Short Communication

Extensive Metabolism of Diltiazem and P-Glycoprotein-Mediated Efflux of Desacetyl-Diltiazem (M1) by Rat Jejunum In Vitro

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ABSTRACT:

The objective of this in vitro study was to investigate both the intestinal metabolism and transport of diltiazem (DTZ) and its major metabolites in rat jejunum. Metabolism experiments were performed with everted sacs, whereas sheets mounted in a symmetrical twin chamber system were used in transport studies. DTZ was rapidly desacylated by the rat jejunum to the principle metabolite desacetyl-diltiazem (M1). In addition, minor amounts of N-demethyl-diltiazem and desacetyl-N-demethyl-diltiazem were formed. Due to the rapid desacylation, it proved difficult to study the transport of DTZ in this model. However, the primary metabolite M1 was shown to be subjected to P-glycoprotein (Pgp)-mediated efflux. The flux rate of M1 was 6- to 7-fold higher from the serosal to the luminal compartment than in the opposite direction. Both coadministration of verapamil and Pgp monoclonal antibody dose dependently increased luminal-to-serosal flux and decreased serosal-to-luminal flux. In conclusion, rat jejunum metabolizes DTZ extensively in vitro, and the major primary metabolite M1 is subjected to Pgp-mediated efflux.

The calcium channel blocker diltiazem (DTZ) undergoes complex metabolism in vivo (Yeung et al., 1990). Desacetylation and N-demethylation are two major metabolic pathways of DTZ. They lead to the formation of desacetyl-DTZ (M1), N-demethyl-DTZ (MA), and the secondary metabolite desacetyl-N-demethyl-DTZ (M2) (Fig. 1). Esterases are responsible for the desacylation of DTZ (Fraile et al., 1996), whereas the N-demethylation is catalyzed by the cytochrome P-450 isoenzyme subfamily CYP3A (Richard et al., 1990).

Several metabolites of DTZ have pharmacological activity. The coronary vasodilating potency of M1, for example, has been shown to be 50% compared with DTZ in dogs (Yabana et al., 1985). The clinical relevance of the metabolites of DTZ has not been determined in humans.

The ATP-dependent efflux pump P-glycoprotein (Pgp) was first described in multidrug-resistant tumors, and later shown to be expressed in a variety of normal tissues, including the gastrointestinal tract (Bellamy, 1996). DTZ has been shown to inhibit intestinal efflux of Pgp substrates in vitro (Hsing et al., 1992; Emi et al., 1997). However, to our knowledge, there are not any studies showing that DTZ itself is a substrate of Pgp.

DTZ is involved in drug interactions at the presystemic level, for example by increasing the bioavailability of cyclosporin A (CsA) (Asberg et al., 1999). The aims of the present study were to investigate intestinal metabolism and transport of DTZ and its major metabolites in the rat jejunum in vitro.

Materials and Methods

Male Wistar rats (250–350 g) obtained from Moellegaard A/S (L. Skensved, Denmark) were anesthetized by i.p. injection of a mixture of barbital/Na 4% (w/v) and pentobarbital/Na 1% (0.4 ml/100 g b.wt.) before the jejunum was isolated. Immediately after removal of the jejunum the rats were sacrificed by decapitation.

For the metabolism experiments each jejunal segment (~5 cm) was prepared as a sac, everted, and filled with buffer to adequate distension (0.5–0.8 m). When Pgp monoclonal antibody was used to inhibit the efflux of M1, the antibody was administered either in the luminal or serosal compartment. To inhibit Pgp in the transport experiments with M1, both compartments were preincubated (30 min) with verapamil (0.15–6 mM) before coadministration of M1 (15 µM) and verapamil (0.15–6 mM). When Pgp monoclonal antibody was used to inhibit the efflux of M1, the antibody was administered at the luminal surface (due to the apical location of Pgp) in increasing concentrations (1–5 ng/µl). Two hours of incubation were needed to generate sufficient amounts of M1 for HPLC analysis in the serosal compartment after luminal administration. The samples were assayed according to a HPLC method previously set up at our laboratory (Christensen et al., 1999).

Neither verapamil nor Pgp monoclonal antibody interfered with the detection of M1. [3H]PEG samples were counted by Tri-Carb/model 1900 TR (Packard Instrument B.V. - Chemical Operations, Groningen, the Netherlands).

DTZ hydrochloride and verapamil hydrochloride were purchased from Sigma (St. Louis, MO). DTZ metabolites were supplied as a gift from Tanabe Seiyaku (Osaka, Japan). Pgp monoclonal antibody (Cat. no. 1378970) was obtained from Boehringer Mannheim Biochemica (Mannheim, Germany), whereas [3H]PEG (MW_average ~ 900, specific activity 2.27 mCi/g) was obtained from DuPont-NEN (Mechelen, Belgium).

Abbreviations used are: DTZ, diltiazem; CsA, cyclosporin A; MA, N-demethyl-DTZ; M2, desacetyl-N-demethyl-DTZ; M1, desacetyl-DTZ; Pgp, P-glycoprotein; PEG, polyethylene glycol.
Results and Discussion

M1 was the most prominent metabolite formed during incubations of DTZ in everted rat jejunal sacs (Fig. 2A). Esterases in the rat jejunum have been shown to be loosely connected to the brush border (Hillestad et al., 1982), and the rapid transformation of DTZ to M1 might reflect deacetylation of DTZ before uptake into the epithelial cells. The rate of DTZ N-demethylation was only one-fifth the desacetylation reaction (Fig. 2B), which may be due to the need of transport into the enterocytes for N-demethylation in contrast to the epithelial desacetylation. The N-demethylation of DTZ, involved in MA and M2 formation, is catalyzed by members of the CYP3A enzyme subfamily (Pichard et al., 1990), which are located intracellularly in mature villous enterocytes (Kolars et al., 1992).

In our procedure, a mixture of barbital and pentobarbital was used as anesthetic. Barbiturates are well known as inducers of drug-metabolizing enzymes, and it has been shown that phenobarbital treatment (4 days) caused increased metabolism of DTZ in the rat in vivo (Yeung et al., 1996). However, it is unlikely that the short time exposure of barbiturates in this study should cause any significant metabolic interference.

The extensive hydrolysis of DTZ made it difficult to study the transport of DTZ across the rat jejunum, and DTZ was in fact only recovered as metabolites (mainly M1) in the compartment opposite to the administration compartment (data not shown). However, the jejunal metabolism of the major metabolite M1 was modest (about 90% recovered after 4 h of incubation, data not shown), and it was shown that the flux rate of M1 was about 6- to 7-fold higher from the serosal to the luminal compartment than in the opposite direction (Fig. 3). The Pgp inhibitor verapamil and Pgp monoclonal antibody reduced this flux ratio (serosal to luminal flux/luminal to serosal flux) in a dose-dependent manner due to both increased luminal-to-serosal flux and decreased serosal-to-luminal flux (Fig. 3). At the highest inhibitory concentrations of verapamil and Pgp monoclonal antibody, the flux ratio of M1 was below the flux ratio of $[^3]$HPEG (data not shown), which is believed to be exclusively exchanged by passive diffusion.

The model used was not appropriate for disclosing if DTZ is subjected to intestinal Pgp-mediated efflux. However, as there is minor structural dissimilarity between DTZ and M1, it is reasonable to assume that the former also is secreted by Pgp. Nevertheless, because metabolites often have relevant pharmacological effects, as is the case for M1 (Yabana et al., 1985), an interesting finding of this study is that drug metabolites can be substrates of Pgp. In addition, the observations that M1 is a substrate of Pgp and that MA is a more potent inhibitor of CYP3A4 than DTZ (Sutton et al., 1997) emphasize the possible importance of these metabolites in pharmacokinetic interactions between DTZ and coadministered drugs.

DTZ is involved in pharmacokinetic interactions at the presystemic level, for example, by increasing the oral bioavailability of CsA in

![Fig. 1. Chemical structures of DTZ and three major metabolites.](image-url)
The flux rates are calculated as percentage of M1 recovered in the compartment opposite to the administration compartment after 120 min of incubation. Flux ratios (flux serosal to luminal compartment/flux luminal to serosal compartment) are indicated on the figure. (Experiments in absence of inhibitor n = 3, mean ± S.D.; experiments in presence of inhibitor n = 1).

renal transplanted patients (Asberg et al., 1999). Because oral bioavailability of CsA seems to be affected by the level of intestinal Pgp expression (Lown et al., 1997), and the DTZ-CsA interaction might be restricted to the intestine (Preuner et al., 1998), our results suggest that Pgp may be implicated in this interaction. However, this hypothesis needs to be evaluated in additional studies.

The oral bioavailability of DTZ in humans is about 30% after a single dose of 120 mg (Bianchetti et al., 1991). In the rat, oral bioavailability seems to be even lower (Lee et al., 1991), which might be due to a greater esterase activity and/or higher level of Pgp expression in the rat intestine compared with the human intestine. Thus caution is required in applying the present results in the rat to humans. However, the findings have general implications and may serve as a useful basis for future hypotheses about DTZ pharmacokinetics in humans.

In conclusion, DTZ was shown to be subjected to rapid desacetylation with subsequent Pgp-mediated efflux of the desacetylated metabolite (M1) by the rat jejunum in vitro.

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References


