First-Pass Metabolism of Diltiazem in Anesthetized Rabbits: Role of Extrahepatic Organs

Marc Lefebvre, Walid Homsy, Gilles Caillé, and Patrick du Souich[1,2]

Received April 19, 1995; accepted September 13, 1995

Purpose. The aim of this study was to assess *in vivo* which organs contribute to the first-pass metabolism of diltiazem.

Methods. Anaesthetized rabbits received diltiazem into the thoracic aorta (TA) (1 mg/kg), jugular vein (JV) (2 mg/kg), portal vein (PV) (4 mg/kg) or small intestine (SI) (5 mg/kg). Serial blood samples were withdrawn from the abdominal aorta to assay diltiazem, N-demethyl-diltiazem (MA) and deacetyldiltiazem (M_1).

Results. The area under diltiazem plasma concentration curve/time ($AUC_{0-\infty}$) normalized by the dose was $AUC_{TA} \approx AUC_{JV} > AUC_{PV} > AUC_{SI}$. Intestinal and hepatic diltiazem availability was 43 and 33%, respectively. The systemic availability of oral diltiazem was 12%. Diltiazem given into the SI and PV generated primarily MA, and injected into the JV and TA produced mainly M_1.

Conclusions. In rabbits, the intestine and the liver contribute to the first-pass metabolism of diltiazem, and the amount and species of metabolites generated depend upon the route of administration of diltiazem.

KEY WORDS: diltiazem; first-pass metabolism; intestine; liver; lungs.

INTRODUCTION

Diltiazem is a calcium channel blocker used in angina pectoris and hypertension (1). Diltiazem undergoes substantial first-pass metabolism (1), resulting in a systemic bioavailability of around 30 to 40% (2,3). Diltiazem is extensively metabolized in the organism (4), with a systemic clearance ranging from 11.5 to 21.3 mL/min/kg by virtue of an elevated intrinsic clearance (2). Several metabolites are generated through O-deacetylation, N-demethylation and O-demethylation, which may be further metabolized by conjugation with glucuronides or sulphates (1,5).

Over the last decade, the ability of extrahepatic tissues to metabolize endogenous and exogenous substrates has attracted much interest. This is because cytochrome P-450-dependant mono-oxygenase activity has been reported in virtually every mammalian organ (6,7), specially in the liver, the intestinal mucosa, the lungs and the kidneys (8,9).

It has always been assumed that diltiazem metabolism takes place in the liver (1). However, since N-demethylation of diltiazem, the major metabolic pathway in man, is carried out by a form of the CYP3A subfamily (10), and this isozyme is widely distributed in the organism, essentially in the small intestine, pancreas, kidneys, skin, testis, and ovaries (11), it raises the possibility that several organs could contribute to the metabolism of diltiazem. Therefore, it appeared of interest to document the contribution of the small intestine and the lungs, relative to the liver, to the first-pass metabolism of diltiazem.

MATERIALS AND METHODS

Animals

The experiment was carried out in 31 New Zealand male rabbits (Ferme Cunicole, Québec, Canada) with a mean body weight of 2.4 ± 0.1 kg (mean ± SEM). The animals were house in metabolic cages with access to water and food *ad libitum* for at least one week prior to the study to be acclimatized to the environment. The rabbits were fasted for 24 hours, and before the experiment the animals were anaesthetized with intravenous pentobarbital (30 mg/kg) (Nembutal Sodium, Abbott Laboratories Ltd., Toronto, Ontario); additional doses (5 mg/kg) of pentobarbital were injected during the experiment as needed. A tracheostomy was performed and the rabbits were ventilated with oxygen-enriched air (21 mL/cycle, 50 cycles/min) (Harvard Apparatus, Boston, Massachusetts). A catheter PE 50 (Intramedic Becton Dickinson and Co., Parsippany, New Jersey) was introduced through the femoral artery via the right leg into the abdominal aorta, until approximately the renal arteries. A solution of heparinized saline was kept in the arterial catheter to ensure permeability. Finally, a laparotomy was done to each rabbit to inject diltiazem into the portal vein, the small intestine, or as sham intervention.

Experimental Protocol

To ensure that the doses of diltiazem used generated first-order kinetics, and to document the effect of anaesthesia, the pharmacokinetics of diltiazem (Marion Merrell Dow Research, Laval, Québec) were assessed following the intravenous injection of 1, 2, 4 and 8 mg/kg diluted in NaCl 0.9% in 2 min to non-anaesthetized rabbits (4 groups of 2 rabbits each). Blood samples (2 mL) were drawn from the central artery of the ear prior to and at 5, 15, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min following the administration of diltiazem. Blood samples were centrifuged (4°C, 4000 rpm, 10 minutes), plasma was separated and stored at (−20°C) until diltiazem was assayed in always less than 2 weeks.

To assess the contribution of the small intestine, liver and lungs to the first-pass metabolism of diltiazem, the drug was injected prior to and after the organ for study. To this purpose, rabbits were divided into four groups, one for each route of administration, and diltiazem was injected into the thoracic aorta (TA) (1 mg/kg), the jugular vein (JV) (2 mg/kg), the portal vein (PV) (4 mg/kg), or into the first 30 cm of the small intestine (SI) (5 mg/kg). The dose of drug injected was corrected for the salt, i.e. 1 mg of diltiazem = 1.088 mg of

[1] Département de Pharmacologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada.

[2] To whom correspondence should be addressed at Département de Pharmacologie, Faculté de Médecine, Université de Montréal, C.P. 6128, Succ. Centre-ville, Montréal, Québec H3C 3J7, Canada.

0724-8741/96/0100-0124$09.50/0

diltiazem.HCl. The dose selected for each site of administration was such as to obtain first-order kinetics and in addition, plasma concentrations of diltiazem in the range of those observed in humans. The injection of diltiazem into the TA was carried out through a catheter inserted into the left carotid artery with the tip placed into the TA. When diltiazem was administered prior to the lungs, liver or into the intestine, the right JV, the PV or the first part of the SI, respectively, were isolated and the drug was directly injected using a 26 gauge needle (Becton Dickinson and Co, Parsippany, New Jersey). Each animal received by infusion a solution of NaCl 0.9%, containing $NaHCO_3$ (4.2 g/L), at a rate of 50 ml/h, to maintain blood pH and to compensate for losses of water and blood during the experimental procedure. Arterial partial pressure of O_2 (PaO_2) and CO_2 ($PaCO_2$) and pH were controlled prior to and at 5, 15, 30, 60, 120, 180 and 240 min following the administration of diltiazem using a BGM, model 1312 pH/blood Gas Analyzer (Instrumentation Laboratory, Lexington, Massachusetts). Arterial blood pressure was monitored continuously through the catheter located into the abdominal aorta, using a pressure transducer (P-1000A, E&M Instrument Co. Inc., Houston, Texas) coupled to a chart recorder (Physiograph Four-A, E&M Instrument Co., Inc., Houston, Texas).

To assay diltiazem and metabolites, 1.5 to 2 mL of blood was drawn from the abdominal aorta prior to and at 5, 15, 30, 60, 90, 120, 150, 180, 240, 300 and 360 minutes after the administration of diltiazem. Plasma concentrations of diltiazem, N-demethyldiltiazem (MA) and deacetyldiltiazem (M_1) were determined by HPLC (12). Other metabolites could not be assayed after the administration of a single dose of diltiazem. The assay limit for diltiazem and its metabolites was 2.5 ng/mL.

To assess the amount of diltiazem absorbed, six hours after the intestinal administration of diltiazem the first part (0 to 60 cm) of the small intestine was cut and washed with 20 mL of a solution of NaCl 0.9%, and the recovered solution was frozen (−20°C) until analysis. Preliminary studies demonstrated that beyond 60 cm, diltiazem was not found in measurable concentrations.

Pharmacokinetics

The following pharmacokinetic parameters of diltiazem were determined and compared between each group: the area under the plasma concentration-time curve ($AUC_{0-\infty}$), estimated by the trapezoidal method, the apparent half-life (t1/2), obtained from the ratio of ln 2/Z, where the disposition rate constant (Z) is the slope of the terminal phase of the logarithm of the concentration-time profile of the drug times 2.303.

The organ (org) bioavailability (F) of diltiazem, i.e. intestine, liver and lungs, was calculated dividing the $AUC_{0-\infty}$ estimated when the drug was administered before the organ (AUC_{aff}) by that computed when it was administered after the organ (AUC_{eff}) (7):

$$F_{org} = AUC_{aff}/AUC_{eff} \times 100$$

The systemic availability of diltiazem (F) after oral administration was estimated using the following equation: $F = F_{intestine} \cdot F_{liver} \cdot F_{lungs}$ (9). The extraction (E) of diltiazem by an organ was estimated using the equation: $E_{org} = 1 - F_{org}$. For all calculations, the $AUC_{0-\infty}$ was corrected by the dose.

To assess the relative amount of MA and M_1 generated by diltiazem biotransformation when administered by the various routes, the $AUC_{0-\infty}$ of each metabolite was calculated by the trapezoidal method, and the ratio of the metabolite's $AUC_{0-\infty}$ over the dose or diltiazem $AUC_{0-\infty}$ were estimated.

Statistical Analysis

Differences of diltiazem and metabolites kinetic parameters between each group were estimated using one-way analysis of variance for parallel groups. The value of p was determined using Dunnett's distribution table and the threshold of significance was established at $p < 0.05$.

RESULTS

Arterial gases and pH remained rather stable throughout the experiment in all groups of rabbits. Mean (± S.D.) values of PaO_2, $PaCO_2$ and pH at the beginning and the end of the experiment were 115 ± 16 mm Hg, 19 ± 5 mm Hg and 7.537 ± 0.106 vs. 95 ± 22 mm Hg, 20 ± 5 mm Hg and 7.503 ± 0.098, respectively. Mean arterial pressure was 82 ± 11 mm Hg at the outset vs. 63 ± 15 mm Hg, 6 hours after diltiazem administration.

When diltiazem was injected to conscious rabbits, the $AUC_{0-\infty}$ increased proportionally to the dose up to 4 mg/kg. The clearance of diltiazem was similar for the 1, 2 and 4 mg/kg doses, i.e. 65 to 75 mL/min/kg, but was smaller for the 8 mg/kg dose (54 mL/min/kg). The t1/2 of diltiazem was not modified by the dosage (around 100 min). Based on these preliminary studies, we have assumed that the clearance of diltiazem for each individual organ was concentration independent at the doses used. Following diltiazem injection into the JV of anaesthetized rabbits, the kinetic parameters of diltiazem, as well as the $AUC_{0-\infty}$ of the generated MA and M_1, closely resembled those assessed in conscious animals (Table I). These results indicate that anaesthesia and surgical procedures did not modify the pharmacokinetics of diltiazem.

Six hours after the intestinal administration of diltiazem, the recovery of the drug from the intestine averaged 0.005% of the dose administered. Small amounts of MA and M_1, i.e.

Table I. Mean (±SEM) Pharmacokinetic Parameters of Diltiazem, MA and M1 Following the Intravenous Administration of Diltiazem to Anesthetized and Conscious Rabbits

Pharmacokinetic paramaters Dose (mg/kg)	Anesthetized rabbits 2	Conscious rabbits 2
$AUC_{0-\infty}$ Dilt (μg · min/mL)	25.2 ± 2.5	29.0 ± 5.3
Cl Dilt (mL/min/kg)[a]	84 ± 9	73 ± 12
t½ Dilt (min)	115 ± 9	94 ± 1
$AUC_{0-\infty}$ MA (μg · min/mL)	3.4 ± 0.9	5.0 ± 1.7
$AUC_{0-\infty}$ M1 (μg · min/mL)	5.5 ± 1.1	6.2 ± 2.2

[a] Clearance of diltiazem: Dose/$AUC_{0-\infty}$.

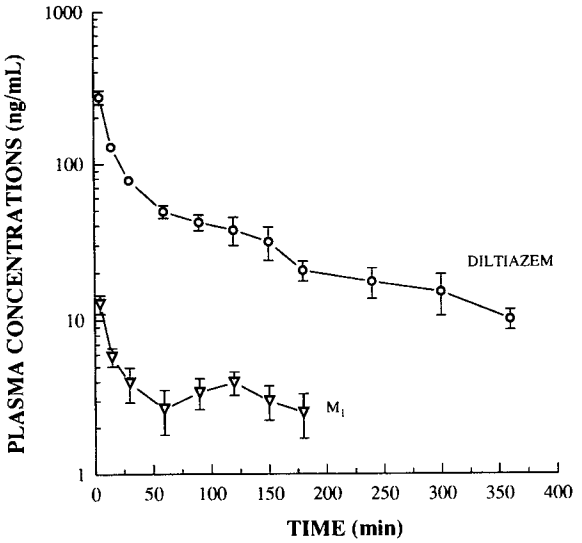

Fig. 1. Mean plasma concentrations of diltiazem and M_1 following a single dose of diltiazem (1 mg/kg) injected into the thoracic aorta of anaesthetized rabbits.

approximately 400 and 200 ng (0.0033% and 0.0017% of the dose) respectively, were detected in the small intestine.

The decay of plasma concentrations of diltiazem and its metabolites was not affected by the dose or the route of administration (Figures 1 to 4). The correction of diltiazem $AUC_{0-\infty}$ by the dose demonstrates that the normalized $AUC_{0-\infty}$ of diltiazem injected into the intestine or the PV were smaller than those calculated following diltiazem administration through the PV, JV and TA, or the JV and TA, respectively (Table II). The normalized $AUC_{0-\infty}$ of diltiazem injected into the JV was 16% smaller than that calculated when diltiazem was given into the TA, but this difference did not reach statistical significance (Table II).

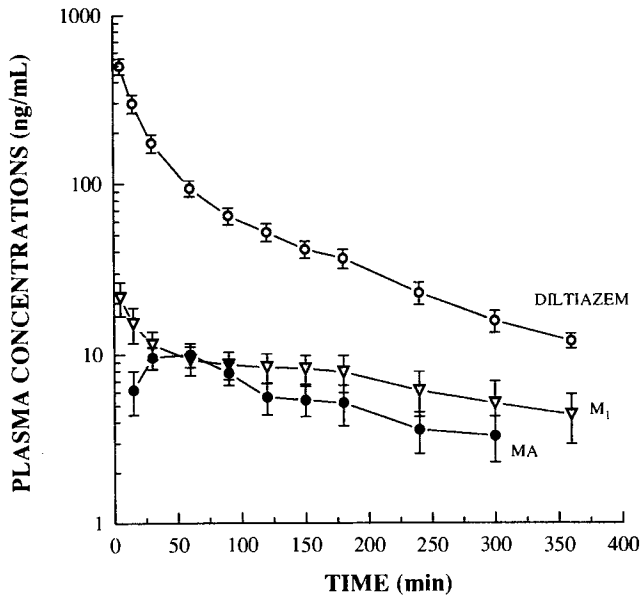

Fig. 2. Mean plasma concentrations of diltiazem, MA and M_1 following a single dose of diltiazem (2 mg/kg) injected into the jugular vein of anaesthetized rabbits.

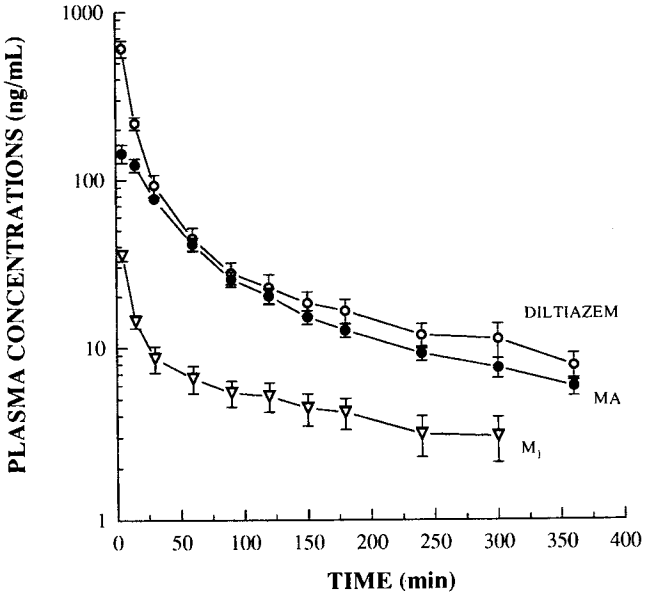

Fig. 3. Mean plasma concentrations of diltiazem, MA and M_1 following a single dose of diltiazem (4 mg/kg) injected into the portal vein of anaesthetized rabbits.

The percentage of the dose of diltiazem extracted by the intestine or the liver was 57% and 67%, respectively. Therefore, the availability of diltiazem injected prior the intestine or the liver was 43 and 33%, respectively. Following oral administration of diltiazem, the systemic bioavailability was 12%.

The dose-normalized $AUC_{0-\infty}$ of MA generated by diltiazem injected into the SI, the PV and the JV were similar, but greater than that estimated after the injection of diltiazem into the TA. On the other hand, when diltiazem was injected into the SI, the ratio of MA over diltiazem $AUC_{0-\infty}$

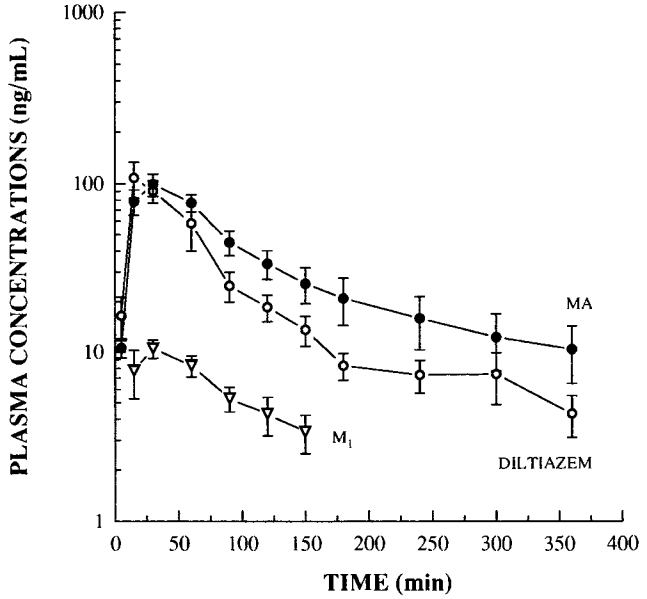

Fig. 4. Mean plasma concentrations of diltiazem, MA and M_1 following a single dose of diltiazem (5 mg/kg) injected into the small intestine of anaesthetized rabbits.

Table II. Mean (±SEM) Area Under Diltiazem, MA, and M1 Plasma Concentrations as a Function of Time ($AUC_{0-\infty}$) Normalized by the Dose, Following the Administration of Diltiazem Through the Thoracic Aorta (TA), Jugular Vein (JV), Portal Vein (PV) and Small Intestine (SI) to Anesthetized Rabbits

Route of injection Dose (mg/kg)	TA (n = 8) 1	JV (n = 7) 2	PV (n = 8) 4	SI (n = 8) 5
			DILTIAZEM	
$AUC_{0-\infty}$ (μg min/mL)	15.1 ± 1.4	25.2 ± 2.5[a]	16.8 ± 2.0	9.2 ± 1.5[b]
$AUC_{0-\infty}$/Dose × 10^{-3} (kg min/mL)	15.1 ± 1.4	12.6 ± 0.5	4.2 ± 0.5[a,b]	1.8 ± 0.3[a,b,c]
			MA	
$AUC_{0-\infty}$/Dose × 10^{-3} (kg min/mL)	0.2 ± 0.1	1.7 ± 0.5[a]	2.6 ± 0.2[a]	2.9 ± 0.6[a]
Ratio[d] (%)	1.2 ± 0.1	15 ± 5[a]	66 ± 7[a,b]	165 ± 24[a,b,c]
			M1	
$AUC_{0-\infty}$/Dose × 10^{-3} (kg/min/mL)	2.0 ± 0.6	2.7 ± 0.6	0.8 ± 0.2[b]	0.4 ± 0.1[a,b]
Ratio[d] (%)	12 ± 3	25 ± 7[a]	21 ± 2[a]	25 ± 5[a]

[a] $p < 0.05$ compared to TA value.
[b] $p < 0.05$ compared to JV value.
[c] $p < 0.05$ compared to PV value.
[d] $AUC_{0-\infty}$ of MA or M1/$AUC_{0-\infty}$ of diltiazem.

was almost three times greater than the ratio estimated when diltiazem was injected into the PV, difference that was even greater when compared with the JV and TA routes of administration (Table II).

The dose-corrected $AUC_{0-\infty}$ of M_1 generated by diltiazem injected into the JV was similar to that computed when diltiazem was injected into the TA, and both these values were higher than the dose-normalized $AUC_{0-\infty}$ of M_1 for the PV and SI routes. On the other hand, the ratio of M_1 $AUC_{0-\infty}$ over diltiazem $AUC_{0-\infty}$ after diltiazem administration into the SI, the PV and the JV routes were similar, although twice that calculated when diltiazem was injected into the TA (Table II).

DISCUSSION

The minute amounts of diltiazem, MA and M_1 detected in the intestine, six hours after the intestinal administration of diltiazem, suggest that the absorption of the drug was almost complete. The presence of MA and M_1 in the intestine may be explained by several mechanisms, such as intraluminal oxidation of diltiazem, secretion of the metabolites into the lumen from the intestine following the oxidation of diltiazem into the intestinal mucosa (13), or by secretion of the metabolites into the lumen of the intestine from the mesenteric circulation, following the metabolism of diltiazem by the liver and other organs.

Dose-corrected $AUC_{0-\infty}$ of diltiazem decreased progressively as diltiazem was injected into the PV or the SI (Table II), indicating that the small intestine and the liver did contribute to diltiazem first-pass metabolism. The injection of diltiazem into the SI, the PV and the JV generated similar dose-corrected $AUC_{0-\infty}$ of MA, but higher than when administered into the TA, suggesting that all presystemic organs contributed to the production of MA. The dose-corrected $AUC_{0-\infty}$ of M_1 was smaller when diltiazem was administered into the SI or the PV than into the JV or TA; this observation could be explained by a combination of factors, i.e. systemic organs contribute to the formation of M_1, in the intestine and liver, N-demethylation is the predominant route of metabolism of diltiazem, and after its formation in the intestine and the liver, M_1 is rapidly degraded in these organs.

The intestine is able to conjugate certain compounds, such as fenoterol, phenol, naphthol, morphine-like analgesics and salbutamol (6,14,15). Intestinal decarboxylation, ester hydrolysis, O-deethylation and N-demethylation have also been demonstrated (7,9,16). CYP3A subfamily of cytochrome P-450 is found in large amounts in the small intestine (11), and this subfamily of enzymes appears to carry out diltiazem N-demethylation in human and rabbit, i.e. generating MA (10). For the first-pass metabolism of a drug administered orally, the intestine may play a relatively important role, due to its strategic position. For instance, 57% of the 5 mg/kg oral dose of diltiazem is extracted by the intestine, i.e. 2.85 mg/kg, compared with the liver who extracted 67% of the remaining 43% of the oral dose, i.e. 1.44 mg/kg.

The liver extracted diltiazem more efficiently than other organs, which is in agreement with *in vitro* studies (17). The greater amount of CYP3A in the liver compared with that found in other organs (11) explains the predominant role of the liver in the metabolism of diltiazem. Following diltiazem injection into the SI or the PV, the ratios of MA and M_1 $AUC_{0-\infty}$ over diltiazem $AUC_{0-\infty}$ were greater than those estimated when diltiazem was injected into the TA (Table II). Since following the SI and PV routes, dose-normalized MA and M_1 $AUC_{0-\infty}$ were equal, the present results suggest that in the intestine and the liver, diltiazem generates metabolites other than MA and M_1 or alternatively, the metabolism of diltiazem in the intestine does produce primarily MA and M_1, but these metabolites are further metabolized. The latter alternative is supported by *in vitro* studies showing that intestinal homogenates generate MA and M_1, and liver ho-

mogenates MA, but both tissues can biotransform MA and M_1 (17). Therefore, we postulate that the intestinal mucosa and the liver are able to metabolize diltiazem to MA and M_1, and possibly to other non-detected metabolites, which may be further biotransformed in the intestine and/or in the liver.

In vivo first-pass uptake of diltiazem by the rabbit's lungs was of lesser importance than reported for other lipophilic drugs, such as propranolol, fentanyl, meperidine and morphine (18,19). Most compounds removed by the lungs are basic amines with a pKa greater than 8, carry a charged group at physiological pH, which is frequently cationic, have a moderate-to-high lipophilic nature, and present a hydrophobic moiety (19-21). We may speculate that both, the low pKa of diltiazem (around 7.5) and as a consequence, a small cationic charge at physiological pH, and the fact that the lungs contain small amounts of CYP3A (11), determine the low pulmonary first-pass uptake of diltiazem.

Flow dependency of drug clearance is assumed when its intrinsic clearance is higher than the blood flow to the organ; however, when several organs contribute to the metabolism of the drug, the estimated intrinsic clearance will be the sum of the intrinsic clearances for each organ. As a consequence, the intrinsic clearance of a specific organ contributing to the metabolism of diltiazem may be equal to or lower than the blood flow to the organ, in which case the clearance will be affected not only by changes in blood flow, but also by changes in intrinsic clearance, and hence protein binding.

In conclusion, in the rabbit, the intestine and the liver account for most of the first-pass metabolism of an oral dose of diltiazem yielding mainly MA by N-demethylation. The lungs appear to have a reduced contribution to the first-pass metabolism of diltiazem. These results indicate that the pharmacokinetic profile of diltiazem and its metabolites depend upon the route of administration of diltiazem. Finally, the dependence of diltiazem clearance upon blood flow to an organ may be less important than assumed in the literature, and as consequence, diltiazem clearance could also be influenced by changes in the intrinsic clearance and in the protein binding.

ACKNOWLEDGMENTS

The authors are grateful to Nordic Merrell-Dow Recherche for the studentship given to M. Marc Lefebvre and for the gracious gift of MA and M_1, and to Mrs Lucie Héroux for her technical assistance. Supported by the Medical Research Council of Canada (Grant # MA-10874).

REFERENCES

1. M.M.T. Buckley, S. Grant, K.L. Goa, D. McTavish and E.M. Sorkin. Diltiazem; a reappraisal of its pharmacological properties and therapeutic use. *Drugs* 39:757-806 (1990).
2. M.S. Smith, C.P. Verghese, D.G. Shand and E.L.C. Pritchet. Pharmacokinetic and pharmacodynamic effects of diltiazem. *Am. J. Cardiol.* 51:1369-1374 (1983).
3. P.K.F. Yeung, S.J. Mosher, M.A. Quillam and T.J. Montague. Species comparison of pharmacokinetics and metabolism of diltiazem in humans, dogs, rabbits and rats. *Drug Metab. Dispos.* 18:1055-1059 (1990).
4. P.K.F. Yeung, S.J. Mosher and P.T. Pollak. Pharmacokinetics and metabolism of diltiazem in rabbits after a single intravenous or single oral administration. *Eur. J. Drug Metab. Pharmacokin.* 16:69–74 (1991).
5. J. Sugihara, Y. Sugawara, H. Ando, S. Harigaya, A. Etoh and K. Kohno. Studies on the metabolism of diltiazem in man. *J. Pharmacobiodyn.* 7:24-32 (1984).
6. J.R. Bend and C.J. Serabjit-Singh. Xenobiotic metabolism by extrahepatic tissues: relationship to target organ and cell toxicity, In JR. Mitchell and MG. Horning (eds.), *Drug Metabolism and Drug Toxicity,* Raven Press, New York, 1984, pp. 99-136.
7. M. Mistry and J.B. Houston. Quantitation of extrahepatic metabolism. *Drug Metab. Dispos.* 13:740-745 (1985).
8. H. Vainio and E. Hietanen. Role of extrahepatic metabolism in drug disposition and toxicity. In P. Jenner and B. Testa (eds.), *Concepts in Drug Metabolism,* part A, Marcel Dekker, New York, 1980, pp. 251-284.
9. M.K. Cassidy and J.B. Houston. In vivo capacity of hepatic and extrahepatic enzymes to conjugate phenol. *Drug Metab. Dispos.* 12:619-624 (1984).
10. L. Pichard, G. Gillet, I. Fabre, I. Dalet-Beluche, C. Bonfils, J.P. Thenot and P. Maurel. Identification of the rabbit and human cytochromes P-450IIIA as the major enzymes involved in the N-demethylation of diltiazem. *Drug Metab. Dispos.* 18:711-719 (1990).
11. D.R. Krishna and U. Klotz. Extrahepatic metabolism of drugs in humans. *Clin. Pharmacokin.* 26:144-160 (1994).
12. G. Caillé, L. Dubé, Y. Théorêt, F. Varin, N. Mousseau and I. McGilveray. Stability study of diltiazem and two of its metabolites using a high performance liquid chromatographic method. *Biopharm. Drug Dispos.* 10:107-114 (1989).
13. K.F. Ilett, L.B.G. Tee, .PT. Reeves and R.F. Minchin. Metabolism of drugs and other xenobiotics in the gut lumen and wall. *Pharmac. Ther.* 46:67-93 (1990).
14. A.S.J. Koster, G.A. Hofman, A.C. Frankhuijzen-Sierevogel and J. Noordhoek. Presystemic and systemic intestinal metabolism of fenoterol in the conscious rat. *Drug Metab. Dispos.* 13:464-470 (1985).
15. S. Perreault, L. Dumont, V. Villière, H. Ong, A. Adam and P. du Souich. Hepatic and extrahepatic metabolism of salbutamol in anesthetized rabbits. *Drug Metab. Dispos.* 21:485-491 (1993).
16. G.M. Pacifici, M. Franchi, P.G. Gervasi, V. Longo, P. di Simplico, A. Temellini, and L. Giuliani. Profile of drug-metabolizing enzymes in human ileum and colon. *Pharmacology* 38:137-145 (1989).
17. W. Homsy, M. Lefebvre, G. Caillé and P. du Souich. Metabolism of diltiazem in hepatic and extrahepatic tissues of rabbits: in vitro studies. *Pharmac. Res.* 12:609-614 (1995).
18. D.M. Geddes, K. Nesbitt, T. Trail and J.P. Blackburn. First-pass uptake of ^{14}C propranolol by the lung. *Thorax* 34:810-813 (1979).
19. D.L. Roerig, K.J. Kotrly, E.J.Vucins, S.B. Ahlf, C.A. Dawson, and J.P. Kampine. First-pass uptake of fentanyl, meperidine and morphine in the human lung. *Anesthesiology* 67:466-472 (1987).
20. A.G.E. Wilson, R.D. Pickett, T.E. Eling and M.W. Anderson. Studies on the persistence of basic amines in the rabbit lung. *Drug Metab. Dispos.* 7:420-424 (1979).
21. U.S. Ryan and C.J. Grantham. Metabolism of endogenous and xenobiotic substances by pulmonary vascular endothelial cells. *Pharmac. Ther.* 42:235-250 (1989).