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BIOPHARMACEUTICAL EVALUATION OF ORAL TABLET ATENOLOL (100 and 50 mg) ON LOCAL POPULATION

OBAID ALI, ROOHI OBAID*, ZAFAR SAIED SAIFY**
AND SYED WASEEMUDDIN AHMED***
Central Drugs Laboratory, Ministry of Health, Govt. of Pakistan
**Drugs Control Administration, Ministry of Health, Govt. of Pakistan*
***Department of Pharmaceutical Chemistry, ****Department of Pharmacognosy,*
Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan

An open-label, randomized study was designed to determine the bioavailability (BA); pharmacokinetic (PK) and pharmacodynamic (PD) behaviour of Atenolol 50 mg (two pills) and 100 mg (one pill) tablet manufactured by a national pharmaceutical industry. Peak plasma concentration (C_{max}): 1.33 ± 0.31 ug/ml, time to peak plasma concentration (T_{max}): 2.2 ± 0.27 hours, AUC (area under the plasma concentration-time curve) 6.34 ± 2.1 ug-hr/ml for 100 mg tab and C_{max}: 1.07 ± 0.23 ug/ml, T_{max}: 2.5 ± 0.35 hours, AUC 4.97 ± 1.09 ug-hr/ml for 50 mg (two pills) tab were observed. The BA and PK parameters such as C_{max}, T_{max}, are comparable to previous studies, although significant decrease in diastolic and systolic blood pressure (mmHg) upto a certain limit for a considerable duration was observed. However, relation between PK and PD may not be established due to regulatory biochemical feedback mechanism.

Keywords: Bioavailability, pharmacokinetic and pharmacodynamic.

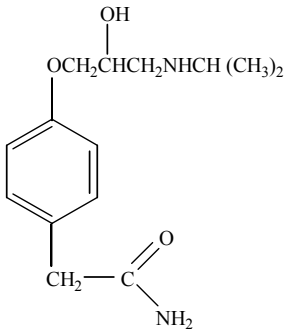
INTRODUCTION

Cardiovascular risk

Cardiovascular disease (CVD) is a leading cause of mortality and is responsible for one-third of all global deaths. Nearly 85% of the global mortality and disease burden from CVD is borne by low- and middle-income countries. In India, for example, approximately 53% of CVD deaths are in people younger than 70 years of age; in China, the corresponding figure is 35%. The majority of the estimated 32 million heart attacks and strokes that occur every year are caused by one or more cardiovascular risk factors – hypertension, diabetes, smoking, high levels of blood lipids, and physical inactivity – and most of these CVD events are preventable if meaningful action is taken against these risk factors. (Integrated Management of Cardiovascular Risk Report of a WHO meeting Geneva, 9–12 July 2002).

Atenolol

$C_{14}H_{22}N_2O_3$
Relative Molecular Mass = 266.3
CAS 29122-68-7
Chemical Name:
(RS)-4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide



Atenolol

Physical properties of atenolol

Below is a table of some of the physical properties of Atenolol.

Melting point	152-155°C
Dissociation constant (pK_a)	9.6 @ 24°C
Partition coefficient (Log P (Octanol))	0.23
Solubility:	
Water	Sparingly soluble
Ethanol/Methanol	Soluble
Ether	Practically insoluble

Pharmacology

Atenolol is a beta-adrenaceptor antagonist, or a more commonly known as a beta-blocker. Beta-blockers are

Corresponding Author: Obaid Ali, Assistant Drugs Controller, Government of Pakistan, Ministry of Health, Central Drugs Laboratory, DHA Phase 11, Karachi email, mobaidali@yahoo.com

competitive inhibitors and interfere with the action of stimulating hormones on beta-adrenergic receptors in the nervous system. Beta-blockers can be subdivided into two distinct groups, known as $beta_1$ and $beta_2$. $Beta_1$ blockers mainly affect the heart, $beta_2$ blockers mainly affect receptors in bronchial tissue.

Reported pharmacokinetics
Atenolol is well absorbed after oral administration, it undergoes extensive hepatic (first pass) metabolism. Its bioavailability is 40%. It has a large volume of distribution and is less completely metabolised before its excretion via urine. Because of hepatic metabolism, a part of the oral dose may not reach the peripheral circulation, so the plasma half-life doesn't correlate with the duration of the therapeutic effects, which is relatively long lasting. This is because the plasma 1/2 life decreases exponentially, following first order kinetics while the effect decreases linearly following zero order kinetics.

Pharmacokinetics
Pharmacokinetic is the study of drug movement in the body over the time during the drug's absorption, distribution and elimination (excretion and biotransformation).

Bioavailability
The concept of bioavailability developed in last three decades and has become more important in order to ensure ultimate quality of product. This concept is based on assumption that the measurement of certain parameters like serially obtained blood or urine concentration of drug following drug administration can be correlated with clinical efficacy.

The bioavailability is defined as: "it is the degree to which the drug is absorbed from the pharmaceutical formulation into the body". For drug products not intended to be absorbed into the blood stream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or moiety may become available at the site of action (FDA-USA, 1997).

The extent of absorption is measured by the bioavailability, or fraction of dose absorbed measured by the area under the concentration-time profile (AUC), whereas the rate of absorption is roughly assessed by measuring the maximum plasma or blood level, C_{max}, of a compound (Mangione, 1998). Although C_{max} is a function of both the rate and extent of absorption, and some have argued that T_{max} would be a more pure measure of the rate of absorption, C_{max} is a clinically relevant parameter in most cases.

Many excipients can affect the absorption of drugs. In addition, any sensitivity the patient may have to the various colorants and preservatives present must be taken into account.

Need of bioavailability study
When the patient on a sustained drug formulation ends, other companies are allowed to market generic formulation of the drug if the company can prove the bioequivalence (FDA 1992 F.D.A. (1997) Draft Guidance for industry).

Bioequivalence
Two or more chemically or generic equivalent products of the same preparation can be said to be bioequivalent, if they do not differ (<20%) significantly in their bioavailability characteristics. This bioequivalent drug is assumed that they will be therapeutically equivalent and can be used interchangeably (F.D.A., 1997; Draft Guidance for Industry).

MATERIALS AND METHODS

In vitro and *in vivo* trials were conducted according to international guidelines. The dissolution test was performed by the rotating paddle method (USP Apparatus II). Reliable, validated BP apparatus by experienced physician is used to monitor the systolic and diastolic blood pressure. Bioavailability of test product 1 was evaluated by plotting blood free concentration profile of drug at different intervals after oral administration followed by pharmacokinetic calculations.

Subjects
For evaluation of 100 mg tablet (test product 1): A subject panel of 5 healthy adults, male, human volunteers of an average age of 25.8 ± 5.50, ranging from 20 to 33 years, an average weight of 73.8 ± 15.93 Kg ranging from 58 Kg to 100 Kg and average height 69.0 ± 1.73 inches ranging from 68 inches to 72 inches participated in the study.

For evaluation of 50 mg tablet (test product 2): A subject panel of 5 healthy adults, male, human volunteers of an average age of 21.0 ± 2.55, ranging from 17 to 23 years, an average weight of 60.6 ± 10.31 Kg ranging from 50 Kg to 74 Kg and average height 65.6 ± 2.30 inches ranging from 63 inches to 68 inches participated in the study.

Selection of volunteers
Selection criteria for volunteer inclusion in this study was:
- No history of allergic tendencies and reaction to Beta blocking agents or any other ingredient used in the formulation of test product.
- With normal blood counts, normal liver and kidney function tests, and without abnormalities in physiology, urine and blood analysis.
- Volunteers should be free of any treatment or any drug for at least a month prior to study.
- Absence of any chronic or pathological disease (No history of Asthma).

The panel members were given a general medical examination to establish good health. The following selection criteria were used for this purpose.

❖No congenital disease.	❖No underline hypertension
❖Response of the following were checked and found normal.	
✓Hepatic	✓Renal
✓Gastrointestinal tract	✓Psychiatric
✓Respiratory tract	✓Metabolic
✓Neurological	✓Cardiovascular

Exclusion criteria

Subjects with any current or past medical condition that might significantly affect their pharmacokinetic and pharmacodynamics response to the administered drug were the limiting factors in the study.

All volunteers were thoroughly informed about the aims and objectives of the study, the drug to be tested, and the hazards/side effects of the drug atenolol and also their rights to separate themselves from the study at any stage without mentioning any reason. Informed consent was also obtained from each subject selected and their willingness to participate in the study.

Restrictions

No volunteer was allowed to take any prescription or OTC drug one week prior to dosing and during the study period, in order to avoid interference with the kinetic behaviour of atenolol in the body or in the determination of drug in the blood. The volunteers were instructed to report the investigator about the illness/side effects and the treatment undertaken. No volunteer took any drug for at least one month prior to and during the study.

Study design (Pharmacokinetics & Pharmacodynamics)

Study was designed according to described objectives and conducted on a single occasion. Volunteers were given a single dose of test drug i.e. test product 1 tablet or Test product 1® (two pills) nationally manufactured with water (FDA, 1997; Draft Guidance for Industry).

Single dose-single period study

In this study, each subject is successively exposed to a single treatment on a single occasion. Blood samples (3 ml) were collected through intravenous route at 0.0,0.5,1.0,1.5, 2.0, 2.5, 3.0,4.0, 6.0, 8.0, 12.0 and 24.0 hours interval and blood pressure (mm Hg) was monitored at 0, 60, 120, 180, 240 and 360 minutes interval after drug administration. A controlled non-fatty diet, juices, refreshment and milk were served to all volunteers.

Chromatographic condition: As reported by Urech *et al*., 1990.

Table 1

Analytical results of tablet atenolol 50 and 100 mg tablets

	Test product 1	**Test product 2**
Identity	Positive	Positive
Color	White	Orange
Appearance	Round, slightly biconvex with broad edges, film coated	Round, biconvex, film coated
Disintegration time	06 min. 50 sec.	07 min. 10 sec.
Mean weight	160.35 mg	326.55 mg
Minimum weight	155.8	315.0 mg
Maximum weight	163.10	343.4 mg
Diameter	8.05 – 8.15 mm	9.55 – 9.65 mm
Thickness	3.50 – 3.60 mm	4.85 – 5.00 mm
Assay	99.60%	102.14 %
Dissolution	Complies with the standard requirement of the USP	Complies with the standard requirement of the USP

Table 2a
Systolic blood pressure (mm Hg) profile of healthy human volunteers after administration of 100 mg of atenolol tablet (test product 1)

Subject identity	Time (in minutes)					
	0	60	120	180	240	360
A	120	114	110	110	110	110
B	130	120	118	112	110	114
C	120	120	108	108	110	106
D	120	116	110	114	114	110
E	116	114	108	110	106	106
Mean	121.2	116.8	110.8	110.8	110.0	109.2
± SD	5.2	3.0	4.1	2.3	2.8	3.3

Table 2b
Systolic blood pressure (mm Hg) profile of healthy human volunteers after administration of 100 mg of atenolol tablet (test product 2)

Subject identity	Time (in minutes)					
	0	60	120	180	240	360
G	110	106	100	100	100	100
H	116	100	100	100	104	106
I	124	114	102	100	102	105
K	120	120	106	100	100	100
L	120	112	98	96	96	100
Mean	118.0	110.4	101.2	99.2	100.4	102.2
± SD	5.3	7.7	3.0	1.8	3.0	3.0

Table 3a
Diastolic blood pressure (mm Hg) profile of healthy human volunteers after administration of 100 mg of atenolol tablet (test product 1)

Subject identity	Time (in minutes)					
	0	60	120	180	240	360
A	76	70	70	70	68	64
B	80	80	76	70	70	78
C	80	76	74	70	70	64
D	76	76	70	70	70	70
E	70	70	68	70	70	70
Mean	76.4	74.4	71.6	70.0	69.6	69.2
± SD	4.1	4.3	3.3	0.0	0.9	5.8

Instrument
HPLC-10Avp attached with class VP recorder (Shimadzu) and C_{18} u Bondapak 3.9 mm X 300 mm column, was used.

Procedure
The volunteers were asked to report to the investigator after an overnight (12 hours) fast. Drug was administered to all volunteers according to the study design. Blood samples

Table 3b
Diastolic blood pressure (mm Hg) profile of healthy human volunteers
after administration of 100 mg of atenolol tablet (test product 2)

Subject identity	Time (in minutes)					
	0	60	120	180	240	360
G	70	70	70	60	60	64
H	80	70	70	68	70	74
I	80	76	74	70	68	70
K	80	76	70	66	66	60
L	70	64	60	60	62	60
Mean	76.0	71.2	68.8	64.8	65.2	65.6
± SD	5.5	5.0	5.2	4.6	4.1	6.2

Table 4a
Blood free concentration (ug/ml) of atenolol following a single oral dose of 100 mg tab.
(test product 1) when given to healthy human volunteers (n= 5 ± SD)

Subject identity	Hours											
	0	0.5	1	1.5	2	2.5	3	4	6	8	12	24
A	0	0.02	0.02	0.432	1.1	1.273	0.89	0.88	0.273	0.131	0.037	0
B	0	0.013	0.022	0.368	1.599	1.751	1.112	0.885	0.691	0.482	0.297	0
C	0	0.02	0.104	0.265	1.525	1.051	0.881	0.818	0.812	0.497	0.159	0
D	0	0.022	0.219	0.398	1.1	0.932	0.839	0.657	0.348	0.318	0.132	0
E	0	0.022	0.028	0.414	1.007	0.862	0.804	0.756	0.333	0.145	0.047	0
Mean	0	0.019	0.079	0.375	1.266	1.174	0.905	0.799	0.491	0.315	0.134	0.0
± SD	0	0.004	0.086	0.066	0.274	0.358	0.121	0.095	0.243	0.176	0.105	0.0

Table 4b
Blood free concentration (ug/ml) of atenolol following a single oral dose of 100 mg tab.
(test product 2) when given to healthy human volunteers (n= 5 ± SD)

Subject identity	Hours											
	0	0.5	1	1.5	2	2.5	3	4	6	8	12	24
G	0	0.028	0.04	0.417	0.541	0.754	0.863	0.462	0.175	0.118	0.048	0
H	0	0.023	0.133	0.502	1.036	1.013	0.934	0.781	0.366	0.188	0.067	0
I	0	0.018	0.02	0.483	0.601	0.894	0.763	0.44	0.428	0.23	0.132	0
K	0	0.016	0.021	0.521	1.192	1.445	0.979	0.989	0.129	0.183	0.078	0
L	0	0.016	0.024	0.306	0.526	1.114	0.767	0.814	0.65	0.403	0.132	0
Mean	0	0.02	0.05	0.45	0.78	1.04	0.86	0.7	0.35	0.22	0.09	0
± SD	0	0.01	0.05	0.09	0.31	0.26	0.1	0.24	0.21	0.11	0.04	0

were collected through disposable heparinzed syringes under aseptic technique, at different intervals of time. The samples were centrifuged, serum collected and immediately frozen. A controlled breakfast, lunch and juices were served to all volunteers at different times as specified in study design. Vital signs of volunteers including blood pressure, respiratory rate, oral temperature and pulse rate were checked pre dosing and closely monitored throughout the day by a consulting physician.

Data quality assurance

Data/samples obtained through out the entire study were recorded on the subjects' "In Process Control Sheet for analysis of atenolol" and on "Sample Collection and Volunteer Monitoring Sheet". Steps were taken to ensure accurate and reliable data including the selection of research associate, review of protocol, procedure and report with the principle investigator and associated personnel at an investigator meeting. Labels of specific nomenclature with different colors were used for the differentiation of each volunteer sample.

Table 5a

Bioavailability and pharmacokinetic parameters following the administration of single oral dose of 100 mg tablet (test products) when given to healthy human volunteers (n=05 ± SD)

Volunteers identity	C_{max} (ug/ml)	T_{max} (hours)	AUC (ug. hr/ml)
A	1.27	2.5	4.65
B	1.75	2.5	9.24
C	1.53	2	7.76
D	1.1	2	5.66
E	1.01	2	4.38
Mean	1.33	2.2	6.34
± SD	0.31	0.27	2.1

Table 5b

Bioavailability and pharmacokinetic parameters following the administration of single oral dose of 100 mg tablet (test product 2 tab.) when given to healthy human volunteers (n= 05 ± SD)

Volunteers identity	C_{max} (ug/ml)	T_{max} (hours)	AUC (ug. hr/ml)
G	0.86	3	3.32
H	1.04	2	5.06
I	0.89	2.5	4.85
K	1.45	2.5	5.25
L	1.11	2.5	6.36
Mean	1.07	2.5	4.97
± SD	0.23	0.35	1.09

Chemicals

- Methanol
- Distilled water
- Potassium di hydrogen phosphate
- Phosphoric acid
- Atenolol standard
- Sodium octane sulfonate
- Acetonitrile

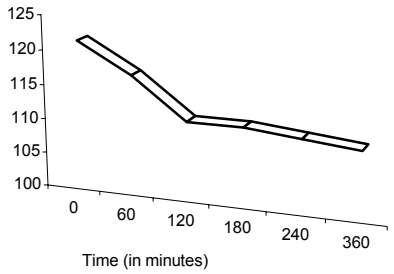


Fig. 1a: Mean systolic blood pressure (mm Hg) after drug administration.

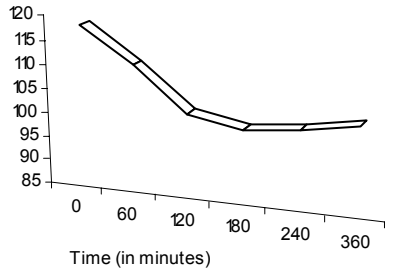


Fig. 1b: Mean systolic blood pressure (mm Hg) after drug administration.

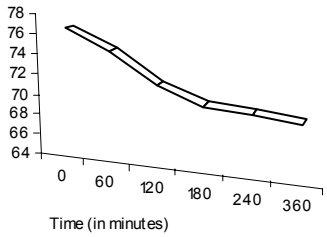


Fig. 2a: Mean diastolic blood pressure (mm Hg) after drug administration.

All chemicals used in the study were procured from Merck, a reliable company in the manufacturing and marketing of high-grade chemicals.

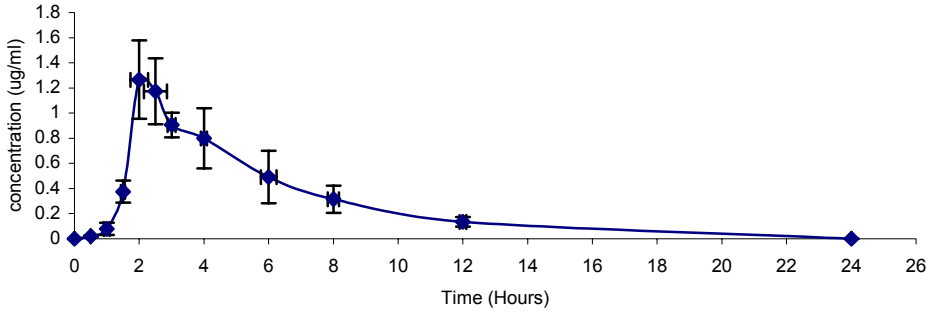


Fig. 3a: Mean (n=05) ± SD plasma concentration of atenolol following a single oral dose of tablet atenolol 100 mg (test product 1) to the healthy, human volunteers.

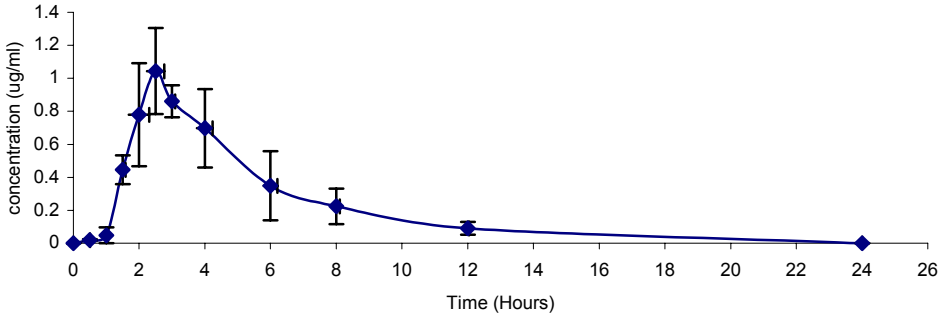


Fig. 3b: Mean (n=05) ± SD plasma concentration of atenolol following a single oral dose of tablet atenolol 100 mg (test product 2) to the healthy, human volunteers.

RESULTS

The plasma drug concentration levels, (fig 3a and b; table-4 a and b) systolic blood pressure (mm Hg) (fig 1a and b; table 2a and b), diastolic blood pressure (mm Hg) (fig. 2a and b; table 3a and b) and dissolution (table 1) were monitored and analyzed statistically in order to evaluate, pharmacological endpoint dissolution behavior (Table-1), bioavailability pharmacokinetic (fig. 4a and b; table 5a and b) and other parameters of the studied formulation. MS Excel 98 program was used for the calculation of peak concentration (C_{max}), time to peak concentration (T_{max}) while area under the curve (AUC) was calculated by trapezoidal method. Other pharmacokinetic values were calculated by manual technique using basic mathematical tools.

DISCUSSION AND CONCLUSION

Three basic following pharmacokinetic and pharma-codynamic parameters were calculated for the tested product i.e. tablet test product 1, test product 2 (a conventional release oral formulations).

1. Area under curve (AUC)
2. Peak concentration C_{max}
3. Time to peak concentration T_{max}
4. Pulse rate
5. Systolic blood pressure
6. Diastolic blood pressure
7. Dissolution test

The bioavailability and pharmacokinetic parameters such as Cmax, Tmax, obtained in this study (table 5a and b; fig.3a

and b, 4a and b) are comparable to previous studies (Lin Lin Wu *et al*., 2003; AHFS Drug Information 1999; Leonetti, *et al.*, 1980 and Wu *et al*., 2002). The significant decrease in diastolic and systolic blood pressure (mm Hg) upto a certain limit for a significant duration provides valuable information for the clinician and researcher although relation between pharmacokinetic and pharmacodynamic may not be established on healthy volunteers due to regulatory biochemical feedback mechanism. Different biochemical measurements will be required to establish the relationship. This is consistent with the previous studies reported by Amery *et al*., 1977 and Kirch *et al*., 1982. Our study provides evidence and confidence to the physician and health care provider in generic atenolol investigated formulation.

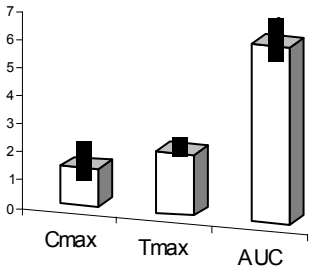


Fig. 4a: Test product 1 Mean (n=5) Pharmacokinetics.

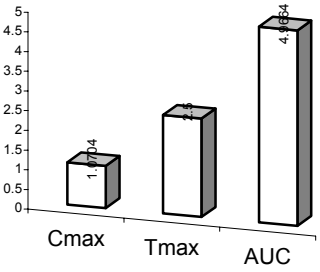


Fig. 4b: Test product 2 Mean (n=5) Pharmacokinetics.

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