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Report

Performance of Diltiazem Tablet and Multiparticulate Osmotic Formulations in the Dog

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The *in vivo* performance of two extended-release (ER) osmotic formulations of diltiazem were evaluated in the beagle dog. Both ER formulations had similar bioavailabilities (*F*) as the diltiazem solution. Although *F* was somewhat variable following ER administration, this variability may be related to the drug entity since intra- and interanimal variability of orally administered diltiazem solutions was substantial. Deconvolution of the ER plasma diltiazem data with absorption data from the orally administered diltiazem solutions provided an estimate of the *in vivo* drug release from the ER formulations. The two ER formulations, designed with different *in vitro* release profiles, reflected these differences *in vivo*, with nearly identical respective *in vivo* and *in vitro* release profiles.

KEY WORDS: diltiazem; controlled release; deconvolution; dog; pharmacokinetics.

INTRODUCTION

Diltiazem is a potent calcium-channel blocker. By inhibiting calcium influx, diltiazem inhibits the contractile process of cardiac and vascular smooth muscle, thereby dilating the main coronary and systemic arteries (1). For the management of Prinzmetal variant angina and chronic angina pectoris, diltiazem has been available in the United States until recently only as a conventional dosage form, with 60 to 90 mg administered four times daily (2). If the dosing frequency were decreased, patient compliance might be improved, leading to improved therapy. Accordingly, the development of a sustained-release diltiazem formulation was initiated. This report summarizes the pharmacokinetics of intravenously and perorally administered solutions and the *in vivo* release rates of two extended-release osmotic formulations of diltiazem in the beagle dog. The *in vivo* release rates are compared with *in vitro* dissolution data.

MATERIALS AND METHODS

Dosage Forms

Two different extended-release (ER) dosage forms based on controlled porosity osmotic pump principles (3) were tested: a unit-dose osmotic tablet formulation and an osmotic multiparticulate formulation. The zero-order release rate of the ER tablet in phosphate buffer (0.05 *M*, pH 7.5) was calculated to be 7.3%/hr, releasing approximately 60% of the drug in 8 hr.

The ER multiparticulate formulation consisted of spherical particles (diameter range, 0.8–1.2 mm) which in phosphate buffer (0.05 *M*, pH 7.5) released 65% of the drug at ~11%/hr over a 6-hr period.

Analytical Method

Reagents and Standards. *N*-Desmethyldiltiazem and *N*-ethyl-*N*-desmethyldiltiazem were prepared from diltiazem (4). Acetonitrile, methanol, ethyl acetate, and methyl t-butyl ether were Burdick and Jackson high-purity solvents. Reagent-grade ammonium carbonate, dipotassium phosphate, and phosphoric acid were obtained from Mallinckrodt.

Instrumentation and Chromatographic Conditions. The HPLC system was equipped with an SSI Model 300 pump, a Perkin-Elmer ISS-100 autosampler, and a Kratos Model 783 spectrophotometric detector. Data were collected on an HP1000 minicomputer using Nelson Analytical Model 6000 software. Separations were performed on a Rainin Dynamix Microsorb CN column (4.6 mm × 25 cm with 5-cm guard column). The mobile phase was a solution composed of 1 part by volume acetonitrile/methanol (3:1) and an aqueous solution of dipotassium hydrogen phosphate (10 m*M*, adjusted to pH 7 with phosphoric acid). The column was operated at 30°C with a flow of 2.0 ml/min and the effluent was monitored at 237 nm. Retention times were 6.8 min for diltiazem, 9.9 min for the metabolite *N*-desmethyldiltiazem, and 8.5 min for the internal standard.

Sample Preparation. Samples were processed quickly after thawing from a −20°C freezer to avoid hydrolysis of diltiazem. In a 5-ml centrifuge tube, 1.0 ml of plasma sample and 0.10 ml of internal standard (1 μg/ml in acetonitrile) were vortexed briefly, and 0.5 ml of 0.1 ammonium carbonate and 4 ml of 3:1 ethyl acetate/methyl t-butyl ether were added. The samples were extracted using a multitube vortexer. Af-

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ter centrifugation, the upper layer was transferred from each tube into a second set of tubes containing 0.2-ml portions of an aqueous solution of phosphoric acid (0.2%). These tubes were vortexed to back-extract, then centrifuged, and the lower layer was taken cleanly for prompt analysis by injection of 50-μl aliquots into the HPLC.

Standard Samples. Working standard solutions were prepared by dissolving 10 mg each of diltiazem and *N*-desmethyldiltiazem in 10 ml of acetonitrile and sequentially diluting with acetonitrile. These solutions were stable when stored at −20°C. Drug-free plasma was spiked to contain 300, 100, 20, and 5 ng of diltiazem/ml. These samples were processed as above to generate the standard curve.

Data Processing. Peak areas of diltiazem, *N*-desmethyldiltiazem, and the internal standard (*N*-ethyl-*N*-desmethyldiltiazem) were measured for spiked plasma standards. Linear regression ($r^2 > 0.99$) of area ratios versus concentration produced standard curves for quantitation of both diltiazem and *N*-desmethyldiltiazem.

Animal Studies

Six beagle dogs (18.0 ± 1.7 kg) were administered either ER tablets, ER multiparticules, a peroral solution on three occasions, or an intravenous (i.v.) solution on two separate occasions.

Intravenous administration. In the i.v. administration, 1 ml diltiazem solution (containing 18.3 mg diltiazem and 9 mg NaCl) was injected into each of the six dogs via the foreleg vein (i.v.-1). To mimic the peroral studies where it was desirable to maximize gastrointestinal retention of the peroral formulation, each dog was fed approximately 50 g of dog food before and every hour following diltiazem administration. Blood samples (3 ml) were taken from either the foreleg or the jugular vein immediately prior to dosing and 10, 20, 40, 60, 90, and 120 min and 4, 6, and 8 hr after administration and were centrifuged for the isolation of plasma and stored at −20°C until assayed. The i.v. administration was repeated (i.v.-2) in the same dogs approximately 5 months later.

Peroral Dosing with Solution Administration. One milliliter of solution (OS), containing 55 mg of diltiazem, was administered by peroral syringe into the back of the throat to the same six dogs. Blood samples (3 ml) were taken prior to dosing and at 0.5, 1, 2, 3, 4, 5, and 6 hr after administration, and they were assayed as described above. The OS administration was repeated in the same dogs approximately 2 and 5 months later.

Peroral Dosing with Extended-Release Dosage Forms. One ER tablet containing 110 mg diltiazem was administered to each dog. Blood samples (3 ml) were taken prior to dosing and at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hr after administration and were assayed as described above. On a separate occasion, one capsule containing 110 mg diltiazem in the ER multiparticulate formulation was similarly administered. Blood samples were taken prior to dosing and 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hr after administration and assayed as described above.

Pharmacokinetics

Individual sets of i.v. diltiazem concentrations (C) were fitted to a two-compartment model [see Eq. (1)] using SIPHAR (SIMED, Créteil, Cedex-France) and a weight of 1 (5):

$$C_{IV} = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where t is time, and α and β are fast and slow rate constants, respectively. Best fits were determined by using Akaike criteria and by minimizing the coefficient of variant (CV) associated with computed parameter estimates.

The parameter "area under the concentration versus time curve" (AUC) for individual dogs was calculated by the trapezoidal method and Eq. (2):

$$AUC_{last-\infty} = C(last)/\beta \quad (2)$$

where C(last) is the last plasma diltiazem concentration and β is the terminal disposition rate constant for the individual dog. Systemic clearance (Cl) was calculated by Eq. (3):

$$Cl = dose/AUC \quad (3)$$

Steady-state volume of distribution (V_{ss}) was estimated by Eq. (4):

$$V_{ss} = dose\ (AUMC/AUC^2) \quad (4)$$

where AUMC is the area under the first moment curve. The individual plasma diltiazem concentrations following administration of the peroral solutions and ER dosage forms were deconvoluted (SIPHAR) against the i.v. data using the point area method (5). The software interpolates the existing data to arrive at equally spaced and identical time intervals following OS, ER, and i.v. during the absorption phase. Next SIPHAR calculates AUC for each interval of the OS and ER data and deconvolutes this against the i.v. concentration corresponding to each interval, to arrive at the fraction absorbed during each interval. By visually inspecting the fraction absorbed versus time data plotted on linear or log-linear coordinates, an absorption rate order can be approximated. For the ER data, the relationship appeared zero order, and the slope yielded the *in vivo* release rate ($k_{0,\ abs}$); for the OS-2 data, the relationship was more obscure (see below). The ER data were similarly deconvoluted against the OS data, again resulting in *in vivo* release rates ($k_{0,\ in\ vivo}$). The apparent *in vivo* release rates of the ER dosage forms were estimated by linear regression of the initial slope of the percentage diltiazem absorbed versus time curves.

Comparisons were made with ANOVA and differences (least significant difference or Student–Newman–Kuel) were considered significant at $P < 0.05$ (6). All data are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Every i.v. plasma concentration versus time profile exhibited nearly identical biexponential characteristics (Table I). The terminal elimination half-life ($t_{1/2,\beta}$) in the i.v. study (2.7–3.2 hr) was similar to the 2.2- to 3-hr half-life previously reported by others (7,8). The V_{ss} (114–124 liters) in this study was also similar to that reported (8) earlier (137 ± 20 liters); however, the Cl was slightly less (500–566 vs 830 ± 86 ml/min).

The initial OS plasma data set (OS-1) and those obtained following repeated studies in the same dogs 2 months (OS-2)

Table I. Selected Pharmacokinetic Parameters Following Intravenous and Peroral Administration of Diltiazem Solutions[a]

	i.v.-1	i.v.-2	OS-1	OS-2	OS-3
Dose (mg)	18.3	18.3	55.0	55.0	55.0
AUC (hr · ng/ml)	753 ± 180	820 ± 95.6	712 ± 216	1223 ± 450*	1166 ± 428**
F (%)	—	—	31.5 ± 9.6[b]	50.5 ± 19.0[c]	47.7 ± 17.7[c]
β (hr^{-1})	0.26 ± 0.06	0.24 ± 0.09	0.31 ± 0.05	0.28 ± 0.03	0.26 ± 0.03
$t_{1/2,\beta}$ (hr)	2.7 ± 0.5	3.2 ± 1.2	2.29 ± 0.40	2.46 ± 0.48	2.68 ± 0.41
C_{max} (ng/ml)[d]	380 ± 119	482 ± 115	215 ± 63.7	329 ± 115	238 ± 132
T_{max} (min)	10 ± 0	10 ± 0	45 ± 16	40 ± 15	85 ± 84
Cl (ml/min)	566 ± 145	500 ± 61.8	—	—	—
V_{ss} (L)	114 ± 32.1	124 ± 40.1	—	—	—

[a] i.v.-1 and i.v.-2 refer to replicate intravenous studies; OS-1, OS-2, and OS-3 refer to triplicate peroral studies. See Materials and Methods for details.
[b] Relative to i.v.-1.
[c] Relative to i.v.-2.
[d] Largest measured plasma diltiazem concentration measured.
* Different from OS-1; $P < 0.01$.
** Different from OS-1; $P < 0.05$.

and 5 months (OS-3) later varied substantially (Fig. 1). Significant differences were attributed (two-way ANOVA) to both intra- and interanimal variability. The AUC calculated from OS-2 was 72% greater ($P < 0.01$) than that of OS-1, suggesting a greater bioavailability for OS-2 than for OS-1. As can be seen in Table I, the AUC from OS-3 was intermediate to the earlier two OS data sets. Although in the same comparison C_{max} appears to be different, statistical significance was not achieved. T_{max} was not different: the abnormally large T_{max} value for OS-3 was due to one outlying value which, when excluded, yielded a T_{max} for OS-3 (54 ± 39 min) comparable to that for OS-1 and OS-2. The $t_{1/2,\beta}$ values for OS-1, OS-2, and OS-3 were also comparable. Although the bioavailability of OS-1 (31.5%) was considerably lower than the 55% reported by others (8), the bioavailability of OS-2 (50.5%) was comparable to the literature value. The wide range in the relative bioavailability of the three OS data sets underscores the magnitude of intra-animal variability in studies with this drug. Since the experiments were completed over an 18-month period, an attempt to minimize any possible contributions of environment on animal physiology (and diltiazem pharmacokinetics) was made by time-matching the studies. Specifically, i.v.-1, OS-1, and the ER tablet studies were completed in a close time range, whereas OS-2 and the ER multiparticulate studies were completed later. The i.v.-2 and OS-3 experiments were then completed shortly after the multiparticulate study and were found not to be different from i.v.-1 and OS-2 (respectively).

The mean diltiazem plasma concentrations following the ER tablet and multiparticulate administration are shown in Figs. 2 and 3, respectively. For comparison purposes, included in each of these figures are the simulated concentrations following the four-times-a-day (every 6 hr) peroral administration of a solution containing 27.5 mg diltiazem.

The mean relative bioavailability of the ER tablet was 93% (compared to OS-1). The mean relative bioavailability of the ER multiparticulate was 109, 64, and 67% when com-

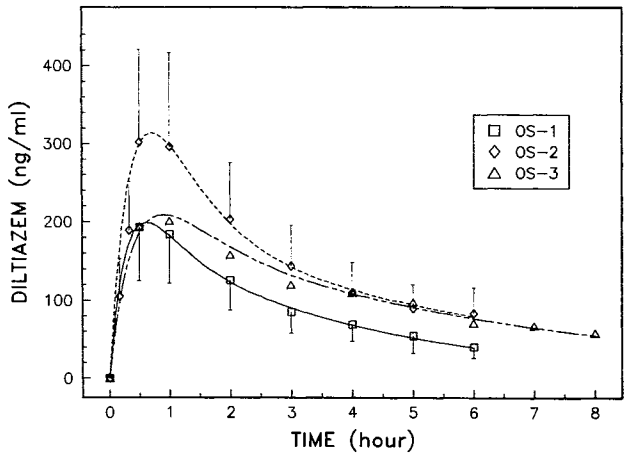

Fig. 1. Plasma diltiazem concentrations following triplicate (OS-1, OS-2, OS-3) peroral administration of 55.0 mg diltiazem on three separate occasions. The continuous lines are best-fit generated curves and the mean ± SD are shown for OS-1 and OS-2. The SD bars for OS-3 were omitted for clarity but are of the same magnitude as that shown for other curves.

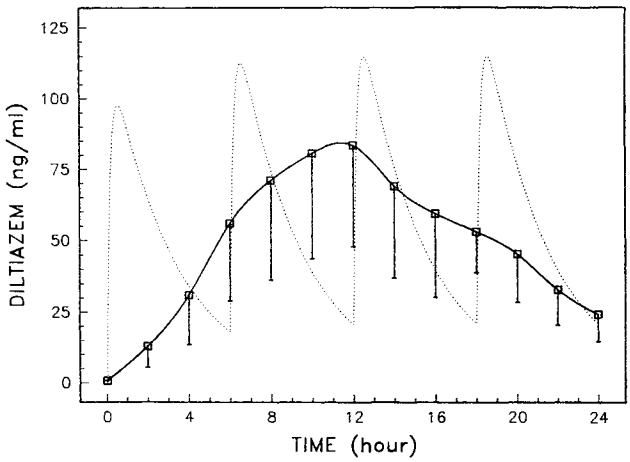

Fig. 2. Plasma diltiazem concentrations following peroral administration of 110 mg diltiazem as an osmotic tablet formulation. The dotted line is a simulation of 27.5 mg diltiazem administered as a solution, every 6 hr. Means ± SD are shown.

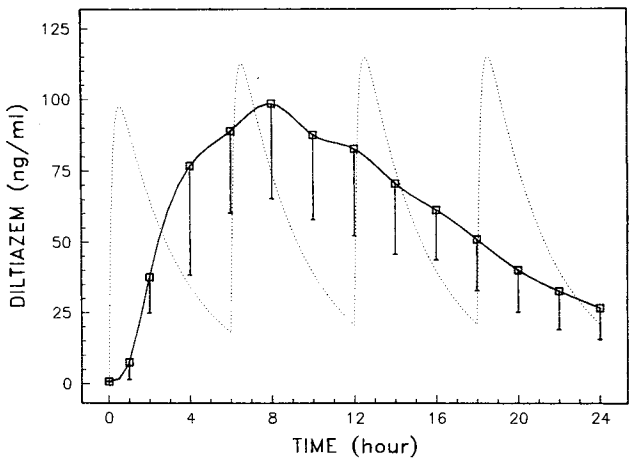

Fig. 3. Plasma diltiazem concentrations following peroral administration of 110 mg diltiazem as an osmotic multiparticulate formulation. The dotted line is a simulation of 27.5 mg diltiazem administered as a solution, every 6 hr. Means ± SD are shown.

pared with OS-1, OS-2, and OS-3, respectively. Due to the faster initial release of drug from the multiparticulate formulation, the plasma diltiazem concentrations from the multiparticulate formulation were significantly greater than those from the tablet formulation at 2 and 4 hr, resulting in a significant difference in T_{max} between the two formulations (Table II). The absolute bioavailabilities of the two ER formulations were similar.

N-Desmethyl Diltiazem

The AUC of a major active metabolite (10), *N*-desmethyl diltiazem (N), following peroral solution administration of diltiazem was individually calculated for each dog by the trapezoidal method. Following peroral solution, tablet, and multiparticulate administration, the AUC values (scaled to the OS dose and corrected for diltiazem bioavailability) were 519 ± 268, 684 ± 261, and 611 + 261 hr · ng/ml, respectively. Thus, the ER formulations apparently did not alter the formation of N or the cumulative activity of diltiazem plus N.

Absorption Profiles

In one OS study (OS-2), additional early blood sampling was completed in an attempt to better characterize the absorption process. Following numerical deconvolution of OS-2 with i.v.-2, a visual inspection and linear or log-linear regression of the individual initial percentage diltiazem absorbed curve was performed in an attempt to assign a zero- or first-order rate to the absorption process (Fig. 4). Despite this additional early sampling, absorption was sufficiently rapid that only three or four data points occurred during the absorption phase, and an unambiguous assignment of absorption rate order was not possible.

Also shown in Fig. 4 are the absorption profiles for the ER tablet and multiparticulate formulations. These curves are the result of numerical deconvolution of the ER formulation with the appropriate i.v. data and clearly demonstrate that the formulations provide a prolonged absorption.

Regression analysis of the ER tablet and multiparticulate formulations profile provided zero-order absorption rate ($k_{0,abs}$) estimates of 6.5 ± 1.2 and 7.9 ± 0.7%/hr, respectively.

In Vivo Release Rate

The individual percentage diltiazem absorbed profiles obtained by numerically deconvoluting individual ER tablet data with OS-1 data from the same dog are shown in Fig. 5.

The percentage diltiazem absorbed vs time data from 2 to 10 hr were individually examined by linear regression to provide estimates of apparent *in vivo* release rates. As summarized in Table II and graphically shown in Figure 5, the percentage diltiazem absorbed profiles favorably compare with the *in vitro* release profile. Although the *in vivo–in vitro* correlation was good (r^2 = 0.91 ± 0.04), there appeared to be a longer lag time for release *in vivo* (1.4 ± 1.3 hr) than *in vitro* (approximately 0.75 hr). When this apparent *in vivo* lag time was incorporated into the *in vivo* analysis, the *in vivo–in*

Table II. Selected Pharmacokinetic Parameters Following Peroral Administration of Osmotic Formulations

	Tablet	Multiparticulate
Dose of diltiazem (mg)	110	110
AUC (hr · ng/ml)	1326 ± 479	1555 ± 572
F (%)	30 ± 10[a]	32 ± 10[b]
C_{max} (ng/ml)	92.7 ± 38.1	101 ± 34.8
T_{max} (hr)	10.7 ± 1.6	6.7 ± 2.4*
$k_{0,\,abs}$ (%/hr)[c]	6.5 ± 1.2	7.9 ± 0.7
$k_{0,\,in\ vivo}$ (%/hr)[c]	6.3 ± 1.2	8.0 ± 0.7
$k_{0,\,in\ vitro}$ (%/hr)[d]	7.3	10.7
$T_{lag,\,in\ vivo}$ (hr)[c]	1.4 ± 1.3	0.25 ± 0.81
$T_{lag,\,in\ vitro}$ (hr)[d]	0.75	0.50

[a] Relative to i.v.-1.
[b] Relative to i.v.-2.
[c] Estimated by deconvolution; see text.
[d] Average of duplicate measurements.
* $P < 0.05$.

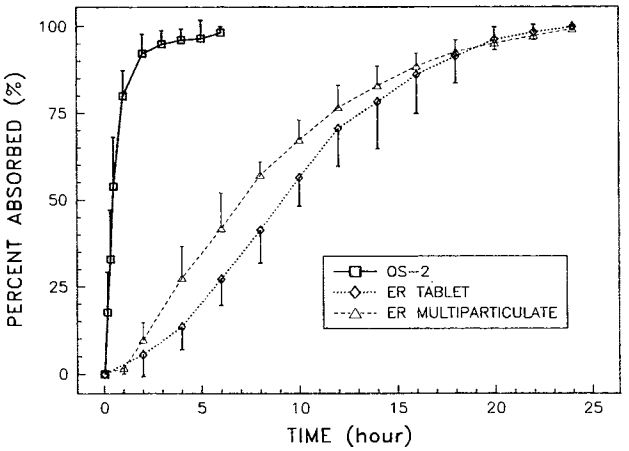

Fig. 4. Absorption profiles following peroral administration of diltiazem as a solution (OS-2) and ER tablet and microparticulate formulations. Means ± SD are shown.

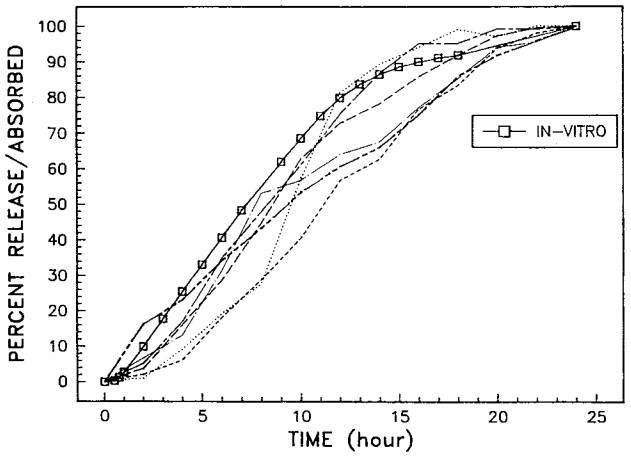

Fig. 5. Individual percentage diltiazem absorbed versus time profiles following administration of ER tablet formulation. For comparison purposes the *in vitro* release data for the tablet formulation in phosphate buffer (pH 7.5) are also shown.

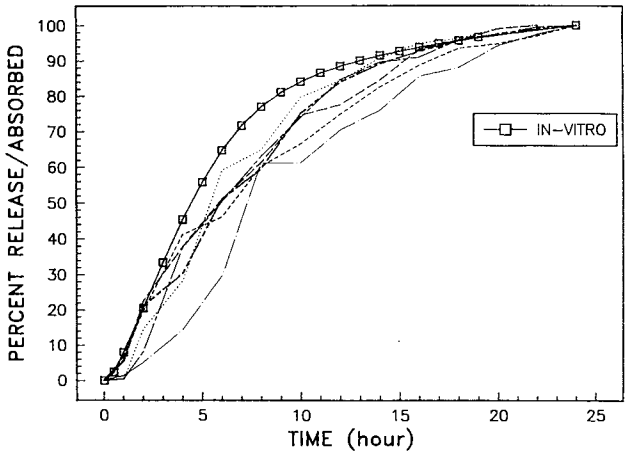

Fig. 6. Individual percentage diltiazem absorbed versus time profiles following administration of ER multiparticulate formulation. For comparison purposes the *in vitro* release data for the ER multiparticulate formulation in phosphate buffer (pH 7.5) are also shown.

vitro correlation was significantly ($P < 0.001$) improved ($r^2 = 0.96 \pm 0.03$).

The values of $k_{0,\ in\ vivo}$ and $k_{0,\ abs}$ following numerical deconvolution of the ER tablet data with OS data (6.3 ± 1.2%/hr) and i.v. data (6.5 ± 1.2%/hr) were compared. The value of $k_{0,\ abs}$ is a less precise *in vivo* estimate of the zero-order release rate, since it is contaminated by an absorption process. The value of $k_{0,\ in\ vivo}$ is not contaminated by an absorption process and, therefore, is a more precise estimate of k_0, *in vitro*. Since $k_{0,\ abs}$ and $k_{0,\ in\ vivo}$ were comparable, this implies that the appearance of diltiazem in the systemic circulation is not limited by absorption processes throughout the intestine.

The individual percentage diltiazem absorbed profiles were obtained by numerically deconvoluting individual ER multiparticulate data with OS-2 data from the same dog (Fig. 6). An analogous linear regression analysis was completed for the multiparticulate formulation for hr 1 through hr 8 ($r^2 = 0.96 \pm 0.02$). The apparent *in vivo* release rates were nearly identical to the *in vitro* release rate, and no increase in lag time was observed (Table II). A comparison of the values of $k_{0,\ in\ vivo}$ and $k_{0,\ abs}$ following numerical deconvolution of the ER multiparticulate data with OS data (8.0 ± 0.7%/hr) and i.v. data (7.9 ± 0.7%/hr) again suggested that diltiazem absorption was not the rate-limiting process. Although the deconvolution techniques require the assumption of linearity and time invariance, the results suggest that drug release and absorption of diltiazem from either ER formulation occur throughout the gastrointestinal tract and that drug release was the rate-limiting process.

In conclusion, the apparent *in vivo* release rate and lag time using a peroral reference dose show a greater variability for diltiazem than suggested by *in vitro* tests. The present study showed that the apparent *in vivo* performance of the osmotic formulations was in reasonably good agreement with that predicted by *in vitro* dissolution methods. Although in the dog both osmotic ER formulations had similar bioavailabilities as the solution, it remains to be shown that this agreement can be reproduced in man.

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