T_{max} (Tenolol®-Tenormin®) were -0.33 and -0.06 hr for 50 mg and 100 mg, respectively. These values were well within the stipulated bioequivalence ranges of T_{max} differences (± 20% of the T_{max} of the reference formulation) of ± 0.58 hr and ± 0.78 hr for 50 mg and 100 mg preparations, respectively. Based on the result of this study, 50 mg and 100 mg of Tenolol® were bioequivalent to the innovator (Tenormin®) with respect to the extent and rate of absorption.

Key words : atenolol, bioequivalence

Address correspondence and reprint request to : Maleeya Manorot, M.D., Division of Clinical Pharmacology, Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand. Tel : 945358, Fax : 217144

INTRODUCTION

The generic pharmaceutical industry in Thailand has expanded enormously during the last 10-20 years. The growth has been driven by a number of factors, in particular the need to contain public spending on health care, including drug products. The protection of consumers demands that a generic drug product that is intended as a substitute for, or to be interchangeable with, the drug product of the pioneer or innovator pharmaceutical company, must be 'equivalent'[1,2]. Substitution of a generic drug product for an innovative drug product requires that the products must not only be pharmaceutically equivalent, but also bioequivalent. In general, for a generic product to be regarded as bioequivalent with the innovative product, any difference in the rate and extent of absorption of the active moiety to systemic circulation and thus the site of drug action must be judged clinically insignificant. The fundamental reason for performing bioequivalence testing is to ensure, as far as possible, the quality of generic drug products. In particular, such testing is intended to establish that there are not likely to be any differences in safety and efficacy between a generic and an innovative drug product; i.e., the products are therapeutically equivalent. Thus, in essence, bioequivalence is considered a surrogate of therapeutic equivalence[1,2].

Atenolol is a β-blocker widely used for the treatment of hypertension, arrhythmias, angina pectoris and acute myocardial infarction[3-5]. It is a synthetic, cardioselective β_1-adrenergic receptor antagonist without intrinsic sympathomimetic activity. Because of its cardioselectivity, it has been shown to produce comparable therapeutic effects with less adverse effects than propranolol. Therefore, atenolol is considered a preferable agent in patients with bronchospastic diseases, diabetes mellitus and occlusive peripheral vascular disease[6,7].

At present, two dosage forms of oral atenolol preparations (50 mg and 100 mg) are available in Thailand both as the innovative (Tenormin®, Zeneca Limited) and the generic preparation (Tenolol®, Siam Pharmaceutical). Since the bioavailability studies of the generic (Tenolol®) and the innovative preparations of atenolol in Thai volunteers had never been reported, this study was thus conducted to study the pharmacokinetics and to assess the bioequivalence of a single oral 50 and 100 mg of innovative and generic atenolol preparations in healthy Thai volunteers.

MATERIALS AND METHODS

Subjects

Twelve healthy volunteers (seven male and five female) aged between 21-47 years old (mean ± SD = 31.08 ± 10.91) participated and completed this study. Their average weight and height were 60.08 kg (48.0-75.0 kg) and 161.13 cm (150.0-170.0 cm), respectively. All were in good health on the basis of history, physical examination, electrocardiography, urinalysis, and laboratory investigations. The laboratory tests included complete blood count with differentials, blood urea nitrogen, creatinine, fasting blood sugar, total protein, albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol and bilirubin. None had a history of bronchial asthma, diabetes mellitus, peripheral vascular disease or cardiovascular disease. Female subjects were not pregnant, confirmed by urine pregnancy test. Subjects with known contraindication or hypersensitivity to β-adrenergic blocking agents were excluded as were those with known history of alcoholism or drug abuse. The purpose and the procedure of the study were informed to subjects verbally. After given written informed consent, all subjects were enrolled in the study.

Study drugs

Test drugs : Siam Pharmaceutical, Bangkok, Thailand
Tenolol® 50 mg (Lot No. Z2562)
Tenolol® 100 mg (Lot No. 22ZA013)

Reference drugs : Zeneca Limited, Macclesfield Cheshire, United Kingdom
Tenormin® 50 mg (Lot No. LO. 949)
Tenormin® 100 mg (Lot No. PO.9808)

Study design

Each subject received one tablet of either 50 or 100 mg Tenolol® or Tenormin® orally by a randomized double-blind crossover design. The washout period between each treatment was at least one week to ensure the total elimination of the previous dosing. On the study day, subjects were admitted to the Clinical Pharmacology Unit of the Department of Pharmacology, Faculty of Medicine, Chiang Mai University at 7 A.M. after an overnight fast. Baseline supine blood pressure and heart rate were measured by automate sphygmomanometer. A peripheral intravenous catheter was

placed into a forearm vein using aseptic technique for blood sample collection. Thereafter, subjects were randomized to receive one tablet of either 50 or 100 mg Tenolol® or Tenormin® with 200 ml water. Ten ml of whole blood was collected just before the dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 15 and 24 hours after dosing of 50 mg atenolol preparations and before the dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24 and 30 hours after dosing of 100 mg atenolol preparations. The blood samples were immediately centrifuged and the plasma was stored at -20 °C until analysis. Supine blood pressure and heart rate were recorded at each blood sampling time. Exercise was not allowed during the study period. Juice was served two hours after dosing and lunch was served after completing the four-hour blood sampling. Meal and fluid intake were identical for all study period. Drugs, alcohol or caffeinated beverage were not allowed during the study period.

Drug assay

Plasma concentration of atenolol were determined by high-pefomance liquid chromatographic (HPLC) method using model LC-10A pump HPLC (Shimadsu, Japan) with RF-10 AXL spectrofluorometric detector and CTO-10A/10AC column oven. The methods being developed were modified from the solid phase extraction procedure and HPLC technique[8]. Chromatographic analysis was carried out at 40° C, using a 150 x 4.6 nm Inersil C_8 column (GL Sciences Inc., Tokyo, Japan), a mobile phase of $NH_4H_2PO_4$ (7.5 mM, adjusted to pH 4.85) and acetonitrile in the ratio of 9.3: 0.7 (flow rate 1 ml/min), and detected by fluorescence detector with excitation and emission wavelength of 230 nm and 310 nm, respectively. The retention time for atenolol was approximately 3.48 minutes. Calibration standards in distilled water containing 15-1500 ng/ml of atenolol were used to establish calibration curves for assay validation and for clinical assay (least squares quadratic regression analysis). From a regression equation obtained from a standard calibration curve, the area under the peaks were used to calculate atenolol concentrations in plasma. Assay recovery was determined by comparing the peak area of atenolol samples in distilled water with the peak area of atenolol in plasma. Mean atenolol recovery from plasma were 85% and the lower limit of quantitation was 10 ng/ml. The percent correlation coefficient (%CV) of inter- and intra-assay validation were less than 4%.

Statistical methods and data analysis

Plasma concentration-time data were analyzed by model-noncompartmental pharmacokinetic method. Maximal plasma concentration (C_{max}) and time to reach the maximal plasma concentration (T_{max}) were obtained directly by visual inspection of each subject's plasma concentration-time profile. Other pharmacokinetic parameters including plasma elimination half-life ($t_{1/2}$), area under the plasma concentration-time curve (AUC $_{0-\infty}$), and mean residence time (MRT) were derived with the use of TOPFIT 2.0, a pharmacokinetic and pharmacodynamic data analysis program for PC. Bioequivalence testing comprised equivalence assessment with respect to the rate (C_{max} and T_{max}) and extent of absorption (AUC) [1, 9, 11]. The C_{max} and AUC were analyzed statistically by logarithmically (ln) transformed the data and performed a three-way analysis of variance (ANOVA), while T_{max} was performed as the absolute difference (untransformed data). Thereafter, using the variance estimate (VAR) obtained from the analysis of variance, calculated the parametric 90% confidence interval (CI) from the following formulation[1, 9].

$$(\mu_A-\mu_B) = (X_A-X_B) \pm t^{v}_{0.1}\sqrt{\frac{2VAR}{n}}$$

Where $\bar{X}_A$, $\bar{X}_B$ were the observed means of the logarithmically (ln) transformed parameters (either C_{max} or AUC) for the test product (A) and the references (B), VAR was the error variance obtained from the three-ways ANOVA (the residual mean square of a three-way crossover study), n was the number of subjects and $t^{v}_{0.1}$ was the tabulated two-tail t value for 90% CI and v was the number of degree of freedom of the mean square from the analysis of variance. The antilogarithm of the confidence interval would express the bioequivalence as a ratio of the test (Tenolol®)/the reference products (Tenormin®). The bioequivalence interval of 0.8-1.25 for the ratio of the average AUC values (Test/Reference) is accepted by the Division of Bioequivalence of the United States Food and Drug Administration (FDA) and by the Canadian as well as the European authorities[1]. The FDA also accepts this range for C_{max} ratio values, however, the European guidelines suggest a wider acceptance range of 0.7-1.43 proposed by Steinijans et al. for C_{max} ratio[1,10]. Regarding analysis of T_{max}, the stipulated bioequivalence range of T_{max} difference (Test-Reference) was ± 20% of the T_{max} of the reference preparation [11]

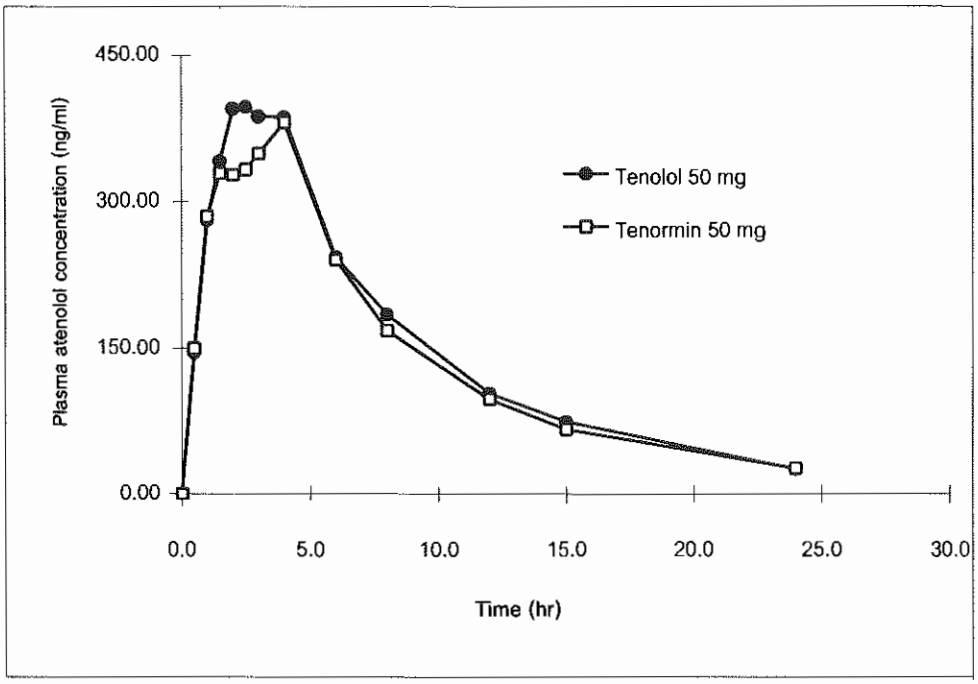

Figure 1 Mean plasma concentration-time curves following a single oral dose of 50 mg Tenolol and Tenormin.

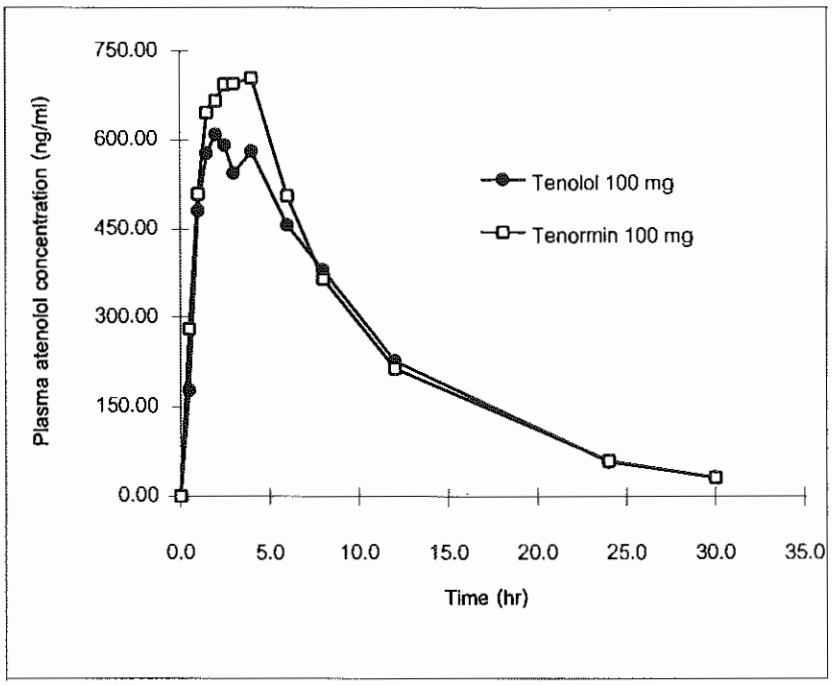

Figure 2 Mean plasma concentration-time curves following a single oral dose of 100 mg Tenolol and Tenormin.

RESULTS

Twelve healthy subjects completed this study without any serious adverse effects. The mean plasma concentration-time profiles of 50 mg and 100 mg of Tenolol® v.s. Tenormin® were shown in Figure 1 and 2, respectively. The means (±SD) of each pharmacokinetic parameter of the two formulations of 50 mg atenolol were shown in table 1. Both formulations of atenolol were rapidly absorbed after oral administration with the mean T_{max} (hr) of 2.54 ± 1.08 and 2.88 ± 1.23 for Tenolol® and Tenormin®, respectively. At this time point the mean C_{max} (ng/ml) of Tenolol® and Tenormin® were 503.33± 111.22 and 436.50 ± 154.60, respectively. The mean value of $AUC_{0-\infty}$ (ng.hr/ml) Tenolol ® was 3,882 ± 756 comparable to the value of 3,639 ± 932 for Tenormin®. The means of each pharmacokinetic parameter of the two formulations of 100 mg atenolol were shown in table 2. The mean values of T_{max} (hr), C_{max} (ng/ml), $AUC_{0-\infty}$ (ng/ml) of Tenolol® and Tenormin® were 3.53 ± 1.93 and 3.58 ± 1.47, 759.17 ± 215.29 and 880.50 ± 399.60, 8,244 ± 1,638 and 7,710 ± 2,562, respectively.

Table 3 illustrates 90% CI and point estimate of C_{max} and $AUC_{0-\infty}$ of 50 mg (Tenolol®/Tenormin®) as well as the T_{max} differences of (Tenolol®-Tenormin®). The mean and 90% CI of the ratio (Tenolol®/Tenormin®) of C_{max} and $AUC_{0-\infty}$ were 1.17 (1.00-1.34) and 1.08 (0.96-1.19), respectively. The mean T_{max} differences of Tenolol®-Tenormin® was -0.33 hr.

Table 4 illustrates 90% CI and point estimate of C_{max} and $AUC_{0-\infty}$ of 100 mg (Tenolol®/Tenormin®) and as well as the T_{max} differences of (Tenolol®-Tenormin®). The mean and 90% CI of the ratio (Tenolol®/Tenormin®) of C_{max} and $AUC_{0-\infty}$ and were 0.94 (0.77-1.12) and 0.98 (0.82-1.14), respectively. The mean T_{max} differences of Tenolol®-Tenormin® was -0.06 hr.

Table 1 Pharmacokinetic parameters after a single 50 mg oral preparation of the generic and innovator atenolol in 12 healthy Thai volunteers. Data expressed as mean ± SD.

Pharmacokinetic parameters	Tenolol®	Tenormin®
T_{max} (hr)	2.54 ± 1.08	2.88 ± 1.23
C_{max} (ng/ml)	503.33 ± 111.22	436.50 ± 154.60
$AUC_{0-\infty}$ (ng.hr/ml)	3,882 ± 756	3,639 ± 932
$MRT_{0-\infty}$ (hr)	8.76 ± 1.05	8.98 ± 1.35
$t_{1/2}$ (hr)	5.93 ± 0.71	6.21 ± 1.05

Table 2 Pharmacokinetic parameters after a single 100 mg oral preparation of the generic and innovator atenolol in 12 healthy Thai volunteers. Data expressed as mean ± SD.

Pharmacokinetic parameters	Tenolol®	Tenormin®
T_{max} (hr)	3.53 ± 1.93	3.58 ± 1.47
C_{max} (ng/ml)	759.17 ± 215.29	880.50 ± 399.60
$AUC_{0-\infty}$ (ng.hr/ml)	8,244 ± 1,638	7,710 ± 2,562
$MRT_{0-\infty}$ (hr)	9.40 ± 1.14	9.31 ± 1.24
$t_{1/2}$ (hr)	6.08 ± 0.75	6.47 ± 0.90