In vitro Pharmaceutical Equivalence Study of Three Brands of Atenolol Tablets Available in Bangladesh

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ABSTRACT: The aim of the present work was to assess the pharmaceutical equivalence of three brands of atenolol (50 mg) tablets available in Bangladesh using *in vitro* dissolution study. The dissolution study was carried out using the paddle apparatus according to the guidelines of United States Pharmacopoeia (USP). The dissolution profiles of three locally manufactured atenolol tablets were determined and compared with the dissolution profile of atenolol tablet from innovator's company. All samples attained more than 85% dissolution within 10 minutes. Mean dissolution values were employed to estimate difference factor (f_1) and similarity factor (f_2). Difference factor (f_1) and similarity factor (f_2) were used to assess *in vitro* bio-equivalency among the three brands. Other general quality assessment parameters such as hardness, friability and disintegration time were also determined. All brands complied with the official specifications for hardness, friability and disintegration time. The study indicated that all brands can be prescribed interchangeably.

Key words: Atenolol, pharmaceutical equivalence, *in vitro*, dissolution.

INTRODUCTION

Hypertension is the most common cardiovascular disease in the world. The prevalence of hypertension increases with age. About 50% of people between the ages of 60 to 69 years old have hypertension, and the prevalence is further increased beyond age 70 in USA.[1] Hypertension is also common in our subcontinent. In India and Bangladesh, an increasing trend of hypertensive disease has been reported.[2] Elevated arterial pressure causes pathological changes in the vasculature and hypertrophy of the left ventricle. As a consequence, hypertension is the principal cause of stroke, a major risk factor for coronary artery disease and its attendant complications myocardial infarction and sudden cardiac death. Hypertension is a major contributor to cardiac failure, renal insufficiency and dissecting aneurysm of the aorta.[3]

The antihypertensive drugs are used to treat hypertension. There are different classes of antihypertensive drugs available all over the world. These drugs lower blood pressure by different mechanisms. The most widely used antihypertensive drugs are the β-adrenoceptor blockers, the centrally acting drugs, the ACE inhibitors and the angiotensin II receptor antagonists. Atenolol is a β-adrenoceptor blocker.[4] Atenolol is widely used as antihypertensive agent. It is widely used because of good patient acceptability and cardio-protective potentiality. This drug is also increasingly used for the treatment of angina pectoris, cardiac arrhythmias and myocardial infraction.[5]

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The bio-pharmaceutical characteristic of atenolol is described as sparingly soluble in water in British Pharmacopoeia (BP). On the basis of studied bio-pharmaceutical data, atenolol could be clearly classified into Bio-pharmaceutics Classification System (BCS) Class III.[6] BCS Class III drugs have high solubility and low permeability.[7]

Pharmaceutical equivalence is the condition in which drug products containing the identical quantity of active ingredient in an identical dosage form, meet all applicable standards of identical strength, quality, purity and potency. The following criteria should be considered in the determination of pharmaceutical equivalence - (i) identical amount of active ingredient(s); (ii) same dosage form or comparable dosage form (e.g. Tablets versus Capsules); (iii) same route of administration.[8]

Determination of pharmaceutical equivalence of tablets can be done by comparing the amount of active ingredient, dissolution time, hardness, friability and disintegration time of the test product against the reference product (innovator product).[9]

In recent years, FDA has placed more emphasis on a dissolution profile comparison in the area of post-approval changes and biowaivers. Under appropriate test conditions, a dissolution profile can characterize the product more precisely than a single point dissolution test. A dissolution profile comparison between pre-change and post-change products for scale-up and post approval change (SUPAC) related changes, or with different strengths helps assure similarity in product performance and signals bioinequivalence.[10]

Atenolol tablets are widely used in Bangladesh due to its effectiveness and affordable price. To the best of our knowledge, no reports are available on the pharmaceutical equivalence of various atenolol tablets manufactured in Bangladesh. The availability of numerous brands of atenolol tablets in drug market of Bangladesh makes physicians in a difficult situation to choice a suitable brand or to use of effective alternative brand.

Hence the present study was set out to assess the *in vitro* pharmaceutical equivalence of atenolol tablets manufactured in Bangladesh. The purpose of the study was to determine dissolution profiles of locally manufactured atenolol tablets and to compare those profiles graphically with drug from innovator's company (as reference standard). In addition to that the results were evaluated statistically using difference factor (f_1) and similarity factor (f_2). This study would provide a rationale for the interchangeability of the selected brands with the innovator brand.

MATERIALS AND METHODS

Drugs and chemicals. Standard atenolol was a kind gift from Healthcare Pharmaceuticals Ltd., Gazipur, Bangladesh. Three brands of atenolol (50 mg) tablets were purchased from the local market of Dhaka city. They were randomly designated as A, B and C. Tablet Tenormin 50 mg (AstraZeneca, the innovator company) was designated as reference innovator (RI). Chemicals and all other reagents were of analytical grade and were purchased from local suppliers.

Preparation of 0.1N acetate buffer, pH 4.6. 0.1N acetate buffer, pH 4.6 was prepared by mixing 44.9 parts (v/v) of 0.1N sodium acetate with 55.1 parts (v/v) of 0.1N acetic acid solution and adjusted with diluted acetic acid to a pH of 4.6.

Preparation of stock solution of atenolol. A stock solution (100 mL) of 50 µg/mL was prepared by dissolving 0.05 g of atenolol in 0.1N acetate buffer, pH 4.6 and made up to the mark volume with the same solvent. Then 10 mL from this was diluted with 0.1N acetate buffer at pH 4.6 and finally the volume was adjusted up to 100 mL with the same solvent. The resulting solution is called stock solution of 50 µg/mL. The stock solution was then diluted to the desired strength by 0.1N acetate buffer at pH 4.6.

Preparation of calibration curve. Serial diluted solutions of 5.0, 7.5, 10.0, 12.5, 15.0 µg/mL of atenolol were prepared from the stock solution (50 µg/mL) with 0.1N acetate buffer, pH 4.6. The absorbances were taken at 218 nm using a UV-Visible spectrophotometer (Model UV-800 Shimadzu, Japan). A plot of absorbance versus

concentration of atenolol was made from which the regression equation was calculated.[11]

Hardness test. The hardness was determined with an automatic tablet hardness tester (Model HDT-300F, Logan Instrument Corp., USA). Six atenolol tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded.

Friability test. Twenty atenolol tablets of each brand were weighed and subjected to abrasion by using a friability tester (Model FIB-2S, Logan Instrument Corp., USA) at 25 rev/min for 4 minutes. The tablets were then weighed and percentage friability was calculated.

Disintegration test. Six atenolol tablets of each brand were used for the test in distilled water with an automatic disintegration tester (Model DST-3, Logan Instrument Corp., USA) employing plastic discs. The disintegration time was taken as the time when no particles remained on the basket of the tester.

Dissolution test. The dissolution test was carried out using a dissolution tester (Model UDT-804, Logan Instrument Corp., USA) according to USP guidelines in 6 replicates for each brand.[12] The dissolution medium was 900 mL of 0.1N acetate buffer, pH 4.6 which was maintained at 37°±0.5°C. The dissolution tester was operated at 50 rpm. In all the experiments, 5 mL of dissolution sample was withdrawn at 5, 10, 15, 30 and 45 minutes and replaced with equal volume of 0.1N acetate buffer, pH 4.6 to maintain sink condition. Samples were filtered, diluted and the absorbences were taken at 218 nm using spectrophotometer where 0.1N acetate buffer, pH 4.6 used as blank. The concentrations of samples were determined from the calibration curve of pure atenolol. The percent dissolutions were computed. The data were tailored and computed the means.

Dissolution profile comparison using graph. The percent dissolutions of the samples and reference innovator were graphed versus time.

Determination of 50% and 90% dissolution. The time required for 50% dissolution ($T_{50\%}$) and 90% dissolution ($T_{90\%}$) were determined as they are used as good indicators for dissolution.[13]

Dissolution profile comparison using difference factor and similarity factor. A model independent mathematical approach was used to compare the dissolution profiles of the samples and the reference product using two factors, difference factor (f_1) and similarity factor (f_2). Mean dissolution values were employed to estimate difference factor (f_1) and similarity factor (f_2). The f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensures sameness or equivalence of the test results and the reference product. The following equations were used to calculate difference factor (f_1) and similarity factor (f_2) for the studied tablets.[14]

$$f_1 = \{[\sum_{t=1} n\,|R_t - T_t|] \div [\sum_{t=1} n\,R_t]\}\,x\,100$$

$$f_2 = 50x\ \log\left\{\left[1 + (1/n)\sum_{t=1} r(R_t - T_t)^2\right]^{-0.5} x\,100\right\}$$

Where, n is the number of time points, R_t is the dissolution value of reference product at time 't' and T_t is the dissolution value for the test product at time 't'.

Data analysis. The data were express as mean±standard deviation (SD).

RESULTS AND DISCUSSION

Hardness is referred to as non-compendial test. It may influence other quality parameters such as friability and disintegration. The crushing strength about 4 kp is the minimum requirement for a standard tablet.[15] Tablets of all brands were found to be satisfactory for hardness. Hardness was found to be within 4.55 to 6.13 kp for all brands. The results are shown in table 1.

Friability test is included in the United States Pharmacopoeia.[12] The standard specification for friability is not more than 1%. It was found to be less than 1% for each brand of tablets (Table 1).

Disintegration times of all the brands were within the limit. The British Pharmacopoeia specifies that uncoated tablets should disintegrate within 15 minutes and film coated tablets within 30 minutes.[16] All atenolol tablets were disintegrated in less than 2

minutes (Table 1). The calibration curve has good correlation ($r^2 > 0.9999$).

The United States Pharmacopoeia specifies that the amount of atenolol dissolved should not be less than 80% of the labeled amount in 30 minutes.[12] All brands complied with the specification. The dissolution mean values of the test products and reference innovator were shown in table 2.

The results of dissolution studies were presented graphically in figure 1. All the tested brands released more than 85% drugs within 10 minutes.

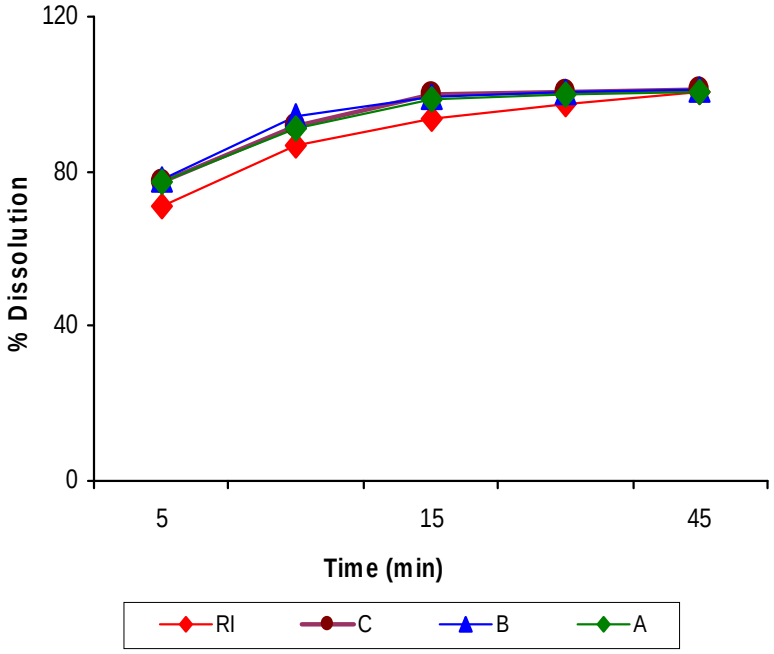

Figure 1. Dissolution profiles of different brands of atenolol tablets.

Table 1. Hardness, % friability and disintegration time of different brands of atenolol tablets.

Formulation	Hardness (kp)	% Friability	Disintegration time (minutes)
A	6.13 ± 0.37	0.097	1.23 ± 0.02
B	4.55 ± 0.15	0.044	0.43 ± 0.01
C	5.06 ± 0.39	0.038	1.36 ± 0.01
RI	5.32 ± 0.50	0.052	1.44 ± 0.02

Table 2. Mean percent dissolution of different brands of atenolol tablets.

	Brands of Tablets			
Time (minutes)	RI	A	B	C
5	70.76 ± 1.89	76.97 ± 2.91	78.16 ± 3.33	77.37 ± 1.64
10	86.58 ± 1.48	92.04 ± 2.66	94.40 ± 1.27	91.39 ± 1.34
15	93.67 ±1.47	99.86 ± 1.21	99.57 ± 1.19	98.68 ± 1.55
30	97.26 ± 1.43	100.60 ± 1.9	100.55 ± 0.77	99.81 ± 0.97
45	100.62 ± 1.22	101.17 ± 0.45	100.86 ± 0.67	100.26 ± 0.75

RI = Reference Innovator

Table 3. $T_{50\%}$, $T_{90\%}$, f_1 and f_2 values of different brands of atenolol tablets.

Formulation	$T_{50\%}$ (min)	$T_{90\%}$ (min)	Difference factor (f_1)	Similarity factor (f_2)
A	<5	<10	4.84	65.21
B	<5	<10	5.48	61.93
C	<5	<10	4.14	67.08

Similarity factor (f_2) has been adopted by the Food and Drug administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA) to compare dissolution profiles.[14,17] Two dissolution profiles are considered similar and bioequivalent, if f_1 is between 0 and 15 and f_2 is between 50 and 100.[14] In this study, parameters like f_1, f_2 and $T_{50\%}$, $T_{90\%}$ values were derived from the dissolution profiles of the different test brands of atenolol tablets. A $T_{90\%}$ of 30 minutes is satisfactory and is an excellent indicator of good dissolution.[13]Table 3 showed that brands A, B and C had $T_{50\%}$ values less than 5 minutes and $T_{90\%}$ values less than 10 minutes. Table 3 showed that f_1, f_2 values of different test brands in comparison of brand RI and it was observed that brands A, B and C had f_1 values less than 15 and f_2 values more than 50. They, therefore, were similar with brand RI and may be used interchangeably.

CONCLUSION

The results obtained from the *in vitro* pharmaceutical equivalence study of three brands of atenolol (50 mg) tablets showed that atenolol tablets of tested brands were equivalent to the brand of reference innovator. It can be inferred that these brands may have similar bioavailability and may be prescribed interchangeably.

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