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Pharmacokinetics and Haemodynamic Effect of Diltiazem in Rats: Effect of Route of Administration

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Abstract

Diltiazem is a calcium antagonist widely used for the treatment of angina and hypertension. Previous studies in patients have shown that the haemodynamic effects of diltiazem are greater after parenteral rather than oral administration. The rat has been used as an animal model to determine the effect of the route of administration on the pharmacokinetic and haemodynamic effects of diltiazem.

The results showed that plasma concentrations of diltiazem were more than 10 times higher after the intra-arterial dose. The plasma concentrations of the major metabolites were also higher after intra-arterial administration, although only for deacetyl diltiazem (M_1) did the difference reach statistical significance ($P < 0.05$). The haemodynamic effects (on blood pressure and heart rate) of diltiazem were considerably greater after intra-arterial administration; this was attributed mainly to the much higher plasma concentrations of diltiazem. The hypotensive and chronotropic effects of diltiazem were similar; E_{max} and EC50 for diastolic blood pressure were $72 \pm 19\%$ and 4.4 ± 5.9 $\mu g\ mL^{-1}$; for heart rate they were $77 \pm 32\%$ and 10.0 ± 11.7 $\mu g\ mL^{-1}$, respectively.

The haemodynamic effects of diltiazem are much greater after intra-arterial administration, mainly because of the much higher plasma concentrations of the drug. The contribution by the metabolites would be minimal after this route of administration.

Diltiazem is a calcium antagonist widely used in the treatment of angina and hypertension (Medical Letter 1993, 1994; Weir 1995). In man it is extensively metabolized via deacetylation, *N*-demethylation, *O*-demethylation and oxidative deamination yielding many metabolites, some of which have potent pharmacological activity (Kiyomoto et al 1983; Yabana et al 1985; Yeung et al 1991b). More recently, we have shown that when deacetyldiltiazem (M_1) and deacetyl-*N*-monodesmethyl diltiazem (M_2) were injected separately into rabbits, they induced a significant hypotensive effect although their effects on heart rate were highly variable (Yeung et al 1996b, 1997a,b). Thus their contribution to the effect of the parent diltiazem should be taken into account, especially if the active metabolites are present at high concentrations. Previous studies have shown that diltiazem undergoes extensive first-pass metabolism in man (Hoglund & Nilsson 1989; Caille et al 1991; Yeung et al 1993) and animals (Yeung et al 1991c; Homsy et al 1995; Lefebvre et al 1996). These have contributed to much lower and variable plasma concentrations of diltiazem after oral doses compared with intravenous administration in both man and animals (Hermann et al 1983; Yeung et al 1991c). The haemodynamic and dromotropic effects of diltiazem are also considerably higher after intravenous administration (Schwartz & Abernethy 1987). This paper characterizes and compares the pharmacokinetic and haemodynamic effects of diltiazem in rats after intra-arterial (i.a.) and oral (p.o.) administration.

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Materials and Methods

Chemicals

Diltiazem and its metabolites were generous gifts from Tanabe Seiyaku (Japan) via Hoechst Marion Roussel Canada Research (Laval, QC, Canada).

Racemic metabolites *O*-desmethyldiltiazem (M_x) and *N,O*-didesmethyldiltiazem (M_B) were kindly provided by Dr P. S. Farmer of the College of Pharmacy, Dalhousie University, Halifax, Nova Scotia, Canada (Li et al 1992). Solvents were high-performance liquid chromatography (HPLC) grade (BDH, Halifax, Nova Scotia, Canada) and other chemicals were reagent grade (Fisher Scientific, Ont., Canada).

Study protocol

The study protocol was approved by the Dalhousie University Committee on Laboratory Animals. Male Sprague-Dawley rats, 300–600 g, were obtained from Canadian Hybrid Farms, Nova Scotia, Canada. They were housed separately in steel metabolic cages for one week before the study for acclimatization to the environment; food (Co-Op, NB, Canada) and water were freely available. An in-dwelling polyethylene catheter (0·040 in o.d.; Dow Corning, Midland, MI) was implanted into the right carotid artery of each animal under halothane anaesthesia for blood sampling and intra-arterial administration as previously described (Tsui et al 1991). The animals were left to recover fully before the experiment (1–2 days), and randomly divided into three groups (n = 6 for each). One group received 20 mg kg^{-1} diltiazem via a rapid intra-arterial infusion (0·5–1 mL over 5 min), another received the same dose of diltiazem by gastric intubation (oral route). The control group received normal saline (1 mL) by rapid intra-arterial infusion. Blood samples (0·3 mL) were obtained from each rat via the catheter at 0 h (just before dosing) and at 0·16, 0·25, 1·0, 1·5, 2, 3, 4, 6, 8, and 10 h post-dose. After removal of each blood sample the animal received 0·3 mL normal saline to replenish the fluid loss. The blood samples were immediately centrifuged (1720 *g*, 4°C, 10 min) to separate the plasma, which was stored at −20°C until analysis. Plasma concentrations of diltiazem and its major metabolites were determined by HPLC as previously described (Yeung et al 1989, 1996a). The assay can measure less than 10 ng mL^{-1} diltiazem and each of its major metabolites with intra- and inter-assay variations < 5%. All plasma samples were analysed within 3 months of collection to avoid possible sample deterioration (Caille et al 1989; McLean et al 1991; Yeung et al 1991a). In addition, arterial blood pressure (b.p.) (both systolic and diastolic b.p.) and heart rate were recorded at each blood sampling time by use of a Sorenson pressure transducer (Abbott Laboratories, IL). The measurement was taken from an average of 10 s recording. Mean blood pressure was calculated from the equation mean b.p. = diastolic b.p. + (systolic b.p. − diastolic b.p.)/3 (Berne & Levy 1983).

Data analysis

The pharmacokinetic parameters maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}) and apparent terminal half-life t½ were, where appropriate, calculated from non-linear curve fitting using a two-compartment model for intra-arterial bolus or first-order oral absorption (Rstrip, MicroMath Scientific Software, Salt Lake City, UT). The area under the plasma concentration–time curve (AUC) from time 0 to the last sampling time and the area under the first moment curve (AUMC) were calculated by the trapezoidal method (Rstrip). The systemic clearance (CL) of diltiazem was calculated from the equation CL = D/AUC, where D was the intra-arterial dose; and the mean residence time (MRT) from the ratio AUMC/AUC. The volume of distribution at steady-state (Vdss) was equal to CL × MRT (Gibaldi & Perrier 1982).

Relationships between diltiazem plasma concentrations and haemodynamic effects (systolic, diastolic and mean b.p. and heart rate) were evaluated by non-linear regression using the inhibitory E_{max} model (PCNONLIN, V. 3·0, SCI Software, Apex, NC) Because the blood pressure (systolic and diastolic b.p.) decreased in both drug-treated and control rats haemodynamic data from the control animals were subtracted from those from the treated rats before use for modelling of the effects of the drug (i.e. percent change = percent change in drug-treated rats − mean percent change in control rats, where percent change = ([individual time data]/[data obtained before injection]) × 100). Plasma concentration and haemodynamic variables were fitted for each animal using the equation $E = E_0 - (E_{max} \times C_p/(EC50 + C_p))$; where E_0 is the effect before administration of diltiazem, E_{max} the maximum effect (both expressed as percent of control), EC50 the concentration inducing 50% of the maximum effect and Cp the concentration of diltiazem in plasma (Holford & Sheiner 1981; Gabrielsson & Weiner 1994). Effects of diltiazem and route of administration were evaluated by factorial design analysis of variance. Dunnett's multiple range tests were used to assess the differences between haemodynamic data before and after drug administration. The haemodynamic effects of diltiazem compared with control at each sampling time were evaluated by means of unpaired *t*-tests. Differences were considered sig-

nificant when $P < 0.05$ (Systat; Systat, Evanston, IL).

Results

Preliminary analysis of the pharmacokinetic data has been published elsewhere (Tsui et al 1994). The metabolic profiles of diltiazem were qualitatively similar for intra-arterial and oral doses. The plasma concentrations of diltiazem were more than ten times higher after intra-arterial administration and measurable up to 6 h post-dose whereas for most of the animals studied they were measurable only in the first 1 to 2 h after the oral administration. The mean oral bioavailability was 15%. The plasma concentrations of the metabolites were also higher after the intra-arterial dose, although the difference was significant only for M_1 (Figure 1).

The resting mean b.p. measured before administration of diltiazem were 115 ± 8 and 110 ± 7 mm Hg ($P > 0.05$), respectively, after intra-arterial and oral administration; the respective resting heart rates were 416 ± 13 and 401 ± 40 beats min^{-1} ($P > 0.05$). Diltiazem significantly reduced systolic and diastolic b.p. and heart rate for almost 2 h post dose when given intra-arterially, but after oral administration only reduced diastolic b.p. significantly for the first hour post-dose. Plots of diastolic b.p. against time after intra-arterial and oral administration of diltiazem are shown in Figure 2. Differences attributable to the effect of the route of administration were significant for diastolic b.p. only ($P = 0.017$).

The hypotensive effects observed were much greater after intra-arterial administration (maximum reduction of diastolic b.p. 54 ± 8 compared with $9 \pm 4\%$). The model-predicted E_{max} for systolic and diastolic b.p. and heart rate after intra-arterial diltiazem were 35 ± 9, 72 ± 19, and $77 \pm 32\%$, respectively; the corresponding EC50 values were 3.1 ± 3.5, 4.4 ± 5.9 and 10.0 ± 11.7 $\mu g\ mL^{-1}$, respectively (Table 1). A plot of concentration–effect relationships using the group mean data for each of these haemodynamic variables is shown in Figure 3. Because of the relatively low plasma concentrations of diltiazem compared with those of its metabolites, and because it was measurable only in the first 1·5 h

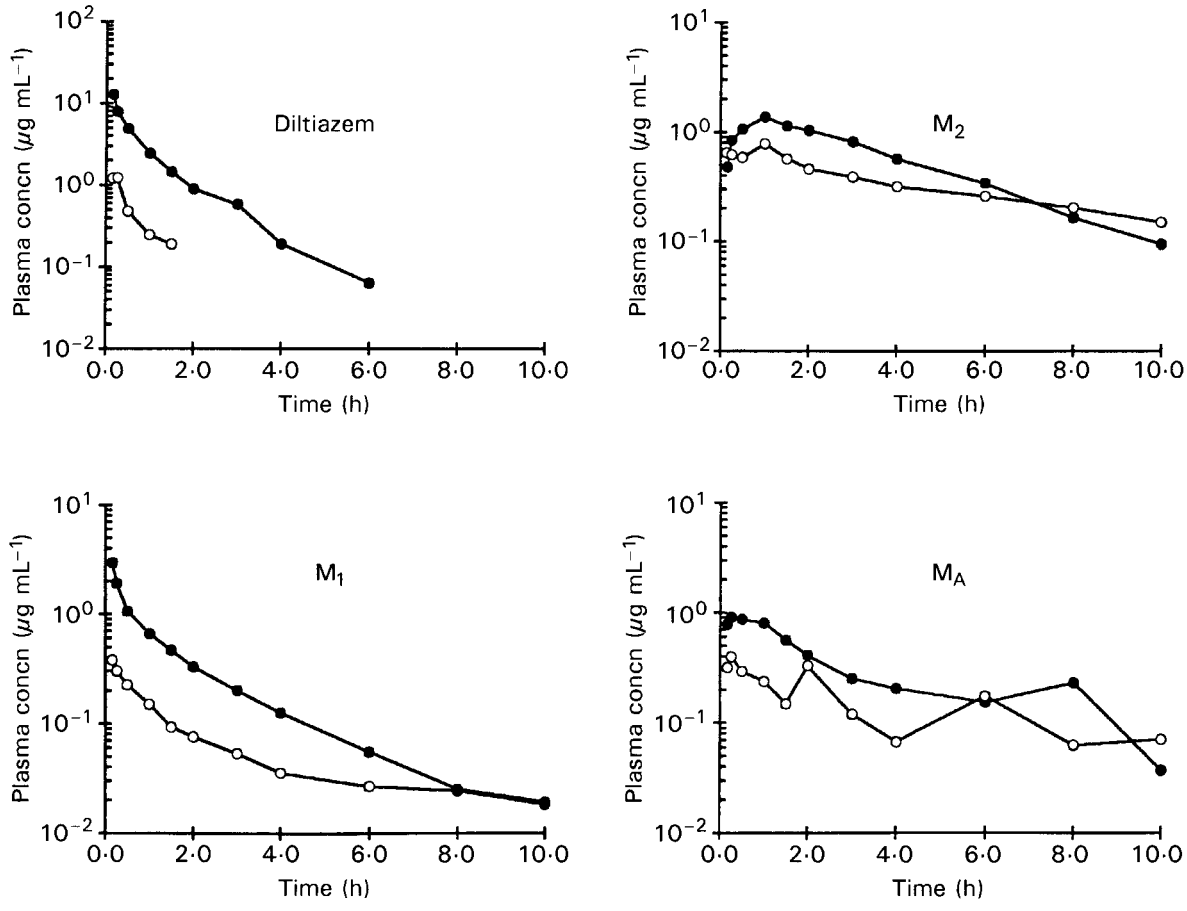

Figure 1. Plasma concentration-time plots for diltiazem and its major metabolites after a single 20 mg kg^{-1} intra-arterial (●) or oral (○) dose of diltiazem.

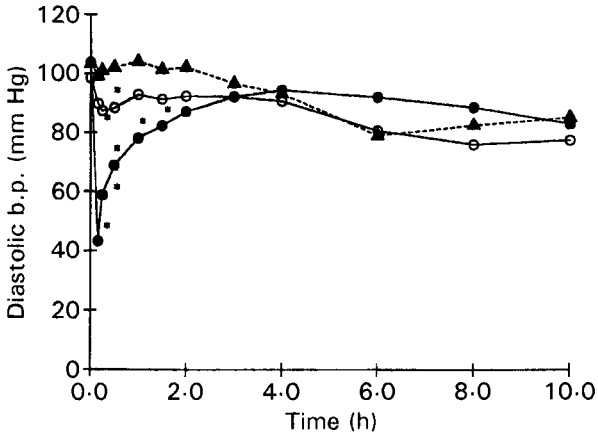

Figure 2. Diastolic blood pressure in control rats (▲) and in rats after a single 20 mg kg^{-1} intra-arterial (●) or oral (○) dose of diltiazem. *$P < 0.05$ significantly different from result for control.

after oral administration (Figure 1), concentration–effect relationships were not evaluated after oral administration. A decrease in diastolic b.p. (but not systolic b.p. or heart rate) was also observed in the control animals, although this became significant only after 6 h.

Discussion

As reported previously, plasma concentrations of the metabolites relative to diltiazem were much higher after oral administration (Tsui et al 1994), which suggests that diltiazem undergoes extensive first-pass metabolism after this route of administration. On the other hand, the plasma concentrations of the metabolites were higher after the intra-arterial dose, although only for M_1 was the difference statistically significant (Tsui et al 1994). These results indicate that after oral administration diltiazem undergoes extensive 'sequential' first-pass metabolism during passage through the gastrointestinal tract and liver (Lee et al 1991; Lefebvre et al 1996). Extra-hepatic metabolism of diltiazem, particularly in the blood and lungs, is also extensive after parenteral administration (Fraile et al 1996).

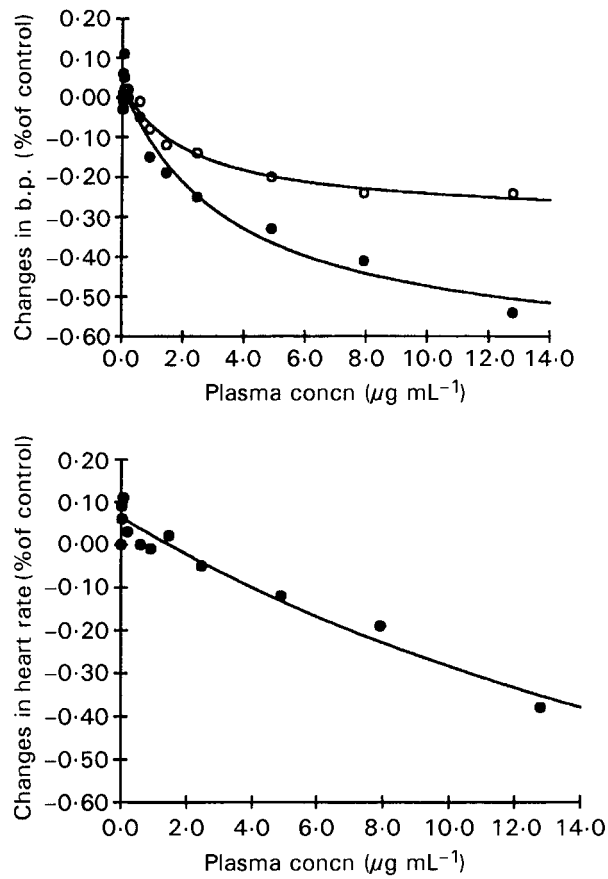

Figure 3. Concentration–effect relationships for systolic and diastolic blood pressure (top figure, ○ and ●, respectively) and heart rate (bottom). The solid lines are the percentage changes predicted by the inhibitory E_{max} model using group mean data.

The baseline haemodynamic results (b.p. and heart rate) reported in this study (Table 1) are

Table 1. Haemod-ynamic effect of diltiazem in rats after a single 20-mg kg^{-1} dose.

	Route of administration	Value before drug administration*	E_{max} (% change from control)	EC50 ($\mu g\ mL^{-1}$)
Systolic blood pressure	Intra-arterial	138 ± 6	35 ± 9	3·1 ± 3·5
	Oral	132 ± 7		
Diastolic blood pressure	Intra-arterial	104 ± 9	72 ± 19	4·4 ± 5·9
	Oral	99 ± 7		
Mean blood pressure	Intra-arterial	115 ± 8	57 ± 13	4·0 ± 5·2
	Oral	110 ± 7		
Heart rate	Intra-arterial	416 ± 13	77 ± 32	10·0 ± 11·7
	Oral	401 ± 40		

Blood pressure in mm Hg, heart rate in beats min^{-1}; values are means ± s.d. of results from six rats.

comparable with those reported in other studies (Downing et al 1987; Harkness & Wagner 1989), indicating that the experiment was adequately designed. The plasma concentration–effect relationships were adequately characterized by the inhibitory E_{max} model (Holford & Sheiner 1981). Similar results were obtained when the inhibitory sigmoidal E_{max} model was used, yielding a 'Hill Factor' of $1{\cdot}1 \pm 0{\cdot}3$, which supports the use of the current model.

The haemodynamic effect of diltiazem was much greater after the intra-arterial dose than after the oral dose. The duration of the effect was also much longer after the intra-arterial dose (Figure 2). This is attributed mainly to the much higher plasma concentrations of diltiazem ($>$ 10-fold difference) after this route of administration (Figure 1). It has been shown in rabbits that many of the metabolites, e.g. M_1 and M_2, have hypotensive effects comparable with those of the parent diltiazem (Yeung et al 1996b, 1997a,b). However, because the concentrations of the metabolites were much lower than those of diltiazem, and because that of M_1 alone was significantly higher after the intra-arterial dose, the haemodynamic effects contributed by the metabolites after the intra-arterial dose would be minimal. Their contribution after oral administration would be considerably more because of their greater concentrations relative to diltiazem (Figure 1), although the haemodynamic effects were relatively minor.

In man, diltiazem lowers both systolic and diastolic b.p. but has a minimal effect on heart rate except a transient reflex tachycardia immediately after drug administration (Joyal et al 1986). The anti-hypertensive effect is also greater after an intravenous administration (Schwartz & Abernethy 1987). When given separately by rapid intravenous injection a single 5 mg kg^{-1} dose of diltiazem, M_1, or M_2 significantly reduces systolic and diastolic b.p. in rabbits, but their effects on heart rate are highly variable (Yeung et al 1996b, 1997a,b). The mean E_{max} and EC50 of the hypotensive effect of diltiazem in rabbits in these experiments were 40% and $1{\cdot}6$ $\mu g\ mL^{-1}$, respectively (Yeung et al 1997a,b). In rat, however, diltiazem significantly reduces both b.p. ($E_{max} = 57 \pm 13\%$; $EC50 = 4{\cdot}0 \pm 5{\cdot}2$ $\mu g\ mL^{-1}$) and heart rate ($E_{max} = 77 \pm 32\%$; $EC50 = 10{\cdot}0 \pm 11{\cdot}7$ $\mu g\ mL^{-1}$) (Table 1 and Figure 3). These results are consistent with those from previous studies which also showed that the hypotensive and chronotropic effects of diltiazem are very similar in this animal (Downing et al 1987). The different haemodynamic effects could be attributed to the much higher plasma concentrations in the rats (greater than 5-fold difference) and possible inherent species differences of the haemodynamic response to diltiazem.

The significant effect of route of administration on the pharmacokinetics and haemodynamic response is clearly demonstrated in this study. Considerably higher plasma concentrations of diltiazem, and greater and more prolonged haemodynamic effects, are attained after intra-arterial administration. Diltiazem has similar effect on the diastolic b.p. and heart rate in rats, although it has a more potent hypotensive effect in man and rabbits.

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