Comparative in vitro dissolution and in vivo bioequivalence of two diclofenac enteric coated formulations

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Abstract

The aim of this study was the comparison of in vitro dissolution and in vivo bioavailability of two different brands of diclofenac sodium (CAS 15307-86-5) enteric coated tablets in healthy male Iranian volunteers in a single-dose, randomized, open-label, single blind study, which was conducted according to a crossover design in healthy volunteers. A washout interval of two weeks was selected between administrations to each subject in this study. Serial venous blood samples over 10 h after each administration to measure diclofenac sodium concentration in serum were obtained, and placed into tubes containing sodium heparin. Then the plasma was separated and kept frozen at -20 °C for subsequent analysis with a modified HPLC method with UV detection. In addition, the in vitro dissolution study was performed on the brands. For the test and reference formulation, mean Cmax values were 2257.3 (ng/ml) and 2156 (ng/ml), respectively. The mean AUC0-τ and AUC0-∞ were 5726.1 (ng·h/ml) and 5917.8 (ng·h/ml) for the test and 5689.9 (ng·h/ml) and 5967.4 (ng·h/ml) for the reference formulation, respectively. Results show that the 90% confidence intervals for the ratio of test and reference products in Cmax (101.4–114.9 %), AUC0-τ (96.3–109.1 %) and AUC0-∞ (94.7–107.3 %) were all within the 80–125% interval proposed by the FDA and EMA. Both formulations released >80% of drug within 30 min in buffer pH = 6.8 medium. Therefore the diclofenac sodium enteric coated tablets of the test and reference formulations are bioequivalent in terms of rate and extent of absorption.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the drugs most commonly used to reduce inflammation and pain. NSAIDs inhibit cyclooxygenase-2 at the inflammation, but unfortunately most of them inhibit gastric mucous cyclooxygenase-1, which produces gastric damage. Diclofenac sodium or, sodium [o-(2,6-dichloro-phenyl)-aminophenyl] acetate (CAS 15307-86-5) is a non-selective cyclooxygenase 1 and 2 inhibitor when tested in vitro, but a slightly preferential cyclooxygenase-2 inhibitor when tested ex vivo [1, 2]. It is approved for long term therapy in patients with osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, acute gout, following some surgical procedures and soft-tissue inflammation in dosages ranging from 75 to 150 mg/day, depending on the indication. Diclofenac sodium is well absorbed orally and dissolves in the intestinal fluid [3]. It is generally known that the drug gets into blood within 30 min and reaches the maximum blood concentration (Cmax) within 1.5–2.5 h following oral administration of an enteric coated tablet. The oral bioavailability is around 60% with an excretion half-life between 1.1 and 1.8 h [4]. Diclofenac sodium is characterized by rapid systemic clearance, which necessitates two or three daily doses [5]. There are substantial evidences that the method of manufacture and the final formulation of the drug can markedly affect the bioavailability of the drug. On the other hand there are several drug products containing the same amount of diclofenac sodium marketed by different pharmaceutical manufacturers. In fact the World Health Organization (WHO) and all drug regulat...
tory agencies support commercialization of generic medicines because they control costs and are irreplaceable therapeutic options in countries lacking the innovator product [6]. However, it is necessary to examine brand drug products in regard to in vitro dissolution and in vivo bioequivalence, and interchangeable uses with the innovator product. Therefore in the present study in vitro dissolution behavior and also the rate and extent of absorption of two commercial brands of enteric coated tablets of diclofenac sodium were determined and compared following oral administration in healthy Iranian volunteers.

2. Methods

2.1 In vitro dissolution study

A comparative in vitro dissolution study was conducted ahead of the in vivo bioequivalence studies according to the procedure described in the US Pharmacopeia. It was ensured that the in vitro dissolution data were acceptable as per the regulatory guidelines for conducting bioequivalence studies. The dissolution study was carried out on 12 units each of the test and reference formulations. The paddle rotation speed was maintained at 100 rotations per minute at 37 ± 0.5 °C. The test was carried out using 900 mL of 0.1 N HCl for 1 h followed by pH 6.8 phosphate buffer for the next hour as dissolution media. Samples of 5 ml were withdrawn at predetermined time intervals (30, 60, 60, 70, 75, 90, 105 and 120 min) and replaced with the same volume of fresh buffer. Each sample solution was filtered, diluted and the absorbance reading determined at 276 nm using a spectrophotometer (Shimadzu 160, Kyoto, Japan) against the blank. The concentrations were thereafter determined from the calibration curve. The mean dissolution values at each time interval were used to calculate the difference factor (f1) and similarity factor (f2) using the standard mathematical equations (7,8):

\[ f_1 = 50 \times \log \left( \frac{1}{n} \sum_{i=1}^{n} \left( R_i - T_i \right) ^{2} \right) ^{-0.5} \]

\[ f_2 = \left( \frac{\sum_{i=1}^{n} \left( \frac{R_i}{T_i} \right) - 1}{\sum_{i=1}^{n} \frac{1}{\left( \frac{R_i}{T_i} \right)}} \right) \times 100 \]

where n is the number of dissolution sample times, R and T are the individual or mean percent dissolved at each time point, and t stands for the reference and test dissolution profiles, respectively.

2.2 Subjects and study design

In this investigation 24 male healthy volunteers were recruited with informed consent in an open label, single-dose, randomized study with a crossover design and a washout period of two weeks between the two phases in accordance with the guidelines of the Declaration of Helsinki (World Medical Assembly 1964) as last revised in Seoul (2008). The study protocol was approved by the local ethics committee of Tabriz University of Medical Sciences, Iran. Subject selection was according to certain established criteria for inclusion into or exclusion from the study. For examples any volunteers being smokers, having allergies to the drug, being overweight or taking any other medications prior to the study were excluded. Two brands of diclofenac sodium enteric coated tablets were selected as a reference (batch no. U0011) and test (Eixir Pharmaceutical Co, Boroujerd, Iran, batch no. 059-01 06) formulations. Subjects were all Iranians and their average age and weight were 23.2 ± 1.6 years and 67.3 ± 8.6 kg, respectively. They were examined for their health conditions. Two tablets (equivalent to 25 mg diclofenac sodium) of the test and the reference formulations were randomly given to the volunteers. Seven milliliters of blood taken from subject's forearm veins serially at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 10 h after each administration. All blood samples were collected into heparinized tubes and plasma samples were separated and kept frozen at a temperature below -20 °C for subsequent analysis.

2.3 Plasma sample analysis

In different studies many methods for determination of diclofenac sodium plasma concentrations were utilized. Among them validated high-performance liquid chromatography (HPLC) either with UV [1, 9], fluorimetric [2], or electro-chemical detection [1, 3, 5, 10-17]; gas chromatography–mass spectrometry [11] and thin-layer chromatography (TLC) [5] have been reported. In this study the analytical procedure for determination of diclofenac sodium in plasma was adopted from the method of El-Sayed and co-workers and validated for specificity, accuracy, precision and sensitivity [18]. For extraction of diclofenac sodium, on 1 ml of plasma 50 μL of the internal standard (200 μg/ml of naproxen) and 200 μL of HCl 1 N was added, then all samples were extracted with 5 ml hexane/isopropyl alcohol (99/1) by vortexing for 5 min. After centrifugation for 5 min at 1000 g, the upper organic phase was transferred to a 7 ml glass evaporation tube. The tubes were placed in a 45 °C water bath. After dryness the residue reconstituted with 200 μL of the mobile phase by vortex mixing for 20 s. 150 μL of the resulting solution was injected onto the HPLC column. The mobile phase was a mixture of acetonitrile and water (45:55 % v/v), pH = 3.3 adjusted with acetic acid. The analytical column used for chromatographic separations was Shimpack C18, 5 μm (150 × 4.6 mm) with a Shim pack C18, 5 μm 4.6 × 20 mm guard column (Shimadzu, Kyoto, Japan). The flow rate was 2 ml/min and the detector wavelength was set at 282 nm. Under these conditions the retention times for diclofenac sodium and the internal standard (naproxen) were 4.2 and 2.3 min respectively. All plasma samples were measured in the same chromatographic run. Each run had a separate daily calibration. Four quality control samples with concentrations within the calibration range were used in triplicates (n = 3) to determine the accuracy and precision of the method [19, 20]. Calibration curves were obtained by plotting the diclofenac sodium to naproxen peak area ratio against the concentrations of the standard solutions.

2.4 Data analysis

The plasma concentration-time data for each volunteer was presented in a graphical form. For oral dosage form, the bioavailability of the drug is most often described by measuring the area under the plasma drug concentration-time curve (AUC), the time for peak drug concentration, T\text{\text{max}} and the peak drug concentration, C\text{\text{max}}. The area under the plasma concentration-time curve from time zero to t (AUC\text{\text{0}}-t), AUC\text{\text{0}}-t, AUC\text{\text{0-\text{\alpha}}} and (t_{1/2}) were calculated as previously described [21-28].

3. Results and discussion

3.1 In vitro dissolution study

Dissolution curves are shown in Fig. 1. From the dissolution profiles, it is clear that there were almost no drug release from both enteric coated formulations in the acidic medium as expected. However, in the phosphate buffer medium (pH 6.8), more than 80 % of drug was re-
Fig. 1: Dissolution profiles of diclofenac sodium. Open and solid circles are the data for reference and test formulations, respectively.

leaked within the first 30 min. Moreover the difference factor \( f_1 \) of 10.8 (acceptable limit 0–15) and the similarity factor \( f_2 \) of 57.7 (acceptable limit 50–100) were calculated [8]. A comparative in vitro dissolution study provides a basis for predicting the likelihood of achieving a successful in vivo bioequivalence performance. This in vitro dissolution study showed that the test and reference formulations were comparable, indicating interchangeability of the two brands.

3.2 Method validation

This analytical method was linear in the 10 to 4000 ng/ml range; with a coefficient of correlation (\( r \)) greater than 0.99 and sensitivity (LOD) of 5 ng/ml of diclofenac sodium in plasma. The recovery of diclofenac sodium was 81.54, 88.5, and 90.1% at a concentration of 20, 200, and 1000 ng/ml, respectively. Recovery values for the extraction procedure were calculated by comparing chromatographic responses obtained from spiked extracted plasma and drug free plasma samples spiked with the same concentration immediately after extraction. Recovery of the internal standard was 92%. Moreover the intra-assay accuracy of the method ranged from 97.6 to 99%, while the inter-assay accuracy ranged from 100.4 to 104.7%. A representative chromatogram of different diclofenac sodium standard concentrations in plasma samples is shown in Fig. 2.

3.3 Pharmacokinetic study

The mean concentration-time plots after administration of reference and test formulations to 24 healthy volunteers are shown in Fig. 3. In Table 1, the averages of pharmacokinetic parameters after administration of both formulations to 24 subjects are reported. The mean maximum serum concentrations of 2257.3 (ng/ml) and 2156.4 (ng/ml) were obtained for the test and reference formulation, respectively. In only three volunteers a second peak phenomenon was observed which can be attributed to diclofenac transformation to the tetrahydrate with lower solubility [29]. The respective values for \( T_{\text{max}} \) were 2.13 h and 2.21 h, and the amount of the \( AUC_0^\infty \) and \( AUC_0^\infty \) for the test formulation were 5723.1 (ng · h/ml) and 5917.8 (ng · h/ml) respectively, the obtained values for the reference formulation were 5689.9 (ng · h/ml) and 5967.4 (ng · h/ml), respectively.

Fig. 2: Representative chromatogram of different diclofenac sodium standard concentrations in plasma samples.
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3.4 Statistical analyses

Because of the variability inherent in human subjects, pharmacokinetic parameters were analyzed statistically. On the other hand, a statistical confidence interval was used to provide an estimated range that is likely to contain the mean pharmacokinetic value if the drug was given to the entire population [30, 31]. Therefore, in the present study, bioequivalence between the formulations was determined by calculating 90% confidence intervals (90% C.I.) for the ratio of \( C_{\text{max}} \), \( AUC_0^\infty \), and \( AUC_0^\infty \) values for the test and reference formulations [24, 32]. The multivariate analysis, accomplished through analysis of variance (ANOVA), was used to assess group and period effects, and values are shown in Table 2. These were all inside the acceptance limits for bioequivalence (80%–125%) and therefore the results supported the bioequivalence. On the basis of FDA and EMA guidelines, the recently accepted criterion for bioequivalence for most dosage forms requires that the mean pharmacokinetic parameters of the test dosage form should be within 80% to 125% of the reference dosage form using the 90% confidence interval. Thus, from the results obtained it is evident that the diclofenac sodium enteric coated tablets of test and reference formulations are bioequivalent in terms of rate and extent of absorption. Therefore, they are expected to have the same safety and efficacy profiles when administered under the conditions listed in the product labeling.

4. Conclusion

The in vitro dissolution study indicated suitability of the test formulation for use in the in vivo bioequivalence studies. The in vivo studies in healthy human subjects demonstrated that the generic enteric coated test tablet, diclofenac sodium 25 mg, is bioequivalent to the reference formula in terms of rate and extent of absorption.

Conflict of Interest

This study was sponsored by Exir Pharmaceutical Co, Boroujerd, Iran. The authors report no conflicts of interest in this work.
References


