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Pharmacokinetics of diltiazem and deacetyldiltiazem in rats

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Summary

Diltiazem (DTZ) was given intravenously (i.v.), orally (p.o.) and hepatoportally (p.v.) in solution form to rats in order to assess the pharmacokinetic behavior of DTZ and its major metabolite, deacetyldiltiazem (DAD). The plasma half-life at postdistributive phase ($t_{1/2,\beta}$), total body (plasma) clearance (CL_t) and volume of distribution at steady-state (Vd_{ss}) of DTZ were 38.3 min, 90.3 ml/min per kg and 3595 ml/kg, respectively, for a 3 mg/kg i.v. dose. DAD was also given to rats through the i.v. route and its plasma pharmacokinetics was compared with that of DTZ. The $t_{1/2,\beta}$, CL_t and Vd_{ss} of DAD were 43.3 min, 82.4 ml/min per kg and 4042 ml/kg, respectively, for a 3 mg/kg i.v. dose. There was no significant difference in the pharmacokinetic parameters between DTZ and DAD. 20% of the i.v. dose was found to be metabolized to DAD in the body. Most of an oral dose of DTZ (30 mg/kg) was extracted during its passage through the GI tract and only 15% of the dose was transported into the portal venous blood as an intact form. DTZ administered by the p.v. route (10 mg/kg) suffered hepatic extraction and 37% of the dose could reach the systemic circulation. As a consequence, bioavailability of only 6% could be obtained for the oral DTZ.

Introduction

Diltiazem (DTZ) has been widely used as a calcium antagonist (Sato et al., 1971) in the treatment of angina pectoris, arrhythmia and hypertension (Chaffman and Brogden, 1985). DTZ has been reported to be extensively and rapidly absorbed from the gastrointestinal (GI) tract, and to be highly metabolized in the liver of rats and mice (Meshi et al., 1971; Sakuma et al., 1971; Nakamura et al., 1987). Their conclusions were based on total radioactivity data in the systemic blood after oral administration of [^{14}C]DTZ. Oral bioavailability of a drug calculated on the basis of total radioactivity in the blood will be overestimated when the drug suffers first-pass metabolism before entering the systemic circulation. The aim of this study was to characterize the pharmacokinetic behavior of DTZ and DAD, a major metabolite of DTZ (Morselli et al., 1979; Rovei et al., 1980; Hermann et al., 1983; Sugihara et al., 1984; Sugawara et al., 1988a,b). Plasma concentration profiles of DTZ and DAD after intravenous (i.v.), hepatoportal venous (p.v.) and oral (p.o.) administration were examined and

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their absolute bioavailabilities were compared by high-performance liquid chromatography (HPLC). The pharmacokinetics of DAD, which displays 30–50% pharmacological activity of DTZ (Rovei et al., 1980), was also studied and compared with that of a DTZ after i.v. administration of DAD to rats.

Materials and Methods

Chemicals and animals

DTZ and DAD were kindly donated by Han-Il Pharmaceutical Co. (Seoul, Korea). Imipramine (IMP), an internal standard in HPLC, was donated by Hwan-In Pharmaceutical Co. (Seoul, Korea). Methanol (Merck), acetonitrile (Merck) and *t*-butyl methyl ether (Aldrich) were of HPLC grade. All other reagents were of analytical grade and used as purchased. Male Wistar rats weighing 200–260 g were used in all experiments after fasting 24 h before the experiments.

Intravenous (i.v.) administration of DTZ and DAD

Rats were fixed at supine position during the experiments. Under light ether anesthesia, femoral arteries and veins of the rats were cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, U.S.A.) for blood sampling and drug administration, respectively. After complete recovery (1 h) from anesthesia, 0.3% (w/v) DTZ solution in saline was administered i.v. to the femoral vein at a dose of 1 mg/kg (3 mg/kg). Blood samples (250 μl) were collected into heparinized tubes from the femoral artery through the catheter at 0, 5, 10, 20, 30, 45, 60, 90, 120, 150 and 180 min after the dose. Heparinization was conducted by treating the tubes with 10 μl of heparinized saline (150 IU/ml) and successively drying the saline. Plasma samples were separated by centrifuging the blood samples at $6000 \times g$ for 1 min and assayed for DTZ and DAD.

0.3% (w/v) DAD solution in saline was also administered i.v. at a dose of 1 ml/kg (3 mg/kg), and blood samples (250 μl) were collected and assayed for DAD concentration in plasma. All the other procedures were identical to those for DTZ study.

Portal venous (p.v.) and oral (p.o.) administration of DTZ

Under light ether anesthesia, the abdomen was opened through a midline incision and the tip of an injection needle (25 gauge) attached to a 1 ml syringe through PE-50 tubing was inserted into the portal vein and fixed with surgical glue (Aron Alpha, Sankyo Co., Japan). The needle was bent at an angle of 120° for convenience of insertion. The femoral artery was then cannulated with PE-50 for blood sampling. After the suture of the abdomen and complete recovery from anesthesia, 1% (w/v) solution of DTZ in saline was administered at a dose of 1 ml/kg (10 mg/kg) through the p.v. catheter. Blood samples (250 μl) were withdrawn at 0, 5, 10, 20, 40, 60, 90, 120, 150 and 180 min after the dose. Plasma samples were separated by centrifuging the blood samples at $6000 \times g$ for 1 min and stored at -20°C for DTZ and DAD (Montamat et al., 1987).

DTZ was also administered orally to another group of rats. Under light ether anesthesia, the femoral artery was cannulated as described above. After complete recovery from anesthesia, 1% (w/v) solution of DTZ in distilled water was administered orally at a dose of 3 ml/kg (30 mg/kg). Oral administration was performed by insertion of a round-tip needle which was connected to a 1 ml syringe. Blood samples were collected and assayed for DTZ and DAD. All other procedures were identical to those for the p.v. study.

HPLC assay of DTZ and DAD

DTZ and its major metabolite DAD in plasma samples were assayed by modifying the reported HPLC method (Goebel and Kolle, 1985). 50 μl of internal standard solution (2 μg/ml of IMP in methanol) was taken in a polypropylene tube and evaporated to dryness under a gentle stream of nitrogen. To the residue, 100 μl of plasma sample was added and vortexed. Subsequently, it was extracted with 3 ml of *t*-butyl methyl ether by vortexing for 5 min. After centrifugation, the tubes were placed in a dry ice bath and 2.5-ml aliquots of the unfrozen upper organic phase were transferred to another tube. They were then

back-extracted with a 100 μl of 0.01 N HCl by vortexing for 1 min. From the HCl layer, 30-μl aliquots were taken and injected into the HPLC system.

The HPLC system consisted of a precision isocratic pump (model SP 8810), C_{18} reversed-phase column (μ-Bondapak, 10 μm silica, 300 × 3.9 mm i.d., stainless steel, Waters, P/N 27324) and a UV absorbance detector (model 757, Applied Biosystems). The mobile phase was a mixture of methanol, acetonitrile, 0.04 M ammonium bromide and triethylamine (40:24:36:0.1 v/v). The pH of the mobile phase was adjusted to 6.4 using 2 N-HBr. The flow rate of the mobile phase was 1.0 ml/min and the wavelength of the detector was 237 nm. DTZ and DAD were appropriately separated from IMP and plasma-derived substances with retention times of 6 and 5 min, respectively. Recoveries of DTZ and DAD were more than 90% and their calibration curves were linear over the concentration range of 20–1000 ng/ml. Their detection limits were 20 ng/ml.

Pharmacokinetic analysis

A linear pharmacokinetic model was assumed throughout the study. Total body plasma clearance (CL_t), distribution volume at steady-state (Vd_{ss}) and pharmacokinetic half-life ($t_{1/2,\beta}$) of DTZ and DAD were calculated using the respective i.v. data as follows:

$$CL_t = D/AUC \quad (1)$$

$$Vd_{ss} = \text{Dose } AUMC/AUC^2 \quad (2)$$

$$t_{1/2,\beta} = 0.693/\beta \quad (3)$$

where D, AUC, AUMC and β respectively denote dose, area under the plasma concentration-time curve from time zero to infinity, area under the moment of the plasma concentration-time curve from time zero to infinity, and apparent elimination rate constant obtained from the slope of log-linear portion (β-phase) of the plasma concentration-time curve. AUC and AUMC were calculated by the trapezoidal rule after fitting the plasma concentration data to the conventional two-compartment model using the MULTI program (Yamaoka et al., 1981). β was also obtained via the fitting.

The fraction of an i.v. dose of DTZ which was converted to DAD (f_m) was calculated from the plasma DTZ and DAD concentration profiles after i.v. administration of DTZ as follows (Rowland and Tozer, 1989):

$$f_m = \{AUC_{DAD}(CL_t)_{DAD}\}/\{AUC_{DTZ}(CL_t)_{DTZ}\} \quad (4)$$

The fraction of DTZ transported (F, availability = 1 − extraction ratio) across the liver (F_{liver}) and GI(F_{GI}) was calculated from the following relationships based on the drug disposition model (Gillette and Pang, 1977; Sugiyama, 1985) using AUC data after i.v., p.v. and p.o. administration. Since only 0.1% of an i.v. dose was reportedly recovered from the bile of rats (Meshi et al., 1971), DTZ was presumed not to be subject to enterohepatic recirculation in this model.

$$F_{liver} = D_{iv}AUC_{pv}/D_{pv}AUC_{iv} \quad (5)$$

$$F_{GI} = D_{pv}AUC_{po}/D_{po}AUC_{pv} \quad (6)$$

The bioavailabilities of DTZ administered through the p.v. and p.o. routes were calculated by comparing the AUC of the routes with the AUC of the i.v. route after dose normalization. F_{liver} in Eqn 5 also indicates the bioavailability of DTZ administered through the p.v. route.

Statistical analysis

One-way analysis of variation (ANOVA) with Tukey's multiple range comparison procedure was used to compare the pharmacokinetic data between DTZ and DAD, and a p value of 0.05 or less was considered to be significant.

Results and Discussion

Plasma levels of DTZ and DAD after i.v. administration of DTZ (3 mg/kg) to rats were plotted as a function of time and are shown as solid curves in Fig. 1. The profiles of DTZ showed

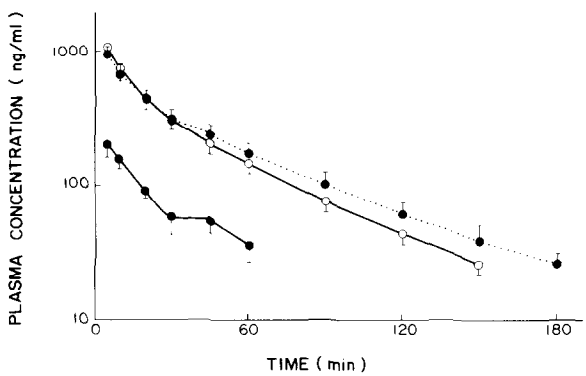

Fig. 1. Plasma concentration-time profiles of DTZ (○) and its major metabolite DAD (●) after i.v. administration of DTZ (solid lines, $n = 6$), and that of DAD (●, dotted lines) after i.v. administration of DAD ($n = 5$) to rats. The doses of DTZ and DAD were identical at 3 mg/kg. Each point represents mean ± SD of the experiments.

a biexponential decay resulting in the spontaneous appearance of DAD in plasma. The plasma profile of DAD after i.v. administration of DAD (3 mg/kg) is also shown as a dotted curve in Fig. 1. The plasma profile of DAD after DTZ administration was nearly parallel to that of DTZ, although it was much lower than that of DTZ. This indicates that DTZ is spontaneously metabolized to DAD in the body, and that the overall plasma profile of DAD is dominated by the elimination process of DAD. The plasma profile of DAD after DAD administration was almost superimposable over that of DTZ after DTZ administration, indicating their similar pharmacokinetics.

Pharmacokinetic parameters of DTZ and DAD are listed in Table 1. There were no significant differences in the parameters between DTZ and DAD. $t_{1/2,\beta}$ of DTZ was comparable to that reported by Nakamura et al. (1987), viz., 56 min. The CL_t of DTZ in this study (90.3 ml/min per kg) was much greater than the values reported previously in humans (Hermann et al., 1983; Kolle et al., 1983) and dogs (Piepho et al., 1982). Values of only 21 (Hermann, 1983) or 11.5 ml/min per kg (Kolle et al., 1983) in humans and 46.1 ml/min per kg in dogs (Piepho et al., 1982), were reported indicating a species-related difference in the pharmacokinetics of DTZ, since there were reportedly no significant differences in the plasma protein binding of DTZ (approx. 70–80%) among the species (Piepho et al., 1982). However, the Vd_{ss} of DTZ was consistent with the results in humans (Hermann et al., 1983; Kolle et al., 1983; Smith et al., 1983) and dogs (Kohno et al., 1977). Therefore, the species-related difference in CL_t could be attributed to the difference in elimination rates among the species.

TABLE 1

Pharmacokinetic parameters of DTZ and DAD following respective i.v. administration to rats (3 mg/kg) [a]

Parameters	DTZ ($n = 6$)	DAD ($n = 5$)
$t_{1/2,\beta}$ (min)	38.3 ± 1.9	43.3 ± 8.6
CL_t (ml/min per kg)	90.3 ± 15.0	82.4 ± 6.2
Vd_{ss} (ml/kg)	3595 ± 808	4042 ± 104
f_m [b]	0.2 ± 0.0	

[a] Expressed as mean ± SD.

[b] Fraction of an i.v. dose of DTZ converted to the metabolite, DAD, was calculated according to Eqn 4 after i.v. administration of DTZ (3 mg/kg).

In contrast to the general properties of metabolites, the pharmacokinetic parameters of the metabolite (DAD), particularly CL_t, were very close to those of the parent drug (DTZ). Therefore, DAD as well as DTZ is also expected to be accumulated in the plasma upon multiple dosing of DTZ. In spite of rapid formation of DAD from DTZ, the fraction of i.v. DTZ metabolized to DAD (f_m) amounted to only 20% (Table 1), implying the subsequent metabolism of DAD (Morselli et al., 1979; Rovei et al., 1980; Hermann et al., 1983).

Fig. 2 shows the plasma profiles of DTZ and DAD after p.v. and p.o. administration of DTZ. The plasma levels of DAD after p.v. administration of DTZ were very close to those of DTZ, indicating extensive metabolism of DTZ to DAD during its first pass through the liver. The plasma levels of DAD after p.o. administration of DTZ appeared even higher than those of DTZ, implying that DTZ is also metabolized to DAD during its passage through the membrane of the GI tract. Oral absorption of DTZ seemed to occur rapidly, since the time to reach the maximum plasma concentration, T_{max}, was below 20 min for both DTZ and DAD. Nakamura et al. (1987) failed to observe the absorption peak of DTZ

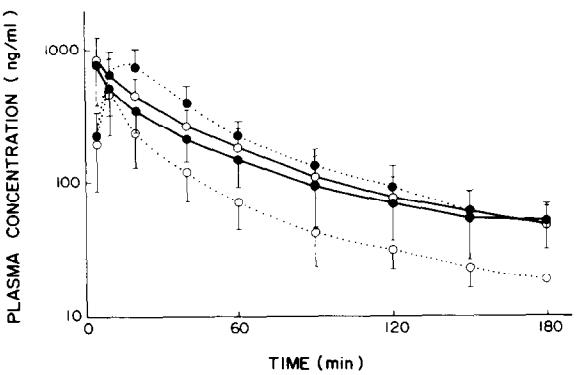

Fig. 2. Plasma concentration-time profiles of DTZ (○) and DAD (●) after p.v. (solid lines, $n = 7$) and p.o. (dotted lines, $n = 6$) administration to rats. The doses through the p.v. and p.o. routes were 10 and 30 mg/kg, respectively. Each point represents mean ± SD of the experiments.

after oral administration of DTZ, since they collected the first blood sample at 30 min after administration.

The bioavailabilities of DTZ administered through the p.v. and p.o. routes were calculated to be 37% (±16%, $n = 7$) and 6% (±2%, $n = 6$), respectively. The fractions transported through the liver (F_{liver}) and GI tract (F_{GI}) calculated according to Eqns 5 and 6 were 0.37 (±0.16, $n = 7$) and 0.15 (±0.07, $n = 6$), respectively, when expressed as mean ± SD of the experiments. F_{liver} clearly indicates considerable hepatic metabolism of DTZ to DAD. F_{GI} indicates either incomplete absorption of DTZ from the GI lumen or metabolism of DTZ in the GI mucosa, although the contribution of each process to the F_{GI} value was not analyzed in this study. The metabolism in the GI mucosa is consistent with our recent work (Lee et al., unpublished observations). This means that most of the oral DTZ is extracted during its passage through the GI tract and only 15% of the dose is transported through the GI tract into the portal venous blood in an intact form. DTZ transported from the GI lumen into the portal venous blood will undergo hepatic extraction of 63%. As a consequence, only 6% (0.15×0.37) of an oral dose of DTZ can reach the systemic blood circulation. Conclusively, high bioavailability of oral DTZ could not be expected due to the extensive first-pass extraction of DTZ before it entered into the systemic circulation.

The bioavailabilities of oral DTZ reported previously to be 44% (Kolle et al., 1983) and 24–74% (Hermann et al., 1983) in humans are much higher than that for rats in the present study (6%). The larger CL_t for the rat as compared to humans might contribute to the difference.

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