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RESEARCH PAPER

Comparative Bioavailability of Diltiazem in Prolonged-Release Oral Preparations

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ABSTRACT

This study was conducted to compare the bioavailability of two prolonged-release pharmaceutical forms containing 300 mg of diltiazem. The test formulation is a new design of tablets with a hydrophilic matrix, and the reference formulation is capsules containing prolonged liberation microgranules, in the same dose, that are commercially available in the pharmaceutical market. Diltiazem plasma concentrations were analyzed by high-performance liquid chromatography (HPLC), which involves solid-phase extraction for plasma sample preparation. Twelve healthy volunteers participated in the study, which had a single-dose, two-treatment, two-sequence-crossover, randomized design. The preparations were compared using pharmacokinetic parameters such as the area under the plasma concentration-time curve $AUC_{(0-36)}$*, peak plasma concentration* C_{max}*, and* $C_{max}/AUC_{(0-36)}$ *ratio as a measure for the absorption rate. No statistically significant difference was observed for any of the parameters, and the 90% confidence intervals calculated for the ratio of the logarithmically transformed* $AUC_{(0-36)}$ *and* $C_{max}/AUC_{(0-36)}$ *values of both formulations were within the bioequivalence limit of 0.80–1.25. Moreover, an in vitro study of dissolution according to USP 23 was conducted, and the in vitro parameters were calculated.*

Key Words: *Bioavailability; Bioequivalence; Diltiazem; Matrix hydrophilic*

INTRODUCTION

Diltiazem, a drug belonging to the benzothiazepine group, possesses pharmacological effects that are related to its ability to inhibit the influx of calcium ions during membrane depolarization in vascular and cardiac smooth muscle (1,2). The drug is effective as an antiarrythmic and antihypertensive agent in sustained-release formulations of 120–240 mg, when it is administered in a single daily dose (3,4). Diltiazem has a hepatic first step, which decreases the oral bioavailability by approximately 50%; it is saturated partially with the increase of levels of drug in plasma (5). Therefore, its bioavailability is improved with designed formulations containing 300 mg.

In this article, the bioequivalence of two prolonged-release pharmaceutical forms containing 300 mg of diltiazem hydrochloride was evaluated using a high-performance liquid chromatography–ultraviolet (HPLC-UV) method previously developed (6). The test formulation is a new design of prolonged-release tablets with 300 mg of diltiazem that utilizes hydrophilic matrices (7,8), and the reference formulation capsule contains prolonged liberation microgranules in the same dose, which are commercially available in the pharmaceutical market.

The hydrophilic matrix used is hydroxypropylmethylcellulose 2208 (Methocel K-100); its advantage over prolonged liberation microgranules lies on its high resistance to release inconsistencies and drug "dumping" since it is a relatively simple system that can put up with variations in ingredients, production methods, and use conditions. Matrix systems are also relatively easy to formulate. Tablets are manufactured with existing conventional equipment and processing methods and, therefore, are more economical.

EXPERIMENTAL

Materials

Two marketed prolonged-release oral formulations containing 300 mg of diltiazem hydrochloride were studied. They were designated as the test formulation (tablets) and the reference formulation (capsules). The diltiazem hydrochloride standard was a gift from Bagó Laboratory (Argentina).

The following chemicals and solvents were chromatographic grade: methanol, acetonitrile, triethylamine, glacial acetic acid, ammonium acetate (J. T. Baker, Phillipsburg, USA). Millipore membranes, type HV, with diameters of 50 and 13 mm and a pore size of 0.45 μm (Millipore, Bedford, MA) were used to filter the mobile phase and the samples. The samples were prepared using solid-phase extraction columns containing 100 mg of sorbent RP 18 (Varian).

Equipment

The chromatographic system consisted of a Konik KNK 500G chromatograph with a double-piston serial pump, equipped with a programmer for the KNK 029-375 microprocessor (Konik, Barcelona, Spain), a Rheodyne 7125 sample injector with a fixed loop of 20 μl capability (Rheodyne, Cotati, CA), and a KNK 029-254 helium bubbling degasificator. In addition, a Lichrocart RP-18 reversed-phase column (125 mm × 4 mm i.d., particle size 5 μm) was used (Merck, Darmstadt, Germany). A variable wavelength UV-visible detector, model 204 (Linear, Nevada, USA), was used, and the integrator was a Datajet model SP 4600 (Spectra Physics, San José, CA). For the preparation of samples, a Socorex 100–1000 μl micropipette was used (Socorex, Switzerland). Drugs and reagents were weighed on a Mettler Toledo AG 204 balance (Metler, Greifensee, Switzerland), and dissolution profiles were obtained using Sotax AT7 dissolution equipment (Sotax AG, Basel, Switzerland). A Waters vacuum manifold for solid-phase extraction (Waters Corporation, Milford, MA) was also used.

Chromatographic Conditions

The mobile phase consisted of a mixture of methanol, 0.04 M ammonium acetate, and acetonitrile (29:38:23) and 0.04% triethylamine (pH 7.4) adjusted with glacial acetic acid, filtered, and deaerated prior to use.

The HPLC-UV system was operated isocratically at ambient temperature with a flow rate of 1.2 ml/min. The detector wavelength was set at 240 nm, attenuation 8, and chart speed 0.25 using height peak (HP) as the integration parameter.

Standards Preparation

Diltiazem hydrochloride 25 mg was dissolved in 25 ml of methanol and the solutions for the calibration curve were obtained by serial dilutions with distilled water. These solutions were added to 2 ml of drug-free human plasma, obtaining different concentrations in plasma (25, 32, 80, 100, 160 ng/ml).

Extraction Procedures

The samples were submitted to solid-phase extraction to eliminate the interferences of the plasma, to increase the sensitivity of the method, and to concentrate them. This process was appropriately validated for two concentration levels of diltiazem, and the system consisted of a column with 100 mg of RP-18 sorbent and an extraction camera for vacuum. The steps of the extraction procedure were as follows: The columns were activated by washing them with two 3-ml portions of acetonitrile, followed by two 3-ml portions of 0.04 M ammonium acetate. Then, 2 ml plasma samples were slowly passed through the columns to obtain a bigger interaction with the filler and to optimize the cleaning of the sample. The columns were washed with a 3-ml portion of methanol:water (20:80 v/v). At this point, the columns were air dried for 3 min. The eluates corresponding to each step were injected into the chromatograph to verify extraction specificity. The compound of interest was eluted with 500 μl of methanol; the aliquot was injected into the HPLC system twice. During the in vivo study, we applied the same extraction process to the predose (T_0) blood samples to check the noninterference of endogenous substances.

Analytical Method Validation

The analytical method was validated by evaluating linearity through the calibration curve obtained with five concentration levels (25, 32, 80, 100, 160 ng/ml) of standard diltiazem hydrochloride in drug-free human plasma. By means of analysis of linear regression, the correlation coefficient, the intercept, and the slope were calculated. To confirm the adjustment to the linear pattern, an analysis of the response factor (relation between the signal and the concentration), a residual analysis and a Bartlett's test for determining the overall data homoscedasticity were carried out. The limit of quantification (LOQ) was 20 ng/ml.

The precision of the system was calculated as the coefficient of variation (CV) of six injections of the same standard solution of diltiazem in drug-free human plasma. The precision of the method was evaluated as the CV for five samples of the same concentration of standard diltiazem hydrochloride in drug-free human plasma. The extraction efficiency of the method was determined by comparing the height peak obtained by injection of standard diltiazem hydrochloride solutions prepared with methanol into the chromatograph to those obtained after the plasma extraction procedure; it is expressed as the absolute recovered percentage of four samples of drug-free human plasma containing 25 and 160 ng/ml. The stability of diltiazem in drug-free human plasma under experimental conditions was investigated. The influence of two freeze-thaw cycles at three concentration levels (25, 160, 400 ng/ml) was examined twice. The height peaks obtained for each concentration after the first and second cycles were related.

In Vitro Dissolution Study

Dissolution testing was conducted on six individual dosages of the test and reference products from the same lots used in the in vivo bioequivalence study. Testing was according to the USP 23 (9) method for diltiazem hydrochloride extended-release capsules for products labeled for dosing every 24 h; we used the paddle method, employing water at 37°C as the dissolution medium and an agitation speed of 100 rpm. At 1, 4, 10, and 15 h, 5-ml samples were obtained and replaced by the same quantity of the release medium.

The samples were centrifuged at 3500 rpm for 15 min and analyzed by UV at 237 nm. The diltiazem dissolved at each time interval was expressed as a percentage of the labeled dose.

In Vivo Study

Twelve healthy volunteers who were nonsmokers, with no alcoholism antecedents, and without hypersensibility to the drug or chemically related substances were selected for this study; they were both sexes (5 females and 7 males), and ages ranged from 20 to 50 years. At admission, a complete clinical

examination consisting of a routine blood test, an electrocardiogram, immunologic controls, and a pregnancy test was carried out. No other drugs were allowed from at least 96 h before the study, and neither alcohol nor beverages containing xanthines were consumed for 48 h prior to each study period and until the last blood sample was collected.

Each subject gave written consent to confirm that participation was voluntary.

After an overnight fast (at least 12 h), subjects received a single dose (300 mg) of the test product or the reference product with 200 ml of water. Blood samples were taken at 0 h (predose) and 1, 2, 4, 6, 8, 10, 12, 14, 18, 21, 24, and 36 h postdose. Plasma, separated by centrifugation (1500 rpm) from whole blood, was frozen at –20°C until the time of analysis.

During the study, the subjects were cardiologically examined for safety reasons: Blood pressure and heart rate were measured at 0 h (predose) and 1, 4, 8, 18, and 24 h after morning treatment. Furthermore, electrocardiograms were recorded at 0, 2, 6, and 24 h after intake of the formulations. The subjects received a protein-standardized, low-fat diet to standardize food intake and avoid possible nondesired interactions between the food intake and the formulations. The study design was a single-dose, two-treatment, and two-sequence crossover with a washout period of at least 15 days. Subjects were randomly assigned to the two dosing sequences.

In Vitro Parameters

The following dissolution parameters were obtained from the dissolved percentage for each dosage unit tested at each time interval: dissolution constant *Kd*, time in which 50% of the labeled drug is dissolved $t_{50\%}$, efficiency of the dissolution (ED%) (10), mean dissolution time (MDT) (11).

In Vivo Parameters

The following pharmacokinetic parameters were obtained from individual plasma profiles: area under the curve from T_0 to the last sample $ABC_{(0–36)}$, area under the curve from T_0 to infinity $ABC_{(0–\infty)}$, plasma maximum concentration of diltiazem C_{max}, $C_{max}/ABC_{(0–36)}$ ratio as a measure for the absorption rate (12–14), time required to reach the maximum concentration T_{max}, elimination constant of first order K_e, time of half-life $t_{1/2}$, and mean residence time (MRT) (15,16).

Statistical Analysis

Differences in the height peaks obtained in the freeze-thaw cycles of plasma and dissolution kinetics parameters were analyzed using the Student *t* test. Differences in pharmacokinetic parameters were analyzed using analysis of variance (ANOVA) to $ABC_{(0–36)}$, $ABC_{(0–\infty)}$, $C_{max}/ABC_{(0–36)}$, *Ke*, and $t_{1/2}$, and Wilcoxon test to T_{max} and MRT for paired data. The 90% confidence interval based on the test of the interval hypothesis (17) for C_{max}, $AUC_{(0–36)}$, and $C_{max}/AUC_{(0\text{-}36)}$ was performed to establish bioequivalence criteria. The values were logarithmically transformed prior to statistical analysis.

RESULTS AND DISCUSSION

Typical chromatograms obtained from blank plasma, blank plasma spiked with standard diltiazem hydrochloride (45 ng/ml), and plasma of a volunteer 6 and 24 h after the administration of diltiazem are represented in Fig. 1. Blank human plasma indicated noninterfering peaks.

A linear response was observed for the analytical method developed for diltiazem, in a concentration range of 25–160 ng/ml, with a coefficient of correlation $r = 0.9888$. The intercept a and the slope b with the respective confidence intervals of 95% were $a = 0.9266 \pm 0.86$, $b = 0.0803 \pm 0.009$.

To confirm the regression analysis and the linearity, an analysis of the response factor was carried out, which was maintained constant along the range of concentrations of the calibration curve. The analysis of residuals followed an aleatory pattern of distribution along the range of concentrations of the calibration curve. In the same sense with the Bartlett test, differences between observed and critical values evaluated by means of the χ^2 test were not significant ($P > .05$). These analyses confirmed that the model used for this analytical method was linear.

The system precision was 0.99%, and the method precision was 2.5%.

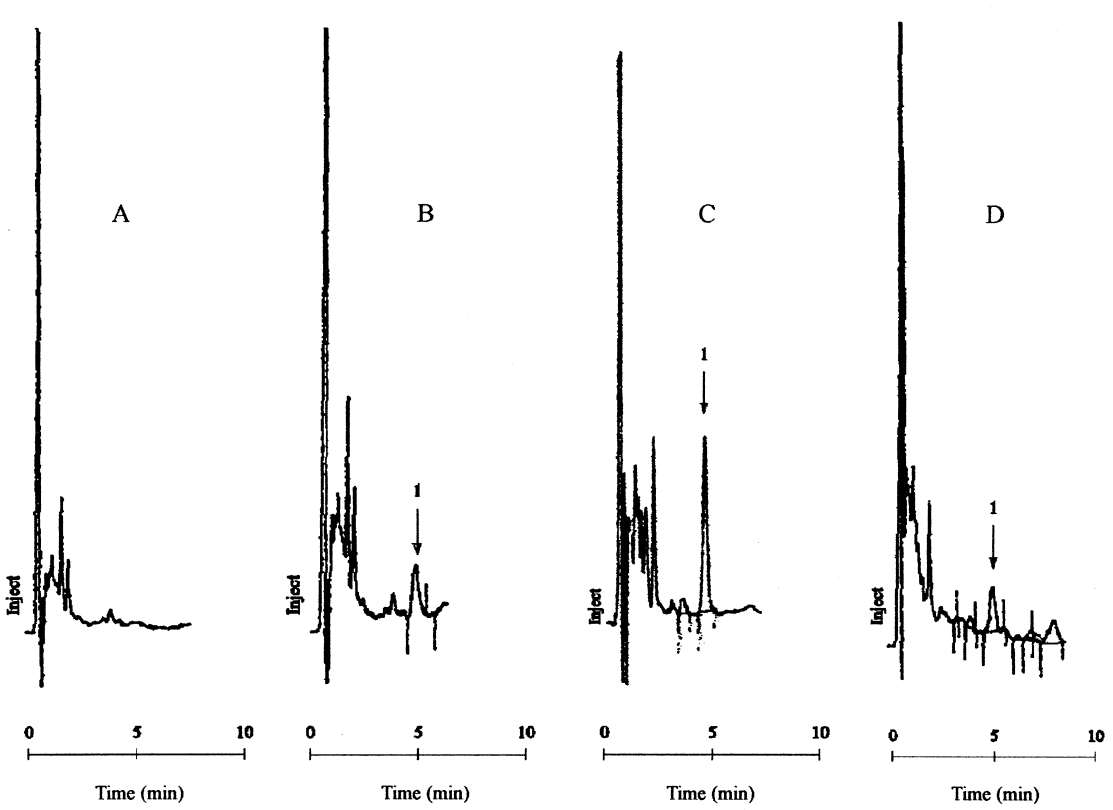

Figure 1. Typical HPLC chromatograms of diltiazem (1). (A) Plasma from a volunteer before a 300-mg oral dose of diltiazem; (B) plasma spiked with standard diltiazem (45 ng/ml); (C) plasma from a volunteer 6 h after the administration of a 300-mg oral dose of diltiazem; (D) plasma from a volunteer 24 h after the administration of a 300-mg oral dose of diltiazem.

The absolute recovery was about 95% ± 2.05% (standard error of the mean, SEM) independent of the concentration.

As no appreciable differences were observed between two freeze-thaw cycles ($P > .01$) (Table 1), it was concluded that these cycles could be tolerated by diltiazem.

The results obtained in the dissolution for both formulations are presented in Table 2, and they show that the formulations comply with the test as the individual percentage obtained at the times specified (1, 4, 10, 15 h) and conform to the Acceptance Table Under Drug Release Test <724> of USP 23 (9). Kinetics dissolution parameters are shown in Table 3. There were no significant differences ($P > .05$) for all parameters.

Table 1

Freeze-Thaw Stability of Diltiazem in Human Plasma

Concentration (μg/ml)	HP Mean 1st Cycle of Freezing	HP Mean 2nd Cycle of Freezing	Ratio[a]
0.025	145 ± 1.3	159 ± 0.9[b]	1.10
0.16	811 ± 4.3	903 ± 4.5[b]	1.11
0.4	2855 ± 8.5	3035 ± 9.1[b]	1.06

Each value represents the mean ± SEM ($N = 3$).
HP, height peaks.
[a]Ratio of HP mean between both cycles.
[b]No statistical difference ($P > 0.01$).

Table 2

Mean Percentages of the Labeled Amount of Diltiazem Dissolved

Time (h)	Reference	Test
0	0	0
1	14.39 ± 0.91	18.10 ± 0.27
4	29.56 ± 1.20	43.32 ± 0.33
10	75.14 ± 1.10	69.43 ± 0.46
15	94.57 ± 1.08	89.23 ± 0.54

Each value represents the mean $\pm$ SEM ($N = 6$).

Table 3

Kinetics Parameters of Dissolution

Formulation	Kd[a] (h^{-1})	$t_{50\%}$[b] (h)	% ED[c]	MDT[d] (h)
Reference	0.109 ± 0.002	6.6 ± 0.08	54.09 ± 0.93	6.42 ± 0.07
Test	0.126 ± 0.002	5.48 ± 0.09	55.74 ± 0.23	5.63 ± 0.06

Each value represents the mean $\pm$ SEM ($N = 6$).
[a]Dissolution constant.
[b]Time in which 50% of the amount of the labeled drug is dissolved.
[c]Efficiency of the dissolution.
[d]Mean dissolution time.

Figure 2 shows the mean plus or minus the SEM plasma concentration-time profile for both formulations. We did not find differences due to the age or sex of different volunteers when checking their blood levels of diltiazem.

Pharmacokinetic parameters calculated for both formulations are shown in Table 4. There were no significant differences ($P > .05$) for any of the parameters except T_{max} ($P < .05$). Results obtained for C_{max} may be due to one of the pharmacokinetic

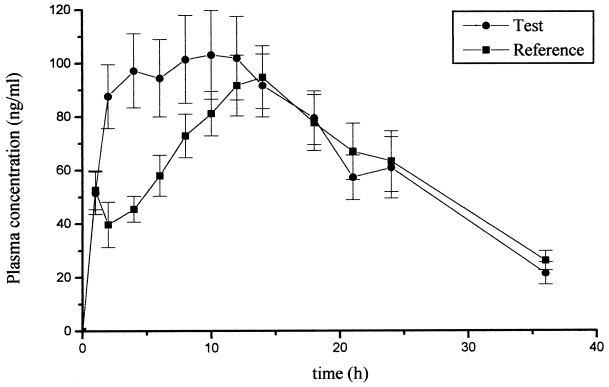

Figure 2. Mean plasma ($N = 12$) concentration-time profiles for both formulations.

Table 4

Pharmacokinetic Parameters in Twelve Healthy Volunteers After a Single 300-mg Oral Dose of Diltiazem

Parameters	Test	Reference
$AUC_{(0-36)}$[a]	2449 ± 348.68	2226 ± 270.63
$AUC_{(0-\infty)}$[b]	2854 ± 406.75	2731 ± 370.58
$C_{max}/AUC_{(0-36)}$[c]	$0.049 \pm 1.82 \times 10^{-3}$	$0.046 \pm 2.81 \times 10^{-3}$
C_{max}[d]	120.23 ± 17	101.7 ± 11.43
T_{max}[e]	8 ± 0.88	13.16 ± 1.05[f]
$t_{1/2}$[g]	11.73 ± 1.95	11.61 ± 1.36
Ke[h]	$0.073 \pm 8.8 \times 10^{-3}$	$0.068 \pm 6.69 \times 10^{-3}$
MRT[i]	14.83 ± 0.352	16.35 ± 0.375

Each value represents the mean $\pm$ SEM ($N = 12$).
[a]Area under the curve between the zero hour and the last sample (h·ng/ml).
[b]Area under the curve between the zero hour and infinity (h·ng/ml).
[c]$C_{max}/AUC_{(0-36)}$ ratio (h).
[d]Plasma maximum concentration of diltiazem (ng/ml).
[e]Time of maximum concentration (h).
[f]Statistical difference with Wilcoxon test ($P < .05$).
[g]Time of half life (h).
[h]Elimination constant of first order (h^{-1}).
[i]Mean residence time (h).

characteristics of diltiazem that shows large interindividual variability in plasma concentrations. It is recognized that a drug that undergoes extensive first-pass elimination has a wide interindividual variability in C_{max} and AUC due to the individual variability in the functional status of the hepatic metabolic enzymes (5). For this reason, we used $C_{max}/AUC_{(0-36)}$ ratio as a more unambiguous measure of the absorption rate than C_{max} (12).

The 90% confidence intervals were as follows: 0.72–1.04 for C_{max}, 0.80–1.15 for $AUC_{(0-36)}$, and 0.82–1.05 for $C_{max}/AUC_{(0-36)}$. Considering the bioequivalence limit between 0.80 and 1.25 for the International Organization Guidances, the confidence nterval for $AUC_{(0-36)}$ reflects the extent of drug absorption and complies with this specification. The confidence interval obtained for $C_{max}/AUC_{(0-36)}$ is inside the tolerance region. Therefore, it is possible to accept these intervals as indicative of bioequivalence for both formulations, although the inferior limit of the confidence interval for C_{max} is lower than the inferior limit (0.80) of the tolerance region for a narrow margin.

A good correlation between the in vitro parameters and the percentages absorbed, obtained with the Wagner-Nelson (18) method, was established.

The present study demonstrates that the behavior of this new design containing 300 mg diltiazem in a hydrophilic matrix does not present significant differences against the behavior of the reference formulation either in vitro or in vivo. It can be concluded that both formulations are comparable in both the rate and the extent of absorption, which indicates that the tablets are bioequivalent to the capsules. This suggests the possibility of using one or the other formulation during a dosage regimen without changing the therapeutic results desired; therefore, both formulations can be safely used.

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