Pharmacokinetics of Diltiazem and Its Metabolites in Dogs After Oral Administration of a Multiparticulate Sustained-Release Preparation

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Received July 29, 1992; accepted February 5, 1993

Pharmacokinetics of diltiazem and its six metabolites were compared after oral administration in dogs of a multiparticulate sustained-release diltiazem preparation (HER-SR, QD) and a conventional diltiazem preparation (HER, TID). The plasma concentration of diltiazem, its two active basic metabolites (M1, *N*-monodesmethyl diltiazem; M2, deacetyl diltiazem), and four acidic metabolites [A1, (+)-(2S,3S)-2-(4-methoxyphenyl)-3-acetoxy-4-oxo-2,3,4,5,-tetrahydro-1,5-benzothiazepin-5-acetic acid; A2, 3-deacetyl-A1; A3, *O*-demethyl-A1; A4, *O*-demethyl-3-deacetyl-A1] following several administration routes were determined using high-performance liquid chromatography with UV detector (UV-HPLC). Following the oral administration of HER to dogs, plasma concentrations were in the descending order of A2, diltiazem, M1, and M2. The absolute bioavailability of diltiazem was about 30%. Diltiazem conversion to its metabolites (M1, M2, A2) was 31.0, 2.1, and 14.6%, respectively. Following intraduodenal and mesenteric venous administration of diltiazem, M1 and A2 were produced mainly in the intestine and liver. Oral administration of HER-SR and HER to dogs resulted in almost-identical plasma concentrations of A2, diltiazem, M1, and M2 (descending order). Supported evidence was the effective absorption of diltiazem from all gastrointestinal tract regions and similar formation ratios of diltiazem basic metabolites (M1, M2) from the duodenum, ileum, and colon.

KEY WORDS: diltiazem; sustained-release preparation; pharmacokinetics; metabolism; colon.

INTRODUCTION

Diltiazem is a coronary vasodilator, commonly referred to as a calcium channel blocker or calcium antagonist (1). It has a relatively short half-life of 4–5 hr and is usually administered three or four times daily. If the dosing frequency could be decreased, patient compliance might be improved, leading to improved therapy. Thus, attempts have been made to develop sustained-release preparations with an extended clinical effect. In this report we describe the pharmacokinetics of diltiazem and its metabolites after oral administration of a multiparticulate sustained-release diltiazem preparation (HER-SR; QD) (2) in dogs and compare it with a conventional diltiazem preparation (HER; TID).

After oral administration, diltiazem is metabolized to several basic or acidic metabolites, via pathways including O-deacetylation, N-demethylation, or oxidative deamination (3,4) (Fig. 1), but the pharmacokinetics have not been fully evaluated. Following intravenous administration of diltiazem and its metabolites, and oral administration of HER and HER-SR, plasma concentrations of diltiazem and its six metabolites were determined using a newly developed UV-HPLC method, and the metabolism of HER-SR in dogs is discussed.

EXPERIMENTAL

Materials

Chemicals. Diltiazem hydrochloride (Diltiazem), its metabolites [*N*-monodesmethyl diltiazem, M1; deacetyl diltiazem, M2; (+)-(2S,3S)-2-(4-methoxyphenyl)-3-acetoxy-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-5-acetic acid, A1; 3-deacetyl-A1, A2; *O*-demethyl-A1, A3; *O*-demethyl-3-deacetyl-A1, A4], and internal standard [*cis*-(2*S*,3*S*)-8-chloro-2-(4-hydroxy-phenyl)-3-acetoxy-2,3-dihydro-4-oxo-1,5-benzothiazepin-5-acetic acid] were synthesized in Tanabe Seiyaku Company. Other chemicals were special-grade reagents. As diltiazem preparation, a conventional diltiazem tablet containing 30 mg of diltiazem hydrochloride (HER; TID) and a multiparticulate sustained-release diltiazem capsule containing 100 mg of diltiazem hydrochloride (HER-SR; QD) were used for the study. The HER-SR preparation consisted of both fast (15% of total diltiazem) and slow (85%) release beads.

Animal Experiment

Animals. Four beagle dogs were purchased from Yoshiki-yakko and maintained on a diet of dog chow (Oriental yeast). The same dogs (12.8 ± 0.3 kg, mean ± SE; $n = 4$) were used in both intravenous and oral administration.

Intravenous Administration. An aqueous solution of 10–20 mg of diltiazem or its four metabolites (M1, M2, A1, A2) was administered intravenously to three dogs, and blood samples were withdrawn at 0, 3, 6, 9, 15, 30, 60, 120, 180, 240, and 360 min after administration.

Oral Administration. One tablet of HER or one capsule of HER-SR was administered orally to four dogs by compulsive swallowing with 30 mL of water. Blood samples were taken at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, and 24 hr for HER and 0, 1, 2, 3, 4, 6, 8, 10, 13, 15, 17, 19, 21, 24, 27, and 30 hr for HER-SR.

Mesenteric Venous or Intraduodenal Administration. An aqueous solution of 10 and 30 mg of diltiazem was administered into the mesenteric vein and ligated upper intraduodenal loop (20 cm), respectively, of pentobarbital anesthetized dogs. Blood samples were withdrawn from a forelimb vein at 0, 3, 6, 9, 15, 30, 60, 120, 180, 240, and 360 min for the former and from the mesenteric vein at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, and 240 min for the latter.

Absorption and Metabolism of Diltiazem from Several Regions of the Gastrointestinal Tract. An aqueous solution of 30 mg of diltiazem was administered into the ligated intraduodenal, ileac, or colonic loops (20 cm) of pentobarbital-anesthetized dogs. Blood samples were withdrawn from a forelimb vein at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 300 min. Plasma samples were frozen at −20°C.

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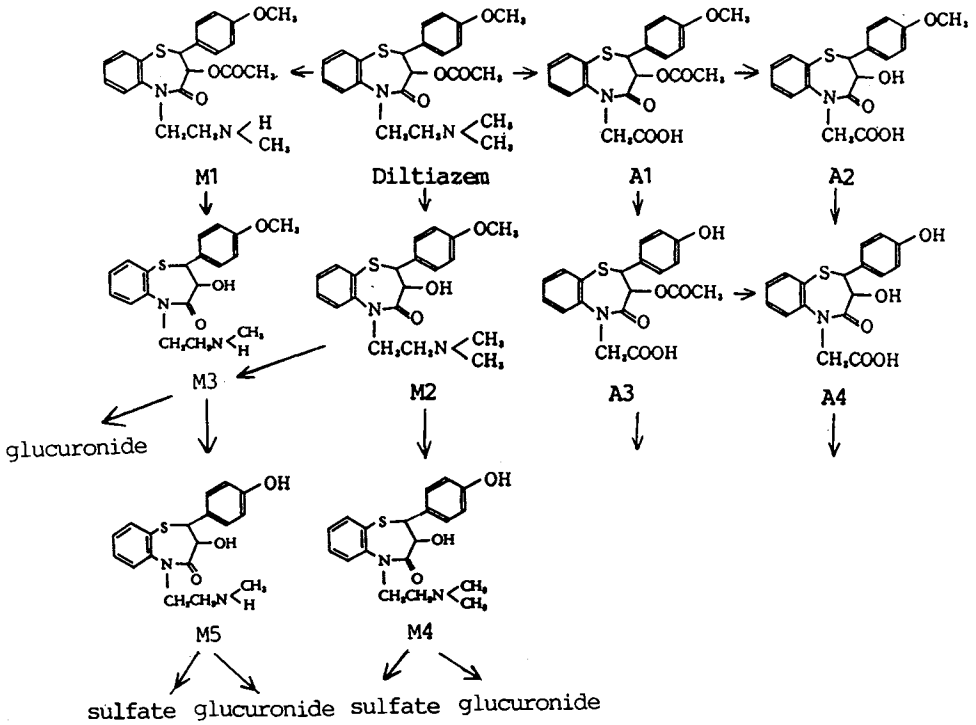

Fig. 1. Major pathways of diltiazem metabolism. Diltiazem, diltiazem hydrochloride; M1, *N*-monodesmethyl diltiazem; M2, deacetyl diltiazem; A1, (+)-(2*S*,3*S*)-2-(4-methoxyphenyl)-3-acetoxy-4oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-5-acetic acid; A2, 3-deacetyl-A1; A3, *O*-demethyl-A1; A4, *O*-demethyl-3-deacetyl-A1.

Determination of Concentrations of Plasma Diltiazem and Its Metabolites

Plasma diltiazem and two basic metabolites (M1, M2) concentrations were determined by high-performance liquid chromatography (Shimadzu LC-3A) with UV detector (5). Plasma diltiazem acidic metabolites (A1, A2, A3, A4) were determined by high-performance liquid chromatography with UV detector as follows.

A 1 mL of plasma specimen was put into a 15-mL test tube containing 0.5 mL of internal standard (400 ng/mL) and 0.5 mL of 1 *N* HCl. To the test tube, 6 mL ether:*n*-octylalcohol (4:1) was added, then the test tube was shaken for 10 min. After 5 min of centrifugation, 5 mL of the organic phase was transferred into a 10-mL tapered tube, and 0.3 mL of a 0.07 *M* phosphate buffer solution (pH 8) was added, then the solution was mixed for 1 min. By this reverse extraction, A1, A2, A3, and A4 were transferred into the aqueous phase. After centrifugation for 5 min, the organic phase was discarded. The buffer solution was washed with 5 mL of *n*-hexane by mixing for 1 min. After centrifugation for 5 min, the organic phase was discarded. This washing procedure was performed again. After the aqueous solution was added with 50 μL of 1 *N* HCl, 200 μL of the aqueous solution was injected into the UV-HPLC. Conditions of HPLC for A1, A2, A3, and A4 were as follows: HPLC, Shimadzu LC-4A; detector, Shimadzu SPD-2AS; column, Spherisorb 5-ODSII, 4.6 ϕ × 250 mm; mobile phase, 0.01 *M* phosphate buffer (pH 6.5): acetonitrile, 154:46 for A1 and A2 and 164:36 for A3 and A4; detection wavelength, 238 nm; flow rate, 0.5 mL/min; and column temperature, 50°C.

Inter- and intraassay coefficients of variation of diltiazem and its metabolites were <10%, and the limit of quantitation of diltiazem and its six metabolites was 5 ng/mL.

RESULTS AND DISCUSSION

Plasma Concentration After Intravenous Administration of Diltiazem to Dogs

The concentrations of plasma diltiazem and its six metabolites (M1, M2, A1, A2, A3, A4) after a single intravenous administration of 20 mg of diltiazem in aqueous solution to three dogs were determined for evaluation of diltiazem metabolism. Detectable plasma concentrations were in the descending order of diltiazem, A2, M2, and A1, while plasma concentrations of M1, A3, and A4 were below assay limits. The percentages of conversion of diltiazem to its metabolites (M2, A1, A2) were calculated to be 6.4, 4.8, and 1.8%, respectively, based on the AUCs of M2, A1, and A2 after intravenous administration of diltiazem or authentic metabolites. Table I shows the AUCs calculated from plasma levels of diltiazem and its metabolites.

Plasma Concentration After Oral Administration of a Conventional Diltiazem Preparation (HER) to Dogs

Table I also shows the AUCs calculated from plasma diltiazem and its four metabolites after an oral administration of a conventional diltiazem tablet (HER) to four dogs. Detectable plasma concentrations in descending order were found for A2, diltiazem, M1, and M2, while plasma concen-

Table I. Comparison of Area Under the Curves of Diltiazem and Its Metabolites

Compound	AUC (ng · hr/mL)[a]					
	iv diltiazem	iv M1	iv M2	iv A1	iv A2	po diltiazem
Diltiazem	360.3 ± 1.2[b]	—	—	—	—	122.7 ± 26.9[d]
M1	—	339.0 ± 36.0[b]	—	—	—	105.1 ± 15.9[d]
M2	31.0 ± 1.0[c]	—	485.5[b]	—	—	10.2 ± 2.9[d]
A1	20.4 ± 4.2[c]	—	—	423.0 ± 60.2[b]	—	—
A2	128.0 ± 16.0[c]	—	—	—	7060.3 ± 1161.0[b]	1032.6 ± 254.0[d]

[a] Corrected to 10-mg dose; values are the means ± SE of three or four dogs.
[b] 0–∞ [AUC = $(A/\alpha) + (B/\beta)$].
[c] 0–6 hr.
[d] 0–∞.

trations of A1, A3, and A4 were below assay limits. The absolute bioavailability of HER calculated from the AUCs of diltiazem after intravenous and oral administration was about 30%. If the pathways of diltiazem metabolism are independent of dose and administration route, the percentages of conversion of diltiazem to its metabolites (M1, M2, A2) can be calculated as 31.0, 2.1, and 14.6%, respectively, based on the AUCs of M1, M2, and A2 after oral administration of diltiazem and intravenous administration of authentic metabolites. This suggested that M1 and A2 were the main metabolites. Also, diltiazem was well absorbed from the intestine, considering both the percentages of its bioavailability and the high conversion of diltiazem to its two metabolites (M1, A2). This was confirmed by the fact that approximately 90% of an orally administered dose of ^{14}C-diltiazem was absorbed in rats (6). Therefore, it appears that lowered bioavailability is due mainly to metabolism during the absorption process.

In order to obtain further information on the metabolism of diltiazem after oral administration, the degree of inactivation of diltiazem in the liver and intestine was examined *in situ*. An aqueous solution of diltiazem was administered to

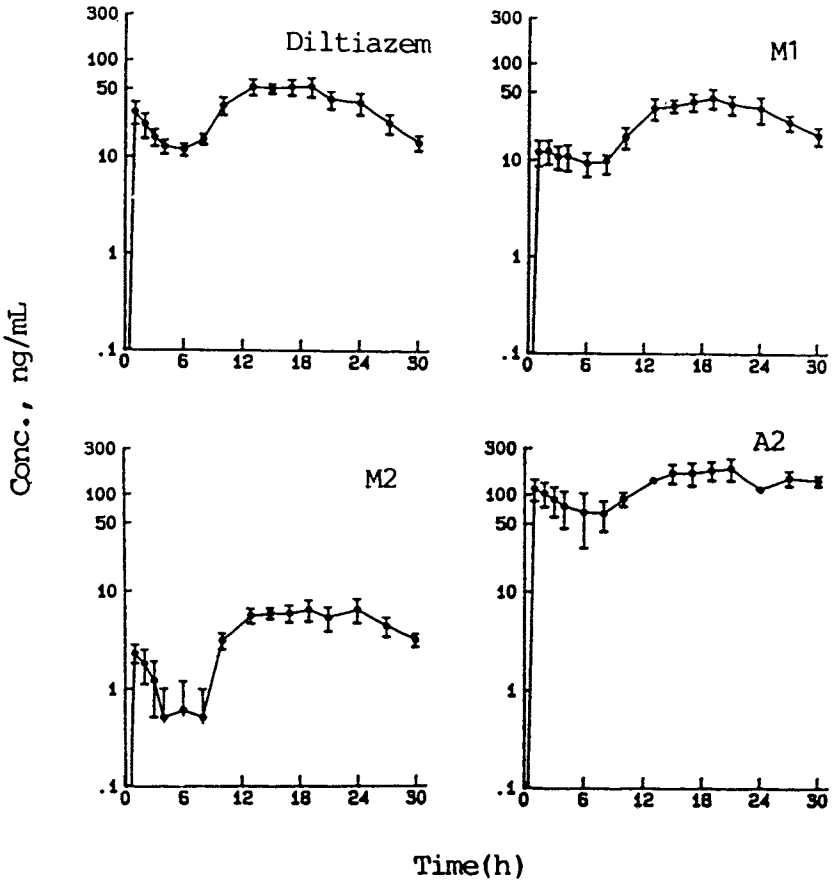

Fig. 2. Plasma concentration of diltiazem and its metabolites after oral administration of HER-SR containing 100 mg diltiazem to dogs. Each point represents the mean ± SE of four dogs.

Table II. Comparison of the Areas Under the Curves of Diltiazem and Its Metabolites

Compounds	AUC (ng · hr/mL)[a]	
	HER-SR[b]	HER[b]
Diltiazem	1071.9 ± 134.0	1226.9 ± 269.3
M1	1008.6 ± 105.3	1050.7 ± 159.1
M2	123.2 ± 18.6	102.0 ± 29.0
A2	7014.3 ± 1317.3	10326.4 ± 2540.3

[a] Corrected to 100-mg dose; values are the means ± SE of four dogs.
[b] 0–∞.

Table III. Absorption and Metabolism of Diltiazem from Several Regions in the Gastrointestinal Tract (n = 2)

Compounds	$AUC_{0-5\ hr}$ (ng · hr/mL)		
	Duodenum	Ileum	Colon
Diltiazem	939.1 (100%)	621.9 (100)	493.6 (100)
M1	272.9 (29.1)	176.7 (28.4)	157.2 (31.8)
M2	86.0 (9.2)	62.1 (10.0)	33.0 (6.7)

the mesenteric vein or the ligated intraduodenal loop (20 cm). Plasma concentrations of diltiazem and its four metabolites (M1, M2, A1, A2) in the systemic circulation and mesenteric vein, respectively, were determined. Diltiazem was metabolized in both the liver (3) and intestine, with M1 and A2 as the main products, although the presence of metabolites in the mesenteric blood must be considered which were due to metabolism in liver after absorption.

Plasma Concentration After Oral Administration of a Multiparticulate Sustained-Release Diltiazem Preparation (HER-SR) to Dogs

The plasma concentrations of diltiazem and its four metabolites (M1, M2, A1, A2) were determined after oral administration of HER-SR. Figure 2 shows the results of oral administration of HER-SR. The HER-SR preparation showed prolonged plasma concentrations of diltiazem and its metabolites and a double peak. Fast-release beads were quickly absorbed, while slow-release beads were gradually absorbed after a certain lag time. Plasma concentrations were in the descending order of A2, diltiazem, and M1, being very similar to that of HER. A1 was not detected in plasma following administration of either HER or HER-SR. Table II shows the AUCs calculated from the plasma concentrations as shown in Fig. 2. The comparative bioavailability of HER-SR to HER calculated from the AUCs of diltiazem was about 90%. The AUCs of M1 and M2 were similar in both preparations. The small difference between the AUC of A2 for HER-SR and that for HER was not statistically significant. These results confirmed the similar metabolism of HER-SR and HER.

The HER-SR preparation consisted of both fast (15% of total diltiazem)- and slow (85%)-release beads, as mentioned under Experimental. The slow-release beads show positioned release, the colon being the main receptive site (2,7). Therefore, it seemed useful to examine the metabolism of diltiazem in several regions of the gastrointestinal tract, especially the colon. An aqueous solution of 30 mg diltiazem was administered to the ligated intraduodenal, ileac, or colonic loop (20 cm), and the plasma concentrations of diltiazem and its two basic metabolites (M1, M2) in the systemic circulation were determined. Table III shows the AUCs calculated from the plasma concentrations of diltiazem and its two basic metabolites. Diltiazem was absorbed effectively from all regions of the gastrointestinal tract, the ratio of formation of basic metabolites (M1, M2) from diltiazem being the same for each region (duodenum, ileum, colon). The plasma concentrations of acidic metabolites were not determined, since A2, M1, and M2 showed similar pharmacokinetic profiles between the two preparations. These results suggested that the metabolism of HER-SR is similar to that of HER, although it may be necessary to compare the ratio of metabolite formation after varying administration rates of the drug.

In conclusion, it was found that metabolism of HER-SR was similar to that of HER. Diltiazem was metabolized in both the liver and the intestine, where M1 or A2 was mainly produced. Also, diltiazem was absorbed from all regions of the gastrointestinal tract, and the ratio of formation of the two basic metabolites from diltiazem was the same for each region.

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