THE IN VITRO PHARMACEUTICAL EQUIVALENCE STUDIES OF LOSARTAN TABLETS OF DIFFERENT MANUFACTURERS AVAILABLE IN BANGLADESH

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ABSTRACT

The study was aimed to assess the pharmaceutical equivalence of some losartan potassium tablets of different manufacturers marketed in Bangladesh using in vitro dissolution study. The dissolution was carried out using the apparatus II according to USP guidelines. Other general quality assessment tests like hardness, friability, disintegration time were also determined. All brands complied with the official specification for hardness, friability and disintegration time. The dissolution profiles showed inter brand and intra brand variability. All samples attained more than 85% dissolution within 30 minutes. The results were subjected to statistical analysis to compare the dissolution profiles. A model independent approach of similarity factor (f²) was employed. The data indicated that only two brands may be used interchangeably.

Key words: Losartan potassium, pharmaceutical equivalence, in vitro dissolution.

INTRODUCTION

Blood pressure is the force of blood against the artery walls as it circulates through the body. Hypertension is the constant pumping of blood through blood vessels with excessive force. When blood pressure becomes persistently high then it is 140/90 mmHg or higher. Hypertension is an important public health challenge all over the world. An increasing trend in the prevalence of hypertension has been shown in the studies from India and Bangladesh.[¹,²]

To treat hypertension the antihypertensive drugs are used. There are many classes of antihypertensive drugs available all over the world. The antihypertensive drugs lower blood pressure by different mechanisms. The most widely used antihypertensive drugs are the beta-adrenoceptor blockers, the centrally acting drugs, the ACE inhibitors and the angiotensin II receptor antagonists.[³]

Losartan potassium is one of the widely used antihypertensive drugs in Bangladesh. It is an angiotensin II receptor antagonist. It also reduces the combined risk of cardiovascular death, stroke and myocardial infarction in hypertensive patients with left ventricular hypertrophy and gives renal protection for type 2 diabetic patients with proteinuria.[⁴]

Pharmaceutical equivalence is the condition in which drug products; containing the identical quantity of active substance in an identical comparable 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 substance(s) (e.g. salt or ester), (ii) same dosage form or comparable dosage form (e.g. tablets versus capsules), (iii) same route of administration.
Pharmaceutical equivalence is not same to therapeutic equivalence which requires a product to be pharmaceutically equivalent and to have the same safety and efficacy profile after administration of the same dosage. Drug products are considered to be therapeutic equivalents only if they are pharmaceutically equivalents and if they have the same clinical effect and safety profile after administered to the patients.[5]

According to FDA, therapeutically equivalent drugs are those drug products that meet the following general criteria- (i) they are safe and effective; (ii) they are pharmaceutically equivalents in that they- (a) contain identical amounts of the same active drug ingredient in the same dosage form and route of administration, and (b) meet applicable standards of strength, quality, purity and identity; (iii) they are bioequivalent; (iv) they are adequately labeled and (v) they are manufactured in compliance with current Good Manufacturing Practice regulations. Bioavailability refers to the rate and extent to which the active ingredient or therapeutic ingredient is absorbed from a drug product and becomes available at the site of drug action. Bioequivalence is the equivalent release of the same drug substance from two or more drug products or formulations. This gives an equivalent rate and extent of absorption from these formulations. If a drug product containing chemically identical drug substance is delivered to the site of action at the same rate and extent as another drug product, then it is equivalent and can be substituted for that drug product. Methods used to define bioequivalence and bioequivalence studies include- (i) pharmacokinetic studies, (ii) pharmacodynamic studies, (iii) comparative clinical trials, and (iv) in vitro studies. The choice of study used depends on the site of action of the drug and the ability of the study design to compare drug delivered to that site by the two products.[6]

Despite the considerable use in Bangladesh, to the best of our knowledge, there are no reports on the pharmaceutical equivalence of the various losartan potassium tablets manufactured in Bangladesh. Therefore, in the present study, we set out to assess in vitro pharmaceutical equivalence of some losartan potassium tablets manufactured in Bangladesh. This study will help us to know the scenario of losartan potassium tablets manufactured in Bangladesh in respect of quality, safety and efficacy. The purpose of the study is to determine dissolution profiles of locally manufactured losartan potassium tablets and to compare those profiles statistically with drugs from innovator company (as reference) using similarity factor ($f_2$).

MATERIALS and METHODS

Drugs and chemicals: Standard losartan potassium was a kind gift from Healthcare Pharmaceuticals Ltd., Bangladesh. Three brands of losartan potassium (50 mg) were purchased from local drug store in Dhaka city. They were randomly designated as A, B and C. The local manufacturers are Incepta Pharmaceuticals Ltd., Opsonin Pharmaceuticals Ltd., and Orion Pharma Ltd. Tablet Cozaar 50 mg [Merck Sharp & Dohme, (New Zealand) Ltd.] was the innovator’s product and it was designated as reference innovator (RI). Chemicals and all other reagents were of analytical grade and were purchased from local suppliers.

Preparation of stock solutions of losartan potassium: Hundred milliliter stock solution of 50 μg/mL was prepared by dissolving 0.05 g of losartan potassium in distilled water and made up to 100 mL with the same solvent. Ten milliliter of this solution was taken, diluted with distilled water and finally made up to 100 mL with the same solvent. The stock solution was diluted to the desired strength by distilled water.

Dissolution Medium: Distilled water.

Preparation of Calibration Curve: Serial diluted solutions of 4.0, 4.5, 5.0, 5.5, 6.0 μg/mL of losartan potassium were prepared from a stock solution (50 μg/mL) in distilled water. Absorbances were taken at 201 nm using a UV-Visible spectrophotometer (Model UV-800 Shimadzu, Japan). A plot of absorbance versus concentration of losartan potassium was made from which the regression equation was calculated.

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

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

Disintegration test: Six tablets of each brand were used for the test in distilled water at 37°C with an automatic disintegration tester (Model DST-3, Logan Instrument Corp.) 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.) according to USP guidelines in 6 replicates for each brand. The dissolution medium was 900 mL of distilled water which was maintained at 37.0±0.5°C. In all the experiments, 10 mL of dissolution sample was withdrawn at 10, 20, 30 and 45 minutes and replaced with equal volume of distilled water to maintain sink condition. Samples were filtered, diluted and the absorbance reading determined at 201 nm using spectrophotometer using distilled water as blank. The concentration was determined from the calibration curve of pure losartan potassium. The percent dissolutions were computed. The data were tallied and computed the means. The percent dissolutions of the samples and reference innovator were graphed against time. The values for T₉₀ and T₄₀ were determined as they are used as guides for dissolution.

**Analysis of similarity factor:** The dissolution profiles were analyzed by a mathematical model, similarity factor (f₂). Mean dissolution values were employed to estimate the similarity factor (f₂). A factor value of 50 or greater (50-100) ensures sameness or equivalence of the two products. The following equations were used to calculate similarity factor (f₂).

\[
f₂ = 50 \times \log \left[ \frac{100}{\sqrt{1 + \frac{2(R_t - T_i)^2}{n}}} \right]
\]

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

**Statistical analysis:** The results were expressed as mean ± standard deviation (SD), where, n= 5.

**RESULTS and DISCUSSION**

Hardness is referred to as non-compendial test. It could influence other parameters such as friability and disintegration. A force of about 4kg is the minimum requirement of a satisfactory tablet. The tablets of all brands were satisfactory for hardness. Tablets hardness was found to be within 6.28 to 8.44 kg (Table 1).

Friability test is included in the USP. The standard specification for friability is 1%. Friability for all the brands was below 1% (Table 1). Disintegration times of all the brands were within the limit. The USP specifies that uncoated tablets should disintegrate within 15 minutes and film coated tablets in 30 minutes. All losartan potassium tablets were film coated and disintegrated in <16 minutes (Table 1).

The calibration curve as shown in Figure 1 has good correlation (r² = 0.9961). The USP specifies that the amount of drug released (dissolution) should not be less than 80% of the labeled amount in 30 minutes. All brands complied with the specification. The dissolution mean values of the generic and reference innovator in water were shown in Table 2. The results of dissolution studies were presented in Figure 2. Both inter- and intra-brand variations in dissolution profiles were observed. Brand A released more than 90% drug within 30 minutes, brand B released less than 90% drug within 30 minutes and brand C released more than 95% drug within 30 minutes. From these data it was clear that although hardness, friability and disintegration time were almost similar within different brands but the brands differ in case of drug release. Similarity factor (f₂) has been adopted by FDA and the European Agency for the Evaluation of Medicinal Products to compare dissolution profiles. Two dissolution profiles are considered similar and bioequivalent, if f₂ is between 50 and 100. A T₉₀ of 30 minutes is satisfactory and is an excellent goal. In this study parameters like T₅₀, T₉₀ and f₂ were derived from the dissolution profiles of the different brands. Table 3 showed the f₂ values of different brands in respect of brand RI. For brand A and brand C, f₂ values were more than 50. So they are similar with brand RI and can be used interchangeably. For brand B, f₂ value was less than 50. So it is not similar with brand RI and can not be used interchangeably.

**CONCLUSION**

This study has emphasized that pharmaceutical equivalence does not indicate bioequivalence of drug product and one brand substituted with another brand on assumption of pharmaceutical equivalence may not give the desired onset of action and subsequent therapeutic effectiveness. However, in vivo test may be required for final comments regarding the quality of different brands of losartan tablet.

**ACKNOWLEDGEMENT**

We acknowledge Healthcare Pharmaceuticals Ltd., Bangladesh for donation of pure drug sample of losartan.
Table 1: Results of hardness, % friability and disintegration time of losartan tablet.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness ± SD</th>
<th>% Friability</th>
<th>Disintegration Time (minutes) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.44 ± 1.03</td>
<td>0.04</td>
<td>10.29 ± 0.35</td>
</tr>
<tr>
<td>B</td>
<td>6.28 ± 0.28</td>
<td>0.03</td>
<td>6.88 ± 0.30</td>
</tr>
<tr>
<td>C</td>
<td>6.88 ± 0.53</td>
<td>0.34</td>
<td>15.22 ± 0.39</td>
</tr>
<tr>
<td>RI</td>
<td>6.89 ± 0.35</td>
<td>0.06</td>
<td>7.19 ± 0.62</td>
</tr>
</tbody>
</table>

Table 2: Mean percent dissolution of losartan tablet.

<table>
<thead>
<tr>
<th>Time</th>
<th>RI ± SD</th>
<th>A ± SD</th>
<th>B ± SD</th>
<th>C ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>58.94 ± 3.37</td>
<td>49.28 ± 4.77</td>
<td>44.72 ± 2.92</td>
<td>53.59 ± 3.37</td>
</tr>
<tr>
<td>20</td>
<td>85.46 ± 3.86</td>
<td>71.13 ± 4.74</td>
<td>72.21 ± 2.66</td>
<td>84.39 ± 1.08</td>
</tr>
<tr>
<td>30</td>
<td>96.36 ± 2.91</td>
<td>91.68 ± 2.78</td>
<td>85.47 ± 1.21</td>
<td>95.85 ± 1.51</td>
</tr>
<tr>
<td>45</td>
<td>98.63 ± 1.73</td>
<td>99.18 ± 0.82</td>
<td>98.30 ± 1.91</td>
<td>99.97 ± 1.39</td>
</tr>
</tbody>
</table>

Table 3: T_{50\%}, T_{90\%} and $f_2$ of three brands of losartan tablet.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$T_{50%}$ (minutes)</th>
<th>$T_{90%}$ (minutes)</th>
<th>Similarity factor ($f_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&gt; 10</td>
<td>&lt; 30</td>
<td>52.26</td>
</tr>
<tr>
<td>B</td>
<td>&gt; 10</td>
<td>&gt; 30</td>
<td>47.58</td>
</tr>
<tr>
<td>C</td>
<td>&lt; 10</td>
<td>&lt; 30</td>
<td>76.21</td>
</tr>
</tbody>
</table>

Figure 1: Calibration curve of losartan tablet for calculation of dissolution profiles.
**Figure 2**: Comparison of dissolution profiles of different brands (A-C) of losartan tablet with innovator product (RI).

**REFERENCES**