EFFECT OF PHENOBARBITAL PRETREATMENT ON THE PHARMACOKINETICS AND METABOLISM OF DILTIAZEM IN RATS*

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SUMMARY

In order to study the effect of cytochrome P-450 isozyme induction on the pharmacokinetics and metabolism of diltiazem (DTZ), male Sprague-Dawley rats weighing 300-600 g were randomly assigned to two groups. The enzyme induction group (n=4) received phenobarbital 60 mg/kg i.p. once daily for 4 days, whereas the control group (n=6) received normal saline for the same duration. Each rat then received a single oral dose of DTZ in solution (20 mg/kg). Blood samples (0.5 ml) were collected from each rat via an implanted polyethylene catheter (0.040" i.d.) in the right carotid artery at 0 (just before dosing), 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 10 h post-dose. Arterial plasma concentrations of DTZ and its metabohtes M_A, M_1, M_2, M_4 and M_6 were determined by HPLC. Pharmacokinetics parameters were calculated using non-linear regression. The results showed that both mean C_{max} and AUC of DTZ were lower (871.6 vs 79.8 ng/ml; 1171 vs 101.9 ng-h/ml), but the mean C_{max} of the primary metabolites M_1 and M_A was higher after phenobarbital (M_1 413.0 vs 648.9 ng/ml; M_A 683.0 vs 814.8 ng/ml). The highest increase was seen in the mean C_{max} and AUC of the secondary metabolite M_2 (837.5 vs 2585.7 ng/ml; 3312.1 vs 13156.5 ng-h/ml). In contrast, plasma concentrations of the O-desmethylated metabohtes M_4 and M_6 did not increase after phenobarbital. These results suggest that both deacetylation and N-

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demethylation of DTZ in rats are catalyzed by drug metabolizing enzymes inducible by phenobarbital.

KEY WORDS

diltiazem, metabolism, phenobarbital, enzyme induction, rats

INTRODUCTION

Diltiazem (DTZ) is a calcium antagonist widely used in the treatment of angina and related disorders /1,2/. 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 /3-7/. However, the contribution of these metabolites to the overall clinical safety and efficacy of DTZ is not clear.

In humans, the most abundant basic metabolite is N-monodesmethyl DTZ (M_A), followed by O-desmethyl DTZ (M_X) and N,O-didesmethyl DTZ (M_B), deacetyl DTZ (M_1), deacetyl N-monodesmethyl DTZ (M_2), deacetyl N,O-didesmethyl DTZ (M_6) and then deacetyl O-desmethyl DTZ (M_4) after p.o. doses of DTZ (Fig. 1) /6,8/. It has been shown that pretreatment of rats with phenobarbital increased deacetylation of DTZ *in vitro* /9/. More recently, various inducers of cytochrome P-450 isozymes including phenobarbital, phenylbutazone and rifampicin increased N-demethylation of DTZ in human and rabbit microsomes and in cultured human hepatocytes *in vitro* /10/. This study determined the *in vivo* effect of enzyme induction by pretreatment with phenobarbital on the pharmacokinetics and metabolism of DTZ in rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing approximately 300 g, were obtained from Canadian Hybrid Farms, Nova Scotia, Canada.

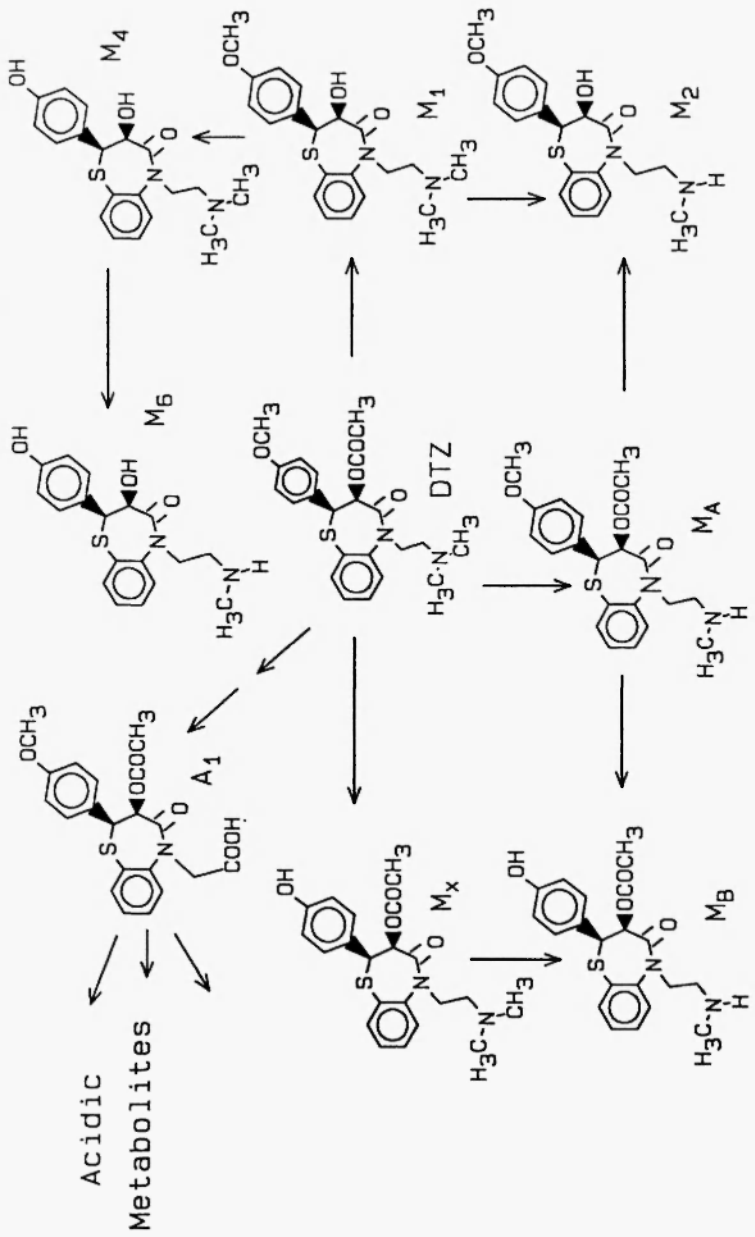


Fig. 1: Major phase I metabolism pathways of DTZ.

Chemicals

DTZ and metabolites M_A, M_1, M_2, M_4, and M_6 were kindly provided by Tanabe Seiyaku Co. (Japan) via Nordic Marion Merrell Dow Research Inc. (Laval, QC, Canada). Sodium phenobarbital was received as a gift from Parke-Davis Canada (Ont., Canada). Racemic M_X and M_{13} were synthesized as previously described /11/. Solvents were HPLC grade (BDH Chem., Halifax, N.S., Canada), and all other chemicals were reagent grade (Fisher Scientific, Canada).

Protocol

A catheter made of silastic tubing (0.037" O.D., Dow Corning, Midland, MI, USA) was implanted into the right carotid artery of each study animal under halothane anaesthesia for blood sampling as previously described /12/. The animals were divided into two groups (Control n = 6; Treatment n = 4), and housed in metabolic cages for one week prior to the study in order to acclimatize them to the environment. They had access to food (Co-Op, N.B., Canada) and water *ad libitum*.

The animals were fasted overnight before the day of experiment. Each control animal received 20 mg/kg p.o. DTZ as hydrochloride salt in 10 mg/ml solution (gavage). Blood samples (0.3 ml) were obtained from each rat via the catheter at 0, 0.25, 0.5, 1.0, 2, 3, 4, 6, 8, and 10 h post-dose. The blood samples were immediately centrifuged (3000 rpm, 4°C, 10 min) to separate plasma, which was stored at -20°C until analysis. For the enzyme induction group, each animal received sodium phenobarbital i.p. in isotonic saline 60 mg/kg (approximately 1 ml) once daily for 4 days before receiving oral DTZ. Blood samples were collected and stored as described for the control animals. All the samples were analysed within 3 months after collection. Plasma concentrations of DTZ, M_A, M_1, M_2, M_4 and M_6 were determined by HPLC as previously described /13/.

Data analysis

Pharmacokinetic parameters C_{max}, t_{max} and $t_{½}$ were obtained from non-linear curve fitting using a two-compartment model with terms appropriate for first order oral absorption (Rstrip®, MicroMath, Utah, USA). AUC from 0 to the last sampling time was calculated by the trapezoidal method. Differences of pharmacokinetic parameters

between the control and phenobarbital treatment groups were evaluated by Student's unpaired *t*-test, and considered significant when $p < 0.05$ (Systat, Systat Inc., Evanston, IL, USA).

RESULTS

Following a single oral dose of DTZ in solution, the mean C_{max} of DTZ in the control group was 870 ng/ml which occurred at about 12 min post-dose. The mean $t_{½}$ of DTZ was 1.4 h. The most abundant plasma metabolite was M_2, followed by $M_6 > M_A > M_1 > M_4$. The mean apparent terminal $t_{½}$ of the metabolites was longer than that of DTZ (Table 1).

In the phenobarbital treatment group, the mean C_{max} and AUC of DTZ were less than 10% of the control group ($p < 0.05$ for AUC), while the plasma concentrations of the metabolites M_1 and M_2 were much higher than the control group, although only the difference of M_2 was statistically significant ($p < 0.05$). In contrast, the concentrations of M_A, M_4 and M_6 were lower in the phenobarbital treated group although only the difference of C_{max} of M_6 was statistically significant (Fig. 2). No statistically significant differences were seen in the apparent $t_{½}$ or t_{max} between the two groups (Table 1).

DISCUSSION

As has been reported previously /14/, there is a marked quantitative species difference in the metabolic profile of DTZ. While M_A is the major metabolite in the plasma of humans and dogs following oral administration, the major metabolite in rats is M_2 which is a secondary metabolite of DTZ (Table 1). Contrary to these observations, Hussain and co-workers /15/ have recently reported that M_1 was the most abundant metabolite when DTZ was administered directly into the portal vein using an isolated perfused rat liver model, although the N-demethylated metabolites such as M_A, M_2 and M_6 were also present in the perfusates. These apparent discrepancies could indicate that N-demethylation of DTZ is a predominant route occurring at the gastro-intestinal mucosa in this species.

The effect of phenobarbital on the first-pass metabolism of DTZ was remarkable in that it reduced plasma concentrations of DTZ by more than 10-fold ($p < 0.05$) after a single oral dose of DTZ. This was

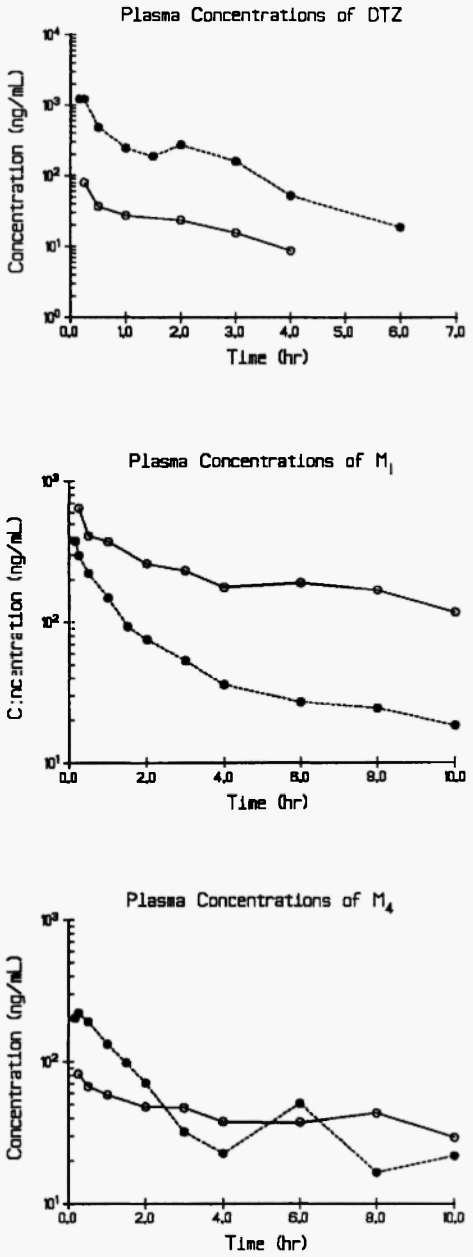
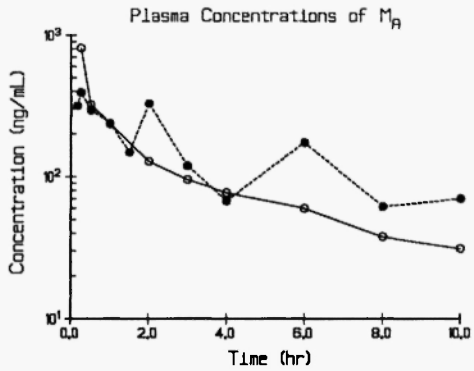
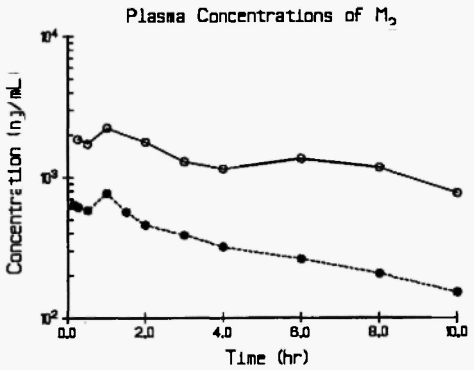


Fig. 2: Mean plasma concentration-time plots of DTZ and its major basic metabolites in rats (● control; ○ phenobarbital treatment).





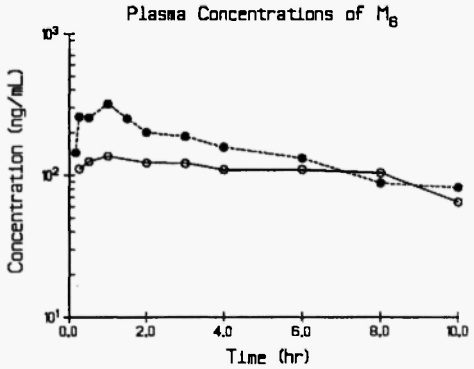


TABLE 1

Pharmacokinetic parameters of DTZ and its metabolites in rats before and after phenobarbital pretreatment

Drug/ Metabolite		Cmax (ng/mL)	t½ (h)	$AUC_{(0\text{-last})}$ (ng-h/mL)	tmax (h)
DTZ	A*	871.56 ± 1063.56**	1.40 ± 1.17	1171.18 ± 353.53**	0.21 ± 0.29
	B**	79.81 ± 54.68	3.17 ± 3.23	101.89 ± 51.28	0.09 + 0.08
M_1	A	412.97 + 133.99	2.65 ± 1.13	556.44 + 129.22	0.15 ± 0.12
	B	648.94 + 313.14	2.82 ± 1.51	2107.77 ± 2135.28	0.45 ± 0.67
M_A	A	683.00 + 486.35	9.09 + 10.26	1360.75 ± 1217.37	0.54 ± 0.71
	B	814.79 ± 597.19	3.79 ± 1.56	965.78 ± 326.80	0.06 + 0.06
M_2	A	837.53 ± 513.48**	5.35 ± 3.59	3312.07 ± 1484.04**	1.43 ± 1.62
	B	2585.73 ± 1117.47	4.15 ± 2.33	13156.5 ± 7181.16	0.40 ± 0.50
M_4	A	256.77 + 169.42	4.74 ± 1.90	485.03 ± 323.57	0.60 + 0.36
	B	87.62 ± 48.61	4.00 + 0.43	422.96 ± 467.75	0.22 + 0.19
M_6	A	339.02 ± 107.95**	10.71 ± 13.44	1528.28 ± 470.93	0.89 ± 0.54
	B	165.08 ± 57.01	9.94 ± 10.50	1060.55 ± 332.48	0.89 ± 0.69

*A = Control; **B = Phenobarbital treated group
***Value represents mean ± SD
****$p < 0.05$ between A and B

accompanied by a considerable increase in the concentrations of M_1 and M_2 resulting from induction of cytochrome P-450 and other enzymes (e.g. esterases) which catalyse the deacetylation and N-demethylation reactions. It has been shown by *ex vivo* experiment that pretreatment of rats with phenobarbital increased metabolism of DTZ to M_1 by more than three-fold by induction of specific types of

microsomal esterases /9/, and that phenobarbital induced cytochrome P-450 (CYP3A) isozymes which catalysed N-demethylation of DTZ to M_A in primary cultures of human hepatocytes /10/. The results obtained from the present study support the notion that both N-demethylation and deacetylation of DTZ are catalyzed by enzymes inducible by phenobarbital. It is not certain, however, whether the phenobarbital inducible esterases are restricted to hepatic tissue only, or are also found in other tissues (e.g. intestine, erythrocytes, plasma, lung, etc.) /16/. The lack of an effect on the apparent $t_{½}$ of DTZ may suggest that phenobarbital increases oral clearance more than systemic clearance of DTZ. Furthermore, the fact that there were no statistically significant differences in the plasma concentrations of M_1 or M_A and their apparent $t_{½}$ between the phenobarbital treated animals and the controls can be attributed to an increase in further metabolism or sequential first pass metabolism of these primary metabolites to M_2 (Table 1).

In contrast to N-demethylation or deacetylation, it appeared that the effect of phenobarbital on O-demethylation of DTZ was minimal as the concentrations of the O-desmethylated metabolites M_4 or M_6 were lower in the phenobarbital treated group although only the difference of C_{max} of M_6 was statistically significant ($p < 0.05$). However, since these O-desmethylated metabolites could be further metabolized by a phenobarbital inducible glucuronidation which was not tested in the present study /17/, these results must be interpreted with caution. The effect of phenobarbital on oxidative deamination of DTZ was not investigated in this study.

In summary, this study has confirmed that phenobarbital induced N-demethylation and deacetylation of DTZ in rats *in vivo*; its effects on other metabolic pathways such as O-demethylation, oxidative deamination and conjugative reactions and their tissue specificities need to be further investigated. On the basis of these data, drugs which are inducers or inhibitors of cytochrome P-450 isozymes or esterases may interact with DTZ metabolism in concurrent therapy.

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