Drug Development and Industrial Pharmacy, 25(3), 357–360 (1999)

COMMUNICATION

Comparative Bioavailability Study of Two Atenolol Tablet Preparations

Kok Khiang Peh, Kah Hay Yuen,* Jia Woei Wong, and Weng Tuck Toh

School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Penang, Malaysia

ABSTRACT

A study was conducted to compare the bioavailability of a generic product of atenolol (Normaten FC) with the innovator product, Tenormin. Twelve healthy adult volunteers participated in the study conducted according to a randomized, two-way crossover design. The preparations were compared using area under the plasma concentration–time curve $AUC_{0-\infty}$*, peak plasma concentration* C_{max}*, and time to reach peak plasma concentration* T_{max}*. No statistically significant difference was obtained between the* T_{max} *values and the logarithmic transformed* $AUC_{0-\infty}$ *and* C_{max} *values of the two products. Moreover, the 90% confidence interval for the ratio of the logarithmically transformed* $AUC_{0-\infty}$ *values of Normaten FC over those of Tenormin was found to lie between 0.82 and 0.98, while that of the logarithmically transformed* C_{max} *values was between 0.82 and 1.09, both being within the bioequivalence limit of 0.80–1.25. The values of elimination half-life* $t_{1/2}$ *between the two products were also found comparable and not significantly different statistically. The* $t_{1/2}$ *values obtained in our study were slightly longer than those reported in the literature for other population groups.*

INTRODUCTION

Atenolol is a cardioselective beta-1 adrenoceptor blocker devoid of intrinsic sympathomimetic and membrane-stabilizing activities (1,2). This compound has been widely prescribed for treating hypertension, cardiac arrhythmia, and angina (3,4). As there is generally a great demand for atenolol, the expiration of its patent has resulted in the manufacturing of many cheaper generic versions of the drug. These generic products, however, have to be proved bioequivalent to the innovator product before they can be safely used to replace the innovator in

* To whom correspondence should be addressed.

treating patients. To establish bioequivalence of generic products, a single-dose, fasting, two-way crossover bioavailability study using healthy human volunteers is generally accepted as the established method for evaluating bioequivalence.

Hence, the aim of the present study was to compare the bioavailability of a generic product of atenolol with the innovator product, Tenormin. The pharmacokinetic data of atenolol was generated mainly from studies conducted on Caucasian subjects. This information may not be applicable to our local population of Asian origin; thus, an attempt was also made to study the pharmacokinetics of atenolol in our local population.

MATERIALS AND METHODS

Products Studied

Normaten FC tablets (50 mg; batch no. 9209072) were supplied by Xepa-Soul Pattinson, Malaysia. Tenormin tablets (50 mg; lot no. SU 315) were purchased from a local pharmacy. Both atenolol and metoprolol tartrate standards were obtained from the National Pharmaceutical Control Bureau of Malaysia. All other solvents and reagents used were high-performance liquid chromatography (HPLC) grade or analytical reagent (AR) grade.

Study Design

This study was approved by the Ethics Committee on Bioavailability Studies (Joint School of Pharmaceutical Sciences, University of Science Malaysia, and Penang General Hospital, Malaysia). After providing written informed consent, 12 healthy adult male volunteers between 24 and 41 years old (mean = 36 years, SD = 6 years) and weighing from 51 to 84 kg (mean = 69 kg, SD = 7 kg) participated in the study. All were judged to be healthy and were not receiving any medication during the study period. The study was conducted according to a single-dose, conventional, two-way, split groups, crossover design with 6 subjects in each of the two treatment groups and a washout period of one week between the two phases. The volunteers were randomly selected to receive two tablets (100 mg) of either Tenormin or Normaten FC. Both products were administered with 150 ml of water in the morning (9:00 A.M.) after a 12-hr overnight fast. Food and drinks were withheld for at least 2 hr after dosing. Lunch and dinner of chicken with rice were served 4 and 9 hr after dosing, and water was given ad libitum. Blood samples of 5 ml volume were collected in vacutainers (containing sodium heparin as an anticoagulant) via an in-dwelling cannula placed on the forearm at 0 (predose), 20 min, 40 min, and 1, 2, 3, 4, 6, 8, 10, 14, and 18 hr after dosing. The 24-hr and 36-hr samples were taken by direct venipuncture. The blood samples were centrifuged at 2000 G for 20 min, and the plasma was transferred to separate glass containers to be kept frozen until analysis.

Analysis of Plasma Levels of Atenolol

Plasma levels of atenolol were analyzed using an HPLC method reported by Buhring and Garbe (5) with slight modification. The HPLC system consisted of a Gilson model 307 pump and a Gilson 121 Filter Fluorometer equipped with a Hitachi D-2500 Chromato-integrator. The fluorometer was operated at an excitation wavelength of 228 nm and an emission wavelength of 356 nm. A LiChrosorb Si-60 (5 μm, 125 × 4 mm internal diameter) column was used for the chromatographic separation. The mobile phase used acetonitrile:distilled water:1 M ammonium phosphate buffer (pH 4) at a proportion of 6:89:5. Analysis was run at a flow rate of 0.5 ml/min, which was increased to 1.0 ml/min at 5.5 min during assay of each sample. The increase in flow rate during each run was necessary to expedite the elution of contaminating compounds with longer retention times than the compound of interest.

Prior to analysis, the drug was extracted from the plasma using the following procedure. A 0.5-ml aliquot of plasma sample was accurately measured into a 10-ml glass tube with a Teflon-lined cap, followed by an addition of 50 μl of 4 μg/ml aqueous metoprolol tartrate internal standard solution, 0.1 ml of 1 M sodium hydroxide solution, and 5 ml of extracting solvent consisting of 75:25 dichloromethane and *n*-butanol. After vortexing for 1 min, the mixture was centrifuged at 3500 rpm for 10 min, and approximately 4.5 ml of the extracting solvent was transferred into a 5-ml reactivial and evaporated to dryness under a gentle stream of nitrogen gas.

Before injecting onto the HPLC column, the residue was washed with *n*-hexane to remove lipophilic contaminants. This was carried out by first reconstituting the residue with 100 μl of 1 M acetic acid, followed by the addition of 3 ml of *n*-hexane. The mixture was vortexed for 1 min, and the organic layer was discarded. Any remaining *n*-hexane was removed under a gentle stream of nitrogen. Finally, 50 μl of the reconstituted sample was injected onto the column.

Pharmacokinetic Analysis

The two products were compared using peak plasma concentration C_{max}, time to reach peak plasma concentration T_{max}, and area under the plasma concentration–time curve $AUC_{0-\infty}$, estimated from the plasma concentration–time profiles of the two products. The values of C_{max} and T_{max} were obtained directly from the plasma data (6), while the $AUC_{0-\infty}$ was calculated by adding the area from time zero to the last sampling time t (AUC_{0-t}) and the area from time t to infinity ($AUC_{t-\infty}$). The former was calculated using the trapezoidal formula and the latter by dividing the last measurable plasma drug concentration with the elimination rate constant k_e. The k_e was estimated from the terminal slope of the plasma concentration–time curve after logarithmic transformation and application of linear regression (7), while the elimination half-life $t_{1/2}$ was calculated using $\ln 2/k_e$. For each of the parameters ($AUC_{0-\infty}$, C_{max}, and $t_{1/2}$), the values obtained for the two products were analyzed statistically using an analysis of variance (ANOVA) procedure appropriate for the study design (8). The $AUC_{0-\infty}$ and C_{max} values were logarithmically transformed prior to the statistical analysis. On the other hand, the T_{max} values were analyzed using the Wilcoxon signed-rank test for paired samples. A statistically significant difference was considered at $p < .05$.

RESULTS AND DISCUSSION

Figure 1 shows the mean plasma atenolol concentration–time profiles of Tenormin and Normaten FC. It can be seen from Fig. 1 that the two profiles were quite comparable, and no lag time in absorption was observed. Peak plasma concentrations were achieved at about 3 hr after dosing, with Normaten FC showing a slightly shorter time to reach peak plasma concentration.

Individual values of T_{max}, C_{max}, and $AUC_{0-\infty}$ are given in Table 1. The parameters T_{max} and $AUC_{0-\infty}$ are related to the rate and extent of absorption, respectively, while C_{max} is related to both processes (9). The extent of absorption is a key characteristic of a drug formulation, and therefore the *AUC* is an important parameter for analysis in a comparative bioavailability study. However, the other two parameters, T_{max} and C_{max}, are also important features of the plasma level profile that are related to the therapeutic use of many drugs (10) and hence are also considered in the analysis. The mean T_{max} values for Normaten FC and Tenormin were 2.4 ± 0.3 hr and 2.8 ± 0.3 hr, respectively, being in good agreement with those reported in the literature (11,12). The slight difference between the mean T_{max} values of Normaten FC and Tenormin was not found to be significantly different statistically ($p > .10$). Also, no statistically significant difference was obtained between the logarithmic transformed C_{max} values ($p = .4564$), as well as the logarithmically transformed $AUC_{0-\infty}$ values ($p = .0603$) of the two products. Moreover, the 90% confidence interval for the ratio of the logarithmically transformed $AUC_{0-\infty}$ values of Normaten FC over those of Tenormin was found to lie between 0.82 and 0.98, while that of the logarithmically transformed C_{max} values was between 0.82 and 1.09, both being within the acceptable bioequivalence limit of 0.80–1.25 (10,13). On the basis of these results, it can be concluded that Normaten FC and Tenormin are comparable in both the rate and extent of absorption, indicating that Normaten FC is bioequivalent to Tenormin.

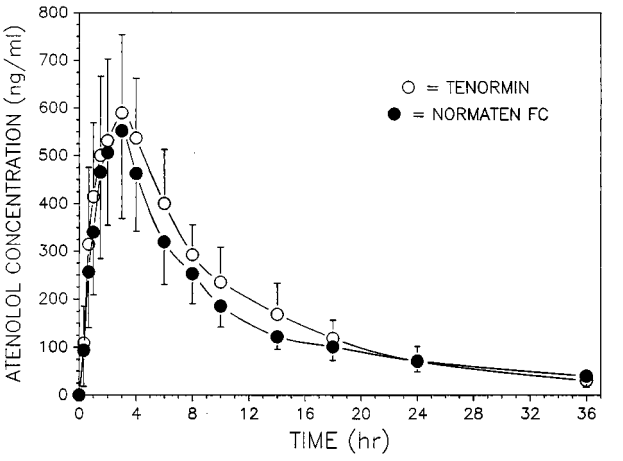

Figure 1. Curves of mean plasma atenolol concentration versus time of Tenormin and Normaten FC (mean ± SD, $N = 12$).

Relatively wide intersubject variation was observed in the $AUC_{0-\infty}$ values obtained, which can be attributed to differences in body weight and drug disposition among the volunteers. However, the intrasubject variation, estimated using the mean square error obtained from the ANOVA analysis (14), appeared to be small. The intrasubject coefficient of variation was estimated to be 12.8%. Based on this value, the 12 volunteers used in the present study were found sufficient to provide a power $(1 - \beta)$ of greater than 80% for detecting a statistically significant difference between the $AUC_{0-\infty}$ of the two products at a type 1 error rate (α) of 0.05 if the true difference is equal to or greater than 20% (6).

Table 1

Numerical Values of T_{max}, C_{max}, $AUC_{0-\infty}$, *and* $t_{1/2}$

	Tenormin				Normaten FC			
Subjects	T_{max} (hr)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (hr.ng/ml)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (hr.ng/ml)	$t_{1/2}$ (hr)
S1	1.0	571.8	5343.4	8.5	2.0	609.5	6084.9	9.0
S2	3.0	554.9	6435.5	9.2	1.5	502.4	5545.1	11.3
S3	4.0	727.8	8239.4	4.7	3.0	508.4	6661.4	9.1
S4	3.0	893.8	7903.7	6.0	2.0	579.4	6080.7	3.9
S5	3.0	835.8	6264.7	5.5	2.0	638.2	5332.9	8.3
S6	2.0	749.9	6745.8	7.4	1.5	849.0	7406.8	10.1
S7	1.5	584.7	5760.7	9.2	1.0	418.9	4586.6	11.7
S8	3.0	458.6	5461.6	6.0	3.0	770.9	5868.8	9.6
S9	2.0	727.6	7103.2	9.9	3.0	835.6	7585.3	7.1
S10	4.0	619.5	6308.2	8.6	3.0	762.8	6297.6	5.4
S11	4.0	575.6	6937.1	14.1	4.0	462.0	5083.5	12.8
S12	3.0	762.0	8460.3	8.1	3.0	707.8	5931.3	7.0
Mean	2.8	671.8	6747.0	8.1	2.4	637.1	6038.7	8.8
(SD)	0.3	129.8	1034.9	2.5	0.9	147.7	880.4	2.6

The $t_{1/2}$ values of the two products are shown in Table 1. The mean values of the two products were closely comparable and were not significantly different statistically ($p = .6475$). The $t_{1/2}$ values ranged between 3.9 and 14.1 hr, with a mean value of approximately 8.5 hr. These values were relatively larger than those reported by other workers (15). This discrepancy may be due to the different population groups employed in the studies. Whereas the volunteers used in our study were of Asian origin, those of the other workers were Caucasians.

CONCLUSIONS

In conclusion, Normaten FC was comparable to Tenormin in both the rate and extent of absorption, indicating that Normaten FC was bioequivalent to Tenormin. The $t_{1/2}$ values of the two products were not significantly different statistically. However, the $t_{1/2}$ values obtained in our study were found to be relatively larger than those reported in the literature, which may be attributed to different population groups employed in the studies.

REFERENCES

1. A. M. Barrett, J. Carter, and J. D. Fitzgerald, Br. J. Pharmacol., 48, 340 (1973).
2. J. D. Harry, M. F. Knapp, and R. J. Linden, Br. J. Pharmacol., 51, 169 (1974).
3. P. A. Majid, W. J. F. Vander Vijgh, P. J. De Feijter, R. Wardeh, E. E. Van der Wall, and J. P. Ross, Eur. J. Cardiol., 9, 419 (1979).
4. W. H. Frishman, N. Eng. J. Med., 306, 1456 (1982).
5. K. U. Buhring and A. Garbe, J. Chromatogr., 382, 215 (1986).
6. D. L. Weiner, in *Statistics in the Pharmaceutical Industry* (C. R. Buncher and J. Y. Tsay, eds.), Marcel Dekker, New York, 1981, p. 205.
7. M. Gibaldi and D. Perrier, *Pharmacokinetics*, 2nd ed., Marcel Dekker, New York, 1982, p. 145.
8. J. G. Wagner, in *Fundamentals of Clinical Pharmacokinetics*, 1st ed., Drug Intelligence Publication, Hamilton, IL, 1975, p. 285.
9. A. Grahnen, Pharm. Int., 5, 100 (1984).
10. W. J. Westlake, in *Biopharmaceutical Statistics for Drug Development* (K. E. Peace, ed.), Marcel Dekker, New York, 1988, p. 329.
11. A. Wolf-Coporda, F. Plavsic, and B. Vrhovac, Int. J. Clin. Pharmacol. Ther. Toxicol., 25, 567 (1987).
12. U.S. Pharmacopoeial Convention, USP DI, in *Drug Information for the Health Care Professional*, 14th ed., Author, Rockville, MD, 1994, p. 550.
13. U.S. Pharmacopoeial Convention, *The United States Pharmacopoeia XXIII*, Author, Rockville, MD, 1995.
14. E. Diletti, D. Haushcke, and V. W. Steinijans, Int. J. Clin. Pharmacol. Ther. Toxicol., 29, 1 (1991).
15. J. D. Fitzgerald, R. Ruffin, K. G. Smedstad, R. Roberts, and J. McAinsh, 13, 81 (1978).