Jejunal Absorption and Metabolism of R/S-Verapamil in Humans

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Purpose. The purpose of this human intestinal perfusion study was to investigate the transport and metabolism of R/S-verapamil in the human jejunum (*in vivo*).

Methods. A regional single-pass perfusion of the jejunum was performed using a Loc-I-Gut® perfusion tube in 12 healthy volunteers. Each perfusion lasted for 200 min and was divided into two periods each of 100 min. The inlet concentrations of verapamil were 4.0 and 40 mg/l in period one and two, respectively.

Results. The effective jejunal permeability (Peff) of both R- and S-verapamil increased ($p < 0.05$) when the inlet concentration was increased consistent with saturation of an efflux mechanism. However, both R- and S-verapamil had high intestinal Peff, consistent with complete absorption. The Peff of antipyrine also increased, but there was no difference in the Peff for D-glucose in the two periods. The appearance of R/S-norverapamil in the intestinal perfusate leaving the jejunal segment was non-linear, presumably due to saturation of the CYP3A4 metabolism.

Conclusions. The increased Peff in parallel with increased entering drug concentration is most likely due to saturable efflux by P-glycoprotein(s) in the human intestine.

KEY WORDS: verapamil; intestinal permeability; P-glycoprotein; absorption; intestinal secretion; metabolism; CYP3A4.

INTRODUCTION

The role of multidrug resistance (MDR) has been extensively studied in numerous cancer cell lines. Verapamil was the first nonchemotherapeutic drug to be used as an inhibitor of MDR *in vitro* (1,2). The molecular basis for MDR is the action of P-glycoprotein(s) as well other efflux proteins such as multidrug resistance-associated protein (MRP) (2). P- glycoprotein(s) are ATP-dependent efflux pumps, located in the membrane, with a broad substrate specificity as a distinct feature (3). In humans two known forms of the P-glycoprotein(s) are named after their corresponding genes MDR-1 and MDR-2 (also known as MDR-3), and are expressed in epithelial and endothelial cells in certain normal human tissues (3–5). Their natural function is probably to reduce the intracellular exposure and eliminate xenobiotics from the body (6). More specifically, in the intestine and at the blood-brain barrier they may decrease the inward transport across these two barriers, increase the outward transport (excretion) from these tissues and increase the degree of metabolism (by apical recycling) when a compound crosses the cell cytosol (6). The function of P-glycoprotein(s) in the intestine and at the blood brain barrier has been elegantly demonstrated in mice (*in vivo*) with homozygously disrupted mdr1a genes [mdr1a(-/-) (7–9).

Direct mechanistic *in vivo* studies in humans showing the role of P-glycoprotein(s) and CYP3A4 for intestinal transport and metabolism of drugs are difficult to perform in humans for obvious reasons. However, recent oral pharmacokinetic studies have estimated the gut wall metabolism to contribute to a significant part of the total first-pass effect of drugs such as verapamil, midazolam, cyclosporine and felodipine (10–14). Fromm et al. reported that the gut wall metabolism of R/S-verapamil increased to about 90% in rifampin induced subjects (11). They suggested that the reduced oral bioavailability was due to induction of CYP3A4 in the enterocytes, since a significant smaller increase was observed in liver clearance (11). However, the reduced bioavailability might also be attributed to decreased absorption and/or increased apical recycling as a consequence of a simultaneously rifampin induced expression of P-glycoprotein(s) in the apical enterocyte membrane. Furthermore, in a human perfusion study with an open perfusion technique intestinal secretion of talinolol (a P-glycoprotein substrate) was reported, but it was not possible to account for the contribution of biliary secretion (15). Other *in vivo* and *in vitro* studies have presented evidence that intestinal secretion is the reason behind the concentration- and dose-dependent absorption of talinolol (16).

Verapamil is a lipophilic model drug (log D 2.7, octanol/H_2O; pH 7.4; MW 455 Da) which has been extensively used as a model substrate for P-glycoprotein(s) in cancer cells (2,17). Verapamil has been reported to have a rather high affinity to P-glycoprotein(s) (2). In the present study the transport and metabolism of R/S-verapamil were investigated using a single-pass perfusion technique (Loc-I-Gut®). The disappearance of drugs from the perfused jejunal segment is used to calculate the effective jejunal permeability (Peff), which has been shown to predict intestinal absorption of drugs with high precision (18). A major advantage with this clinical tool is that intestinal transport and metabolism of drugs and their metabolites is possible to measure adjacent to the tissue where the events actually occurs.

The main purposes of this *in vivo* perfusion human study were to determine jejunal Peff and to investigate the transport mechanism of R/S-verapamil at two different perfusate concentrations. A third aim was to investigate if there was any enantioselectivity and/or non-linearity in the appearance of the R/S-norverapamil in the human jejunum (*in vivo*).

MATERIAL AND METHODS

Subjects

The study was approved by the Ethics Committee and Isotope Committee of the Medical Faculty, University of Uppsala, Sweden. Twelve healthy volunteers participated (eight male and four female) ageing between 21 and 38 years, and weighing between 64–85 kg (males) and 55–64 kg (females). The subjects were given written information about the study and all of them gave their informed consent to participate in

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it. Two subjects (one male and one female) did not complete the study because of substantial distal leakage from the jejunal segment during the perfusion. Prior to the study all participants underwent a full clinical examination and they all had normal clinical and laboratory findings in serum (S) and blood (B) (S-creatinine, S-ASAT, S-ALAT, S-ALP, S-Potassium, S-Sodium, S-Bilirubin, B-Erytrocytes, B-Haemoglobin, B-Trombocytes, B-Leukocytes and HIV). None of the participants received any medication before or during the perfusion experiment other than the drugs under investigation. The study was performed at the clinical research department of the University Hospital in Uppsala, Sweden.

Study Medication

R/S-verapamil for oral human use was a gift from Knoll AG, Darnstadt, Germany. Antipyrine was supplied by Astra Läkemedel AB, Södertälje, Sweden, and was used as an absorption marker. The perfusion solution consisted of antipyrine 10 mg/l, potassium chloride 5.4 mM, sodium chloride 30 mM, mannitol 35 mM, D-glucose 10 mM, PEG 4000 (1.0 g/l) all dissolved in a 70 mM phosphate buffer with pH at 6.5. Each perfusion lasted for 200 min and was divided into two periods (P1 and P2) of 100 min each. The inlet concentration of R/S-verapamil was 4.0 and 40 mg/l in period one (P1, 0 to 100 min) and period two (P2, 100 to 200 min), respectively. Each subject was exposed for the lower concentration of R/S-verapamil in the first period in order to avoid carry over effects caused by the higher concentration. Polyethylene glycol labelled with ^{14}C (^{14}C-PEG 4000) was purchased from Amersham Laboratories, Buckinghamshire, England and added to the perfusion solution as a volume marker with an activity of 2.5 μCi/l.

Experimental Procedure

After an overnight fast of 10 hours a regional single-pass perfusion of the proximal jejunum was performed using a Loc-I-Gut® perfusion tube (Synectics Medical, Stockholm, Sweden). Having applied a local anaesthesia to the oesophagus with a lidocaine spray, the Loc-I-Gut® tube was introduced through the mouth. During insertion there was a teflon coated guide wire inside the instrument to facilitate the passage of the tube into the intestine. The position of the tube was checked by fluoroscopy control and the perfused segment was located in the proximal part of the jejunum. Along with the Loc-I-Gut® instrument, another tube was positioned in the stomach for drainage of gastric juice during the experiment (Salem sump tube, Sherwood Medical, U.K.). Once the perfusion tube was in place, the two balloons were inflated with approximately 26–30 mL of air creating a 10 cm long closed segment. A vacuum pump was connected to the proximal drainage channel of the perfusion tube to drain of any intestinal fluid above the perfused segment (Ameda suction pump type 23, Ameda AG, Zug, Switzerland). The segment was then rinsed with isotonic saline (37°C) for at least 20 min. When stable perfusion conditions were achieved the perfusion solution (37°C) was pumped into the jejunal segment at a flow rate of 2.0 ml/min using a calibrated syringe pump (model 355, Sage Instr. Orion Research Inc. Cambridge, MA, USA). A more extensive description of this perfusion technique is published elsewhere (19,20). The perfusate leaving the segment was quantitatively collected on ice in 10 min fractions and immediately frozen (−20°C) pending analysis. When the perfusion was terminated after 200 min the jejunal segment was rinsed with 120 ml of saline, and the Loc-I-Gut® instrument was removed.

Stability and Adsorption Test of R/S-Verapamil

The stability and adsorption of R/S-verapamil to the materials in the tube were tested by *in vitro* perfusion using the Loc-I-Gut® instrument at 37°C for 200 min. R/S-verapamil was stable in the perfusion solution at 37°C for at least 200 min. Approximately 5% of R/S-verapamil was adsorbed to the perfusion instrument during *in vitro* perfusion at both concentrations. Since the adsorption was the same at both concentrations it was considered to be negligible when making the calculations for the *in vivo* perfusion experiment. The adsorption of R/S-norverapamil was assumed to be same due to the structural similarities.

Analytical Methods

An enantioselective HPLC-method for R/S-verapamil and R/S-norverapamil was performed on a Chiral AGP-column (4 × 150 mm, Chrometech, Stockholm, Sweden), with a Chiral AGP precolumn (3 × 10 mm). The pump was a Shimatzu LC-9A (Kyoto, Japan), and the flow rate applied was 1.0 ml/min. The detection was made with a Jasco FP-920 fluorescence detector (Tokyo, Japan) with an excitation wavelength of 232 nm and an emission wavelength of 310 nm. The analytical condition used for the analysis of the perfusate was a phosphate buffer with an ionic strength of 0.01 and a pH of 7.6 with 22% (V/V) of acetonitril at 30°C, by heating the column and the mobile phase in a heating bath (Grant Instrument Ltd, type JB1, Cambridge, England). The perfusate samples were diluted in mobile phase and 50.1 μl was injected on the column. The limits of quantification (LOQ) (±S.D) for both R- and S-verapamil were 5.5 ± 0.3 ng/ml in perfusate. The LOQ (±S.D) for R- and S-norverapamil were 2.9 ± 0.2 ng/ml. More details of the enatioselective HPLC method is available elsewhere (21). The concentration of antipyrine in the perfusate and perfusion solution was analysed by HPLC and UV detection using a previously validated method (20,22). The concentration of the volume marker ^{14}C-PEG 4000 was determined by liquid scintillation counting (Mark III, Searle Analytic Inc., Des Plaines, Illinois, USA). The perfusate concentration of D-Glucose was analysed using a multichannel analyser (Technicon DAX®, Bayer Diagnostics) at Calab AB, Stockholm, Sweden.

Data Analysis

All calculations of the effective intestinal permeability (Peff), the net water flux (NWF), the fraction absorbed (fa), the appearance ratio (Ar) and the ratio between the enantiomers (Er) were made from the last five steady state concentrations in the outlet perfusate in periods one (60–100 min) and two (160–200 min), respectively. Each sample represents the mean concentration of the aliquots collected for each 10 min interval. The net water flux per cm in the isolated jejunal segment was calculated according to equation 1 for each sample:

$$\text{Net water flux} = \frac{(1 - \text{PEGout/PEGin})\text{Qin}}{\text{L}} \quad (1)$$

where PEGin and PEGout are the concentrations of ^{14}C-PEG

4000 (dpm/ml) entering and leaving the segment, respectively. Qin is the flow rate of the perfusion solution and L is the length of the perfused jejunal segment (10 cm). The concentration of each compound in the perfusate leaving the intestine was corrected for water flux before fa, Peff and Ar were calculated.

The amount that disappeared during the single-passage through the jejunal segment was assumed to have been absorbed (fa):

$$fa = 1 - \left(\frac{Cout \times PEGin}{Cin \times PEGout}\right) \quad (2)$$

where Cin and Cout are the inlet and outlet concentrations of each compound, respectively. The intestinal effective permeability (Peff) was calculated according to a well mixed tank model as shown in equation 3 (20,23):

$$Peff = \frac{(Cin - Cout)Qin}{Cout \times 2\pi rL} \quad (3)$$

The surface of the cylinder ($2\pi rL$) of the jejunal segment was calculated using the intestinal radius (r = 1.75 cm) and length (L = 10 cm) of the segment. That this estimate of the human jejunal radius is accurate for the perfused segment is shown by a recently performed fluoroscopic investigation (Knutson et al., personal communication).

The appearance ratio (Ar) was calculated for each of the enantiomers of norverapamil as shown in equation 4:

$$Ar = \frac{Cout\ (norverapamil)}{Cin\ (verapamil) \cdot fa} \quad (4)$$

where Cout (norverapamil) is the concentration of R- or S-norverapamil in the perfusate leaving the segment, and Cin (verapamil) is the concentration of the corresponding enantiomer of verapamil entering the segment (on the molar basis). The fraction absorbed (fa) during the perfusion was obtained from equation 2. An important assumption for perfusion studies, since we are measuring the disappearance rate of a drug, is that R/S-verapamil has to be absorbed before it can be metabolised by the CYP3A4 enzyme localised in the enterocytes (Fig. 1).

The ratio (Er) between the enantiomer of the norverapamil metabolite in the intestinal lumen was calculated from the outlet concentrations of R- and S-norverapamil according to equation 5:

$$Er = \frac{Cout\ (R\text{ - }norverapamil)}{Cout\ (S\text{ - }norverapamil)} \quad (5)$$

The statistical difference between the two periods was assessed by using a Student t-test for paired data. The data are given as mean values with a 95% confidence interval (CI) unless otherwise stated.

RESULTS

Transport Across the Human Jejunal Barrier *In Vivo*

The effective jejunal permeability (Peff) of both R- and S-verapamil increased ($p<0.05$) in all subjects except one when the concentration of racemic verapamil entering the jejunal segment was increased from 4.0 to 40 mg/l (Fig 2a-b). These two luminal concentrations represent the remaining fractions of a 100 mg dose of R/S-verapamil when 99 and 90% has been absorbed, respectively. The mean Peff increased significantly

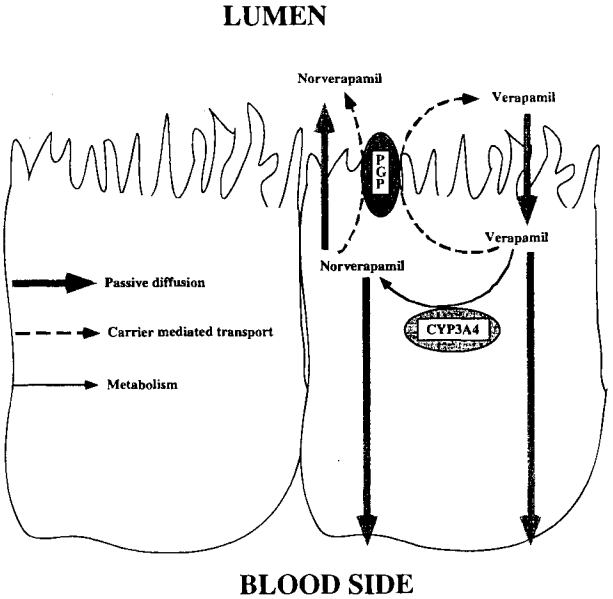

Fig. 1. Schematic figure showing the P-glycoprotein mediated transport and CYP 3A4 metabolism of R/S-verapamil in the human jejunum. It is assumed that the drug has to be absorbed before it can be metabolized inside the human enterocyte. The formed metabolite may diffuse both into the vena porta and the intestinal lumen. The metabolite may as well be transported by the efflux mechanism.

from 2.7 (95% CI 1.9 to 3.4) to 4.74 (95% CI 3.3 to 6.1) and 2.2 (95% CI 1.6 to 2.8) to 4.7 (95% CI 3.1 to 6.3) • 10^{-4} cm/sec for R- and S- verapamil, respectively (Table I). There was a slight difference in the Peff between R- and S-verapamil at the lower inlet concentration ($p<0.05$), but no difference was observed at the higher concentration (40 mg/l) (Table I).

The individual and mean absorption variables for the antipyrine and D-glucose are given in Figures 3a and b and Table II. The Peff of antipyrine increased from 2.2 (95% CI 1.6 to 2.8) to 4.0 (95% CI 2.6 to 5.5) ($p<0.05$). There was no difference in the Peff for D-glucose in the two periods (Table II). The mean steady-state recovery of PEG 4000 exceeded 95% in both periods, and a net secretion of water was observed in the segment in both periods; this agreed with our earlier studies (Table II) (20,24,25).

Metabolism in the Human Jejunal Tissue *In Vivo*

The concentrations of R/S-norverapamil, formed by CYP 3A4 mediated N- dealkylation, were determined in the outlet jejunal perfusate samples during steady. Individual values of the appearance ratios (Ar) for each enantiomer are shown in Figure 4a-b. The average values of Ar for R-norverapamil/R-verapamil during period one and two were 8.5% (95% CI 6.1 to 10.9%) and 2.5% (95% CI 1.9 to 3.0%) ($p<0.05$), respectively. The corresponding data for S-norverapamil were 14.4% (95% CI 9.5 to 19.2%) and 3.8% (95% CI 3.1 to 4.5%) ($p<0.05$), respectively (Table II, Fig. 4a-b). A higher concentration of S-norverapamil compared to R-norverapamil was observed in the outlet perfusate at both concentrations, but the ratio between the two metabolites did not change (Table II and Fig. 4a-b). The enantiomeric ratio (Er) of the R to the S-form of norverapamil (from equation 5) averaged 70% (95% CI 68 to 73%) and 64% (95% CI 58 to 71%), in periods one and two, respec-

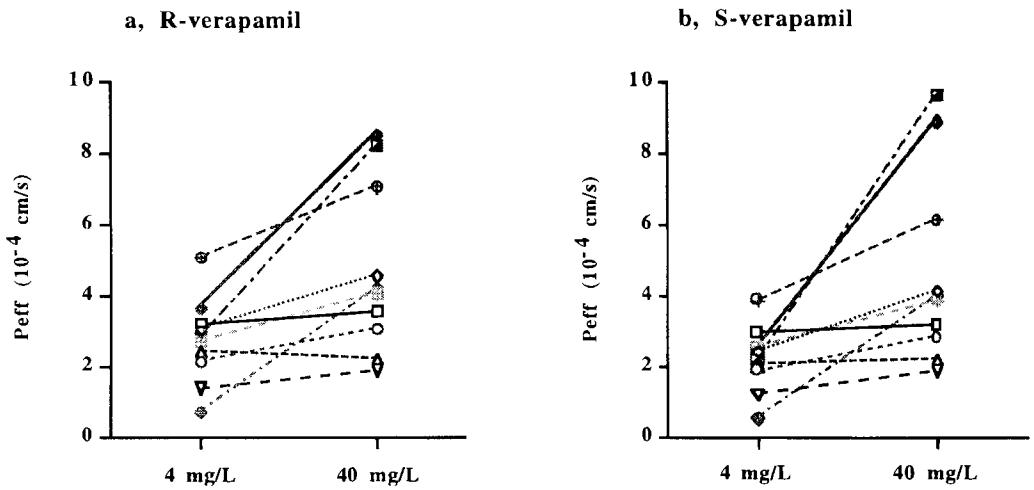

Fig. 2. Individual values of the human jejunal effective permeability (*Peff*) for (a) R- and (b) S-verapamil at the intestinal perfusate concentration of 4.0 (P1) and 40 mg/l (P2).

Table I. Absorption and Metabolic Variables (Mean and 95% CI) for Both Enantiomers of R/S-Verapamil at Two Luminal Concentrations of 4.0 and 40 mg L^{-1} in Ten Healthy Subjects During Jejunal Perfusion. The Metabolic Variables Are Based on the Concentrations of Each Enantiomer of R/S-Norverapamil in the Outlet Jejunal Perfusate

	R-verapamil			S-verapamil			R/S
Period	Peff (10^{-4} cm/sec)	fa (%)	Ar (%)	Peff (10^{-4} cm/sec)	fa (%)	Ar (%)	Er (%)
One	2.71[a]	43[b]	8.5[c]	2.25[a]	39[b]	14.4[c]	70
95% CI	2.00–3.41	34–51	6.1–10.9	1.71–2.79	30–47	9.5–19.2	68–73
Two	4.74	57	2.5	4.69	56	3.8	64
95% CI	3.34–6.13	51–63	1.9–3.0	3.11–6.27	50–62	3.1–4.5	58–71

Note: CI, Confidence interval. The effective permeability (Peff), fraction absorbed (fa), appearance ratio (Ar) and enantiomeric ratio (Er) were all calculated under steady state conditions during the two perfusion periods.
[a,b,c] Groups of variables with the same superscript are statistically different between periods one and two ($p<0.05$).

tively. The levels of the metabolites were low compared to the concentrations of R/S-verapamil entering the jejunal segment. For R- and S-norverapamil they were 2.6% (95% CI 1.8 to 3.3%) and 1.1% (95% CI 0.8 to 1.3%) in period one and two, respectively.

DISCUSSION

In this study the measured jejunal Peff of both enantiomers of R/S-verapamil at 4.0 and 40 mg/l was high enough (>2 • 10^{-4} cm/sec) to predict complete intestinal absorption following

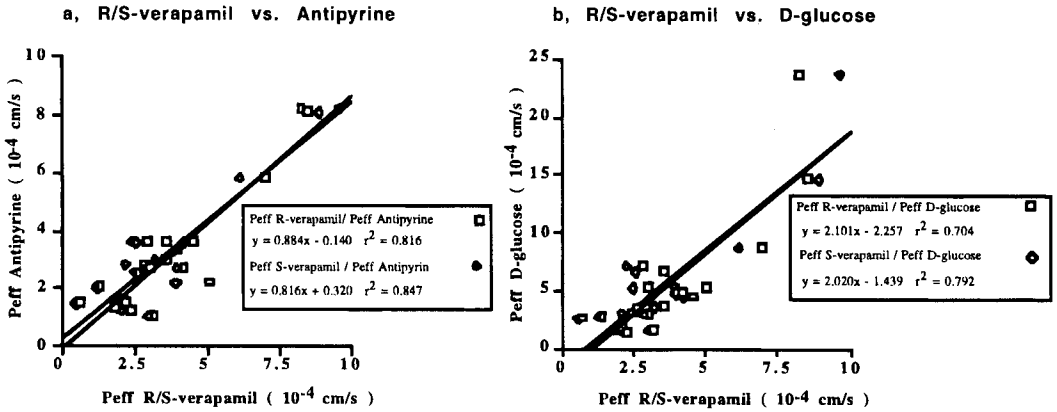

Fig. 3. (a) Correlations between the *Peff* of R/S verapamil versus the *Peff* of Antipyrine. (b) Correlations between the *Peff* of R/S verapamil versus the *Peff* of D-glucose.

Table II. Absorption and Technical Parameters from the Jejunal Perfusion (Loc-I-Gut®)

Parameter	Period one	Period two
NWF (ml/hr/cm)	1.7	2.2
95% CI	1.1–2.4	1.6–2.8
PEGrec.ss (%)	97	96
95% CI	92–102	89–103
Peff Antipyrin	2.2[a]	4.0[a]
95% CI	1.6–2.8	2.6–5.5
fa Antipyrin (%)	40[b]	51[b]
95% CI	33–46	43–59
Peff D-glycose	3.94	7.13
95% CI	2.60–5.27	1.46–12.80

Note: CI, Confidence interval. Net water flux (NWF), recovery of the ^{14}C-PEG4000 during steady state (PEGrec.ss), fraction absorbed (fa) and effective permeability were all calculated during steady state conditions in the two perfusion periods.
[a,b] Groups of variables with the same superscript are statistically different between periods one and two ($p<0.05$).

oral dosing (26). Consequently, even if R/S-verapamil are substrates for P-glycoprotein(s) located in the apical enterocyte membrane and it would not affect their quantitative transport across the apical enterocyte membrane. Both enantiomers of R/S-verapamil are classified as high permeability drugs according to the recently proposed biopharmaceutical drug classification (30).

The human jejunal Peff for both R- and S-verapamil increased when the inlet concentration was increased from 4.0 to 40 mg/l, consistent with a saturable efflux mechanism mediated by P-glycoprotein(s) in human jejunum. Two further observations made in humans support the conclusion that R/S-verapamil is a substrate for P-glycoprotein(s) located in the human jejunum *in vivo:* (1) The Peff for D-glucose did not differ between the two periods, and (2) the Peff for R/S-verapamil was even higher, approximately $7 \cdot 10^{-4}$ cm/sec, when a luminal concentration of 400 mg/l was used in separate human perfusion study (27). In addition, we have reported that R/S-verapamil is transported both in lumen-blood and blood-lumen directions in rats (*in situ*), and the rat jejunal Peff increased for both enantiomers when chlorpromazine was added as an inhibitor (28). However, the parallel increase in Peff for antipyrine does not support such a conclusion, unless it is also a substrate for P-glycoprotein. Overall, the data support the existence of a functional MDR in the human jejunal enterocyte, this is most likely mediated by P-glycoprotein(s), but it would probably have at most a minor effect on the quantitative intestinal absorption since the Peff in the concentration region 4.0-400 mg/l is high ($>2 \cdot 10^{-4}$ cm/sec) (26). Although, the small difference in the absorption rate may affect the fraction metabolised when passing across the enterocyte as indicated by the decreased appearance ratio of both R and S-verapamil at 40 mg/l, this would probably be masked by variability in gastric emptying rates.

There was no difference in the jejunal Peff for the R- and S-forms of verapamil in the present human study. This indicates that P-glycoprotein(s) in the human jejunal enterocyte do not discriminate between the two enantiomers, which is also in agreement with data reported from *in vitro* studies using tumour cell lines (29). The lack of stereoselectivity is in accordance with a broad substrate specificity, a distinct feature for the P-glycoprotein(s) (2).

The measured jejunal Peff in this study is a mass transfer coefficient of the epithelial cell. It is suggested that the epithelial Peff of passively transported drugs are more accurately reflected by the diffusion across the complex apical membrane (26). For R/S-verapamil this is probably an interplay between diffusion across the apical membrane and an interaction with the transdomains of the P-glycoprotein(s) (i.e., the binding sites), which then export the drug to the outside of the cell (Fig. 1). Consequently, the extent of absorption (fa) is defined as all the processes involved until the drug is transported across the apical membrane of the enterocyte, and reaches the cytosol adjacent to the cytoplasmic leaflet of the apical membrane. In general, metabolism by CYP 3A4 (and other cytosolic localised enzymes) will certainly influence the fraction reaching the portal vein. However, as long as metabolism occurs inside the enterocytes, it will have a limited effect on the permeability measured using a single-pass perfusion technique. Excluding luminal and brush-border metabolism, R/S-verapamil has to be absorbed to be metabolised by CYP 3A4 inside the enterocyte.

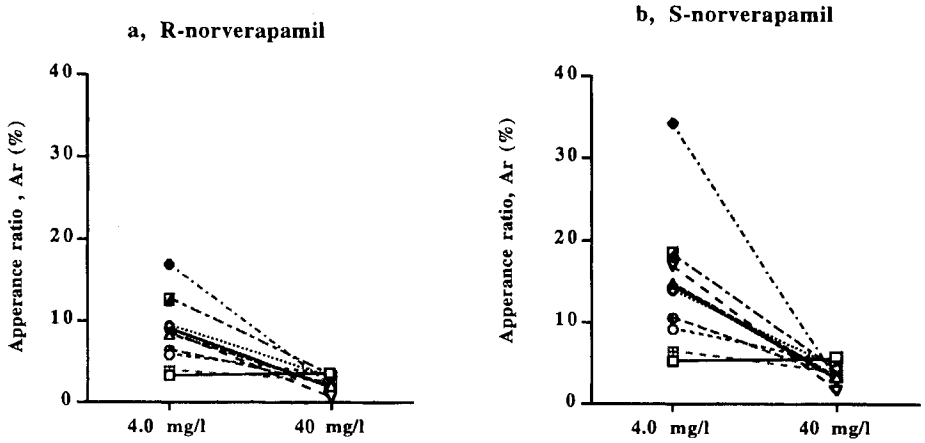

Fig. 4. Individual values of the appearance ratio (Ar) in the human jejunum for (a) R- and (b) S-norverapamil at the intestinal perfusate concentration of 4.0 (P1) and 40 mg/l (P2).

This means that (R/S)-norverapamil is formed intracellularly, and then transported into the portal vein as well as back into the intestinal lumen (efflux) (Fig. 1). The low ratios of the secreted metabolite concentrations to the entering concentration of R/S-verapamil indicate that CYP3A4 metabolism occurring in the jejunal lumen and at the brush-border is not an important limitation of the overall bioavailability. The transport of metabolites back into the intestinal lumen may occur by passive diffusion and/or efflux by P-glycoprotein(s), or some other transporter (Fig. 1) (29). The non-linear appearance rate (Ar) of R/S-norverapamil in the jejunal segment clearly support our suggestion that they are formed in the enterocyte and not in the hepatocyte. It is unlikely that these very low dosing rates of R/S-verapamil would result in saturation of the liver metabolism and subsequent non-linearity of appearance in the jejunal segment during a single-pass perfusion.

Pharmacokinetic studies have reported the importance of the proximal small intestine as a site for extensive presystemic metabolism for drugs like midazolam, cyclosporine, felodipine and R/S-verapamil (10,11,13,14,32). The reduced oral bioavailability and pharmacological effect (atrioventricular conduction) for R/S-verapamil after repeated administration of rifampin was explained by induction of CYP3A4 in the enterocytes since a significantly smaller increase was observed in clearance (11). The effect on bioavailability of R/S-verapamil might also be attributed to decreased absorption and/or increased apical recycling as a consequence of rifampin induced expression of P-glycoprotein(s) in the apical enterocyte membrane. It has been suggested that the induction of CYP3A4 expression is in some way linked to P-glycoprotein mediated transport (33). Recently, Lown et al. reported that grapefruit juice affects the oral pharmacokinetics of felodipine by a down-regulation of protein levels of CYP3A4 in the intestine, while liver CYP3A4 was not affected (32). At the same time the concentrations of P-glycoprotein(s) in the small intestine was not influenced (32). Recently, an oral pharmacokinetic study in 25 kidney transplant recipients suggested that interindividual variation in Cmax and Cl/F were explained by CYP3A4 activity in the liver (erythromycin breath test) and intestinal P-glycoprotein content (34). The fact that intestinal CYP3A4 activity did not influence any pharmacokinetic variables investigated do not completely agree with the discussion above. Instead the author suggested that P-glycoproteins in the apical enterocyte membrane (biopsies from duodenum) determining the presentation rate of the drug for the CYP3A4 enzymes inside the enterocyte and thereby increases the degree of first-pass gut metabolism. Therefore, the contribution the intestinal first-pass metabolism of drugs is still unclear. For instance, the present human study have shown that the appearance ratio (Ar) in the jejunum for one of the metabolites—the CYP3A4 formed R/S-norverapamil—was non-linear due to saturation of the CYP3A4 metabolism rather P-glycoprotein secretion (probably saturated by the higher concentrations of R/S-verapamil). In addition, as we could not observe any major difference in the Peff of R and S-verapamil, the somewhat higher concentration of S-norverapamil compared to R-norverapamil is probably due to a stereoselective gut wall metabolism rather than stereoselective P-glycoprotein transport (35). Future studies regarding molecular mechanisms of transport and metabolism in the human intestine and its importance for oral drug delivery will include regulation of these processes in the human intestine in the healthy state and at various disease states.

CONCLUSIONS

R/S-verapamil is a high permeability drug *in vivo* in the proximal human small intestine throughout the luminal concentration range that is expected to be valid at the intestinal absorption site. The observed concentration dependent intestinal permeability of R/S-verapamil in humans is probably due to saturation of the efflux by P-glycoprotein(s) located in the apical membrane of the human enterocytes, however, P-glycoprotein mediated efflux does not appear to be important to the bioavailability of this compound. First-pass metabolism inside the enterocytes, as demonstrated with the concentration dependent appearance of R/S-norverapamil into the jejunal lumen, appears to be selective for the S-form of verapamil.

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