THE SITE OF ABSORPTION IN THE SMALL INTESTINE DETERMINES DILTIAZEM BIOAVAILABILITY IN THE RABBIT

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ABSTRACT

Purpose. Since the ability of the small intestine to biotransform a drug may decrease in distal segments of the intestine, this study aimed to assess whether the site of administration in the small intestine could affect the systemic bioavailability of diltiazem and its two active metabolites, *N*-desmethyldiltiazem (MA) and desacetyldiltiazem (M1).

Methods. Five mg/kg of diltiazem were administered into the lumen of the proximal (0-30 cm, n=9) or the distal (150-180 cm) small intestine (n=7) of anesthetized New Zealand rabbits. Blood samples were drawn from the femoral artery for 6 hours, and diltiazem, MA and M1 were assayed by HPLC.

Results. The area under the curve ($AUC_{0\rightarrow\infty}$) of diltiazem administered into the distal small intestine was larger than that estimated when diltiazem was given in the proximal segment (14.20 ± 2.82 vs 8.14 ± 0.88 µg.min/ml, $p<0.05$), due to a lower diltiazem oral clearance (440 ± 78 vs 660 ± 55 ml/min/kg, $p<0.05$). The $AUC_{0\rightarrow 360}$ of MA was not affected by the site of diltiazem administration, but the $AUC_{0\rightarrow 360}$ of M1 was increased when diltiazem was administered in the distal segment of the small intestine. Since the molar sum of diltiazem and its active metabolites was 48% greater following administration into the distal segment as compared to the 0-30 cm segment of the small intestine, absorption of diltiazem in distal segments of the intestine may enhance its pharmacological response.

Conclusions. The site of absorption into the intestine modulates the bioavailability of diltiazem and its two active metabolites.

KEY WORDS: Diltiazem, metabolism, intestine, rabbits.

INTRODUCTION

Diltiazem, a calcium channel blocker of the benzothiazepine family, is widely prescribed for the treatment of hypertension and angina (1). Diltiazem is highly extracted by the organism to undergo biotransformation mainly through cytochrome P-450 3A subfamily of isozymes (2). This is reflected by an important first-pass uptake, i.e. diltiazem bioavailability is approximately 30 to 40% (1, 3-5), with less than 4% of an oral dose being excreted unchanged in urine (5-7). In several animal species and in humans, the metabolism of diltiazem generates acidic and basic metabolites, which are further metabolized through oxidation and conjugation pathways (8-12). Four acidic metabolites resulting from the deamination of the dimethylaminoethyl group of diltiazem do not elicit any pharmacological activity (10). On the other hand, some of the basic metabolites retain pharmacological activity as antihypertensives and as coronary vasodilators, i.e. by reference to diltiazem, *N*-desmethyldiltiazem (MA) elicits 33% and 20%, and desacetyldiltiazem (M1) 100% and 50%, respectively (13).

The first-pass uptake of a drug given orally may occur within three potentially active metabolic sites, i.e. the intestine, the liver and the lungs. Although the liver has been acknowledged as the major site of drug metabolism, the presence of enzymes of the cytochrome P-450 system (CYP) and other metabolizing enzymes in extra-hepatic tissues, such as the intestine, suggests that other organs could contribute to the biotransformation of endogenous and exogenous substrates (14,15).

In the gastro-intestinal tract of humans and of animals, the greatest amounts of CYP 3A subfamily and other mixed-function oxydases are localized in the proximal segments of the small intestine, i.e. duodenum and jejunum, and decrease in more distal segments (14,16-18). The distribution of mixed-function oxydase enzymes along the small intestine may entail practical consequences since *in vitro* studies have demonstrated that the rate of biotransformation of propranolol (19) and of diltiazem (20) is slower in terminal regions of the small intestine than in the duodenum and the jejunum. These observations raise the question as to whether, *in vivo*, the site of absorption in the gastrointestinal tract would influence the availability of diltiazem. The present study aimed to assess the effect of the site of absorption on the disposition of diltiazem. To this purpose, anesthetized rabbits received diltiazem into the first 30 cm segment of the small intestine or into the 150-180 cm segment, and the kinetics of diltiazem, MA and M1 have been evaluated. The rabbit was selected as animal model since like in humans, MA is the primary metabolite produced (8) and as such it appears to be the most suitable animal model to study the intestinal metabolism of drugs (21).

MATERIALS AND METHODS

Animal Model

Sixteen male New Zealand white rabbits (2.2 ± 0.1 kg) purchased from La Ferme Cunicole (Mirabel, Québec) were used throughout the study. They were maintained on Purina pellets and water *ad libitum* in individual well ventilated metabolic cages. The animals were kept in their cages for at least one week before any experimental work was done, and then were fasted for 24 hours before surgery.

Experimental Protocol

Following anesthesia with pentobarbital (30 mg/kg), a tracheotomy was performed and the rabbits were artificially ventilated with oxygen-enriched air (21 ml/cycle, 50 cycles/min) (Harvard Apparatus, Boston, MA). Additional pentobarbital was administered as necessary through a catheter inserted in a peripheral vein of the ear. The right femoral artery was cannulated until the abdominal aorta, above the renal arteries, with a PE-50 catheter (Intramedic, Becton Dickinson and Company, Parsippany, NJ) to allow for blood sampling and for arterial pressure measurement. Heparinized saline (179 U/ml) was kept in the arterial catheter to ensure patency.

Under anesthesia, each animal received in a lateral vein of the ear, cannulated with a Butterfly-25 (Venisystems, Abbott Ireland, Sligo, Ireland), a solution of dextrose 5% - sodium chloride 0.9% containing 50 mM of sodium bicarbonate, at a rate of 60 ml per hour. Once surgery was started, the infusion rate was increased to 80 ml per hour due to the higher risk of dehydration through laparotomy. This rate of infusion was chosen to maintain an adequate blood pH and to compensate for losses due to ventilation and blood sampling. The animals were kept warm by placing them on a heated pad during the experiments. Arterial pH, PaO_2, $PaCO_2$ were measured in arterial blood all along the protocol with an automated and computerized 1312 pH/oxygen analyzer (Instrumentation Laboratory, Lexington MA). Throughout the experiment, arterial blood pressure was monitored via a three-way stopcock (Seamless, Division of Professional Medical Products Inc., Ocala, Florida) connected to a pressure transducer and a physiograph (E and M Instruments, Houston, Texas).

Following laparotomy, the intestinal tract was briefly exposed and 5 mg/kg of diltiazem, dissolved in NaCl 0.9%, was injected into the lumen of the proximal small intestine (0-30 cm beyond the pylorus, n=9) and into the lumen of the distal small intestine (150-180 cm beyond the pylorus, n=7). Diltiazem solution was injected in one minute in all rabbits. The dose of 5 mg/kg of diltiazem was chosen because preliminary studies determined that it generated first order kinetics, and because this dose generates in the rabbit diltiazem plasma concentrations in the range of those observed in humans. To characterize diltiazem disposition, blood samples were drawn from the femoral artery prior to and at 5, 10, 20, 30, 45, 60, 80, 100, 120, 180, 240, 300 and 360 minutes

following its administration. The samples were immediately centrifuged (2500xg, 4°C, 10 min), and the plasma was kept frozen at -20°C until assay. Plasma concentrations of diltiazem, *N*-desmethyldiltiazem (MA) and desacetyldiltiazem (M1) were assayed by HPLC, as described elsewhere (22).

In order to evaluate the extent of drug absorption from the gut, at the end of each experiment, the intestinal segment (30 cm) where diltiazem was injected was removed and washed twice with 20 ml of methanol HPLC grade (19,23). In addition, the segment included between 30 and 280 cm, or 180 and 280 cm, was removed and washed with 20 ml of methanol. The resulting eluate was assayed by HPLC for diltiazem and its metabolites (22).

Pharmacokinetic parameters

Initial kinetic parameters were determined by graphic analysis as described by Gibaldi and Perrier (24). Pharmacokinetic parameters were calculated by least-square linear regression analysis of the logarithm of diltiazem concentrations as a function of time plots, describing a two-compartment open model. The area of plasma concentrations of diltiazem, MA and M1 as a function of time curve ($AUC_{0\rightarrow360}$) were estimated by means of the trapezoidal method, with extrapolation to infinity in the case of diltiazem ($AUC_{0\rightarrow\infty}$) using the rate constant of disposition estimated from the terminal linear of diltiazem plasma concentrations. The absorption rate constant (K_a) and the apparent disposition rate constant (ß) were determined by linear least-square regression analysis of the residual and terminal portions of the concentrations of diltiazem. The

apparent half-lives of absorption ($T_{1/2\ abs}$) and of the terminal phase ($T_{1/2}$) were obtained from the ratio of ln $2/K_a$ and of ln 2/ß, respectively. Diltiazem oral clearance (Cl_{oral}) was estimated by using the following equation $Cl_{oral} = D/AUC_{0\rightarrow\infty}$, where D is the administered diltiazem dose.

Statistical analysis

The results are presented as the mean ± S.E.M. Differences between each group were assessed using one-way analysis of variance for parallel groups, and the significance was determined using Dunnett's distribution tables (25). The minimal level of significance was established at $p<0.05$.

RESULTS

Arterial gases and pH remained rather stable throughout the experiment in both groups of rabbits. Mean values of PaO_2, $PaCO_2$ and blood pH at the beginning and at the end of the experiments were, respectively, 128 ± 4 mm Hg, 25.9 ± 0.7 mm Hg and 7.545 ± 0.013 *vs* 147 ± 6 mm Hg, 23.9 ± 1.0 mm Hg and 7.511 ± 0.015. Mean arterial pressure was 82 ± 2 mm Hg once the anesthesia was started *vs* 62 ± 4 mm Hg at the end of the six-hour protocol.

The rate of absorption of diltiazem was not affected by the site of administration: the time to reach diltiazem peak plasma concentration was 20 min, and the absorption half-life was similar (Table I). Absorption was completed after approximately 40 min. Practically the whole dose of diltiazem was absorbed, since only 0.008 ± 0.003% and 0.109 ± 0.061% of the dose were recovered in the lumen of rabbits receiving the drug in the proximal or in the distal small intestine, respectively. When comparing the sites of administration, diltiazem was better absorbed in the proximal segment than in the distal segment, i.e. 0.84 ± 0.30 µg *vs* 15.06 ± 7.54 µg ($p<0.05$) of diltiazem were recovered in the intestinal lumen, respectively. When diltiazem was administered into the proximal segment of the intestine, 1.64 ± 0.40 µg and 0.42 ± 0.25 µg were recovered in the intestine as MA and M1, respectively. On the other hand, following diltiazem administration into the 150-180 cm segment, 6.98 ± 1.66 µg and 3.33 ± 1.78 µg of MA and M1 were recovered in the intestine. The amount of MA recovered from the intestinal lumen was greater when diltiazem was administered distally than proximally. In all

cases, most of the drug or its metabolites were recovered in the eluate of the segment where diltiazem was injected (the first 30 cm).

Following the administration of diltiazem into the distal intestine, mean diltiazem plasma concentrations were higher than those observed when the drug was administered into the proximal intestine (Figure 1). As a consequence, mean $AUC_{0\rightarrow\infty}$ of diltiazem administered in the distal intestine was almost twice the value of the one calculated in animals receiving diltiazem into the proximal intestine (Table I). This difference in $AUC_{0\rightarrow\infty}$ was due to a lower oral clearance when diltiazem was administered in the distal small intestine. MA and M1 were detected in the plasma of rabbits from both groups, MA being the predominant metabolite (Figure 2). The concentrations of MA in plasma were not influenced by the site of administration in the gut, as the $AUC_{0\rightarrow 360}$ of MA were similar between the groups (Table I). However, the ratio of MA $AUC_{0\rightarrow 360}$ over diltiazem $AUC_{0\rightarrow 360}$ was greater in the rabbits receiving diltiazem into the proximal intestine than when administered into the distal intestine, i.e. 1.79 ± 0.14 *vs* 1.33 ± 0.15 ($p<0.05$). M1 plasma concentrations were higher following diltiazem administration into the distal intestine than when given into the proximal intestine (Figure 2 and Table I). M1 $AUC_{0\rightarrow 360}$ ratio over diltiazem $AUC_{0\rightarrow 360}$ in animals receiving the drug into the proximal intestine was similar to the one estimated in animals receiving diltiazem into the distal intestine, i.e. 0.28 ± 0.02 *vs* 0.26 ± 0.02 ($p>0.05$).

The rate constant of disposition (ß) of diltiazem was not influenced by site of administration of diltiazem (Table I). This suggests that the systemic disposition of

diltiazem, i.e. systemic clearance and apparent volume of distribution, was independent of the site of diltiazem absorption in the small intestine.

DISCUSSION

The present study indicates that the administration of diltiazem into the distal parts of the small intestine of rabbits increases diltiazem bioavailability by a factor of almost two, as compared to the bioavailability of diltiazem absorbed in the proximal intestine. An increase in diltiazem bioavailability could theoretically be explained by either a higher amount of drug being absorbed, by a decreased pre-systemic clearance, or by a decreased systemic elimination. Since the amounts of diltiazem recovered in the intestine six hours after its administration were less than 1% of the administered dose, intestinal absorption of diltiazem can be considered complete and equal following both sites of administration. On the other hand, the rate constant of diltiazem disposition calculated in the proximal intestine was similar to the one estimated when diltiazem was given into the distal intestine. Therefore, assuming that diltiazem apparent volume of distribution was not changed between the groups, the systemic clearance of diltiazem should not have been affected by the administration site. Hence, the increase in diltiazem bioavailability when it is absorbed through the distal intestine is due to a decreased pre-systemic extraction of the drug.

The distribution of the metabolic enzymes throughout the body seems to depend on their location within the cell (26). Microsomal enzymes (CYP, *O*-de-ethylases, glucuronyl transferases, etc) are found in greater concentrations in the liver, whereas cytosolic enzymes (esterases, gluthatione *S*-transferases, *N*-sulphotransferases, etc) have an almost even and wide distribution throughout the organism (17,26,27). Furthermore, the regional distribution of the enzymatic activity within one organ may vary

considerably, which could result in significant differences in *in situ* drug metabolism activity (14,15,17,28).

The intestinal epithelium is exposed to the highest concentrations of numerous ingested compounds and of the orally administered drugs. The intestine is the organ containing the highest specific metabolic activity after the liver (28). Thus, xenobiotics may undergo biotransformation within the luminal mucosal cells, considered to be the predominant metabolic site in the gut (29,30). While phase II conjugative activity in the gut is comparable to that of the liver (15,31-33), phase I reactions such as reduction, oxidation and hydrolysis are reported to be lower than in the liver (14,15,29). In general, phase I intestinal enzymatic activity is highest in the mucosal epithelial cells of the duodenum and jejunum, and tends to decrease distally to the pylorus (14,15,29); whereas conjugation enzymes seem to be evenly distributed throughout the gut (14,31).

Total cytochrome P-450 content undergoes a two-fold decrease between proximal and distal small intestine of humans (33). CYP 3A subfamily, 2C8-10 and 2D10 are found in decreasing concentrations in the distal small intestine (14,28,31). Some subfamilies are expressed only in the duodenum, i.e. CYP 1A1 (14,28,34); whereas some other isozymes are detected in duodenum and jejunum only, i.e. CYP 2D6 (14,31). Many isozymes are not expressed at all in the human intestine, i.e. CYP 1A2, 2A6, 2A7, 2B1, 2B2, 2C11, 2E1, 3A5, 3A7, etc (14,15,28,31). In the colon, CYP content is extremely low compared to that of the liver and until now, only a 54-KDa isozyme has been detected (18). The repercussions of this distribution of mixed function oxidase enzymes

along the small intestine are well illustrated with the results of the present study, i.e. the first-pass uptake of diltiazem in the distal portion of the small intestine is at least half the value of that of the proximal small intestine.

Following the administration of diltiazem into the lumen of the proximal intestine the AUC of diltiazem and its active metabolites were smaller than those estimated when diltiazem was given into the distal small intestine. These differences are difficult to explain with the present *in vivo* model because of the fact that the AUC of diltiazem and its metabolites are the result of the first-pass metabolism in the intestine, liver and lungs (20). However, since the molar ratio of the AUC of the metabolites over the AUC of diltiazem was smaller following diltiazem injection in the proximal small intestine than after its injection into the distal small intestine, we may speculate that these differences are secondary to the biotransformation of diltiazem to metabolites other than MA and M1 in the proximal intestine. If this is the case, more diltiazem will be available to generate M1 and MA when diltiazem is administered in the distal small intestine.

The presence of MA and M1 in the intestinal lumen could be explained by two mechanisms not exclusive among themselves. On the one hand, the metabolites may have back-diffused from the epithelial cells of the small intestine. On the other hand, we can not exclude that a small fraction of the dose of diltiazem may have been biotransformed by the bacterial flora of the intestinal lumen. Selected microorganisms normally found in the intestinal tract contain high quantities of deaminase (35,36) and nitro reductase enzymes (37,38). The intestinal microflora can biotransform nitro

compounds such as nitrofurantoin (39), metronidazole (40) and chloramphenicol (41), but there is no evidence that N-demethylation or desacetylation may occur. The fact that greater amounts of MA and M1 were recovered in the distal small intestine favours the possibility that diltiazem was metabolized in the intestinal lumen since distally, the presence of bacterial flora is greater than proximally. Whenever the presence of metabolites into the intestinal lumen is secondary to intraluminal metabolism of diltiazem, it does not change the interpretation of the results of the present study, since the amounts of metabolites recovered represent only 0.09% of the dose of diltiazem given.

In conclusion, the present study confirms *in vivo* that the site of absorption in the small intestine modulates the bioavailability of diltiazem and its metabolites. This is mainly due to regional differences in the ability of the intestine to metabolize diltiazem. This site-dependent intestinal metabolism of diltiazem may explain why in healthy volunteers diltiazem availability was greater when administered in a slow release formulation, where the absorption occurs more distally, than when given in a conventional formulation (42). The site of absorption may entail clinical dynamic consequences, since the molar sum of diltiazem and its two active metabolites was 48% bigger when administered into the distal intestine than when given in the proximal small intestine. In addition, the present results emphasize the need to keep in mind the metabolic activity of the intestine when designing 24-hour modified release formulations of drugs highly extracted by presystemic organs.

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TABLE I. Pharmacokinetic parameters of diltiazem (5 mg/kg) following its administration into the lumen of the proximal (0-30 cm) or distal segments of the gut (150-180 cm).

	PROXIMAL INTESTINE	DISTAL INTESTINE
K_a (min^{-1})	0.1613 ± 0.0357[a]	0.2260 ± 0.0657
$T_{1/2\ abs}$ (min)	6.3 ± 1.5	4.8 ± 1.1
$AUC_{0\rightarrow\infty}$ DTZ (µg.min/ml)	8.14 ± 0.88	14.20 ± 2.82[b]
$AUC_{0\rightarrow 360}$ MA (µg.min/ml)	11.29 ± 1.08	14.26 ± 1.43
$AUC_{0\rightarrow 360}$ M1 (µg.min/ml)	1.75 ± 0.16	2.92 ± 0.42[b]
Cl_{oral}(ml/min/kg)	660.2 ± 54.7	439.9 ± 78.2[b]
ß (min^{-1})	0.0038 ± 0.0003	0.0048 ± 0.0004
$T_{1/2\ el}$ (min)	190 ± 16	152 ± 13

a. mean ± S.E.M.
b. $p < 0.05$ as compared to the proximal intestine values.

Figure 1 Diltiazem plasma concentrations following the administration of a single dose of diltiazem (5mg/kg) into the proximal (Δ) or the distal intestine (▲) of anesthetized rabbits. Values are mean ± SEM.

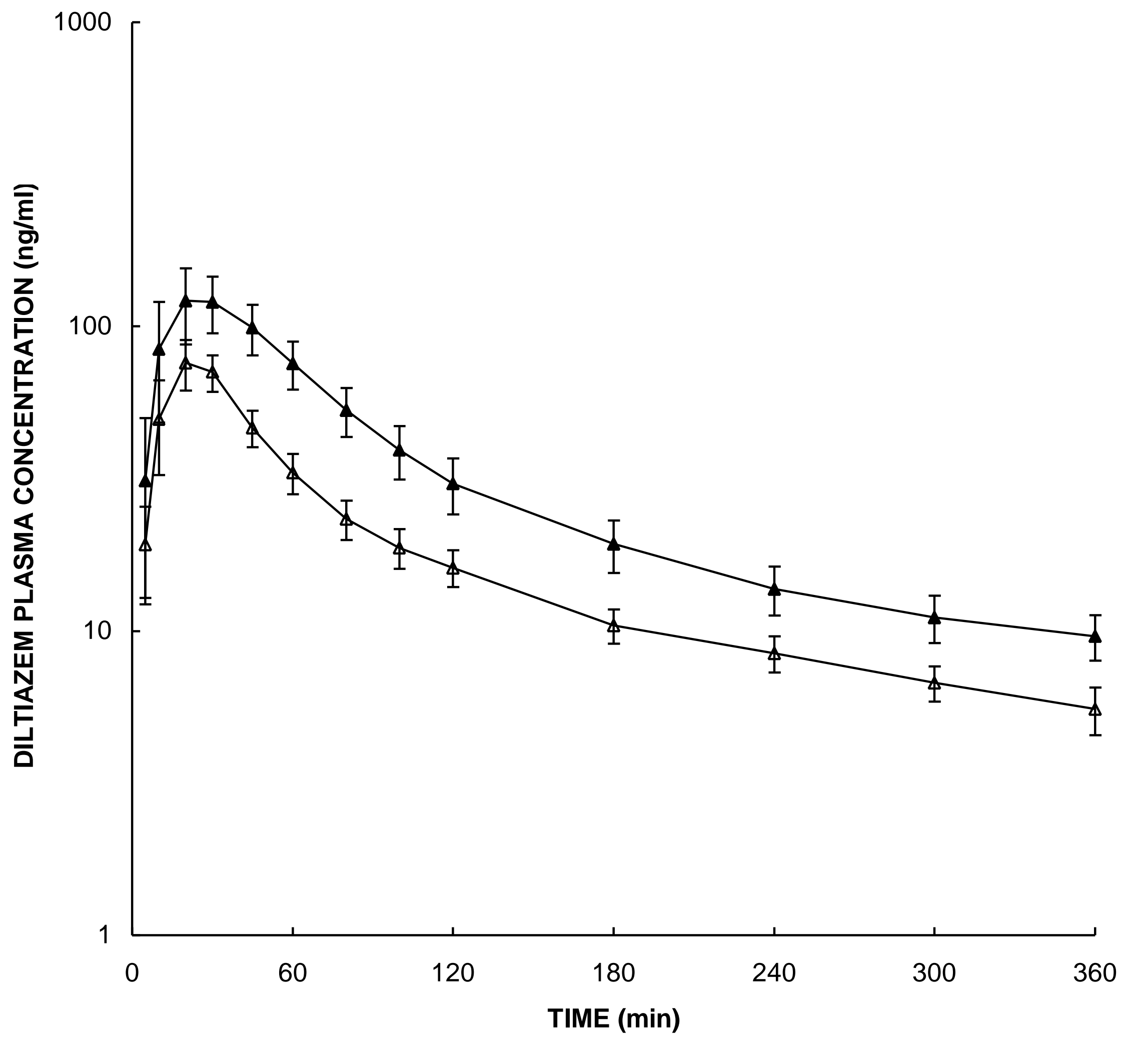

Figure 2 Plasma concentrations of diltiazem metabolites MA (O) and M1 (◊) following the administration of a single dose of diltiazem (5 mg/kg) into the proximal (hollow symbols) or the distal intestine (filled symbols) of anesthetized rabbits. Values are mean ± SEM.