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Pharmacokinetics and Haemodynamic Effect of Deacetyl Diltiazem (M_1) in Rabbits after a Single Intravenous Administration

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ABSTRACT: Deacetyl diltiazem (M_1) is a major metabolite of the widely used calcium antagonist diltiazem (DTZ). In order to study the pharmacokinetic and haemodynamic effects of this metabolite, M_1 was administered as a single 5 mg kg^{-1} dose intravenously (iv) to New Zealand white rabbits ($n = 5$) via a marginal ear vein. Blood samples, blood pressure (SBP and DBP), and heart rate (HR) recordings were obtained from each rabbit up to 8 h, and urine samples for 48 h post-dose. Plasma concentrations of M_1 and its metabolites were determined by HPLC. The results showed that the only quantifiable basic metabolite in the plasma was deacetyl N-monodesmethyl DTZ (M_2). The $t_{1/2}$ and AUC of M_1 and M_2 were 2.1 ± 0.5 and 3.0 ± 1.1 h, and 1300 ± 200 and 240 ± 37 ng h mL^{-1}, respectively. The Cl and Cl_r of M_1 were 60 ± 10 and 0.81 ± 0.63 mL min^{-1} kg^{-1}, respectively. M_1 significantly decreased blood pressure (SBP and DBP) for up to 1 h post-dose ($p < 0.05$), but had no significant effect on the heart rate ($p > 0.05$). The E_{max} and EC_{50} as estimated by the inhibitory sigmoidal E_{max} model were $20 \pm 18\%$ 620 ± 310 ng mL^{-1}, respectively for SBP; $20 \pm 8.3\%$ and 420 ± 160 ng mL^{-1} for DBP. © 1998 John Wiley & Sons, Ltd.

Key words: diltiazem; metabolism; pharmacokinetics; pharmacodynamics; haemodynamics

Introduction

Diltiazem (DTZ) is a calcium antagonist widely used in the treatment of angina and hypertension [1–3]. It is extensively metabolized in humans via deacetylation, N-demethylation, O-demethylation, and oxidative deamination, yielding a host of metabolites, some of which have potent pharmacological activities. Deacetyl DTZ (M_1) is produced from DTZ by deacetylation (Figure 1), and is one of the most abundant metabolites of DTZ in plasma of humans and animal species [4–7]. Metabolism of DTZ to M_1 is catalyzed by specific esterases inducible by phenobarbital, although it is yet to be determined whether these are CYP450 related isozymes [8,9].

The coronary vasodilating property of deacetyl DTZ (M_1) is approximately 50% of that of DTZ as shown in an anaesthetized dog model [10]. However, it is more potent than DTZ in inhibiting platelet aggregation [11], or adenosine uptake by erythrocytes *in vitro* [12]. Thus it may contribute to the overall clinical safety and efficacy of DTZ. When administered intravenously to rats, the disposition kinetics of M_1 was very similar to that of DTZ although the haemodynamic effects were not determined [13]. A more suitable animal model may be the rabbit since the disposition of DTZ in this animal species is closer to that in humans [4]. In light of its potential as a separate therapeutic entity, and its contribution to the effect of DTZ in clinical drug therapy, this study evaluates the disposition of M_1 in rabbits following a single 5 mg kg^{-1} intravenous

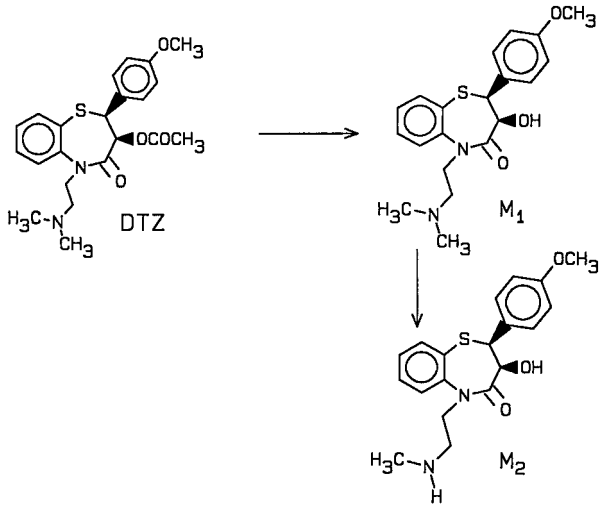

Figure 1. Metabolism of diltiazem to M_1 and M_2

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dose, and its haemodynamic effects in conscious rabbits.

Materials and Methods

Chemicals

DTZ and its metabolites were generously received as gifts from Tanabe Seiyaku (Japan) via Hoechst Marion Roussel Canada Research (Laval, QC, Canada). Racemic metabolites O-desmethyl DTZ (M_x) and N,O-didesmethyl DTZ (M_B) were kindly provided by Dr. P.S. Farmer of the College of Pharmacy, Dalhousie University, Halifax, NS, Canada [14]. Solvents were HPLC grade (BDH, Halifax, NS, Canada), and all other chemicals were reagent grade (Fisher Scientific, Ont., Canada).

Study Protocol

The study protocol was approved by the Dalhousie University Committee on Laboratory Animals. Female New Zealand white rabbits (Riemens Ranch, Ont., Canada) weighing between 3.1 and 4.2 kg were used for the experiments. They were housed in steel metabolic cages for 1 week prior to the study in order to acclimatise to the environment, and had access to food (Co-Op, NB, Canada) and water *ad libitum*. The animals were divided into two groups (treatment $n = 5$; control $n = 3$). Each animal was fasted overnight before the experiment. On the day of the experiment, a 21G 3/4 in needle butterfly catheter (E-Z set®, Desert Medical, Becton Dickinson) attached to a 2 cm long 0.030 in id × 0.065 in od silastic tubing (Dow Corning, Midland, MI, USA) was placed in a central ear artery for blood sampling, blood pressure (SBP and DBP), and heart rate (HR) recording. The animal was allowed to rest in the restraining cage (Nalgene®, Fisher Scientific, Canada) for 0.5 h before dosing. Each animal received either 5 mg kg^{-1} M_1 intravenously (iv) (2–3 mL) via the other ear over 5 min, or the same volume of normal saline (control). Blood samples (1.0 mL) were collected from each animal via the catheter at 0, 0.1, 0.15, 0.25, 0.5, 1.0, 2, 3, 4, 6, and 8 h post-dose into heparinized micro-centrifuge tubes, and urine collected for 48 h post dose. In addition, intra-arterial BP and HR were recorded at each sampling time using a Sorenson™ pressure transducer (Abbott Laboratories, IL, USA) coupled to a Tektronix monitor (model 414) and recorded (model 400, OR, USA). The measurement was taken from an average of a 10 s recording. The blood samples were immediately centrifuged (3000 rpm, 4°C, 10 min) to separate plasma, which was stored at −20°C until analysis by HPLC [4,7]. All the samples (plasma and urine) were analysed within 3 months after collection to avoid possible sample deterioration [15–17].

Data Analysis

Pharmacokinetic parameters C_{max}, t_{max}, and apparent terminal $t_{1/2}$, where appropriate, were calculated from non-linear curve fitting using a two-compartment model (Rstrips®, MicroMath Scientific Software, Salt Lake City, UT, USA). For the parent drug M_1, the data were fitted by a two-compartment model following bolus iv injection. The data for the primary metabolite M_2 were analysed by a two-compartment model after first-order input. Area under the plasma concentration–time curve from zero to the last sampling time (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal method (Rstrip®, MicroMath, UT, USA). Systemic clearance (Cl) for M_1 was calculated from the equation $Cl = D/AUC$, where D was the iv dose. Mean residence time (MRT) for M_1 was calculated from the ratio AUMC/AUC, and ($AUMC_m/AUC_m - AUMC/AUC$) for the metabolite M_2 where $AUMC_m$ and AUC_m were the corresponding areas of the metabolite M_2, respectively [18]. The volume of distribution at steady state (V_{dss}) was equal to Cl MRT [19]. Renal clearance (Cl_r) of M_1 and the metabolite M_2 was calculated from the equation A_e/AUC where A_e was the amount excreted as drug or metabolite in the urine over 48 h, and AUC was the corresponding area for the drug or metabolite, respectively [20,21]. Formation clearance of M_1 to M_2 (Cl_m) was estimated from the produce of $Cl\ A_e/D$, where A_e was the amount of M_2 excreted in the urine over 48 h post-dose and D the iv dose of the parent M_1 [19].

Relationships between plasma concentrations of M_1 and haemodynamic effects (SBP, DBP, MBP, and HR) were evaluated by the inhibitory sigmoidal E_{max} model using non-linear regression (PCNONLIN, Version 3.0, SCI Software, Apex, NC, USA). Due to the fact that the blood pressure (both SBP and DBP) decrease in both drug treated rats and the controls during the experiment, the haemodynamic data obtained from the control animals were subtracted from those of the treated rats before use for modelling of the drug effects (i.e. % change = % change in drug treated rats − mean % change in the control rats, where % change = individual time data/data obtained before injection × 100). Plasma concentration and haemodynamic variables were fitted for each animal using the equation $E = E_0 - (E_{max}\, C_p^n/EC_{50}^n + C_p^n)$; where E_0 was the effect before injection of M_1, E_{max} was the maximum effect (both expressed as percentages of the control), EC_{50} was the effective concentration at 50% of E_{max}, C_p was the plasma concentration of M_1, and n was theoretical measure of the sigmoidicity of the curve (Hill factor) [22,23]. The effect of drug was evaluated by ANOVA following by the Dunnett multiple-range tests for difference between haemodynamic data before and after drug administration, and consid-

Table 1. Pharmacokinetic parameters of M_1 and M_2 in rabbits after a single iv injection of M_1 (5 mg kg^{-1})

Parameters	M_1	M_2
Apparent $t_{1/2}$ (h)	2.1 ± 0.5	3.0 ± 1.1
MRT (h)	1.7 ± 1.0	4.3 ± 3.9
t_{max} (h)	NA[a]	0.74 ± 0.59
C_{max} (ng mL^{-1})	NA	60 ± 17
AUC (ng h mL^{-1})	1300 ± 210	240 ± 37
Cl (mL min^{-1} kg^{-1})	60 ± 10	NA
Cl_m (mL min^{-1} kg^{-1})	NA	0.24 ± 0.14
Cl_r (mL min^{-1} kg^{-1})	0.81 ± 0.63	1.6 ± 1.3
V_{dss} (L kg^{-1})	5.9 ± 3.3	NC[b]

[a] Not applicable.
[b] Not calculated.

ered significant when $p < 0.05$. The haemodynamic effects of M_1 versus control at each sampling time were evaluated by an unpaired t-test, and considered significant when $p < 0.05$ (Systat®, SYSTAT, Evanston, IL, USA).

Results

Following a single iv administration, plasma concentrations of M_1 declined bi-exponentially with an apparent terminal $t_{1/2}$ of 2.1 ± 0.5 h. The Cl and V_{dss} were 60 ± 10 mL min^{-1} kg^{-1} and 5.9 ± 3.3 L kg^{-1}, respectively. M_2 was the only basic metabolite produced in high enough concentrations to allow an adequate characterization of its pharmacokinetics. The maximum plasma concentration (C_{max}) of M_2 was 60 ± 17 ng mL^{-1} and $t_{1/2}$ 3.0 ± 1.1 h. The Cl_r of M_1 and M_2 were 0.81 ± 0.63 and 1.6 ± 1.3 mL min^{-1} kg^{-1}, and MRT were 1.7 ± 1.0 and 4.3 ± 3.9 h, respectively. The results are summarized in Table 1 and Figure 2.

The mean testing MBP and HR measured prior to administration of M_1 were 82 ± 5.5 mm Hg and

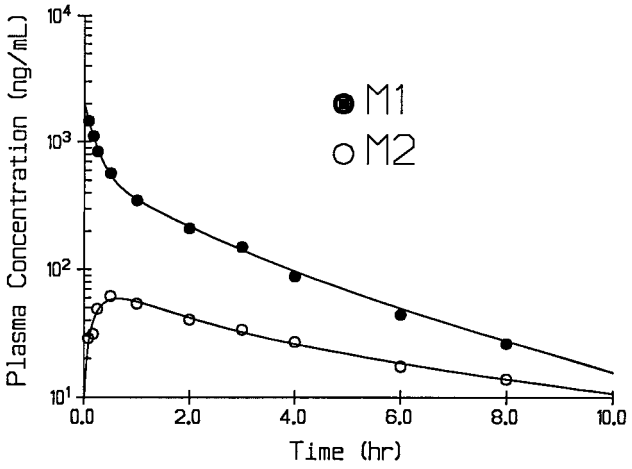


Figure 2. Mean plasma concentration–time profiles of M_1 and M_2 in rabbits after a single 5 mg kg^{-1} iv dose of M_1. The solid lines are concentrations predicted by the model using mean data

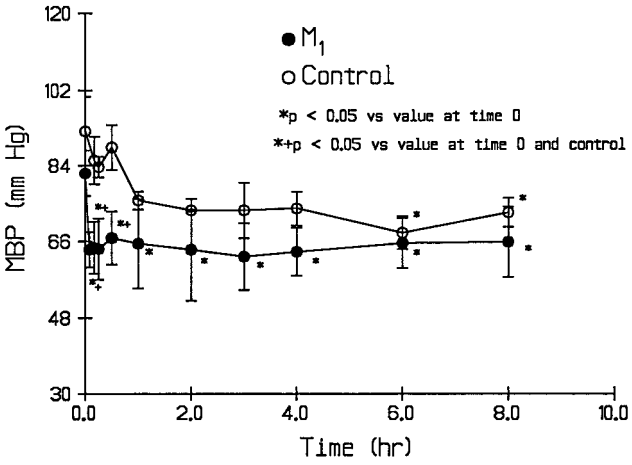


Figure 3. Mean blood pressure in rabbits after a single 5 mg kg^{-1} iv dose of M_1

240 ± 54 beats min^{-1}, respectively. After given iv as a bolus of 5 mg kg^{-1}, M_1 decreased DBP and SBP significantly for the first hour ($p < 0.05$) with a maximum effect occurring at the first sampling time. It has no significant effect on the HR ($p > 0.05$). The BP of the control animals also decreased during the experiment although it was significant only after 6 h ($p < 0.05$) (Figure 3). Using the haemodynamic data after subtracting from the control animals, the maximum hypotensive effect (E_{max}) of M_1 was estimated to be on average about 20%, although there were considerable differences between animals especially for SBP. This was attributed to the large difference between the E_{max} for SBP calculated from the mean data (20%) versus the pooled data (10%). The EC_{50} for SBP and DBP were 620 ± 310 and 420 ± 160 ng mL^{-1}, and the Hill factor n 4.5 ± 5.0 and 7.9 ± 3.7, respectively (Table 2). A plot of the DBP and SBP changes against plasma concentrations of M_1 is shown in Figure 4.

Discussion

The disposition kinetics of M_1 after the iv dose could be described adequately by a two-compartment model yielding a mean value of 4.7 ± 0.7 for the model selection criteria (MSC), which is considered 'very good' for biological data (Rstrips®, MicroMath Scientific Software, Salt Lake City, UT, USA). The mean distribution (α) and terminal (β) $t_{1/2}$ were 0.24 ± 0.16 and 2.1 ± 0.54 h, respectively. The $t_{1/2}$ (β) was shorter than the 3.6 ± 2.3 h reported for DTZ [21]. Unlike DTZ, for which several major metabolites were identified after a single iv dose [21], only M_2 could reach high enough concentrations for quantitative measurement and characterization. Despite the fact that no other metabolites besides M_2 could be quantified in the study, the Cl_r of M_1 was only about 2% of the total Cl, suggesting

Table 2. Haemodynamic effect of M_1 in rabbits after a single iv 5 mg kg^{-1} injection

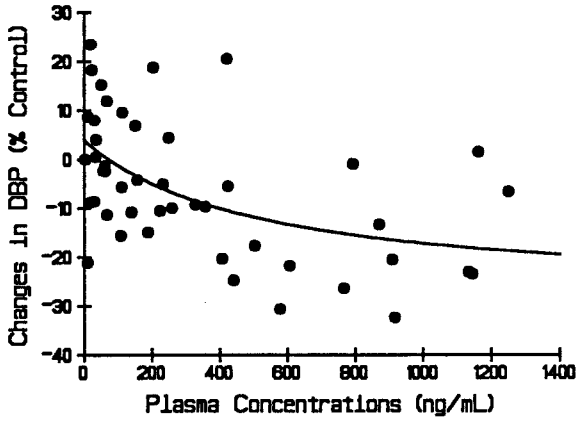
Haemodynamic/pharmacodynamic variables	Effect before drug administration (mm Hg)	E_{max} (% change from control)	EC_{50} (ng mL^{-1})	Hill factor n
SBP	98 ± 4.6	20 ± 18	620 ± 310	4.5 ± 5.0
DBP	74 ± 6.7	20 ± 8.3	420 ± 160	7.9 ± 3.7
MBP	82 ± 5.5	23 ± 12	540 ± 270	6.3 ± 5.1

that it was predominantly metabolized before excretion. The fact that only a very small fraction of M_1 was metabolized to M_2 ($< 1\%$), and no other phase I basic metabolites could be identified, suggested that M_1 may be predominantly biotransformed by phase II metabolism, and oxidative deamination yielding a series of acidic metabolites [24].

The Cl of M_1 was 60 ± 10 mL min^{-1} kg^{-1}, which was similar to the 64 mL min^{-1} kg^{-1} reported for DTZ. On the other hand, the V_{dss} was smaller than that of DTZ (5.9 ± 3.3 versus 6.8 L kg^{-1}) and the Cl_r was greater than that of DTZ (0.81 ± 0.63 versus 0.22 mL min^{-1} kg^{-1}) [21] suggesting that the shorter $t_{1/2}$ of M_1 was attributable to a smaller volume of distribution and a greater renal clearance. It remains to be investigated whether or not protein binding would have a significant effect on the disposition kinetics of M_1. It is interesting to note that the Cl_r of M_1 found in this study was smaller than those reported earlier when DTZ was administered (0.81 ± 0.63 versus 1.5 mL min^{-1} kg^{-1}) [21]. These results suggest that Cl_r of DTZ and its metabolites is concentration dependent such that it decreases at higher concentrations.

The haemodynamic variables (BP and HR) reported in this study are comparable to those reported in other studies in conscious rabbits when data were collected via the carotid artery [25], indicating that the experimental design was adequately set up. The hypotensive effects of M_1 particularly on DBP are clearly shown in this experimental mode. The effects on HR varied greatly between animals and as such the effects were not statistically significant ($p > 0.05$). It is interesting to note that the control animals under the described conditions also showed a decrease in blood pressure (both SBP and DBP) particularly near the end (after 6 h) of the experiment (Figure 3). This could be due to restraining of the animals resulting in an initial fluctuation of the haemodynamic variables, which became settled after 1 h.

It is apparent that M_1 has an anti-hypertensive effect which may contribute to the effect of the parent DTZ in clinical drug therapy, although its effect on HR varies. The disposition kinetics is similar to that of DTZ after iv administration. Further investigations are needed to evaluate its effectiveness after oral administration.



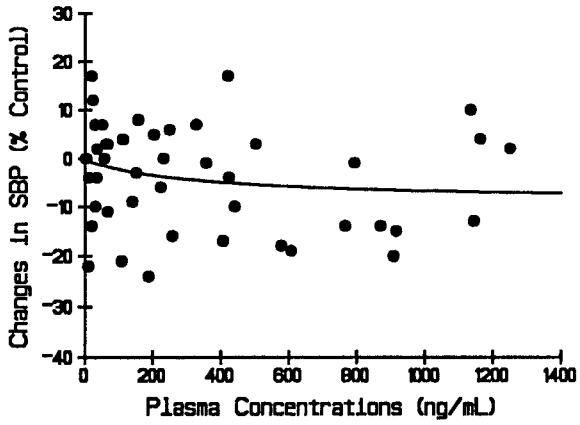


Figure 4. The concentration–effect relationship for DBP and SBP. The solid lines are percentage changes predicted by the model using pooled data

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