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Original Article

Bioequivalence Studies of Two Formulations of Baclofen Tablet in Healthy Volunteers

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Abstract

The relative bioavailability of the test (generic) product 2 × 25 mg baclofen tablets, with respect to the reference product, Lioresal® 2 × 25 mg tablets (baclofen; Squibb) was determined in a single-blind, single dose, randomised, crossover study. The mean values for the variable C_{max} were 737.6 ng/ml for the reference and 739.5 ng/ml for the test product. The mean values for the variable AUC were 3980.3 hr.ng/ml and 4066.7 ng.hr/ml for the reference and test, respectively. The 90% confidence intervals for the "test/reference" mean ratios of the plasma baclofen pharmacokinetic variables C_{max} and AUC_{0-t} (as measures of the rate and extent of absorption of baclofen, respectively) lie between 0.98 and 1.06, which is within the conventional bioequivalence range of 80-125%. The test product (baclofen) is therefore bioequivalent to the reference product (Lioresal®) with respect to the rate and the extent of absorption of baclofen with a strength of 25 mg.

Keywords: Baclofen tablet; Bioequivalence study; Plasma; AUC; C_{max}.

Introduction

Baclofen is used as a skeletal muscle relaxant, acting centrally by inhibiting transmission of reflexes at spinal level, possibly by action at primary afferent fiber terminals resulting in the relief of muscle spasticity. It is also used in the treatment of reversible spasticity, resulting from multiple sclerosis (1). Baclofen is rapidly absorbed from gastro-intestinal tract and the peak plasma concentration is achieved within about 2 h. It is largely excreted in the urine, 80% as unchanged drug and, the rest as metabolites. The elimination half-life has been reported to be 3 h. The bioavailability as well as the clinical pharmacokinetics of baclofen has been reported in previous studies (2, 3).

Bioequivalency studies on generic drug products manufactured in Iran have been conducted by Ministry of Health and Medical Education to assure a high quality of available drug products within the Iranian drug market. The aim of this work, as a part of a general study on the Iranian generic products, was to compare the bioavailability of a baclofen formulation containing 25 mg baclofen, with a reference product of the same strength, in healthy volunteers. Formulation parameters were accounted for by carrying out in vitro characterization of the dosage forms.

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Experimental

Materials and instrumentation

Baclofen and propylthiouracil were supplied by food and drug central laboratories (Tehran, Iran). HPLC-grade acetonitrile and all other chemicals were obtained from Merck (Darmstadt, Germany). Water was obtained by double distillation and purified additionally with a Milli-Q system

A Knauer HPLC system (Germany) employed consisted of Wellchrom K-1001 pump, Rheodyne 7125 injector and K 2600 UV detector connected to Eurochrom 2000 integrator.

In vitro characterization

Weight variation, content uniformity and assay of active ingredient were conducted on 20 tablets of each brand, in accordance to the United States Pharmacopoeia (4), as well as a test procedure for baclofen tablets (5). The dissolution profiles of test and reference baclofen tablets were determined based on the United States pharmacopoeia (4). In here 5 ml samples were removed at intervals of 0, 5, 10, 20, 30 and 40 minutes. Samples were assayed by HPLC at 265 nm. The amount of baclofen dissolved at each time interval was calculated using a calibration curve which had been prepared on the same day of study.

In Vivo Studies

Study Protocol

Twelve healthy, non-smoking, male volunteers (aged 23-42 years, weight 67-85 kg, height 169-180 cm) took part in this study. All the volunteers gave written informed consent after they had received detailed instructions about the aims, restrictions and possible adverse effect which could be experienced as a result of taking the drug. All volunteers were found to be in good physical health, based on physical examinations, hematological and urinary laboratory tests carried out. Subjects did not take any other medications for at least 2 weeks prior to and throughout the entire study. Each subject was fasted overnight prior to the experiment, and food was withheld for 4 h after dosing. Tablets were swallowed with 150 ml of water. A standard lunch was given to all subjects 4 h after dosing. A washout period of 1 week was included between the administration of each product.

Blood Sampling

Blood samples (5 ml) were taken *via* an indwelling venous cannula according to the following time schedule: before drug administration (0 h), and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, 9, and 12 hours after drug administration. Within 10 minutes of collection, blood samples were centrifuged at 3000 rpm for 9-11 min and after clot retraction from each sample; two aliquots of plasma were transferred to labeled tubes. All samples were handled at room temperature before storage at –20ºC, pending baclofen assays.

Assay Performance

Analysis was performed using a modified reversed-phase high-performance liquid chromatographic method reported by Rustum (5). To 0.6 ml plasma in a 5 ml glass tube was added 30 µl water containing propyl thiouracil (100 µg/ml) as the internal standard, and mixed with 900 µl of acetonitrile and saturated salt. The mixture was then vortexed for 30 sec. Next, samples were centrifuged for 10 min and 500 µl of aliquots obtained were taken and evaporated to dryness under nitrogen. The residue was reconstituted in 60 µl acetonitrile, of which 50 µl was injected onto the HPLC column (Knauer, Nucleosil C18, 4 µm, 250 mm × 4.6 mmID). Detection was made by UV detector at 205 nm. The analytical method was fully validated in terms of intra and inter-day variations assessment, linearity, accuracy and the percent age of recovery, according to USP guidelines (4).

Pharmacokinetic Variables

To compare the rate and extent of absorption of baclofen in both studies, the following pharmacokinetic variables were calculated for each volunteer and product, using the actual blood sampling times. The area under the plasma concentration curves (AUC_{0-t}) were calculated with the linear trapezoidal rule. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were obtained directly from the plasma-concentration data. The $AUC_{0-\infty}$ was calculated by dividing the last measured concentration (C_t) by the elimination rate constant and adding the result to the AUC_{0-t}. The elimination rate constant was calculated by least-squares regression, using the last points of each curve.

Statistical Analysis

The test and reference treatments of each study were compared with respect to relevant pharmacokinetic variables using an analysis of variance, with volunteer, product and period effects after a logarithmic transformation of the data. Point estimates and 90% confidence intervals (CI) for the "test/reference" mean ratios of these variables were calculated. Bioequivalence of the test and reference product was assessed on the basis of these CIs, in relation to the conventional bioequivalence range of 80-125%.

Results and Discussion

All products met the pharmacopoeial specifications for weight variation, content assay,

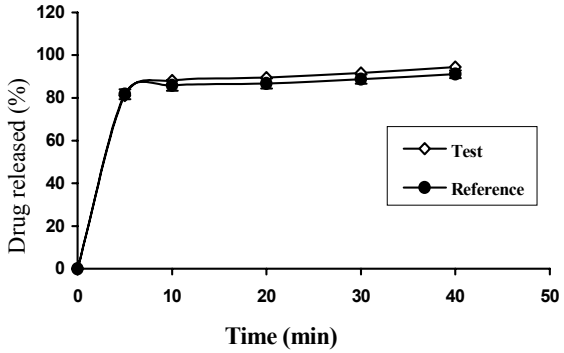

Figure 1. In vitro dissolution profiles of the Lioresal® (reference) and test baclofen tablets 25 mg tablets in water at 37°C (n=3, mean ± SD).

and content uniformity assay. Dissolution behaviors of the two brands studied are shown in Figure 1. All tablets met the United States Pharmacopeia dissolution specifications (4), which indicates that not less than 80% (Q=75%) of the labeled amount of baclofen should be dissolved in 30 min. The analytical method used in this study was found to be linear over a range of 50-1200 ng/ml of drug in plasma. Between-day coefficient of variation (CV) determined from quality control samples processed together with each batch of samples run, were between 4.9% and 11.9% for concentrations ranging between 50 ng/ml, and 800 ng/ml and the accuracy was 96.9-99.2%. Pharmacokinetic variables obtained from this study are summarized in Table 1. The mean plasma baclofen concentrations are represented in Figure 2.

In this study, the mean values for the variable C_{max} were 737.6 ng/ml for reference and 739.5 ng/ml for the test product. The mean values for the variable AUC were 3980.3 hr.ng/ml and 4066.7 hr.ng/ml for the reference and test, respectively. The point estimates (90% CI) of the "test/reference" mean ratio for C_{max}, AUC_{0-t} and T_{max} were 100.7% (97.7-104.1%), 102.3% (99.2-105.8%), and 106.5% (100.3-113.6%).

Table 1. Pharmacokinetic data for baclofen (dose 2 × 25 mg baclofen tablets; n= 12)

	Cmax (ng/ml)	Tmax (hr)	AUC0-t (ng.hr/ml)	AUC0-∞ (ng.hr/ml)
Baclofen (Test)				
Mean	739.5	2	4066.7	4416.0
SD	120.5	0.4	556.0	601.8
RSD (%)	16.3	18.5	13.7	13.6
Lioresal (Ref.)				
Mean	737.6	1.9	3980.3	4280.0
SD	139.5	0.3	573.7	601.8
RSD (%)	18.9	16.58	14.4	14.1

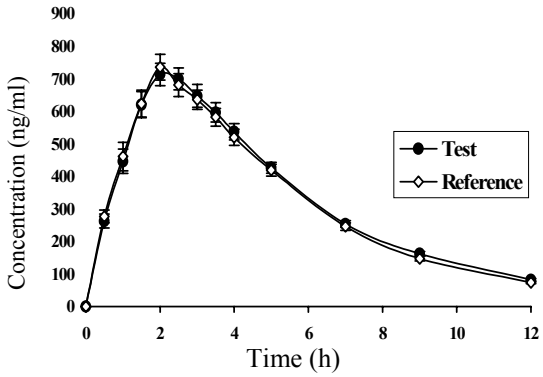

Figure2. Mean Plasma concentration of baclofen obtained from the administration of 2 × 25 mg test and reference (Lioresal®) tablets to 12 healthyvolunteer.

In conclusion, concerning the relative bioavailability of the respective test and reference products, the 90% CIs for the "test/reference" mean ratios of the plasma baclofen pharmacokinetic variables C_{max}, AUC_{0-t} and T_{max} (as measures of the rate and extend of absorption of baclofen, respectively) all fall within the conventional bioequivalence range of 80-125%. The test product (baclofen) is therefore bioequivalent to the reference product (Loiresal) with respect to the rate and extent of absorption of baclofen for 25 mg tablets.

Acknowledgement

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