JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 963–972

Received January 9, 2003
Accepted April 3, 2003
DOI 10.1211/0022357021495
ISSN 0022-3573

Regional transport and metabolism of ropivacaine and its CYP3A4 metabolite PPX in human intestine

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Abstract

The major aim of this study was to investigate the CYP3A4 metabolism and polarized transport of ropivacaine and its metabolite 2′,6′-pipecoloxylidide (PPX) in tissue specimens from the human small and large intestine. Ropivacaine has been shown to be effective in the treatment of ulcerative colitis in human colon. This study was conducted using a modified Ussing-chamber technique with specimens from jejunum, ileum and colon collected from 11 patients. The local kinetics of ropivacaine and PPX were assessed from their concentration–time profiles in mucosal and serosal compartments. The permeability (P_{app}) in the absorptive direction for both ropivacaine and PPX increased regionally in the order jejunum < ileum < colon. Ropivacaine was not found to be subjected to any carrier-mediated intestinal efflux. However, the CYP3A4 metabolite left the human enterocyte in a polarized manner and both the extent of CYP3A4 metabolism of ropivacaine and the extrusion of its metabolite to the mucosal chamber were more efficient in jejunum than in ileum. P-glycoprotein was probably not involved in the metabolite extrusion. No other metabolite than PPX was found. This in-vitro study with human intestinal tissues provides new mechanistic insights into regional transport and metabolism of drugs.

Introduction

The interactive nature of passive and carrier-mediated transport and metabolism of drugs in different regions of the human intestine, and to what extent these processes affect bioavailability, have become increasingly important issues in drug development. Predictions of in-vivo drug metabolism, transport and the function of efflux carriers such as P-glycoprotein (Pgp) are usually carried out using in-vitro models including microsomes and various cell lines (Hochman et al 2001; Obach et al 2001; Mandagere et al 2002). Intestinal microsomes cannot be used to study the interaction of cellular transport and enzymatic activity during the intestinal absorption process. Cell culture models, on the other hand, do not account for the regional aspects of drug permeability and metabolism in man (Ungell et al 1998; Nakamura et al 2002). Furthermore, it is well known that enzymes and efflux proteins are not necessarily expressed in appropriate proportions in cell cultures, and the expression levels of efflux proteins may differ between laboratories due to dependence on culturing conditions (Anderle et al 1998). A difference in gene expression between cultured cells and human tissue has also been reported (Sun et al 2002). The major advantage of using excised human intestinal tissues in studies of efflux and metabolism interplay is that they contain the appropriate array and expression levels of transporters and enzymes as along the human small and large intestine in-vivo. This absorption model is expected to reflect the regional and interindividual variability in activity of these proteins, provided functional tissue viability is maintained throughout the experiment (Söderholm et al 1998; Coleman et al 2001). The effects of regional activity of proteins involved in carrier-mediated transport and metabolism of drugs and metabolites are poorly investigated in the human gastrointestinal tract.

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Acknowledgements: We would like to thank Christel Bergström for ClogP and PSA determinations and Dr Hans von Euler-Chelpin for LC/MS analysis.

CYP3A4 is one of the most important enzymes in man regarding drug metabolism, as approximately 50% of all approved drugs are metabolized by this isoenzyme (Wrighton & Thummel 2000). It is expressed mainly in the liver but intestinal CYP3A4 can also affect the first-pass extraction of orally administered drugs such as midazolam and felodipine (Regårdh et al 1989; Thummel et al 1996). The apical recycling hypothesis suggests a synergistic activity of Pgp and CYP3A4 where the active efflux prolongs the residence time in the enterocyte of the parent drug and thereby increases exposure of the drug to CYP3A4 (Benet et al 1996; Watkins 1997; Ito & Sugiyama 1999; Johnson et al 2001). Previous studies of CYP3A4-mediated intestinal metabolism suggest that the interplay also includes Pgp-mediated active transport of formed metabolites to the intestinal lumen (Gan et al 1996; Hochman et al 2001). Further evidence for the metabolism and efflux interplay is the close vicinity of Pgp and CYP3A4 in the enterocyte and the overlapping substrate specificity (Wacher et al 1995; Watkins 1997; Cummins et al 2001). However, functional evidence from intact human tissue and in-vivo models is still not available to support these hypotheses. In addition, the validity in-vivo is not evident as drugs such as midazolam and felodipine, both CYP3A4 substrates, undergo extensive gut-wall metabolism without being Pgp substrates (Regårdh et al 1989; Thummel et al 1996; Kim et al 1999; Cummins et al 2002).

In this study, excised tissue from three different regions of the human intestinal tract were mounted into modified Ussing chambers and ropivacaine, an amide-type local anaesthetic compound, was used as a model drug. Ropivacaine undergoes CYP3A4-mediated N-dealkylation to 2′,6′-pipecoloxylidide (PPX), according to previous studies of hepatocytes (Ekström & Gunnarsson 1996), but it is not known whether ropivacaine or PPX are subjected to any carrier-mediated transport. Ropivacaine has previously been shown to have beneficial effects in the treatment of ulcerative colitis locally in the colon (Arlander et al 1996; Martinsson et al 1999). Accordingly, there is also an interest in investigating the transport and metabolism of this drug in different regions of the human intestine, especially since it has been reported that efflux activity by Pgp may play an important role in poor response to medical therapy in inflammatory bowel disease (Farrel et al 2000). The activity of efflux proteins has been shown to be region dependent in both rat and human intestine (Makhey et al 1998), whereas the regional aspect of metabolite extrusion has not yet been reported.

The major aim of this study was to investigate the polarized transport and metabolism of ropivacaine and its CYP3A4 metabolite PPX in specimens from human small and large intestines. Ketoconazole and verapamil, well-established inhibitors of CYP3A4 and Pgp, were used to investigate the metabolism and transport mechanism. This is, to our knowledge, the first study in which CYP3A4 metabolism and transport interplay were investigated in a regional perspective in the human intestine.

Materials and Methods

Drugs and chemicals

Ropivacaine hydrochloride and PPX hydrochloride were provided by AstraZeneca R&D (Södertälje, Sweden) (Figure 1). Verapamil was obtained from Knoll AG (Darmstadt, Germany). Ketoconazole was purchased from Sigma Chemicals (St Louis, MO). ^{3}H-D-glucose (1.0 mCi mL^{-1}) was purchased from Amersham Labs (Buckinghamshire, UK), and ^{14}C-mannitol (0.031 μCi mL^{-1}) was purchased from NEN Life Sci. Products (Brussels, Belgium). All other chemicals used were of analytical grade.

Human intestinal segments

Intestinal tissues were obtained from 11 patients, both male and female. Jejunal segments (n = 5) were obtained from patients undergoing gastric bypass or surgery for pancreatic cancer. Ileum (n = 3) and colon (n = 5) segments were obtained from patients undergoing surgery for intestinal cancer. None of the patients had been treated with cytotoxic drugs or radiation therapy before surgery, as these treatments could significantly alter intestinal function and permeability (Nejdfors et al 2000). None of the intestinal specimens showed any signs of inflammation or cancer infiltration. The segments were put in carbogen-bubbled room-temperature Krebs buffered Ringer (KBR) solution, pH 7.4, immediately after resection and were then transported to the laboratory. The study was approved by the Ethics Committee of Lund University (Approval numbers LU 760-00 and LU 761-00). Before surgery the patients were informed about the aim of the study and had to sign an informed consent.

Experimental procedure and study design

The intestinal mucosa was carefully dissected from the serosa muscle layer and mounted in the Ussing diffusion chambers with 1.78 cm^2 exposed tissue area (Navicyte, San Diego, CA). The serosal and mucosal reservoirs of the Ussing diffusion chambers were filled with 6 mL modified Krebs buffer (KBR) (pH 7.4, 290 mOsm kg^{-1}) containing (in mM):NaCl 108, KCl 4.7, Na_2HPO_4 1.8, KH_2PO_4 0.6, $NaHCO_3$ 16, $MgSO_4$ 1.2, Na-pyruvate 4.9, fumarate 5.4, L-glutamate 4.9, D-glucose 11.5 and $CaCl_2$ 1.25. The KBR buffer was continuously bubbled with carbogen (95% O_2 and 5% CO_2) and was circulated by gas lift at 37 °C. The transepithelial potential difference (PD) was measured using a Voltmeter (Millicell-ERS, Millipore, Sweden) equipped with a pair of Ag/AgCl electrodes embedded in 3 M KCl agar, just before the start of the experiment to ensure the viability of the resected segment. The experiment was started (t = 0) within 60 min of division of the blood vessels by replacing the buffer with 6 mL of the test solutions (Table 1) (Nejdfors et al 2000). Donor concentrations of 500 μM ropivacaine and 250 μM

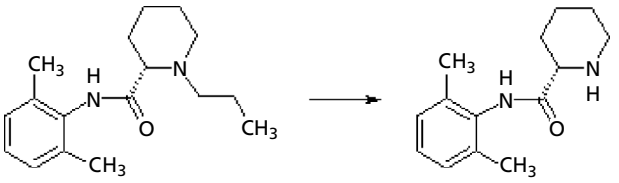

Figure 1 Molecular structures of ropivacaine and the N-dealkylated metabolite PPX. The PPX formation has been shown to be mediated by CYP3A4 in human liver microsomes (Ekström & Gunnarsson 1996). Physicochemical properties are given in the text.

PPX were chosen according to the criteria that concentrations should be clinically relevant, the metabolites should be quantifiable and the concentrations used should not be toxic to the intestinal segments in the chambers. Neither ropivacaine nor PPX bound to the Ussing chamber material under experimental conditions. The donor solution also contained viability and integrity markers, ^{3}H-D-glucose and ^{14}C-mannitol. When verapamil and ketoconazole were used as inhibitors of Pgp and CYP3A4, they were added to both mucosal and serosal compartments. The experiment was continued for 150 min, with a 1-mL sample being withdrawn from the mucosal and serosal reservoirs, respectively, every 30 min. The volume withdrawn was immediately replaced with starting solutions for each chamber. Samples were immediately frozen at −20 °C until analysis.

Chemical analysis

Ropivacaine and PPX were assayed using reversed-phase HPLC. For quantification of ropivacaine, a sample volume of 60 μL was injected onto the column (μ-Bondapak C18 9 × 150 mm; Waters, Milford, MA) at a flow rate of 1.0 mL min^{-1}. The mobile phase consisted of phosphate buffer (pH 8.0, I = 0.05) with 60% v/v acetonitrile, UV detection wavelength 220 nm. Ropivacaine was eluted at 4 min. The variability, expressed as coefficient of variation (CV), was 0.2, 0.03 and 0.3% at quality controls 38.5, 19.5 and 2.45 μM, respectively. The limit of quantification (LOQ) was 0.4 μM (CV 4.1%). For quantification of PPX, a volume of 120 μL was injected onto the column (Symmetry C8 3.9 × 150 mm) with guard column (Symmetry C8 3.9 × 20 mm; Waters, Milford, MA) at a flow rate of 1.0 mL min^{-1}. The mobile phase consisted of phosphate buffer (pH 2.0, I = 0.05) with 23% v/v acetonitrile and 5 mM 1-octane sulfonic acid, UV detection wavelength 210 nm. PPX was eluted at 9.5 min. CV at concentrations of quality controls was 0.4, 0.6 and 4.0% at 4.1, 0.41 and 0.1 μM, respectively, and the LOQ was 0.05 μM (CV 1.0%). Concentrations (d min^{-1} mL^{-1}) of ^{3}H-D-glucose and ^{14}C-labelled mannitol were measured using a liquid scintillation counter (Tricarb 1900 CA, Packard Instruments) after addition of 8 mL scintillation liquid (Ready Safe; Beckman Fullerton, CA). Analysis to verify whether ropivacaine-related metabolites other than PPX were formed was carried out using HPLC with electrospray ionization mass spectrometry (LC/MS). The HPLC system consisted of a binary pump (HP 1100 G1312A, Hewlett Packard) with an autosampler (HP 1100 G1313A, Hewlett-Packard). Separation was achieved on a reversed-phase column (Symmetry C8, 1.0 × 150 mm; Waters, Milford, MA) with guard column (Opti-Guard, 1.0 × 10 mm; Alltech Associates, Deerfield, IL). The mass spectrometer was a Quattro II (Micromass Ltd, Manchester, UK) with an electrospray ion source and cross-flow counter-electrode, cone voltage 30 V and 60 V in alternating scans. A gradient program was used, and the mobile phases consisted of A: 0.03% (v/v) trifluoroacetic acid in deionized water and B: 0.03% trifluoroacetic acid in water–acetonitrile (40:60 v/v) with the content of phase B increasing by 2% every minute. The flow rate was 0.04 mL min^{-1}.

Data analysis

The apparent permeability, P_{app}, for ropivacaine, PPX, ^{3}H-D-glucose, and ^{14}C-mannitol was calculated using equation 1, whereas the extrusion rate of the metabolite PPX to mucosal or serosal compartments, was calculated according to equation 2.

Table 1 Each intestinal segment was divided into eight pieces and included in the following study groups. Ropivacaine (Ropi) (500 μM) or the metabolite PPX (250 μM) were added in the donor compartment. Inhibitors, verapamil (Vera) (500 μM) or ketoconazole (Keto) (10 μM), were added to both donor and reciever compartments. Experiments were performed mucosa to serosa (M-S) and serosa to mucosa (S-M).

Substrate	Inhibitor	Transport direction
Ropi	—	M-S
Ropi	—	S-M
Ropi	+ Vera	M-S
Ropi	+ Vera	S-M
Ropi	+ Keto	M-S
Ropi	+ Keto	S-M
PPX	—	M-S
PPX	—	S-M

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{A \cdot C_0} \quad (1)$$

$$\text{Extrusion rate} = \frac{dQ}{dt} \cdot \frac{1}{A} \quad (2)$$

where dQ/dt is the steady-state appearance rate, A is the exposed tissue area, and C_0 is the donor concentration.

Permeability efflux ratio Er(p) was calculated with equation 3.

$$Er(p) = \frac{P_{app\ S\text{-}M}}{P_{app\ M\text{-}S}} \quad (3)$$

The metabolite extrusion ratios, Er(m), for comparing the relative metabolite extrusion pattern towards the mucosal and serosal side were calculated for each of the transport directions for both jejunum and ileum (see Table 3).

ClogP for ropivacaine and PPX was calculated using a ClogP program (BioByte Corp., Claremont, CA). Three-dimensional conformations of the compounds were generated using MacroModel version 6.5, and polar surface areas (PSA) were calculated using MAREA version 2.4 (provided free of charge for academic users johan.grasjo @farmaci.uu.se).

Statistical calculations were made using analysis of variance with Scheffe's post-hoc test (StatView; Abacus Concepts, Inc., Berkeley, CA). P-value less than 0.05 was considered significant. Data are presented as mean ± s.e.m.

Results

Intestinal permeabilities of ropivacaine and its CYP3A4 metabolite PPX were high ($P_{app} > 1 \times 10^{-5}$ cm s^{-1}) and predicted complete absorption in all three human intestinal regions (Figure 2) (Lennernäs et al 1997; Ungell et al 1998). The $P_{app\ M\text{-}S}$ for both the drug and metabolite increased in the order jejunum < ileum < colon. The $P_{app\ S\text{-}M}$ for both the drug and metabolite was approximately equal throughout the human intestinal regions (Figure 2). Permeability data also indicate that neither ropivacaine nor PPX are subjected to any intestinal efflux transport, as they do not undergo any lumen-directed efflux across the jejunal, ileal or colonic tissues (Figure 2). The P_{app} was higher for ropivacaine than PPX in all three segments, which is in accordance with the lipophilic properties of ropivacaine (ClogP 3.18, PSA 29.0 Å^2), which are greater than those of PPX (ClogP 1.52, PSA 37.4 Å^2). The P_{app} for ropivacaine and PPX was not significantly affected in any intestinal region by addition of verapamil or ketoconazole (Table 2).

The metabolism of ropivacaine to PPX was about 2-fold higher in jejunum than ileum (Figures 3 and 4). In the colon,

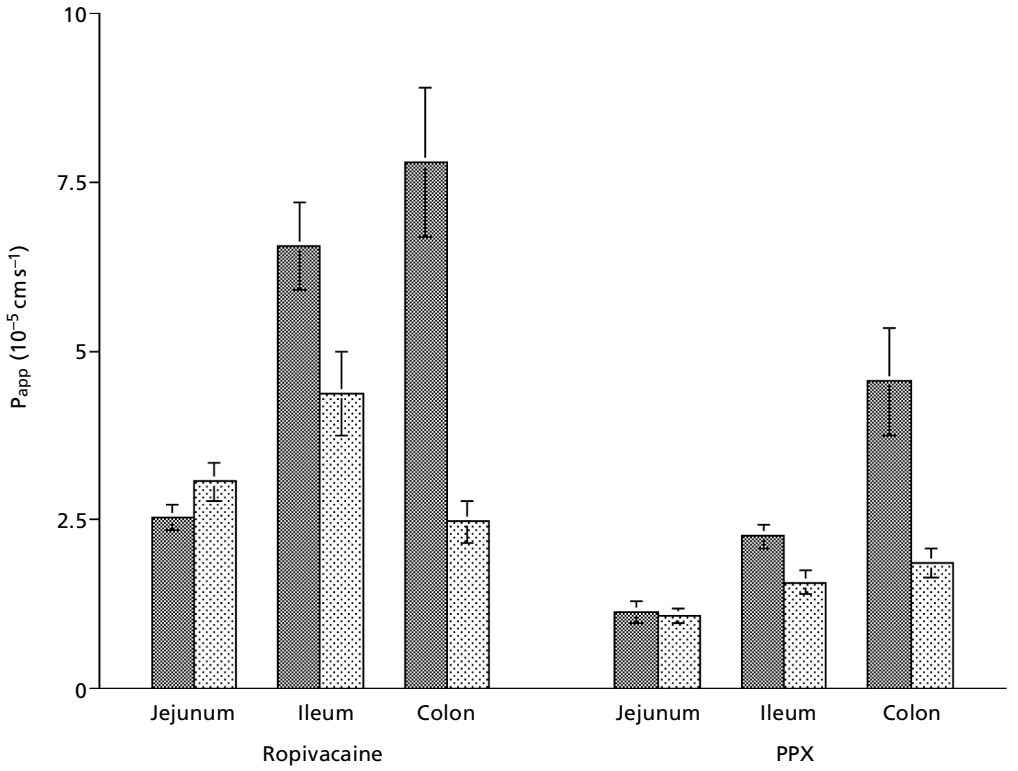

Figure 2 Apparent permeability, P_{app} (cm s^{-1}), mucosa to serosa (M-S) (■) and serosa to mucosa (S-M) (□) in different regions of human intestine for ropivacaine (500 μM) and PPX (250 μM) given as mean ± s.e.m.

Table 2 Intestinal permeability (P_{app}, cm s^{-1}) mucosa to serosa (M-S) and serosa to mucosa (S-M) of ropivacaine, 500 μM, with and without addition of Pgp and CYP3A4 inhibitors, verapamil (Vera), 500 μM, and ketoconazole (Keto), 10 μM, in jejunum, ileum and colon.

		P_{app} (10^{-5} cm s^{-1})	P_{app} (10^{-5} cm s^{-1}) + Vera	P_{app} (10^{-5} cm s^{-1}) + Keto
Jejunum	M-S	2.54 ± 0.18^{c}	2.66 ± 0.26	4.18 ± 0.61
	S-M	3.07 ± 0.29	2.74 ± 0.33	4.37 ± 0.68
Ileum	M-S	6.55 ± 0.64	4.56 ± 0.26	5.30 ± 0.69
	S-M	4.38 ± 0.63	3.93 ± 0.36	4.25 ± 0.25
Colon	M-S	$7.79 \pm 1.11^{a,c}$	7.25 ± 0.55	11.0 ± 0.26^{b}
	S-M	2.48 ± 0.31^{a}	3.48 ± 0.51	4.59 ± 0.72^{b}

[a,b]Significant difference comparing transport directions (M-S) vs. (S-M), $P < 0.05$. [c]Significant difference comparing intestinal regions, $P < 0.05$.

PPX was detected but in amounts too small to be quantified (data not shown). The appearance of PPX in mucosal and serosal chambers showed a linear increase throughout the 150-min experiment, which indicated a maintained metabolic activity and no saturation of the enzyme (Figure 3). The degree of metabolism was less than 10% of the start concentration of the parent drug, which indicates no substrate depletion. No metabolites other than PPX were detected in this study, which was confirmed using LC/MS. Intracellularly formed PPX was predominantly extruded from the intestinal segment to the mucosal chamber when ropivacaine was transported in the M-S direction in both jejunum and ileum (Figure 4). The relative appearance rate of PPX in the mucosal chamber was higher in jejunum than ileum, whereas the ropivacaine transport route-dependent formation of PPX was more pronounced in ileum than jejunum (Table 3). Ketoconazole (10 μM) almost completely inhibited the formation of PPX in both jejunum and ileum, whereas verapamil (500 μM) did not completely inhibit the PPX formation (Figure 4).

The potential difference (PD) and the permeability of ^{3}H-D-glucose were used as viability markers for the human intestinal tissue specimens in this study. Initial PD values (mV) for jejunum, ileum and colon were 5.6 ± 0.36, 9.0 ± 1.1, and 17.8 ± 1.0, respectively, and the P_{app} of ^{3}H-D-glucose demonstrated adequate viability of each intestinal specimen (Figure 5). The P_{app} for D-glucose in the M-S direction was higher in the small intestine than in the colon, which is in accordance with the regional intestinal distribution of glucose transport activity in-vivo (Lee et al 1994). The integrity of the human intestinal segments was evaluated by assessing the mannitol transport demonstrating maintained tissue integrity over the experimental period (Figure 5). The stable absorption rate of D-glucose and mannitol throughout the experiment indicated no escalating changes of intestinal viability and integrity during the experiment, which is in agreement with previous viability studies (Söderholm et al 1998). The inhibitors used, verapamil and ketoconazole, did not affect the transport of D-glucose or mannitol.

Discussion

To our knowledge, this is the first in-vitro study reported where CYP3A4-mediated drug metabolism and transport have been investigated using excised tissue from three different regions of the human intestine. The P_{app} in the absorptive direction for both ropivacaine and its CYP3A4 metabolite, PPX, varied regionally in the order jejunum < ileum < colon. This is consistent with other drugs classified as having high permeability with passive diffusion as the dominating transport mechanism, which has previously been shown for rat intestine (Ungell et al 1998). This observation further validates the rat as an absorptive model with high predictive value for human drug absorption. An explanation for the regional permeability profile might be differences in the lipid composition of the small and large human intestine as this parameter is important for transport of lipophilic compounds (Meddings 1989).

PPX is formed by CYP3A4-mediated N-dealkylation, which has previously been shown for human hepatocytes (Ekström & Gunnarsson 1996). This was confirmed for intestinal specimens in this study by inhibition experiments with ketoconazole, a potent inhibitor of CYP3A4, which almost completely inhibited the metabolism of ropivacaine to PPX. The metabolism was approximately 2-fold higher in the human jejunum than ileum segments, which agreed with previous studies reporting that jejunum has a higher CYP3A4-mediated metabolic activity than ileum (Paine et al 1997). In colon specimens, no metabolite could be quantified, which is in accordance with the weak CYP3A4 expression reported for the human colon (de Waziers et al 1990; Zhang et al 1999). In-vivo in man, ropivacaine undergoes extensive metabolism and the main enzyme is CYP1A2 followed by CYP3A4 (Arlander et al 1998). PPX was the only metabolite quantified when ropivacaine was administered in the various intestinal segments, which confirms that CYP1A2 activity is very low throughout the human intestine (de Waziers et al 1990). Consequently, CYP3A4 is the most important

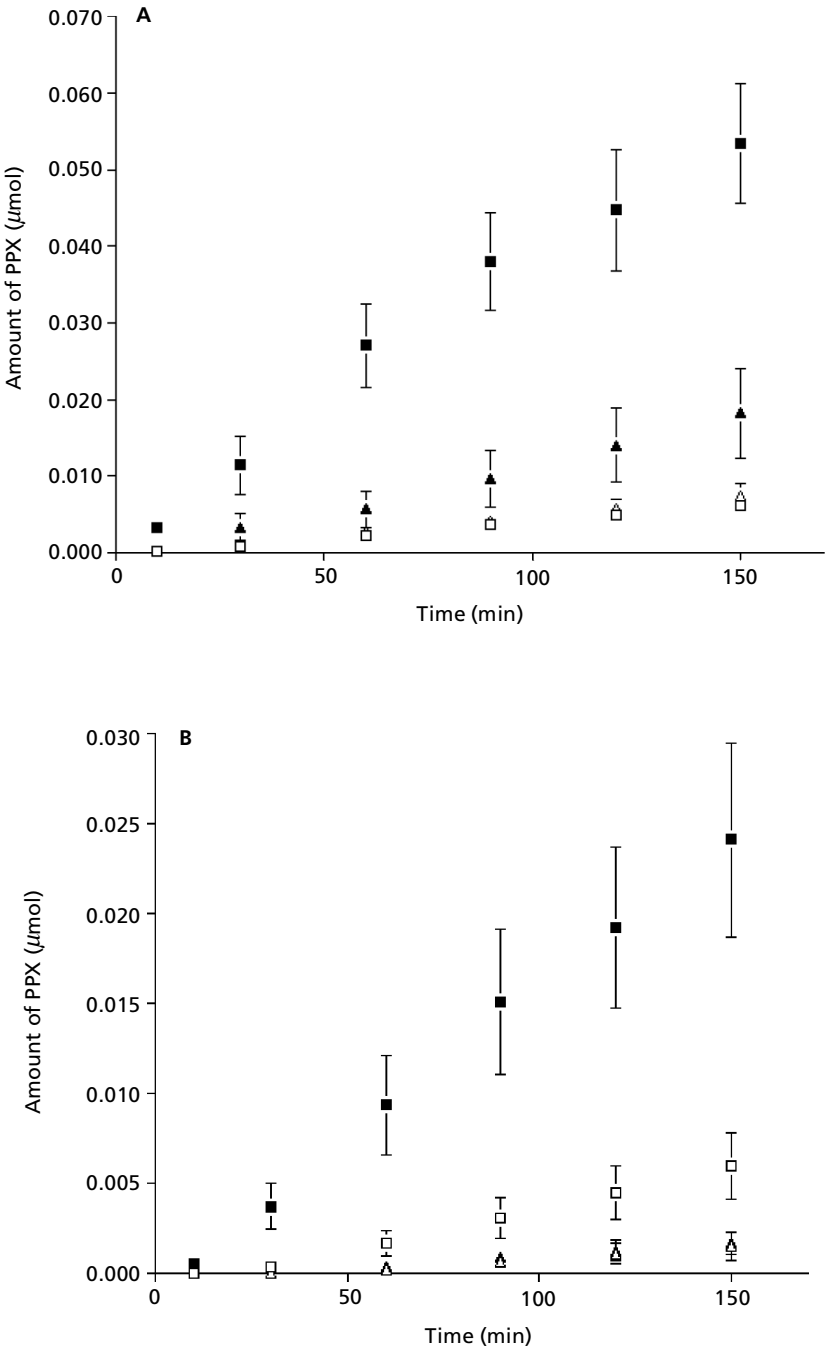

Figure 3 PPX in the mucosal (filled symbols) or serosal (open symbols) compartments (μmol) increasing with time when ropivacaine (500 μM) is transported M-S (squares) or S-M (triangles) for jejunum (A) and ileum (B) given as mean ± s.e.m.

enzyme for local metabolism of ropivacaine in the small intestine.

A polarized transport of intracellularly formed metabolite was found in the small intestinal regions, which is in agreement with previous results from studies using cell cultures and animal tissue (Gan et al 1996; Lampen et al 1998; Hochman et al 2001; Johnson et al 2001). Interestingly, the metabolism and the luminal extrusion pattern of the formed metabolite differed between jejunum and ileum. Jejunum not only presented a more efficient CYP3A4-mediated metabolism than ileum, but also exhibited a relatively more efficient extrusion of metabolite into the intestinal lumen. When ropivacaine was transported in the absorptive direction (M-S), the extrusion of intracellularly formed metabolite was 8.1- and 3.8-fold higher towards the mucosal vs the serosal compartment for jejunum and

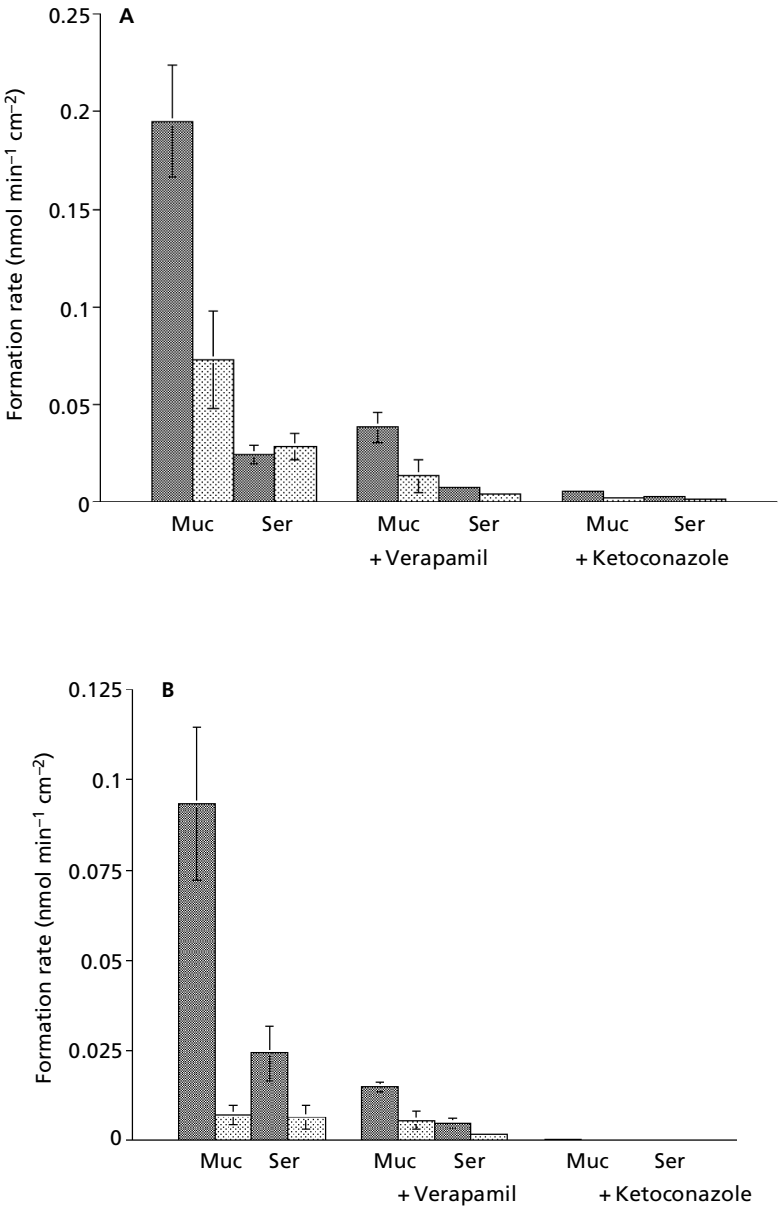

Figure 4 PPX metabolite extrusion rate in jejunum (A) and ileum (B) as ropivacaine (500 μM) is transported mucosa to serosa (M-S) (■) and serosa to mucosa (S-M) (□). Inhibition of PPX extrusion rate to mucosal (Muc) or serosal (Ser) compartments with added verapamil (500 μM) and ketoconazole (10 μM) in both compartments, mean ± s.e.m.

ileum, respectively (Table 3). An efflux ratio of metabolites greater than 3 has previously been suggested for a carrier-mediated efflux mechanism (Cummins et al 2002). The luminal-directed extrusion process of the intracellularly formed metabolite seemed to be more efficient in human jejunum than ileum. This observation might be explained by a more efficient carrier-mediated efflux in the apical membrane, but could also be caused by a greater surface area of the apical enterocyte membrane or a shorter diffusion distance from the CYP3A4 enzyme to the apical membrane. When the bidirectional transport of PPX was investigated separately, there was no evidence for any apical efflux. This could be due to either a saturation of the transporter or the transporter having access to PPX only when it is formed intracellularly. It might also be due to the fact that PPX is not a substrate for any efflux transporter.

CYP3A4 has been suggested to be polarized within the intracellular space towards the apical side (Watkins 1997; Cummins et al 2001), and the binding site for CYP3A4 may even be partly embedded in the membrane (Wang et al 2001). A metabolite, which is generally more hydrophilic than the parent drug, formed by CYP3A4 in a hydrophobic environment might require, or at least be

Table 3 Relative extrusion pattern of intracellularly formed metabolite, PPX, in jejunum and ileum when the parent drug, ropivacaine, is transported mucosa to serosa (M-S) or serosa to mucosa (S-M). Extrusion rate of metabolite is calculated for either mucosal (muc) or serosal (ser) chamber.

	M-S (muc/ser)[a]	S-M (muc/ser)[b]	muc (M-S/S-M)[c]	ser (M-S/S-M)[d]
Jejunum	8.1*	2.6	2.7*	0.8
Ileum	3.8*	1.1	13.0*	3.8

*$P < 0.05$. [a,b]Ratio of mucosal and serosal extrusion of PPX when ropivacaine is transported M-S or S-M. [c,d]Ratio of metabolite extrusion dependence on ropivaciane transport direction, M-S or S-M, when PPX is extruded to the mucosal or serosal compartment.

more efficiently extruded from the membrane with, facilitated transport. Verapamil, an inhibitor of Pgp and, to some extent, also CYP3A4, lowered the total metabolite extrusion of intracellularly formed PPX, probably due to CYP3A4 inhibition. On the other hand, verapamil did not seem to affect the transport profile of the intracellularly formed PPX in the small intestinal regions in this study (Figure 4). This suggests that Pgp is not involved in the polarized transport of intracellularly formed PPX in human jejunal and ileal specimens. Instead its polarized cellular transport might be mediated by another active transport mechanism or passive transcellular diffusion in combination with the cellular organization of the CYP3A4 enzyme.

The metabolite formation and extrusion were also shown to be dependent on the transport route of the parent drug, as the intracellularly formed metabolite was more efficiently extruded to the lumen when ropivacaine was added on the mucosal rather than the serosal side (Figure 4). When ropivacaine was added M-S vs S-M, the extrusion of PPX to the mucosal chamber was 2.7- and 13-fold higher in jejunum and ileum, respectively. This suggests that luminal-directed metabolite extrusion rate is more dependent on drug transport direction in ileum than in jejunum (Table 3). The parent drug transport route dependence for metabolite extrusion shown in this study has previously been explained for verapamil in rat jejunum by polarized transport of the parent drug due to

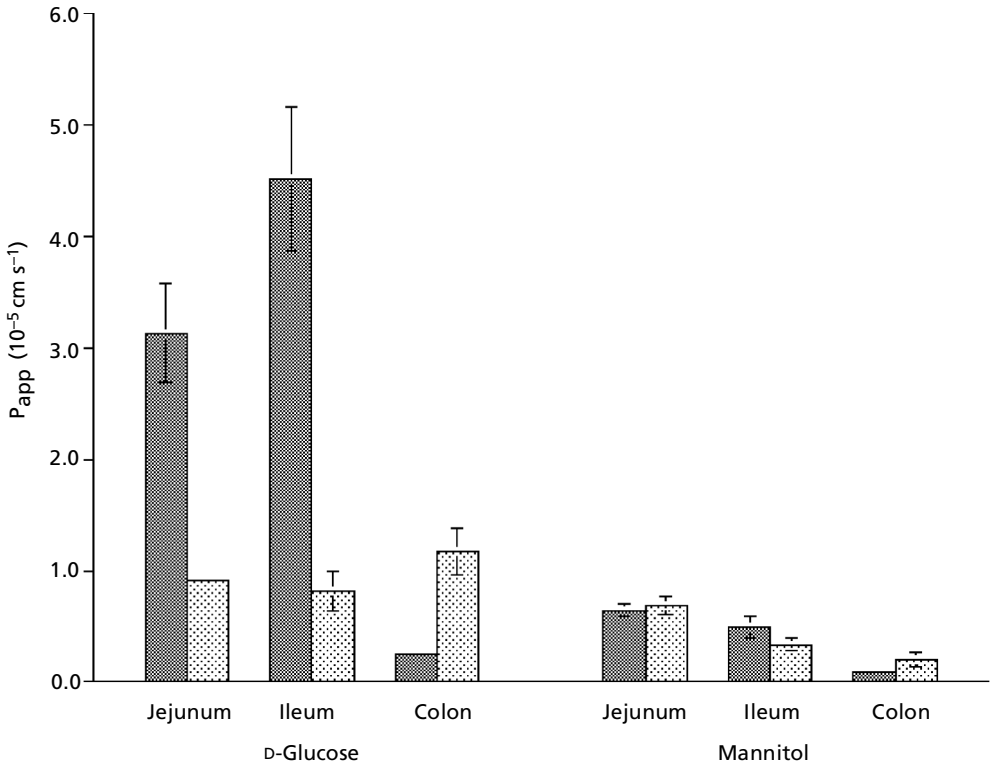

Figure 5 Apparent permeability, P_{app} (cm s^{-1}), mucosa to serosa (M-S) (▩) and serosa to mucosa (S-M) (▨) in the different intestinal regions for D-glucose (viability marker) and mannitol (integrity marker) given as mean ± s.e.m.

Pgp transport causing a differing residence time in the enterocyte (Johnson et al 2001). However, that hypothesis is less plausible for ropivacaine since the metabolite extrusion in the human small intestine was route dependent even though no efflux of the parent drug was observed. Verapamil affected neither permeability nor the extrusion pattern of metabolism in any of the small intestinal segments, suggesting no efflux involvement. This differing route-dependent extrusion of metabolite in jejunum and ileum might be explained by differences in the membrane structure of jejunum and ileum that may affect passive diffusion (Ungell et al 1998) or differences in activity of carrier-mediated processes other than Pgp. The parent drug transport route dependence of ropivacaine metabolism in the human small intestine obviously has another explanation than the apical recycling theory since ropivacaine is not affected by efflux transport.

A polarized metabolism/transport kinetics similar to that of ropivacaine has been shown for felodipine, a substrate for CYP3A4 that is extensively metabolized in-vivo in the human intestine (Regårdh et al 1989; Cummins et al 2002), although not subjected to any efflux in MDCK-MDR1 and Caco-2 cell monolayers (Soldner et al 1999). In CYP3A4-transfected Caco-2 cells, the intracellularly formed CYP3A4 metabolite dehydrofelodipine was preferentially extruded towards the apical side (2.6-fold), which was not affected by Pgp-inhibitors such as ciclosporin or GG918 (Cummins et al 2002). Midazolam has also been shown to exhibit a polarized metabolite extrusion that was not affected by verapamil (Schmiedlin-Ren et al 1997). This study supports previous findings that polarized transport of intracellularly formed metabolite may occur even if there is no involvement of any efflux transporter. The mechanism of the polarized extrusion of metabolites needs further investigation for intracellularly formed metabolites from drugs such as ropivacaine, felodipine, and midazolam.

Conclusion

We found an increased P_{app} for both ropivacaine and PPX in the absorptive direction for the more distal regions (jejunum < ileum < colon). Neither ropivacaine nor the metabolite PPX were found to undergo any intestinal efflux even if the distribution of the CYP3A4 formed PPX was polarized and dependent on parent drug transport route. We also found that both CYP3A4 metabolism and metabolite extrusion to the mucosal compartment were more extensive in jejunum than ileum. Finally, human intestinal specimens in the Ussing-chamber model may be well suited for mechanistic studies of absorption and gut-wall metabolism of drugs, which is important to consider in oral dosage-form design.

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