PHARMACOKINETICS AND COMPARATIVE BIOAVAILABILITY OF TWO DILTIAZEM TABLET FORMULATIONS IN HEALTHY VOLUNTEERS

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ABSTRACT

The pharmacokinetic parameters and bioavailability of diltiazem following a single oral administration of a generic diltiazem 60 mg tablet (Sobhan Pharmaceuticals, Iran) were compared to those of a reference product (Entrydil, Orion Pharmaceuticals, Finland). Twelve healthy male volunteers received a single oral dose of either formulation following overnight fasting in a double blind, randomized, crossover study. Blood samples were collected at selected times during 24 h and diltiazem plasma concentrations were determined with a sensitive HPLC method. Individual pharmacokinetic parameters, $t_{1/2}$, $t_{1/2(abs)}$, K, K_a, T_{max}, C_{max}, Vd/F, Cl/F, AUC_{0-24} and AUC_{0-} were calculated. No significant differences were observed in pharmacokinetic parameters between two formulations. The 90% confidence intervals for the test/reference geometric mean ratios of C_{max}, AUC_{0-24} AUC_{0-} and C_{max}/AUC_{0-} were within the conventional bioequivalence range of 0.8 - 1.25.

In-vitro parameters of mean dissolution time (MDT) and time for 70 % dissolution (T_{70}) were also determined. There was a significant difference between the MDT for two dosage forms ($p<0.0001$). It was concluded that despite of a higher dissolution rate, the test product of diltiazem is bioequivalent to the reference product with respect to the rate and extent of absorption.

Keywords: Bioequivalence, Diltiazem, Dissolution, Pharmacokinetics

INTRODUCTION

Diltiazem is a slow calcium channel blocking agent which acts by interfering with calcium-mediated events in excitation contraction coupling in smooth muscles, particularly arteries and is commonly used in treatment of mild or moderate hypertension and angina (1,2).

Because of the extensive first-pass hepatic metabolism, only 30-40% of diltiazem is available after a single oral dose (3,4). Several factors may contribute to alter the absorption rate of diltiazem. The formulation is of prime importance, since it has been established that the time required reaching the maximal plasma concentration after taking diltiazem with aqueous solutions or as capsules and tablets is 40, 60 and 180 min, respectively (5).

The purpose of this study was to compare in-vitro dissolution and in-vivo bioavalability and pharmacokinetic profiles of diltiazem of a new tablet formulation with a commercial product.

METHODS

In-vitro study

Dissolution data were obtained on 12 tablets of each product using rotating paddles at 75 rpm according to the specifications of the USP XXIV (apparatus II).

The dissolution medium was 900 ml of water at 37°C. Aliquots (5 ml) were taken for analysis at 5 min intervals for 240 min and measured spectrophotometricaly at 240 nm.

The mean dissolution time, MDT, was calculated by moment analysis as described previously (6). The time required for 70% dissolution, T_{70}, was calculated using the Hixon-Crowell cube-root law as applied previously to compressed tablet formulations (7)

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In-vivo study

Subjects

Twelve healthy male volunteers with a mean age of 26.5±1.9 years and a mean weight of 68.4±7.4 kg participated in the study after giving written informed consent. All volunteers had normal examination and clinical laboratory test results, and all were not on any medications for at least two weeks prior to and during the period of the study.

Drug administration and blood sampling

The study was conducted as a double blind, randomized, crossover design in which fasting subjects took a single oral of 120 mg diltiazem hydrochloride (as two tablets) of either test product (Diltiazem 60, Sobhan laboratories, Iran) or reference product (Entrydil 60, Orion pharmaceutical, Finland) with 150 ml of tap water in each period of study.

Blood samples (5 ml) were drawn just before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after drug administration. Samples were centrifuged at 3000 rpm for 10 min and plasma samples were stored at -20°C until analysis.

Sample analysis

Plasma diltiazem concentrations were measured by a validated high performance liquid chromatography (HPLC) method (8). To 1 mL of plasma were added 20 µl of aqueous solution of imipramine as internal standard (10 $\mu g mL^{-1}$) and 4 mL of the solution of n-hexane-ether (50:50 V/V). After vortex mixing for 5 min and centrifugation at 4000 rpm for 15 min the organic phase was separated and acidified with 200 µl of 0.01 M hydrochloric acid. The mixture was vortex-mixed for 5 min and after centrifugation the organic phase was discarded and 50 µl of aqueous phase was injected onto the column. The mobile phase was a mixture of 0.04 M ammonium chloride-acetonitrile-triethyl amine (35:35:30:0.05) which was pumped at a rate of 1.6 mL/min through a µ-Bondapak C18 column and peaks were detected at 237 nm.

Pharmacokinetic analysis

Diltiazem pharmacokinetic parameters were determined by non compartmental methods. Elimination rate constant (K) were estimated by the least-square regression of plasma concentration-time data points lying in the terminal log-linear region of the curves. Half-life was calculated as 0.693 divided by K. The area under the plasma concentration-time curve from time zero to the last measurable concentration at time T (AUC_{0-T}) was calculated using the trapezoidal rule. The area was extrapolated to infinity (AUC_{0-}) by addition of C_T/K to AUC_{0-T} where C_T is the last detectable drug concentration. Peak plasma concentration (C_{max}) and time to peak concentration (T_{max}) were determined by inspection of the individual subject concentration time curves. The relative bioavailability of the test formulation was estimated as the AUC_{0-} ratio of the test to the reference formulation. The Wagner-Nelson method was used to estimate fractional absorption (9). Fractional absorbed data for each subject and treatment were used for estimation of the apparent absorption rate constant (K_a). Absorption half-life ($t_{1/2\ (abs)}$) calculated by $0.693/K_a$.

Apparent oral clearance (Cl/F) and apparent volume of distribution (Vd/F) were calculated by equations 1 and 2 respectively:

$$Cl/F = Dose/AUC_{0-} \quad (1)$$

$$Vd/F = Cl/F.K \quad (2).$$

Statistical analysis

The in-vitro dissolution data were compared by two-tailed student's t-test. Logarithmic transformation of AUC_{0-24}, AUC_{0-}, C_{max}, C_{max}/AUC_{0-} and $t_{1/2}$ were compared by analysis of variance for a crossover design followed by 90% confidence interval test for geometric mean of test/reference individual ratios for each parameter. Arithmetic mean for individual T_{max} differences together with its parametric (ANOVA) 90% CI was also determined. Statistical significance was defined as $p<0.05$.

RESULTS

The bioequivalence of 2 diltiazem formulations following a single oral administration of 120 mg using a randomized 2-way crossover design was investigated. According to in-vitro dissolution data diltiazem was released faster from reference product which exhibited a MDT of 33.6 ± 2.24 min compared with 54.3 ± 3.79 min of the product (Table 1). Statistical analysis showed a significant difference between the

MDT for two dosage forms ($p<0.0001$). Diltiazem were well tolerated following administration of a single oral dose of the test and reference products. No adverse effects were reported and no significant effects on heart rate as well as systolic and diastolic arterial pressure were observed at any time with respect to the basal value.

Table 1. Comparative mean in-vitro dissolution data (± SD) for two diltiazem products (n =12)

Parameter	Product		t-test
	Test	Reference	
MDT (min)	54.30 ± 3.79	33.60 ± 2.24	P < 0.0001
T_{70} (min)	92.88 ± 2.88	96.23 ± 3.15	NS

SD= Standard deviation, MDT= Mean dissolution time, T_{70}= Time for 70% dissolution, NS= Non significant

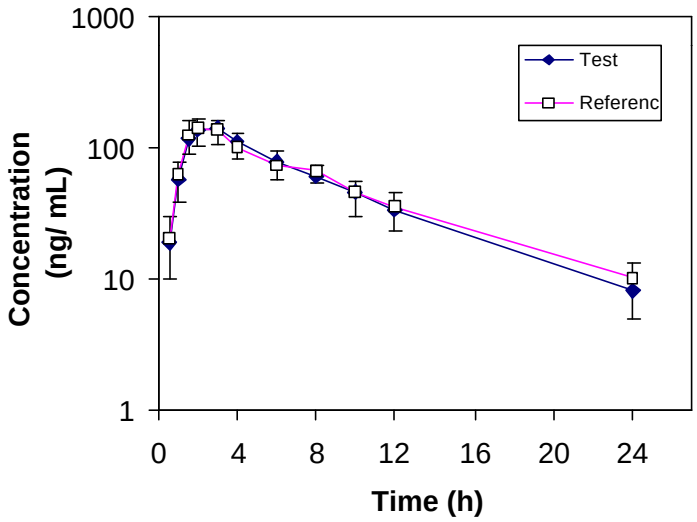

Figure 1. Mean (± SD) plasma concentration-time profiles of diltiazem after administration of 120 mg (2 × 60 mg) oral dose as test or reference product to 12 volunteers.

Table 2. Mean Pharmacokinetic parameters (± SD) of diltiazem following administration of 120 mg (2 × 60 mg) diltiazem in two different oral formulations (n=12)

Parameter	Test product	Reference product	ANOVA
C_{max} (ng mL^{-1})	155.86 ± 19.81	151.74 ± 23.94	NS
T_{max} (h)	2.20 ± 0.62	2.46 ± 0.58	NS
AUC_{0-24} (ng mL^{-1})	1074.95 ± 188.81	1276.05 ± 201.69	NS
$AUC_{0-\infty}$(ng mL^{-1})	1276.00 ± 212.68	1334.22 ± 207.79	NS
$T_{1/2}$ (h)	5.60 ± 1.35	6.04 ± 1.44	NS
$T_{1/2\ (abs)}$ (min)	39.54 ± 15.09	32.96 ± 14.17	NS
K (h^{-1})	0.13 ± 0.04	0.12 ± 0.03	NS
K_a (h^{-1})	1.08 ± 0.51	1.42 ± 0.61	NS
Cl/F(L Kg^{-1} h^{-1}	1.41 ± 0.37	1.44 ± 0.42	NS
Vd/F (L Kg^{-1})	11.75 ± 4.28	12.33 ± 4.15	NS

SD= Standard deviation, ANOVA= Analysis of variance

Table 3. Parametric 90% confidence intervals for the mean pharmacokinetic parameters of diltiazem formulations

Parameter	**Test/Reference**	
	Geometric. mean	90% CI
AUC_{0-24} (ng h ml^{-1})	0.979	0.93 – 1.03
$AUC_{0-\infty}$ (ng h ml^{-1})	0.970	0.90 – 1.04
C_{max} (ng ml^{-1})	1.04	0.96 – 1.14
C_{max}/ $AUC_{0-\infty}$ (h^{-1})	1.07	1.01 – 1.13
Parameter	**Test-Reference**	
	Arithmetic. Mean	90% CI
T_{max} (h)	-0.16	-0.56 – 0.24

CI= Confidence interval

The plasma concentrations of diltiazem were determined using a reported HPLC method (8). The chromatographic method yielded sharp, symmetrical and well-resolved peaks for diltiazem and I.S. without interference from endogenous plasma compounds. Diltiazem and imipramine (I.S.) were eluted after 6.8, and 11.2 min respectively.

The calibration curve for detection of diltiazem was linear over the concentration range of 5-200 ng mL^{-1} (r^2= o.998) and the average recovery was about 90%. The coefficients of variation of intra-day and inter-day reproducibility of the assay were less than 10%. The limit of detection (LOD) with considering signal to noise ratio of 3 was 2 ng mL^{-1} and the limit of quantitation (LOQ) was 6 ng mL^{-1}.

Figure 1 shows the mean plasma concentration-time profiles of diltiazem after administration of both formulations to 12 volunteers. Pharmacokinetic parameters calculated from individual plasma level-time data are shown in table 2. These results are in agreement with previous reports (5,10). No significant differences were observed in C_{max}, T_{max}, $AUC_{0-\infty}$, AUC_{0-24} , $C_{max}/AUC_{0-\infty}$, K_a, K, Cl/F and Vd/F between two formulations ($p>0.05$). In order to determine bioequivalence, the 90% confidence intervals for geometric mean of test / reference, individual ratios of C_{max}, AUC_{0-24}, $AUC_{0-\infty}$ and $C_{max}/AUC_{0-\infty}$ were calculated, all values were within the conventional bioequivalence ranges of 0.8–1.25 (table 3).

The mean and 90% confidence interval of the difference (test-reference) of T_{max} are also shown in table 3. The mean difference was 0.16 h with a 90% confidence interval of 0.56-0.24. The stipulated bioequivalence range for the difference in T_{max} is ± 20% of the T_{max} of the reference product (11), which in this case corresponds to ± 0.49.

DISCUSSION

The pharmacokinetics and bioequivalence of two diltiazem formulations following a single oral dose of 120 mg were studied. The plasma concentration of diltiazem was determined using a sensitive and reproducible HPLC method which followed international standards in validation of the analytical assay.

Diltiazem exhibited a similar plasma concentration pattern after administration of either formulation. Single oral doses of 120 mg of diltiazem resulted in mean maximum concentrations of 155.86 ± 19.8 and 151.74 ± 23.94 ng. mL^{-1} at 1.5-3 h following administration of the test and reference products. The AUC_{0-24} was >80% of the $AUC_{0-\infty}$ in all subjects, indicating adequate sampling time. The mean pharmacokinetic parameters (C_{max}, T_{max}, K, K_a, AUC_{0-24}, $AUC_{0-\infty}$, Cl/F and Vd/F) were in the same order of magnitude as reported values (5, 10).

The 90% confidence intervals for geometric mean of test/reference individual ratios for C_{max}, AUC_{0-24}, $AUC_{0-\infty}$ and $C_{max}/AUC_{0-\infty}$ were within the acceptance limits of bioequivalence.

The parametric point estimate of the mean difference of T_{max} between two formulations (test-reference) was 0.16 h with a 90% confidence interval of 0.56–0.24. Although this interval is slightly wider than stipulated bioequivalence range of ± 0.49 but this slight difference may not affect the conclusion of bioequivalence in the rate of absorption since the other determinants such as C_{max} and $C_{max}/AUC_{0-\infty}$ are similar for both products.

As already mentioned, the in-vitro dissolution profiles of diltiazem from two products were also determined in this study. Although diltiazem was released faster from the reference product with a mean MDT of 33.6 min compared with 54.3 min

for the test product, in-vivo results showed that the differences in the in-vitro dissolution rate were of insufficient magnitude to affect the rate and extent of diltiazem absorption. In conclusion results show that the two studied formulations of diltiazem are bioequivalent regarding the rate and extent of absorption.

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REFERENCES

1. McAllister, R.G., Hamann, S.F., Blouin, R.A. (1985) Pharmacokinetics of calcium entry blockers. Am. J. Cordiol. 55: 30 B - 40 B.
2. Smith, M.S., Verghese, C.P., Shand, D.G., Pritchett, E.L.C. (1983) Pharmacokinetic and Pharmacodynamic effects of diltiazem. Am. J. Cardiol. 51: 1369 - 1374.
3. Ochs, H.R., Knuchel, M. (1984) Pharmacokinetics and absolate bioavailabitity of diltiazem in humans. Klin. Wochenschr. 62: 303 – 306.
4. Roveri, V., Gomeni, R., Mitchard, M., Larribaud, J., Blatrix, C., Thebault, J.J.., Morselli, P.I., (1980) Pharmacokinetic and metabolism of diltiazem in man. Acta. Cardiol. 35: 35 – 45.
5. Du Souich, P.N., Lery, L., Varin, F., Boucher, S., Vazina, M., Pilon, D., Spenard, J, Caille, G. (1990) Influence of food on the bioavailability of diltiazem and two of its metabolites following the administration of conventional tablets and slow release capsules. Biopharm. Drug Disposit. 11: 137 – 147.
6. Tanigawara, Y., Yamaoka, K., Nakagawa, T., Uno, T. (1982) New method for the evaluation of in vitro dissolution time and disintegration time. Chem. Pharm. Bull. 30: 1088 – 1090.
7. Stavchansky, S.A., McGinty, J.W., Bioavailability in tablet technology. In: Liberman HA., Lachman L, Schwartz JB (eds) (1989) Pharmaceutical dosage forms. Vol. 2, Dekker, New York, PP 349 – 564.
8. Zarghi, A., Dadashzadeh, S., Ebrahimian, J.A. (2000) HPLC quantification of diltiazem in plasma from man. Pharm. Pharmacol. Commun. 6: 341 – 343.
9. Wagne,r .JG. (1979) Fundamentals of Clinical Pharmacokinetics Drug Intelligence Publications, Hamilton IL, PP: 173 – 201.
10. Loffreda, A., Matera, M.G., Vacca, C., Motola, G., De Santis, D., Ruggiero, A., Russo, .F, Fici,F., Cantoni, V., Lampa, E., Ross, F. (1999) Bioequivalence assessment of two different tablet formulations of diltiazem after single and repeated doses in healthy subjects. Curr. Med. Res. Opin. 15: 53 - 61.
11. Sauter, R., Steinijans, V.W., Diletti, E., Bohm, A., Schulz, H-U (1992) Presentation of results of bioequivalence studies. Int. J. Clin. Pharmacol.Ther. Toxicol. 30: 233 - 256.