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The influence of caprate on rectal absorption of phenoxymethylpenicillin: experience from an in-vivo perfusion in humans

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Abstract

The aim of this in-vivo perfusion study in humans was to investigate the influence of a penetration enhancer, sodium caprate, on the rectal absorption of phenoxymethylpenicillin and antipyrine. Six subjects, 3 male and 3 female, were included in two separate studies using perfusion solution of different pH (T1 and T2, respectively). Each in-vivo rectal perfusion investigation lasted for 200 min and consisted of two periods of 100 min, the first serving as a control, and sodium caprate being added in the second period in both T1 and T2. The concentrations of phenoxymethylpenicillin, antipyrine and sodium caprate in the outlet perfusate were assayed by HPLC, as was the plasma concentrations of phenoxymethylpenicillin. At pH 6.0 (0–100 min) the fraction absorbed (f_{abs}) and effective permeability (P_{eff}) of phenoxymethylpenicillin were 0.3% and 0.06×10^{-4} cm s^{-1}, respectively, and remained unaffected by the addition of sodium caprate. When the same subjects were perfused at pH 7.4, the f_{abs} and P_{eff} of phenoxymethylpenicillin were 2.4% and 0.11×10^{-4} cm s^{-1} (0–100 min), respectively, also remaining unchanged by addition of sodium caprate (100–200 min). It was possible to determine the plasma AUC of phenoxymethylpenicillin after addition of sodium caprate in three subjects at both pHs; this was in the range of 14.0–62.8 and 56.4-231 (min μmol L^{-1}) at pH 6.0 and 7.4, respectively. Interestingly, there was a correlation between P_{eff} for sodium caprate and the individual plasma AUC and C_{max} of phenoxymethylpenicillin, which indicates that the permeability of the enhancer in the tissue upon which it should act is crucial for achieving an effect. The f_{abs} and the P_{eff} of antipyrine were not affected at either pH when sodium caprate was added to the perfusion solution. In conclusion, the plasma pharmacokinetics of phenoxymethylpenicillin suggested a slightly increased rectal absorption at pH 7.4 in subjects where sodium caprate was transported into the rectal tissue. However, the increased P_{eff} for phenoxymethylpenicillinwas too small to detect from the outlet perfusate, which suggests that sodium caprate alone has a limited effect on the permeability in-vivo across the rectal epithelium when it is presented in a solution.

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Introduction

The clinical pharmacokinetics of rectal administration of antibiotics is rarely reported and is poorly documented in the treatment of infections with antibiotics (Bergogne-Bérézin & Bryskier 1999). Clinically, the rectal route for antibiotic administration is used less frequently today. However, the usefulness of rectal administration of antibiotics is very clear in a number of clinical situations including those in which: oral dosing causes nausea, vomiting or gastric pain;

patients are uncooperative or unconscious; intravenous dosing is traumatic (e.g. in children or in patients in intensive-care units who require multiple drugs and continuous fluid infusions but have few undamaged veins); intramuscular administration of drugs is not well accepted and is painful in ambulatory patients (Bergogne-Bérézin & Bryskier 1999). Obviously, there is still a therapeutic need to develop new and improved rectal dosage forms for antibiotics.

In general, the bioavailability of drugs following rectal administration in humans is determined by various factors such as dissolution, stability, residence time, rectal permeability, first-pass extraction in the liver and the fraction of the rectal blood flow bypassing the liver (van Hoogdalem et al 1991; de Boer et al 1992; Lennernäs et al 1995; Bergogne-Bérézin & Bryskier 1999). A limited number of clinical studies have investigated the factors that affect bioavailability of antibiotics when given rectally to humans (Farouk et al 1984; Sjöwall et al 1984; Bergström et al 1988; van Hoogdalem et al 1988, 1989). β-Lactams have a low bioavailability when administered in a rectal dosage form due to their low rectal permeability. Therefore, rectal dosage forms can be improved (increasing bioavailability and decreasing variability) by adding an absorption-promoting agent to the dosage form (Farouk et al 1984; Sjöwall et al 1984; Bergström et al 1988; van Hoogdalem et al 1988, 1989).

Even though animal and in-vitro studies may be helpful in the development of new rectal dosage forms, there is a need for direct measurement of the effects of absorption-promoting agents on the in-vivo permeability in the human rectum. Unfortunately, perfusion techniques are rare and validated perfusion techniques are of limited accessibility due to methodological problems and unwillingness of subjects or patients (Devroede & Phillips 1970; Böttger et al 1984). Therefore, a new approach to the perfusion of a well-defined regional segment in the human rectum was developed and validated for mechanistic drug absorption studies in-vivo (Raab et al 1992; Lennernäs et al 1995). The effect of an absorption enhancer, when administered in a solution together with drugs into the rectal segment in-vivo, can be measured directly. The advantages over the present in-vivo rectal perfusion technique are the possible reduction of leakage into the test segment, achievement of high and consistent recovery of the perfusion solution, and estimation of the absorption rate of both the drug and the enhancer directly from a segment with a specified absorption surface area (Raab et al 1992; Lennernäs et al 1995). The present clinical method is unique in that it provides an absorption parameter, permeability, which cannot be determined from plasma pharmacokinetics.

Several pharmaceutical adjuvants have been investigated in animal studies and have been found to increase the intestinal permeability of poorly absorbed antibiotics (Tomita et al 1988; Sawada et al 1991). The enhancing effect of sodium salts of saturated straight-chain fatty acids on the rectal absorption of ampicillin and of ceftizoxime has been shown in mice, rats, rabbits and dogs (Kakeya 1985). The fatty acid sodium caprate increased the rat intestinal permeability in-vitro by affecting the para- and transcellular transport routes across the intestinal epithelial barrier (Tomita et al 1988; Sawada et al 1991).

Our main purpose was to investigate the influence of a penetration enhancer, the fatty acid sodium caprate, on the human in-vivo rectal permeability of phenoxymethylpenicillin. At the same time we determined the permeability of antipyrine (a marker for passive transcellular diffusion) as well as that of sodium caprate. Finally, this exploratory perfusion study also investigated the potential usefulness of the rectal perfusion technique, Loc-I-Col, to assess effect of pharmaceutical additives and excipients on in-vivo permeability.

Materials and Methods

Design and positioning of the tube

The segmental rectal in-vivo perfusion in humans was performed using a modified system of a recently developed and, for permeability studies, improved and validated method (Figure 1) (Raab et al 1992; Lennernäs et al 1995). In this study, a tube made of polyvinyl chloride (PVC), with a total length of 40 cm and an inner and outer diameter of 10 and 16 mm, respectively, was used. The tube had three latex balloons attached to its wall. The two distal balloons surrounded the anal canal and functioned to stabilise the position, and a third balloon served to delimit the rectal perfusion segment (Figure 1). The tube consisted of five channels with an inner diameter of 1 mm each. Three of the channels were used to inflate the balloons and two were used for the perfusion of the rectal segment. The segment (8 cm long) was positioned in the mid-upper rectum with the aid of an endoscope (Olympus PQ 20). The positioning of the tube was preceded by an endoscopy of the rectum and sigmoid colon. With the endoscope in place, the tube was slowly inserted into the intestinal lumen with the endoscope acting as guide. Once the tube was positioned, the two anal balloons were inflated first followed by inflation of the proximal segmental balloon.

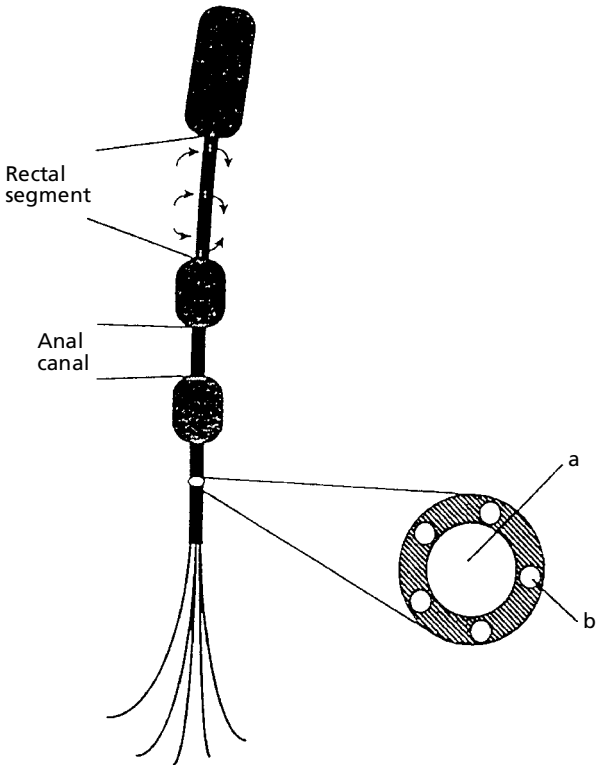

Figure 1 A schematic presentation of the perfusion tube with 3 balloons delimiting the segment located in the mid-upper part of the rectum. Two balloons located on either side of the anal canal controlled the position of the tube. The central channel (a) was used for the introduction of the tube by the endoscope, and decompression of gas and fluids during perfusion. The smaller channels (b) were used to deliver air into the balloons and perfusion fluid to and from the rectal segment. The length of the perfused rectal segment was 8 cm.

Next, the endoscope was withdrawn and the position of the tube in the rectum was checked by fluoroscopy. The main channel of the tube functioned thereafter to decompress gas and fluids from the proximal bowel during the perfusion.

Study design

The study was an explorative randomised cross-over study and the 6 healthy subjects (3 female and 3 male) participated at two different occasions, treatment 1 and 2 (T1 and T2). The time between the treatments (T1 and T2) was at least 2 weeks. The only difference between T1 and T2 was the pH of the perfusion fluids (6.0 in T1 and 7.4 in T2). The study was approved by the Ethics Committee of the Medical Faculty, Uppsala University, and the Medial Product Agency, Uppsala, Sweden. The subjects were prepared for routine flexible recto-sigmoid endoscopy (i.e., 2 days with diet restriction and an oral purgative (Pico-Salax, Ferring, Malmö, Sweden) in the morning and afternoon on the day before examination). In the morning, before intubation, a single dose of pethidine (25 mg) and diazepam (2.5 mg) was given intravenously as routine premedication.

A perfusion experiment lasted for 200 min and consisted of two periods of 100 min each. The first period in each perfusion experiment (P1 in T1 and P3 in T2) served as a control without any enhancer present. Immediately after the first period (100 min), without any wash-out period, the second perfusion solution (P2 in T1 and P4 in T2) containing the absorption enhancer was introduced. The perfusion experiment began with the rinsing of the rectal segment with isotonic saline (37°C) for approximately 15–20 min, using a syringe pump (model 355, Sage Instruments, Orion Research Inc., Cambridge, MA). This single-pass perfusion method applied a flow rate of 2.0 mL min^{-1}. The perfusate leaving the rectal segment was collected quantitatively on ice at 10-min intervals throughout the perfusion experiment (200 min). The syringes and perfusate were weighed, and the samples were frozen immediately and stored at −70°C until analysis. The subjects were recumbent during the 200-min perfusion period. After cessation of the drug perfusion, the intestinal segment was rinsed with approximately 120 mL saline for 3–5 min to prevent further drug absorption. Blood samples were collected from a cannula placed in an arm vein before administration of the drug and at 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 240, 270, 300, 360 and 420 min after the beginning of drug administration. The plasma was separated (2000 *g* for 10 min) and frozen at −20°C and transferred within 24 h to −70°C, where they were stored until analysis.

Study-drug and perfusate composition

The effective rectal permeability (P_{eff}) was determined for phenoxymethylpenicillin, antipyrine and the absorption enhancer sodium caprate in an aqueous buffer solution at two different pHs (6.0 and 7.4). The perfusion solutions were manufactured at the university hospital, Uppsala, just before the perfusion experiment started. The solutions were kept stirred at 37°C during the perfusion experiment. The inlet perfusate concentration was 5 mg mL^{-1} for phenoxymethylpenicillin, 0.02 mg mL^{-1} for antipyrine and 3.5 mg mL^{-1} for sodium caprate. Each subject was exposed to a dose of 2 g of phenoxymethylpenicillin, 8 mg antipyrine, 1 μCi ^{14}C-PEG 4000

and 0.7 g sodium caprate during each perfusion experiment. Glucose, 4–14 mg mL^{-1}, was added to adjust osmolality to approximately 290 mmol L^{-1}.

Phenoxymethylpenicillin, supplied by Astra Läkemedel AB, Södertälje, Sweden, has the following physicochemical properties: pK_a = 2.8 weak acid; log D (octanol/buffer) = −2.7 at pH 7.4; molecular weight = 358. In the study a potassium salt of phenoxymethylpenicillin was used with a solubility of 1 g/1.5 mL. Antipyrine, supplied by Kabi-Pharmacia, Stockholm, Sweden, has the following physicochemical properties: pK_a = 1.5; log D = −0.4 at pH 7.4; molecular weight = 188. Sodium caprate, supplied by Astra Läkemedel AB, Södertälje, Sweden, has a log D value of 4.2–4.8 at pH 7.4. The isotonic control solutions (P1 and P3) consisted of KH_2PO_4 100 mM, NaOH 2 M to adjust pH, polyethylene glycol (PEG 4000; MW = 4000) 1 g L^{-1}, and D-glucose 4–14 mg mL^{-1}, to adjust osmolality to about 290 mOsm L^{-1}. Polyethylene glycol labelled with carbon-14 (^{14}C-PEG 4000) was purchased from Amersham Lab., Buckinghamshire, UK, and added to all perfusion solutions as a non-absorbable volume marker (2.5 μCi L^{-1}).

Stability and adsorption test of phenoxymethylpenicillin and sodium caprate

The stability of phenoxymethylpenicillin in the presence of sodium caprate was studied in the perfusion solutions at both pHs for 180 min at 37°C. The degradation of phenoxymethylpenicillin during that period was 1.9% and 6.1% at pH 6.0 and 7.4, respectively, and was considered not to have any effect on the measured permeability in the perfused rectal segment, as the transit time in the rectal lumen is approximately 10–15 min. The adsorption of sodium caprate to perfusion tubes (polyvinyl chloride) was negligible. Incubation of antipyrine and D-glucose in the perfusion medium at 37°C for 180 min showed no degradation of any compound. The stability of antipyrine and D-glucose was also tested in the rectal perfusate for 60 min at 37°C at a pH of 7.8, and no degradation was found. There was no adsorption of either antipyrine or D-glucose onto the perfusion tubes.

Analytical methods

Perfusate samples

The analysis of the concentrations of phenoxymethylpenicillin, antipyrine and sodium caprate in outlet rectal perfusate were performed by an HPLC method combined with UV detection at 205 nm (an analytical method developed at Astra Läkemedel AB, Sweden). The samples were diluted with phosphate buffer at pH 7.4 and then analysed. The mobile phase consisted of phosphate buffer at pH 7.4, containing tetrabuthylammoniumhydrogen sulfate and acetonitrile. This assay allowed for separating and quantifying the three compounds in the same chromatogram. The limit of quantifications (LOQ) for phenoxymethylpenicillin, antipyrine and sodium caprate in the outlet rectal perfusate were 3.16 μM, 341 μM and 8.3 μg mL^{-1}, respectively, in perfusate samples diluted 11 times.

Perfusate samples (0.5 g) were weighed and the total radioactivity of ^{14}C-PEG 4000 was determined by liquid scintillation counting (dpm) for 10 min (Beckman Instruments, model 244), after the addition of 8 mL Beckman Ready Safe. The radioactivity was corrected for quenching using the internal standard of the instrument. The osmolality of the outlet perfusion solution was measured by the vapour pressure method (Vescor osmometer 5500).

Plasma samples

The concentration of phenoxymethylpenicillin in plasma was assayed by reversed-phase liquid chromatography and post-column derivatisation after protein precipitation and dilution with phosphate buffer at pH 6.0 (an analytical method developed at Astra Läkemedel AB, Sweden). The mobile phase consisted of 40% (v/v) methanol in phosphate buffer at pH 7.4 (ionic strength = 0.1). The post-column derivatisation reagent used was a mixture of NaOH, $HgCl_2$ and EDTA. The LOQ for phenoxymethylpenicillin in plasma was 0.16 μM. The intra-assay variability was found to be 4.8% and 0.8% at 0.50 and 19.7 μM, respectively.

Calculations

The calculations were made from 4–5 steady-state concentrations of each compound in the perfusate leaving the rectal segment during its single pass through the 8-cm segment. Equilibrium in the perfusate within the closed intestinal segment was considered to have been achieved when the concentrations of the solute and the ^{14}C-PEG 4000 in the outlet perfusate reached a plateau at 50–60 min after the start of the perfusion experiment (equation 1). The net water flux per cm of the rectal segment was calculated using equation 1.

$$\text{Net water flux} = (1 - [\text{PEG}]_{out}) \times Q_{in} / ([\text{PEG}]_{in}\ \text{L}) \quad (1)$$

Table 1 Absorption parameters of phenoxymethylpenicillin and antipyrine obtained during a rectal perfusion in humans. The data are generated with and without sodium caprate at two different pHs in the perfusion solution (6.0 and 7.4, respectively).

Time (min)	Phenoxymethylpenicillin		Antipyrine	
	f_{abs} (%)	P_{eff} ($\times 10^{-4}$ cm s^{-1})	f_{abs} (%)	P_{eff} ($\times 10^{-4}$ cm s^{-1})
0–100, pH 6.0 (no caprate)	0.28±2.00	0.06±0.07	15.0±4.0	0.74±0.24
100–200, pH 6.0 (no caprate)	−1.2±0.8	−0.07±0.03	17.0±4.5	0.86±0.26
0–100, pH 7.4 (no caprate)	2.4±1.2	0.11±0.05	17.0±2.2	0.78±0.12
100–200, pH 7.4 (caprate)	1.0±0.9	0.04±0.02	19.0±4.5	1.04±0.34

Data are presented as means±s.e.m.

where $[PEG]_{in}$ and $[PEG]_{out}$ are the entering and leaving dpm mL^{-1} of ^{14}C-PEG 4000. Q_{in} is the perfusion rate entering the rectal segment, which is obtained by dividing the total volume entering the rectal segment by the sampling time (10 min), and L is the length of the rectal segment (8 cm).

The fraction disappearing from the perfusate when it had passed through the rectal segment during the single-pass perfusion is assumed to have been absorbed, and is defined as the fraction absorbed (f_{abs}) of the three investigated compounds in this human perfusion study. The calculations used the ratio of the fluid-corrected concentrations leaving (C_{out}) and entering (C_{in}) the rectal segment at steady-state (equation 2).

$$f_{abs} = (1 - C_{out}\,[PEG]_{out})/C_{in}\,[PEG]_{in} \quad (2)$$

The gradual increase of the outlet perfusate concentrations of PEG 4000 supports our hypothesis that the solution in the isolated segment was well mixed (Lennernäs et al 1995, 1997). Assuming a well-stirred perfused rectal segment, the effective rectal permeability (P_{eff}) of the drug was calculated using equation 3 (Lennernäs et al 1995, 1997).

$$P_{eff} = [Q_{in}\,(C_{in} - C_{out})/C_{out}]/2\,\Pi rL \quad (3)$$

where $2\,\Pi rL$ is the area of the mass transfer surface within the rectal segment that is assumed to be the cylinder area with a length (L) of 8 cm and a radius (r) of 1.75 cm.

The observed maximum plasma concentration (C_{max}) of phenoxymethylpenicillin and the corresponding time taken to reach it (t_{max}) were estimated from the individual plasma concentration–time profile. The area under the plasma concentration versus time curve (AUC_{0-t}) for phenoxymethylpenicillin was calculated using the lin-log trapezoidal rule between zero and the last detectable plasma concentration. The pharmacokinetic parameters from plasma concentrations were calculated with the computer program MKMODEL, version 3.04, 1986 (Cockshott & Haywood 1992).

Interindividual variability is expressed as standard error mean (s.e.m.) throughout the paper. To evaluate differences in absorption between the two experimental periods in each perfusion experiment, one-way analysis of variance, followed by Fisher's contrast test, was used.

Results

In this explorative study we performed 12 successful in-vivo single-pass perfusion experiments in human rectum in 6 individuals (3 female and 3 male).

Perfusate data, pH 6.0 (T1)

The absorption variables, P_{eff} and f_{abs}, for phenoxymethylpenicillin and antipyrine are given in Table 1. The mean (±s.e.m.) P_{eff} for phenoxymethylpenicillin was 0.06±0.07 and −0.07±0.03 ($\times 10^{-4}$ cm s^{-1}) in P1 and P2, respectively. The P_{eff} for antipyrine during P1 and P2 were 0.74±0.24 and 0.86±0.26 ($\times 10^{-4}$ cm s^{-1}), respectively. The f_{abs} and P_{eff} were not possible to measure for sodium caprate, as the fatty acid was not completely dissolved and adhered to the material at pH 6.0.

Table 2 Perfusion parameters obtained during a rectal perfusion in humans. The data are generated with and without sodium caprate at two different pHs in the perfusion solution (6.0 and 7.4, respectively).

	PEG 4000 recovery (%)	Net water flux (mL cm h^{-1})	pH of the perfusate	Osmolality (mOsm L^{-1})
0–100, pH 6.0 (no caprate)	99±4.1	2.1±0.6	6.2±0.04	279±4.1
100–200, pH 6.0 (no caprate)	103±3.7	0.9±0.2	6.2±0.04	279±4.9
0–100, pH 7.4 (no caprate)	89±4.5	0.7±0.2	7.4±0.02	296±1.6
100–200, pH 7.4 (caprate)	98±2.0	0.8±0.1	7.4±0.04	297±2.4

Data are presented as means±s.e.m.

The recovery of the volume marker PEG 4000, net water flux, osmolality and pH are given in Table 2.

Perfusate data, pH 7.4 (T2)

The P_{eff} and f_{abs} for phenoxymethylpenicillin, antipyrine and sodium caprate are given in Table 1. The P_{eff} of phenoxymethylpenicillin in P3 and P4 was 0.11±0.05 and 0.04±0.02 ($\times 10^{-4}$ cm s^{-1}), respectively. The P_{eff} and f_{abs} for antipyrine were similar to those obtained at pH 6.0, which agrees with its unionised state at both pHs (a base, pK_a = 1.5). The f_{abs} and P_{eff} of sodium caprate at pH 7.4 were 25±4.9% and 1.4±0.4 ($\times 10^{-4}$ cm s^{-1}), respectively. The relationship between rectal P_{eff} for phenoxymethylpenicillin, antipyrine and sodium caprate and their log D values (octanol/buffer) at pH 7.4 is shown in Figure 2. The recovery of the volume marker PEG 4000, net water flux, osmolality and pH are given in Table 2. The luminal concentration of sodium was 50–100 mM when sodium caprate was present at both pH 6.0 and 7.4 (P2 and P4).

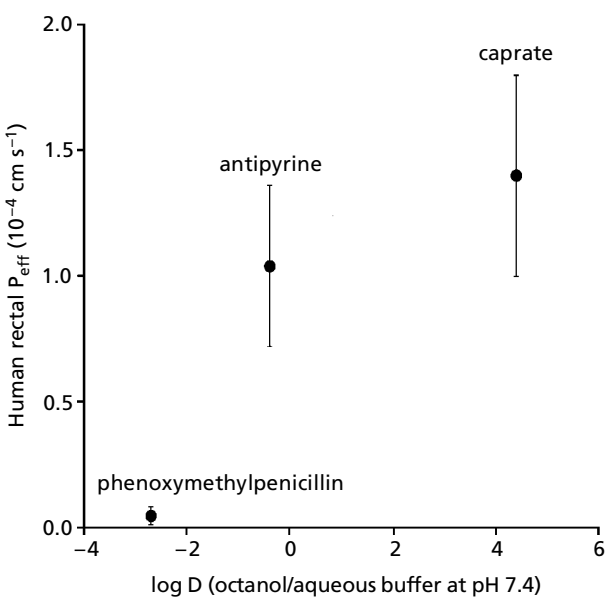

Figure 2 The relationship between measured in-vivo rectal effective permeability (mean±s.e.m.) for phenoxymethylpenicillin, antipyrine and sodium caprate and their partition coefficients at pH 7.4 (log $D_{7.4}$).

Plasma data at pH 6.0 and 7.4 (T1 and T2)

The individual plasma concentration–time profiles of phenoxymethylpenicillin in the three subjects that attained detectable blood concentrations at pH 6.0 and 7.4, respectively, are shown in Figures 3A and 3B. Phenoxymethylpenicillin was only possible to quantify in plasma during the second period (P2 and P4) of either treatment (T1 and T2) (i.e., when sodium caprate was added). Plasma data for phenoxymethylpenicillin for subjects 1, 4 and 7 at pH 6.0, and subjects 1, 6, and 7 at 7.4 were not possible to include in the plasma pharmacokinetic analysis, since no plasma concentrations could be determined above the LOQ. The median C_{max} was 0.29 μM (range 0.23–0.98 μM) at pH 6.0 and 1.24 μM (range 0.24–3.5 μM) at pH 7.4. The median t_{max} was 195 min (range 160–220 min) at pH 6.0 and 195 min (range 180–200 min) at pH 7.4. The AUC_{0-t} for phenoxymethylpenicillin was 14.0–62.8 min μM^{-1} at pH 6.0, and 56.4–231 min μM^{-1} at pH 7.4 (Figures 3 and 4). Figure 4 shows that subjects with the highest plasma AUC_{0-t} for phenoxymethylpenicillin at pH 7.4 also had the most extensive rectal absorption of sodium caprate.

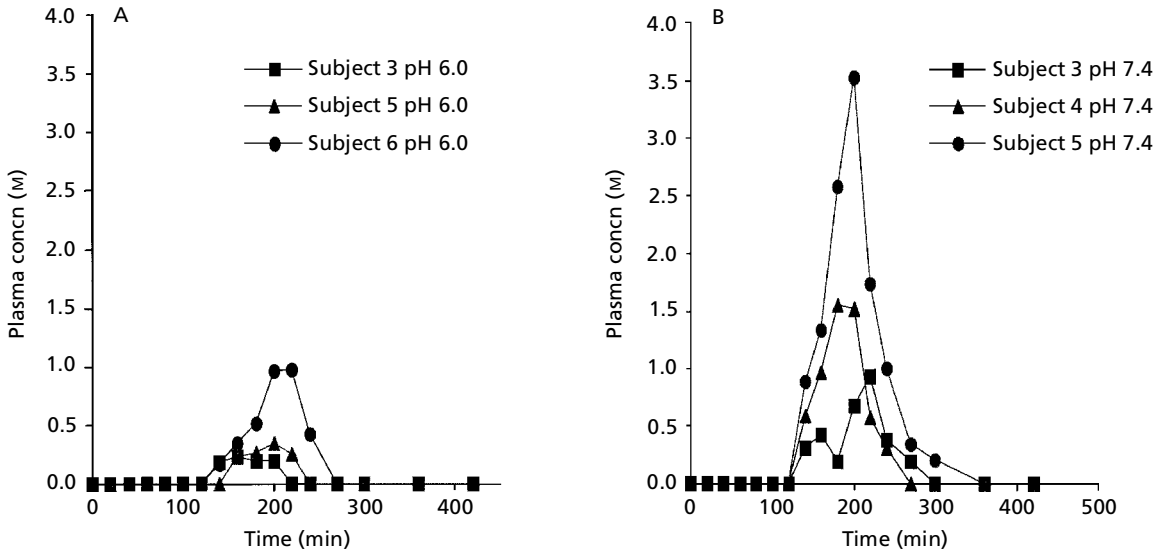

Figure 3 The plasma concentration of phenoxymethylpenicillin during rectal perfusion in humans at pH 6.0 (A) and 7.4 (B). During 0–100 min and 100–200 min the rectum was perfused without and with sodium caprate, respectively.

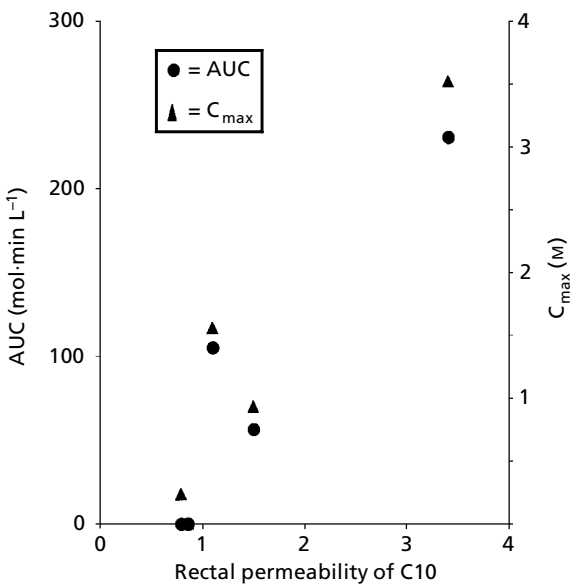

Figure 4 The relationship between the individual values of effective rectal permeability of sodium caprate in human rectum at pH 7.4 and the individual plasma pharmacokinetics (C_{max} and AUC) of a low-permeability drug, phenoxymethylpenicillin.

Discussion

This is the first mechanistic single-pass in-vivo perfusion study of human rectum where we investigated the direct effect of a penetration enhancer on the rectal absorption and bioavailability of a low- and a high-permeability drug. It is important to note that the enhancer is presented to the rectal epithelium in solution when it is active (at pH 7.4) and it is not in direct contact with the rectal epithelium as with a suppository. Therefore, the enhancer's effect did not benefit from any plausible synergistic effect of other pharmaceutical additives that make up a rectal dosage form. In addition to the monitoring of the permeability of phenoxymethylpenicillin we investigated the permeability of the enhancer itself and correlated it to the plasma pharmacokinetics of phenoxymethylpenicillin.

The plasma concentrations of phenoxymethylpenicillin increased slightly when sodium caprate was present in the perfusion solution in 3 out of 6 subjects at both pH 6.0 and 7.4. Furthermore, the plasma AUC and C_{max} values of phenoxymethylpenicillin suggested that sodium caprate was more active as a penetration enhancer at pH 7.4. This observation is promising as this pH best reflects the normal pH in human rectum (Lennernäs et al 1995). The somewhat better effect of sodium caprate at this higher pH is due to its better solubility, and therefore to a higher degree of rectal absorption. This solubility effect is in accordance with the generally poorer effect of sodium caprate (lower AUC and C_{max} values) at pH 6.0. The correlation between P_{eff} of sodium caprate and individual plasma AUC and C_{max} for phenoxymethylpenicillin shown in Figure 4 suggests that the transport of sodium caprate into the rectal tissue is crucial for obtaining an absorption-enhancing effect for

a poorly permeable compound. This is not surprising as the active site for sodium caprate is located in the rectal tissue. However, the effect of sodium caprate on the absorption of the poorly permeable drug phenoxymethylpenicillin in this study was low and erratic regardless of the pH used. For instance, the lack of effect of sodium caprate in subjects 1 and 7 is due to poor rectal absorption of sodium caprate. To summarise, the present in-vivo data suggest that it is important to better characterise factors that determine the interindividual variability of the in-vivo absorption of the absorption-enhancing agent and not only the drug itself.

The in-vivo rectal P_{eff} of phenoxymethylpenicillin was low ($\sim 0.01 \times 10^{-4}$ cm s^{-1}), which is in accordance with its hydrophilic properties (log D = −2.7 at pH 7.4) that predict slow membrane diffusion in-vivo (Stein 1986; Winiwarter et al 1999). The P_{eff} for the other two compounds, antipyrine and sodium caprate, was higher as a result of more lipophilic properties (Stein 1986; Lindahl et al 1996; Winiwarter et al 1999). The correlation between log $D_{pH7.4}$ and the measured rectal P_{eff} for phenoxymethylpenicillin, antipyrine and sodium caprate (Figure 2) supports the suggestion that passive diffusion is the dominating transport mechanism in human rectum in-vivo (Bergogne-Bérézin & Bryskier 1999). By comparison, the permeability in Caco-2 cells is high and passive diffusion is the most likely mechanism (24×10^{-4} and 28×10^{-4} cm s^{-1} at 0.01 and 1.0 μM, respectively) (Frick et al 1998). Frick et al (1998) have reported that phenoxymethylpenicillin has a complete absorption after oral administration and is a class I drug according to the biopharmaceutics classification system (Amidon et al 1995). In this study the P_{eff} of antipyrine was similar at both pH 6.0 and 7.4, which is in accordance with it being a weak base (pK_a = 1.5). The absorption enhancer did not affect the P_{eff} of antipyrine, which is also an expected result for a high-permeability compound.

The measured f_{abs} and P_{eff} for phenoxymethylpenicillin were unaffected when sodium caprate was present in the rectal lumen. This slight discrepancy between plasma and perfusate absorption parameters might be explained by the fact that the increased absorption of phenoxymethylpenicillin was too small to be detected in the perfusate leaving the rectal segment (disappearance from rectum), which also explains the negative values of the absorption variables obtained in some subjects. The rectal perfusion approach of an 8-cm-long segment may be too insensitive to detect small changes in drug absorption for low-permeability compounds. The poor rectal absorption of phenoxymethylpenicillin was also evident from the low plasma concentrations.

It has been reported that the plasma AUC of cefmetazole increased 10 times after addition of sodium caprate (18–20 μM) in rat colon in-situ (Tomita et al 1988). The same group also reported that the P_{app} of urea and thiourea increased 5–6 times in rat colon in-vitro (Ussing chamber) (Sawada et al 1991). It has been suggested that sodium caprate increases paracellular permeability by opening the tight junctions through a widening of the pore radius and intercellular space, contraction of calmodulin-dependent actin microfilaments or contraction of the perijunctional actomyosin ring in the same concentration range applied in this study (Tomita et al 1988; Lindmark et al 1995; Takahashi et al 1997). However, our data from human rectum during in-vivo conditions indicate that the rectal delivery of phenoxymethylpenicillin is improved only minorly by the presence of sodium caprate at a concentration of 3.5 mg mL^{-1} (25 μM). In-vitro, an 8-fold increased P_{app} was found for mannitol in the concentration interval 10–20 μM (Lindmark et al 1995). Our in-vivo study clearly demonstrates the need to validate and correlate in-vitro findings before in-vitro data can be extrapolated to an in-vivo situation. The difference between in-vivo and in-vitro is most likely due to a higher sensitivity of cell cultures than the in-vivo tissue, and this has certainly led to over-interpretation of the in-vivo importance of some in-vitro data (Lindmark et al 1995). In addition, in subjects 1 and 7 we could not detect any plasma concentrations of phenoxymethylpenicillin above the LOQ, which most likely is due to their low absorption of sodium caprate. Accordingly, this finding shows that an individual's absorption of the enhancer from the rectum is critical for the performance of any new rectal drug-delivery approaches for drugs with low permeability. It seems to be crucial to have a consistent absorption of sodium caprate above a certain threshold value to establish a sufficiently high membrane concentration, since sodium caprate displays a steep concentration–effect relationship (all-or-none effect) (Anderberg et al 1992).

Recently, Lindmark et al (1997) demonstrated that ampicillin absorption was increased following rectal administration in suppositories containing sodium caprate. However, the absorption enhancement coincided with non-specific damage to the rectal mucosa. They suggested that other factors, in addition to the presence of sodium caprate, contributed to the absorption-enhancing effect (Lindmark et al 1997). Our rectal perfusion data obtained in-vivo in humans clearly showed that sodium caprate alone was not potent enough to increase the rectal absorption of phenoxymethylpenicillin.

It has also been reported that sodium caprate may affect membrane fluidity and thus inhibit the transport activity of transmembrane efflux proteins such as P-glycoprotein (Lo & Huang 2000). This may also be a potential effect of sodium caprate in human rectum since it has been reported that expression of the MDR1 gene has been found (Mizoguchi et al 1990). However, in this study it seems less likely to be an effect as neither phenoxymethylpenicillin nor antipyrine has been reported to be efflux substrates.

In conclusion, the plasma concentrations of phenoxymethylpenicillin suggested a slightly increased rectal absorption, but highly variable when sodium caprate was transported into the rectal tissue at pH 7.4. However, the increased P_{eff} for phenoxymethylpenicillin was too small to detect from the outlet perfusate, which suggests that sodium caprate alone has a limited effect on the permeability across the rectal epithelium when it is exposed in a solution. This study also demonstrated that the variability in the uptake of the enhancer itself will certainly be a crucial factor for the absorption improvement. Finally, strong in-vivo evidence in this report showed that in-vitro models need to be validated against mechanistic in-vivo methods, otherwise inaccurate predictions may be made.

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