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BIOEQUIVALENCE STUDY OF ATENOLOL: PHARMACOKINETIC AND PHARMACODYNAMIC EVALUATION

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ABSTRACT

This study was designed to assess pharmacokinetic parameters and pattern of pharmacodynamic effects (heart rate and blood pressure) of 100 mg Atenolol tablets in comparison with those of 100 mg Tenormin tablets as reference. A double blind cross over study was carried out among 12 healthy male subjects. A HPLC system using RP-C_{18} column and fluorescence detector was used to assess atenolol in plasma. Heart rate and blood pressure were measured by the trained clinic staff. Peak levels were observed about 2.97h for Atenolol and 3.73h for Tenormin after oral dosing. C_{max} values for both formulations were about 0.49 µg/ml. AUC_{0-24} was about 4.89 µg.h/ml for the test and 5.31 µg.h/ml for the reference group. Atenolol given orally caused a significant reduction in heart rate, systolic and diastolic blood pressure after administration of two formulations ($P<0.05$). It is concluded that two formulations are not significantly different in terms of pharmacodynamic and pharmacokinetic parameters which were studied.

Keywords: Atenolol, Pharmacokinetics, Pharmacodynamics, Blood pressure, Heart rate

INTRODUCTION

Atenolol is a specific β_1-receptor antagonist, used to treat essential hypertension (1). Pharmacokinetics and clinical effects of this drug have been extensively studied (2-5). The object of the present study was to compare the pharmacokinetics and clinical effects of two formulations of atenolol by oral route. Atenolol 100mg generic formulation made by Lorestan Pharmaceutical Company and Tenormin 100mg made by Zeneca were used as test and reference formulations, respectively. The plasma concentrations of atenolol were measured at various time intervals after administration of two formulations (100mg p.o.) and the pharmacokinetic pattern was determined. The pharmacodynamic effects (heart rate, systolic and diastolic blood pressure) of two formulations after drug administration were also investigated.

MATERIAL AND METHODS

Selection of Subjects

12 healthy adult male subjects were selected for the study. The object of the study was fully explained after approval of the protocol by the ethics committee of Daroupakhsh Pharmaceutical Research Center. Subjects who had no history of diabetes, asthma or other respiratory disease, gastro-intestinal, cardiovascular, hepatic, renal or hematological disorders were selected for the study. They were between 30-45 years old (35.6 ± 4.2) and weighed 62-92 Kg (73.4 ± 7.3). They were not allowed to take any medication for two weeks prior to and through the experiment. Subjects underwent a complete physical and laboratory examination 7 days prior to the study.

Drug Administration and Blood Sampling

The form of study was a double blind cross over design. Subjects were given two formulations of the drug; Tenormin 100mg (Zeneca, UK) and generic Atenolol 100mg (Lorestan, Iran).

Subjects were fasted over-night prior to each treatment period. Each subject was given 240 ml of water at the time of drug administration. A light breakfast and a standard meal were permitted 2h and 5h after drug administration, respectively. Blood samples were obtained from an indwelling needle in forearm vein before and at 0.5, 1, 2, 3, 5, 7, 12, 24h after dosing. Heart rate and blood pressure measurements were made before drug administration and at each sampling time. Blood pressure was measured indirectly using a standard mercury manometer

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and a 14cm-wide cuff and the heart rate was taken by a trained clinic staff member.

Blood samples were collected and centrifuged in heparinized tubes. The plasma was removed by means of a disposable pipette and transferred to a sterile tube, which was then frozen and stored at -20°C.

Sample Analysis

Atenolol concentration in plasma was determined by some modifications in the reported method (6). The following HPLC conditions were employed for analysis; column was Spherisorb C_{18} (10µm particle size, 10 cm *x* 4.6 mm I.D.). Mobile phase consisted of water, methanol, acetonitril, acetic acid (49:45:5:1). Flow rate was 0.8 ml/min maintained by a solvent delivery system (Pye-Unicam, PU4003). The column effluent was monitored by a variable wavelength fluorescence detector (Perkin-Elmer, LS-4) set at excitation of 220 nm, emission of 305 nm for the first 14.5 minutes and then at excitation of 242 nm, Emission of 356 nm to the end of run time. The lower limit of the assay of atenolol was 10 ng/ml. The retention times of compounds of interest were 10 and 18 minutes for atenolol and procainamide (used as internal standard) respectively.

Calculations

Pharmacokinetic parameters were obtained by noncompartmental analysis. Apparent first order terminal rate constant (k) was calculated from the terminal portion of the plasma concentration-time curve using least square regression analysis of the logarithm of concentration versus time. Biological half life ($t_{½}$) was calculated by the following relationship:

$t_{½} = \ln(2)/k$

The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule to 24h and then extrapolated to infinity using the terminal rate constant value.

The software designed for pharmacokinetic analysis (Drug-knt) was coded using TurboC ver. 2.01. This software uses linear iterative curve stripping method to solve and fit one and two compartment kinetics. (7)

SPSS 10.0.1 was used for statistical data analysis. 90% confidence interval and paired t-test were used for comparison of the pharmacokineitc parameters ($p<0.05$) in two formulations. ANOVA (General linear model/ Repeated measures method) was used to compare pharmacodynamic effects (heart rate, systolic and diastolic blood pressure) of two formulations.

RESULTS AND DISCUSSION

The serum concentration vs. time curves of the two formulations are shown in Fig 1. A summary of mean values for the pharmacokinetic parameters is provided in Table 1.

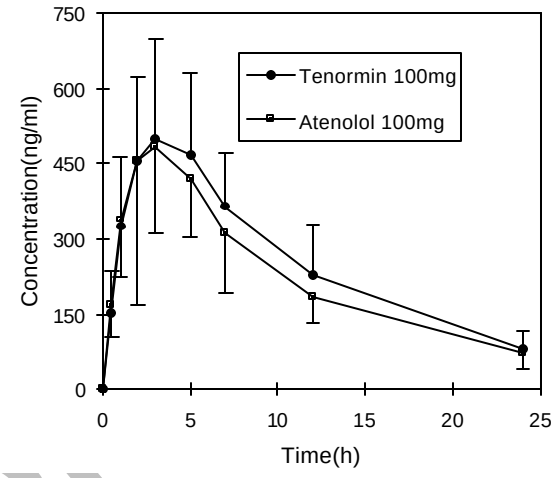

Figure 1. Plasma concentration-time profile of two formulations (Mean ± SD)

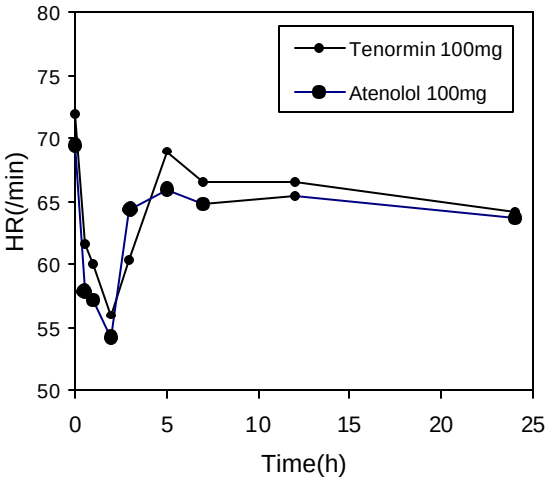

Figure 2.Profile of heart rate versus time (Mean Data)
HR: Heart Rate

No clinically important adverse experiences or drug-related changes in laboratory parameters were noted with either of two formulations.

Peak levels of atenolol were observed about 2.97h for atenolol 100mg and 3.73h for tenormin 100 mg after oral dosing. C_{max} values were about 0.49 µg/ml for both formulations.

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Table 1 - Pharmacokinetic parameters of two formulations (Mean ± SD).

Parameter	Atenolol 100mg (Mean ± SE)	Tenormin 100mg (Mean ± SE)	t-test P<0.05
AUC_{0-24} (ng.h/ml)	4891.19±2027.90	5312.86±2412.84	N.S.
$AUC_{0-\infty}$ (ng.h/ml)	4939.00±2031.90	5362.78±2416.04	N.S.
C_{max} (ng/ml)	491.08±262.61	489.75±214.02	N.S.
T_{max} (h)	2.97±491.08	3.73±1.71	N.S.
k (h^{-1})	0.09±0.02	0.09±0.02	N.S.
$t_{½}$ (h)	8.23±1.92	8.02±2.28	N.S.

AUC_{0-24} (ng.h/ml): Area under the blood level curve (up to last blood sampling (24 hours after drug administration)), $AUC_{0-\infty}$ (ng.h/ml): Area under the blood level curve (up to infinity)), k(h^{-1}): Elimination rate constant, $t_{½}$(h): Elimination half life, N.S. : Not significant

Table 2- 90% Confidence levels for ratios of pharmacokinetic parameters (Test/Reference).

	90% Confidence Interval	
Parameter	Lower Limit	Upper Limit
$AUC_{0-¥}$	0.86	1.06
$AUC_{0-¥}$ *(Log)*	0.91	1.01
C_{max}	0.90	1.19
C_{max} *(Log)*	0.95	1.15
T_{max}	0.81	1.10

Table 3- Comparison of pharmacodynamic effects between the two formulations (ANOVA: General linear model/ Repeated measures method)

Parameter	MD (I-J)	p	LB	UB
Heart Rate	-1.44	0.95	-8.77	5.90
Systolic BP	-1.44	0.97	-10.28	7.41
Diastolic BP	0.09	1.00	-7.11	7.30

I: Atenolol 100mg J: Tenormin 100mg , MD: Mean differences in the two formulations, p: Calculated p-value for MD, LB: Lower bound of 95% confidence interval, UB: Upper bound of 95% confidence interval, BP: Blood Pressure.

AUC_{0-24} was about 4.89 µg.h/ml for the test and 5.31 µg.h/ml for the reference (Table 1). The above parameters were not statistically different for the reference and test formulations based on paired t-test ($p<0.05$).

The 90% confidence levels of C_{max}, T_{max} and $AUC_{0-\infty}$ were also within the acceptable range: of 80-120% of the mean of ratios (test/reference) of the corresponding pharmacokinetic parameters

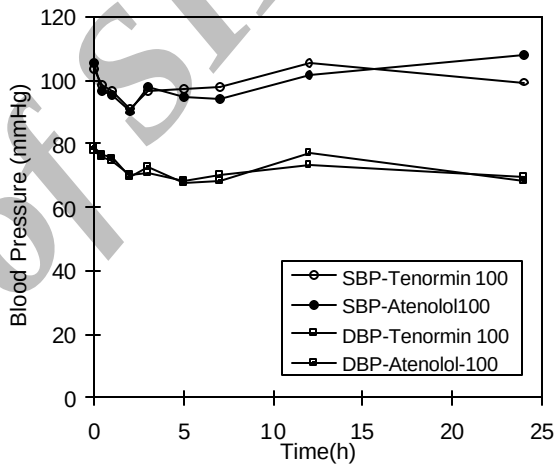

Figure 3. Profile of systolic and diastolic blood pressure versus time (Mean Data)
SBP: Systolic blood pressure, DBP: Diastolic blood pressure

and 80-125% of the mean of ratios (Test/Reference) of the corresponding log transformed pharmacokinetic parameters. (table 2)

Thereafter, two formulations were not significantly different in terms of pharmacokinetic parameters which were studied and therefore they are bioequivalent.

The profile of plasma concentration of the drug was similar to those which were reported previously (2,9,10).

There are reports about additive effects of moderate exercise to the hypotension produced by beta blocker (11-15). Subjects participating in our study were tested at rest and the resulting clinical effects were similar to other studies in which atenolol was tested at rest (2, 10) and

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dissimilar to the reports that had performed clinical tests during exercise tachycardia (3-5). The time courses of the effects of atenolol on heart rate, systolic and diastolic blood pressure are shown in figures 2 and 3.

Both formulations given orally caused a significant reduction in heart rate, systolic and diastolic blood pressure.

Pharmacodynamic effects of two formulations were compared using ANOVA (General linear model/ Repeated measures method). The results showed that heart rate, systolic and diastolic blood pressure were not significantly different for two formulations as p alue was greater than 0.05 (P>0.05). Difference of the means of the above-mentioned pharmacodynamic parameters was about zero and confidence intervals covered zero (Table 3).

These results indicate that both formulations were bioequivalent in terms of pharmacokinetic parameters and did not differ significantly in terms of pharmacodynamic pattern (heart rate, systolic and diastolic blood pressure).

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