Use of Parent Drug and Metabolite Data in Bioavailability Assessment of a Novel Diltiazem HCl Once-Daily Product[1]

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INTRODUCTION

Diltiazem is an established drug for the management of essential hypertension and angina pectoris (1). The drug is readily absorbed but exhibits a low bioavailability due to substantial first-pass metabolism (2). Present regulatory guidelines (3,4) require acceptable drug bioavailability to be demonstrated for ER products relative to the IR formulation under single- and multiple-dose conditions. The necessity of measuring metabolite levels in bioavailability and bioequivalence studies has been a topic of discussion in recent years (5). While metabolite quantification may not be relevant in bioequivalence studies on IR dosage forms, it may be important when ER dosage forms are compared with IR formulations. The relative bioavailability of a novel once-daily ER formulation of DTZ HCl (intended to be marketed as Tiazac® Capsules) compared to IR tablets was evaluated in fasting healthy males following 360 mg single-dose of the test product and 120 mg q8h of the reference. The two principal metabolites of DTZ, desacetyldiltiazem (DEA) and N-desmethyldiltiazem (DEM), were also quantified in this study, and statistical computations of bioequivalence parameters were performed on their data as for the parent drug.

MATERIALS AND METHODS

Drug Products and Reference Standards

Tiazac® capsules[5] (Biovail, Toronto) is a multiparticulate system consisting of polymer-coated beads in hard gelatin capsules. Cardizem® tablets, 120 mg (Marion Labs., Kansas City) was the reference drug product. Diltiazem HCl and DEA were purchased from Sigma and Abic Laboratories, respectively. The DEM was synthesized by the analytical laboratory of the Contract Research Division of Biovail Corporation International; the structure was confirmed by spectroscopy.

Study Design

The protocol was approved by IRB of the Contract Research Division of Biovail Corporation International. Twenty-seven subjects with ages ranging from 19 - 38 years and weighing 66 - 91 kg were admitted into the study. Written informed consents were obtained from the volunteers. Subjects fasted for at least 9 hours.

Each drug product was administered at 7 am with 180 mL of tap water. Two hours after drug administration, subjects were given 355 mL of a non-caffeine-containing soft drink. No further fluid intake was allowed until 4 h after drug administration when water intake was allowed *ad libitum*. Meals were served at 11 am and 7 pm.

Volunteers were randomly assigned to either of the two treatments—single dose of 360 mg Tiazac® Capsules or 120 mg q8h of Cardizem® Tablets. Following a one week washout, each volunteer was administered, in a cross-over design, the alternate formulation. The blood sampling times when subjects ingested Tiazac® were 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 20, 24, 28, 32, 36, 40 and 48 h. The sampling times for Cardizem® were 0, 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 24, 30, 36 and 48 h. Vital signs and ECGs were monitored at predetermined times. Pre-cooled EDTA Vacutainers® were used for sample collection. All blood samples were centrifuged within 15 minutes of collection and the plasma portions were stored frozen at -70 ± 5°C until analysis.

Analytical

The plasma samples were assayed for DTZ, DEA and DEM by an HPLC procedure, with UV detection, which was validated in accordance with guidelines of the analytical method validation conference report (6). Briefly, the extraction procedure, accuracy and precision of the assay are as follows:

To 1 ml of plasma sample was added 100 μL of internal standard solution (propranolol HCl, 100 ng/mL) and 750 μL of 0.1M KH_2PO_4 (pH 7.5). The analytes were extracted with 4 mL of diethyl ether and then back-extracted into an aqueous 0.075% phosphoric acid solution. The aqueous solution (75 - 80 μL) was injected onto the HPLC column.

Calibration curves were linear over the concentration ranges for all three analytes. Lower limits of quantification were 3.1 ng/mL for DTZ and DEM, and 1 ng/mL for DEA. Inter-day coefficients of variation for lower limits of quantitation were 9.5%, 8%, and 10.4 % for DTZ, DEA, and DEM, respectively. Intra- and inter-assay precisions of the method were less than 5 % for all three analytes.

Pharmacokinetic and Statistical Analyses

Data were analyzed in accordance with the Canadian Health Protection Branch's draft bioavailability and bioequivalence guidelines for oral modified-release formula-

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[5] The product was developed in partnership with Galephar, Puerto-Rico.

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tions (3), and according to the recommendations of the workshop on controlled-release dosage forms (4). Pharmacokinetic parameters were computed non-compartmentally. The AUC and C_{max} Metabolite:Parent drug ratios were calculated after conversion to molar units, i.e. nmol.h/L and nmol/L, respectively.

The pharmacokinetic parameters of the two treatments were compared for all three analytes by ANOVA at the 5% level of significance (7) using the SAS General Linear Model Procedure. In the case of AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, $C_{max}/AUC_{0-\infty}$ and MRT, ANOVAs were performed on both the raw and log-transformed data. Arithmetic and geometric 90% confidence intervals were calculated for the aforementioned dose-dependent pharmacokinetic parameters.

RESULTS

Twenty-four subjects completed the study. One subject was dismissed due to vomiting and persistent headache. Two additional subjects were dismissed due to second degree heartblocks.

The mean DTZ, DEA and DEM plasma concentration versus time curves following ER and IR products are shown in Figure 1 and the mean pharmacokinetic parameters are presented in Tables I and II. The mean diltiazem t_{max} of Tiazac® was significant longer than that of Cardizem®. Statistically significantly differences in mean MRT and $C_{max}/AUC_{0-\infty}$ between the two drug products were detected by ANOVA ($p=0.0001$). The diltiazem $AUC_{0-\infty}$ ratio, was 89% (90% geometric confidence interval = 81–96 %).

Desacetyldiltiazem and N-desmethyldiltiazem

The third DEA C_{max} of IR (17.5 ± 10.7 ng/mL) was not significantly different from that of the ER product ($p=0.9935$). The t_{max} and apparent $t_{1/2}$ of DEA obtained for Tiazac® were significantly longer than those of the IR product. Shapes of the plasma concentration—time profiles showed that DEA levels were sustained similar to the parent drug following the administration of ER diltiazem formulation compared to the IR product. The geometric mean test/reference ratio of DEA AUC was 105 % (geometric confidence interval = 95–116 %). Statistically significant differences in the mean pharmacokinetic parameters of DEM between the two products were detected by ANOVA. The DEM geometric mean AUC_{0-t} ratio was 83% (geometric confidence interval = 78–87 %). Mean C_{max}'s and AUC's of DEA and DEM are summarized in Table II.

The DEA inter-subject coefficients of variation for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were 92 %, 103 %, and 85 %, respectively for the ER product. The corresponding coefficients of variation for the IR product were 62 %, 78 %, and 32 %, respectively. In general, the ER and IR products exhibited similar inter-subject differences in diltiazem and DEM pharmacokinetic parameters.

Metabolite: Parent Diltiazem AUC and C_{max} Ratios

The mean ratios for AUC_{0-t} and C_{max} are graphically depicted in Figure 2. Significant differences in mean AUC_{0-t} metabolite:DTZ ratios between the IR and ER diltiazem products were demonstrated for both metabolites. While the diltiazem ER formulation exhibited a higher mean DEA:DTZ AUC_{0-t} ratio compared to IR Cardizem® ($p = 0.003$), a greater mean DEM:DTZ AUC_{0-t} ratio was observed for the IR product ($p = 0.0259$) compared to the ER formulation. Following the administration of 360 mg Cardizem® tablets in three divided doses, DEA accumulated to a greater extent than parent diltiazem.

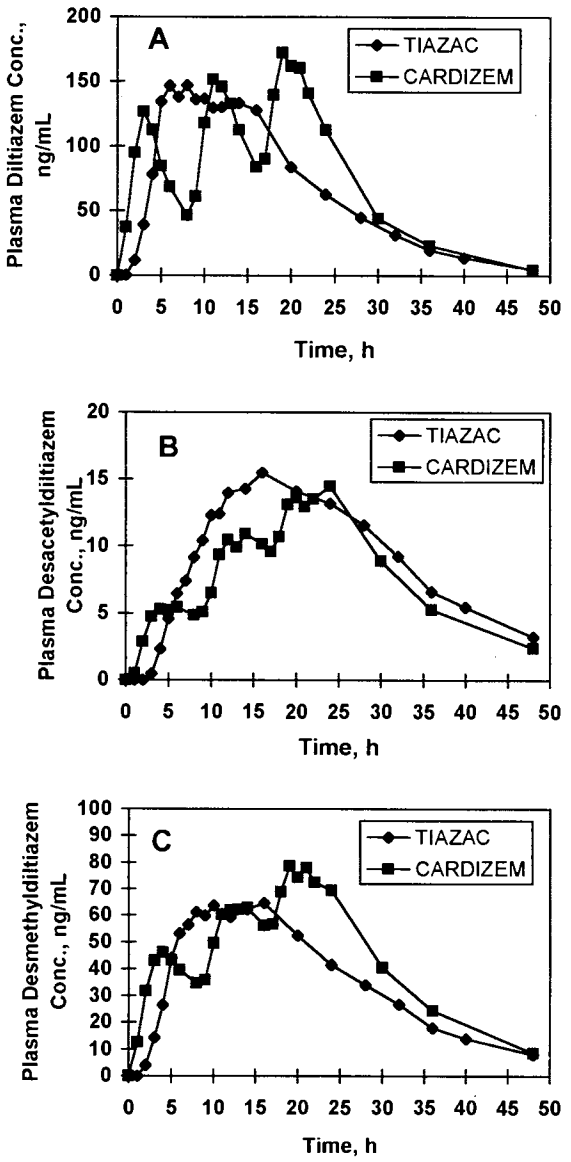

Fig. 1. Mean diltiazem (A), desacetyldiltiazem (B), desmethyldiltiazem (C) plasma concentration versus time curves after administration of 360 mg diltiazem HCl as Tiazac® capsules and 120 mg Cardizem® tablets q8h for 24 h to 24 healthy male volunteers.

DISCUSSION AND CONCLUSION

Tiazac® was found to exhibit extended-release characteristics and did not dose-dump under fasting conditions. Based on the diltiazem mean $AUC_{0-\infty}$ test:reference ratio and

Table I. Pharmacokinetic Parameters of Diltiazem (Expressed as mean (±SD)) Following Administration of One 360 mg Diltiazem HCl Once Daily Extended Release Capsule (Tiazac®) and 120 mg q8h Cardizem® Tablets for 24 h

Dosage form administered	C_{max}[b] (ng/mL)	t_{max}[b] (h)	AUC_{0-t}[b] (ng · h/mL)	$AUC_{0-\infty}$[b] (ng · h/mL)	MRT[b] (h)	$\frac{100 \cdot C_{max}}{AUC_{0-\infty}}$[b] ($h^{-1}$)	$t_{1/2}$[b] (h)	K_{el}[b] (h^{-1})
Tiazac capsules, 360 mg	177.43 (58.98)	9.17 (2.97)	2991.66 (956.35)	3064.12 (972.36)	17.72 (2.16)	6.04 (1.49)	6.51 (1.54)	0.113 (0.029)
Cardizem tabs., 120 mg q8h	135.62[a] (38.64)	3.21 (0.88)	3391.01 (1171.55)	3453.90 (1181.55)	10.13 (3.70)	20.54 (2.17)	4.92 (1.01)	0.147 (0.032)
90% Arith. C.I.	116–145	—	80–96	80–96	163–187	25–33	—	—
90% Geom. C.I.	113–144	—	81–96	81–96	167–202	26–30	—	—
Arith. Ratio (%)	130.83	—	88.2	88.7	184	29	—	—

[a] C_{max} during first dosing interval.
[b] Treatment means were significantly different at 5% level.

90 % geometric confidence interval, the novel formulation was demonstrated to be bioequivalent to Cardizem® in terms of the extent of diltiazem absorption. Tiazac® exhibited a significantly slower rate of diltiazem absorption. There is currently a general understanding that C_{max} and t_{max} are inappropriate measures of rate of absorption for ER drug products (8). In addition to the recently proposed C_{max}/AUC quotient by Endrenyi *et al* (8), MRT had also been advanced as a measure of rate for ER products (9). The comparison of the aforementioned rate metrics between diltiazem ER and IR formulations demonstrated statistically significant differences. This discrimination between the diltiazem ER and IR dosage forms in their MRT and C_{max}/AUC values gives credence to their suitability as metrics of drug absorption rate.

While the inter-subject differences in pharmacokinetic parameters of DEM and diltiazem, as reflected in the %CVs, were similar for both test and reference products, marked differences were observed for the active metabolite, DEA. The data do not allow for a complete understanding of the reason for this difference in variability of DEA plasma levels between ER and IR forms of diltiazem. However, it is reasonable to surmize that due to the slower input rate of the drug from the ER formulation, different degrees of saturation of the deacetylation pathway were attained in different subjects. Conversely, the IR form may have resulted in attainment of more complete saturation in most subjects and therefore less inter-individual differences in plasma levels of DEA compared to the ER formulation. Although the greater inter-subject variability of DEA compared to diltiazem and other metabolites was previously reported (10), the authors are not aware of any report comparing the variability of this metabolite when IR and ER formulations of the parent drug are administered. Greater inter-individual variability in DEA plasma levels relative to DEM was also manifested in the metabolite:diltiazem ratios of AUC_{0-t} and C_{max} (Figure 2).

In general, ER dosage forms may have the potential to produce low bioavailability for a given drug due to slow rate of input. For drugs such as diltiazem which undergo first-pass metabolism and exhibit non-linear kinetics the quantification of major metabolites in plasma, for both test and reference drug products, is useful for bioavailability/bioequivalence studies as it allows accountability for drug input. It is pertinent to mention that the plasma levels of DEA and DEM observed in this study were approximately 10 % and 45 %, respectively, of parent diltiazem. Their contribution to the overall therapeutic effect of the drug may not be considered substantial, but their quantification can aid significantly in accounting for drug input after oral administration.

Table II. Pharmacokinetic Parameters of Desacetyldiltiazem and Desmethyldiltiazem (Expressed as mean (±SD)) Following Administration of One 360 mg Tiazac® Capsule and 120 mg q8h Cardizem® Tablets for 24 h to 24 Healthy Volunteers

	Desacetyldiltiazem			Desmethyldiltiazem		
Dosage form administered	C_{max}[b] (ng/mL)	AUC_{0-t}[b] (ng · h/mL)	$AUC_{0-\infty}$[b] (ng · h/mL)	C_{max}[b] (ng/mL)	AUC_{0-t}[b] (ng · h/mL)	$AUC_{0-\infty}$[b] (ng · h/mL)
Tiazac capsules, 360 mg	17.80 (15.13)	424.81 (392.61)	492.38 (507.11)	74.52 (22.79)	1650.67 (391.97)	1790.58 (434.54)
Cardizem tablets, 120 mg q8h	5.92[a] (1.89)	354.45 (221.70)	416.96 (325.38)	49.32[a] (10.72)	1992.16 (480.01)	2096.29 (508.57)
90% Arith. C.I.	215–382	99–139	100–140	136–166	78–88	80–89
90% Geom. C.I.	214–230	95–116	98–119	135–163	78–87	80–89
Arith. Ratio (%)	300	120	118	151	82.9	85.4

[a] C_{max} during first dosing interval.
[b] Treatment means were significantly different at 5% level of significance.

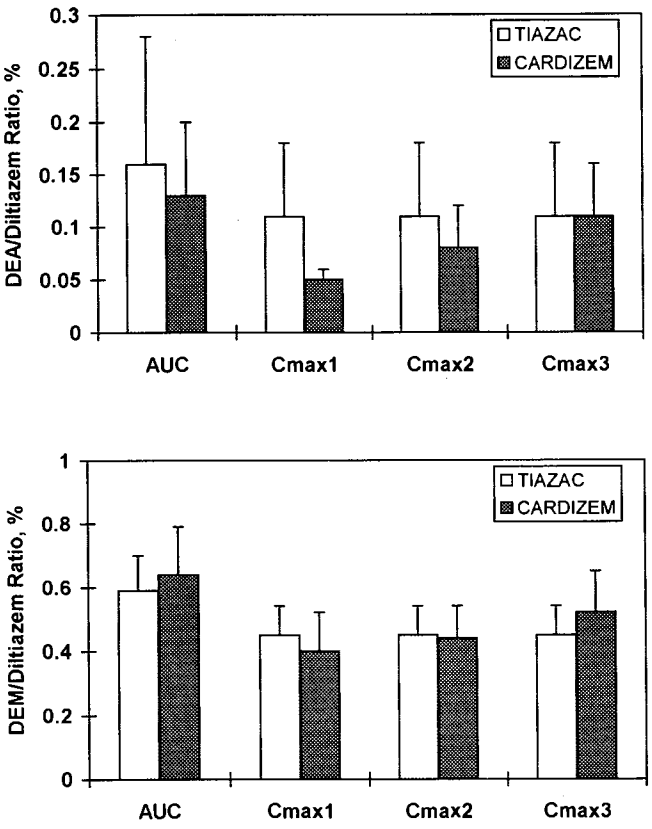

Fig. 2. Mean desacetyldiltiazem:diltiazem (upper panel) and desmethyldiltiazem:diltiazem (lower panel) AUC_{o-t} and C_{max} ratios of 360 mg Tiazac® capsules administered as a single dose and 360 mg Cardizem® tablets administered as 120 mg q8h to 24 healthy male volunteers. C_{max1}, C_{max2}, and C_{max3} represent analyte maximum plasma concentrations during the first, second and third dosing intervals, respectively, of Cardizem® tablets. The bars represent 1 SD.

As demonstrated by parent drug and metabolite data, Tiazac®, a novel diltiazem hydrochloride ER capsule for once-daily administration demonstrated ER characteristics, acceptable bioavailability and did not dose-dump under single-dose, fasting conditions.

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