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Increased Understanding of Intestinal Drug Permeability Determined by the LOC-I-GUT Approach Using Multislice Computed Tomography

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Abstract: This study further evaluated the in vivo single-pass perfusion technique (LOC-I-GUT) in three different ways. First, the intestinal radius of the human small intestinal segment was measured on plain X-ray films; second, evaluation was performed by applying multislice computed tomography investigations; and third, furosemide was used as model drug in a transport study. In total 17 (6 + 4 +7) intubation/perfusion studies were performed in healthy volunteers. Mixobar was used as a positive radiographic contrast agent in the first six volunteers when plain film examination was made, followed by four studies using multislice computed tomography. Mantel area calculations of the perfused segment after X-ray investigations using barium as contrast were determined to be 101.0 ± 2.9 cm^2. Maximal dilatation of the closed segment with room air as contrast and using MSCT revealed a mantel area of 121.30 ± 7.0 cm^2 ($P < 0.01$). Thus, the mantle area increased a further 20% when the bowel was fully distended, reflecting different physiologic distention patterns for air and fluid. A jejunal single-pass perfusion study was performed in a further seven volunteers. In each experiment furosemide was perfused during 200 min, and in the treatment period (100−200 min), fexofenadine was added to the perfusion solution. The mean (±SD) P_{eff} for furosemide was 0.17 ± 0.07 and 0.12 ± 0.09 × 10^{-4} cm/s in the control and treatment period, respectively. This study showed that the calculation of human in vivo permeability is based on physiological values, which are important for the wide application of these in vivo permeability data in physiologically based pharmacokinetic modeling.

Keywords: LOC-I-GUT; MSCT; BCS; absorption prediction; fexofenadine; furosemide

Introduction

The gastrointestinal tract has two major tasks: to efficiently absorb nutrients, fluids and electrolytes, and at the same time prevent potentially noxious substances from entering the body. To optimize these different gastrointestinal functions a multitude of physiological and biochemical mechanisms are integrated and regulated in a very complex pattern. At the present time we understand and realize the meaning and importance of quite a few of these mechanisms in vivo. When it comes to the development of novel and effective oral pharmaceutical products, it is important to recognize that the drug must have biopharmaceutical properties that favor intestinal drug absorption and bioavailability among other factors. Oral drug administration is the most common route for many reasons, and the majority of the drugs also have to pass the intestinal barrier in order to obtain a clinical effect. A rapid, time- and resource-sparing technology to examine human oral absorption by directly determining a value of key pharmacokinetic variables, such as permeability, has

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been a goal of biopharmaceutical scientists for generations. In addition, early discovery and early development teams would benefit from data generated in such in vivo based clinical relevant models. The savings in time and resources clearly justify the continued effort to both improve existing models and investigate/validate new models.[1,2] An accurate quantitative estimation of transport properties across the intestinal wall of the small bowel is possible by using a single-pass perfusion technique, LOC-I-GUT.[3]

Our present knowledge regarding permeability of drugs is one of the cornerstones of the Biopharmaceutics Classification System (BCS).[4,5] The BCS provides a scientific approach for classifying drug compounds based on solubility as related to dose and intestinal permeability in combination with the dissolution properties of the oral immediate release (IR) dosage form.[6] The aim of the BCS is to provide a regulatory tool for replacing certain bioequivalence (BE) studies by accurate in vitro dissolution, solubility and permeability tests and subsequent absorption predictions.[7] These human permeability data have been used to validate various in vitro and in situ models and to be one of the important databases in computer based simulation programs such as Simcyp and Gastroplus.

The introduction of helical computed tomography (CT) has changed CT from a two-dimensional to a three-dimensional imaging modality, as the helical technique produces volume data sets and slices are reconstructed secondarily making virtual endoscopy possible. The high speed of multislice helical CT (MSCT) allows examination of the small intestine within one breath hold and with a spatial resolution of about 3 mm.[8] Motion artifacts by breathing or bowel movement are reduced by the high scanning speed. Of recent interest has been the use of CT for the evaluation of patients with small-bowel disease.[9] However, the CT scan excels in demonstration of the bowel wall and extra luminal structures but lacks the ability to provide sufficient functional information. Nevertheless, the technique will provide detailed and novel imaging that may be considered helpful for the pharmaceutical scientist to demonstrate the physiological space available for the development of novel oral pharmaceutical products.

The present study was undertaken to authenticate and further evaluate the LOC-I-GUT technique and estimate the intestinal radius applying multislice computed tomography investigations comparing determinations by plain X-ray films of the human small intestinal segment. Our aim was to validate and if possible improve the calculations for intestinal drug permeability based on our model for segmental intestinal single-pass perfusion. Through evaluation of the structure and macroscopic anatomy of the perfused jejunal segment during in vivo conditions by using two imaging techniques described above we want to confirm the accuracy of data previously obtained at our laboratory, where the major part of the human permeability data for the BCS system was performed. In parallel, we also investigated if fexofenadine affected the permeability of furosemide. This is of special interest since furosemide has a highly varable absorption and belongs to the BCS class III drugs for which intestinal transporters have been suggested to be of importance besides the passive diffusion.

Experimental Section

Subjects and Position Procedure of the Perfusion Tube. In total 17 subjects were included in the study that was divided into three different parts ($n = 6$, I; $n = 4$, II; and $n = 7$, III). Six healthy volunteers (students and medical staff), three women and three men aged 19−37 years (median 28 years), participated in the first study (I) when a plain X-ray examination was made. In the second part (II) of the study, a MSCT examination was performed in four healthy volunteers (3 male and 1 female, aged 22−32, mean 27 years). Finally a single-pass perfusion study (III) was performed in a further seven healthy volunteers (5 male and 2 female, aged 22−34, median 25 years). This part of the study was an open-label, fixed order, single center study that consisted of two furosemide study days (without fexofenadine (T1) and with (+) fexofenadine (T2)) separated by a

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wash-out period of four weeks. Each subject underwent a full clinical examination and standard laboratory tests for liver, renal and heart disease. They all had normal physical and laboratory values, both before and after the study. None of the subjects enrolled in the study was taking any medication. Smoking and the consumption of alcohol and caffeine containing beverages were prohibited for at least 24 h prior to and during the study. The subjects also had to be abstinent from grapefruit or its juices for at least 3 days prior to and during the study.

The Ethical Committee of the Medical Faculty of Uppsala University had approved the study, and the subjects all gave their informed consent to participation.

Intestinal Perfusion Procedures. All of the subjects ($n = 17$) were investigated in the morning of the study day after a 10 h overnight fast. After the upper throat had been anesthetized locally with lidocaine, a polyvinyl chloride tube with an outer diameter of 16 French (5.3 mm; LOC-I-GUT) was inserted orally into the small intestine. A detailed description of the technique is given elsewhere.[10] Briefly, the tube has six channels and is provided distally with two elongated latex balloons, 40 mm long and 10 cm apart, each separately connected to one of the smaller channels. The two wider channels in the center of the tube are for introduction of air or fluid into, and aspiration from, an uninvolved segment of the proximal jejunum. The two peripheral, smaller channels can be used for administration of marker substances or for drainage. A Teflon-coated guide wire is used for insertion of the tube, and the tip of the tube is provided with an 8 cm long weight made from short tungsten plates to facilitate positioning in the desired part of the small intestine, as checked fluoroscopically. The balloons were inflated with 26−28 mL of air. A separate Salem-sump tube was used for continuous gastric drainage. The subjects were free to choose a comfortable position, either seated or lying down. All tubes used were disposable (Figure 1).

Plain Abdominal X-ray. The first part of this study was made on six healthy volunteers, and the LOC-I-GUT tube was positioned in the proximal part of the jejunum during fluoroscopic guidance, as described above. Mixobar was used as a radiographic contrast agent in the first six examinations, and then a plain abdominal X-ray was made. A total of 60 mL of Mixobar suspension was injected during 30 s, and the plain abdominal X-ray imaging pictures were taken immediately after dosing (Siemens Polydorus 80S). The diameter of the jejunal segment and the estimated volume and cylindrical surface were measured and calculated on the X-ray films taking into account the enhancement factor obtained through the process.

Multislice Computed Spiral Tomography; Virtual Endoscopy of the Small Intestine. In the second part of the study four subjects underwent multislice computed spiral tomography (MSCT) following positioning of the LOC-I-GUT tube in the proximal small intestine. The investigation was performed with a 16-channel scanner (Somatom Sensation 16, Siemens, Forchheim, Germany), and 120 mL of 20 °C air was used as contrast for the first series. The bolus-triggered image acquisition started 4 s after an increase in attenuation of 100 Hounsfield units (HU). The protocol used had a tube potential of 120 kV, tube current 215 mA (typical value, Care Dose was used), rotational time 0.5 s, detector collimation 16 × 0.75 mm, and table movement 12 mm/rotation. Images were reconstructed with an increment of 0.7 mm, image thickness 1 mm, and with a standard abdomen filter. The axial images were viewed using different window settings. The images were transferred to a workstation (Leonardo, Siemens, Forchheim, Germany) and reconstructed: multiplanar reconstruction (MPR), curved planar reconstructions (CPR), maximal intensity projections (MIP), shaded surface display (SSD), and volume-rendering technique (VRT). Three-dimensional reconstructions were stored together with all original slices in the digital archive (IMPAX by AGFA, Mortsel, Belgium). These parameters allow for optimal multiplanar reformatting of the data for detailed investigation of selected small bowel segments not adequately visualized in the axial display. A window width of 1200 HU and a window level of 200 HU were used. This setting provides optimal demonstration of small bowel folds. All CTE images were reviewed on a workstation in both the axial and reformatted coronal and sagittal planes.

The subjects were all instructed in a breath-hold technique. The helical CT scanner required a 30 to 40 s breath-hold. Newer multislice CT scanners are faster, and a breath hold of only 20 s is needed.

For virtual endoscopy, the data were transferred to a workstation and reprocessed on a CT workstation (MxView, Philips Medical Systems). Volume rendering was used for 3D visualization and virtual endoscopy. The user interface allowed inspection of the data in different ways: (1) virtual endoscopy (internal) view and (2) simultaneous cross-reference to the corresponding cross-sectional images. The

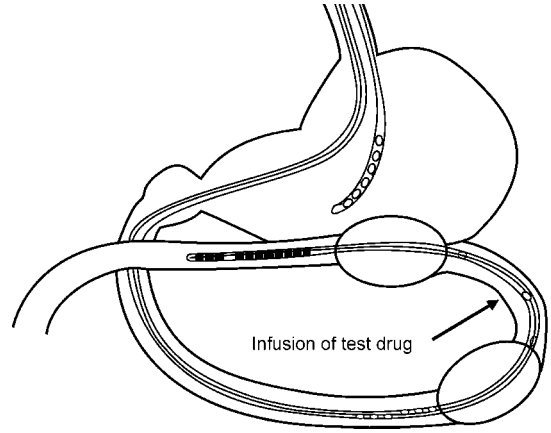

Figure 1. The LOC-I-GUT tube isolated a 10-cm long jejunal segment in the proximal jejunum. The diameter and the mantle area of this isolated intestinal segment were accurately determined using different radiological methods.

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path was planned manually, and the "flight through" visualizing the internal mucosal relief and caliber of the small bowel was stored for later playback.

These techniques include maximum intensity projection, volume rendering, and multiplanar reconstruction (MPR). These CT data sets were also used to create intraluminal images, thereby generating virtual endoscopic studies.

Study Drugs. In the third part of the study three drugs were administered by single pass perfusion. The drugs used were furosemide (Hoechst AG, Frankfurt, Germany) and fexofenadine hydrochloride (Aventis Pharmaceuticals, Kansas City, MO). A low perfusate concentration (10 mg/L, 53 μM) of antipyrine (Astra Läkemedel AB, Södertälje, Sweden) was used as a marker for passive transcellular diffusion in all of the perfusion experiments. ^{14}C labeled polyethylene glycol 4000 (^{14}C-PEG 4000) (2.5 μCi/L, Amersham Pharmacia Biotech, Little Chalfont, England) was used as a nonabsorbable volume marker.

The perfusion solution consisted of potassium chloride 5.4 mM, sodium chloride 30 mM, mannitol 35 mM, D-glucose 10 mM and PEG 4000 1.0 g/L, all dissolved in a 70 mM phosphate buffer with pH 6.5 and osmolality of 290 mOsm/kg. This perfusion buffer has been used as a vehicle in several perfusion studies.[11] In both the control and the treatment period, the jejunal segment was perfused with the perfusion solution containing 66 mg/L (200 μM) furosemide. The total given dose of furosemide was 26 mg (13 mg in each period). In the treatment period (100−200 min), furosemide was administered together with 50 mg/L (97 μM) of fexofenadine (total given dose 10 mg). The drug concentrations in the perfusate were clinically relevant for both drugs.

Drug Perfusion Studies. Once the perfusion tube was in place and the balloons were inflated, a vacuum pump was connected to the proximal drainage channel of the perfusion tube to drain any intestinal fluid above the perfused segment (Ameda suction pump type 23, Ameda AG, Zug, Switzerland). The jejunal segment was then rinsed with isotonic saline (37 °C) for at least 20 min. When stable perfusion conditions were attained, 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 Instruments, Orion Research Inc., Cambridge, MA).[10] The perfusate leaving the jejunal segment during the single-pass perfusion was quantitatively collected on ice at 10 min intervals and immediately frozen at −20 °C pending analysis. Immediately upon completion of the perfusion experiment, the jejunal segment was rinsed with 120 mL of isotonic saline to terminate the intestinal drug absorption process, whereupon the LOC-I-GUT instrument was removed.

Adsorption and Stability Test of Fexofenadine and Furosemide. There was no adsorption of fexofenadine and furosemide to the LOC-I-GUT tube, which was investigated by an in vitro perfusion test in a glass tube for 100 min. Fexofenadine, antipyrine and furosemide were stable in perfusion solution and human jejunal fluid at 37 °C for 180 and 60 min, respectively.

Analytical Methods. Furosemide in the perfusate and perfusion solution was analyzed by HPLC with UV detection. The HPLC system consisted of a Shimadzu LC-9A pump (Kyoto, Japan) and a Spectra 100 UV detector (Thermo separation products). The analytical column was a Hypersil 5 ODS (250 × 4.6 mm i.d. Chrompack). The mobile phase was composed of a phosphate buffer with an ionic strength of 0.05 and a pH of 7.4 with 35% (v/v) acetonitrile. The flow rate was 1 mL/min, and the injection volume was 10 μL. The limit of quantification (LOQ) was set to 1.86 mg/L (coefficient of variation, CV 2.0%), and the standard curves were linear in the range 1.9−91 mg/L. The CV of the intraassay variability ($N = 6$; quality controls containing 14, 35 and 70 mg/L) ranged between 0.5 and 0.7%. The CV of the interassay variability was below 20%. The concentrations of antipyrine were analyzed using an HPLC method with UV detection according to a previously validated method.[12] The total radioactivity of ^{14}C-PEG 4000 in the perfusion solution and the perfusate samples was determined by liquid scintillation counting (Mark III, Searle Analytic Inc., Des Plaines, IL).

The total radioactivity of ^{14}C-PEG 4000 in the perfusion solution and the perfusate samples was determined by liquid scintillation counting (Mark III, Searle Analytic Inc., Des Plaines, Illinois). The osmolality and pH of the perfusion solution and perfusate samples were measured with the vapor pressure method (5500 vapor pressure osmometer, Wescor Inc., Logan, UT) and a pH meter (632 pH-Meter, Metrohm AG, Herisau, Switzerland), respectively.

Data Analysis of Imaging Data. The cylindrical surface area and volume were calculated using standard equations including measured individual values of the radius from both techniques and length of 10 cm of the studied segment.

Data Analysis of Permeability Study. All calculations from the single-pass perfusion experiment for furosemide and antipyrine were made from steady-state concentrations in the outlet jejunal perfusate in the control (T1) and treatment period (T2). Each sample represents the mean concentration of the aliquots collected for each 10 min interval (0−200 min). The net water flux (NWF, mL/h/cm) in the isolated jejunal segment was calculated according to eq 1:

$$\mathrm{NWF} = \left(1 - \frac{\mathrm{PEG_{out}}}{\mathrm{PEG_{in}}}\right)\frac{Q_{\mathrm{in}}}{L} \qquad (1)$$

where $\mathrm{PEG_{in}}$ and $\mathrm{PEG_{out}}$ are the concentrations of ^{14}C-PEG 4000 (dpm/mL) entering and leaving the segment, respectively. Q_{in} (mL/min) is the flow rate of the perfusion solution

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entering the segment, 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 the fraction of drug being absorbed in the segment (f_{abs}) and the permeability (P_{eff}) were calculated.

Since the drugs under study did not bind to the perfusion tube and were stable in the perfusion solution, the amount that disappeared during the single passage through the jejunal segment was considered to be absorbed. The fraction of the drug absorbed in the segment during the perfusion (f_{abs}) was calculated from eq 2:

$$f_{abs} = 1 - \left(\frac{C_{out}\mathrm{PEG}_{in}}{C_{in}\mathrm{PEG}_{out}} \right) \quad (2)$$

where C_{in} and C_{out} are the concentrations entering and leaving the jejunal segment, respectively.

The effective jejunal permeability (P_{eff}) of each drug was calculated according to a well-mixed tank model, as shown in eq 3:

$$P_{eff} = \frac{C_{in} - C_{out}}{C_{out}} \frac{Q_{in}}{2\pi rL} \quad (3)$$

where the cylindrical area representing the jejunal segment ($2\pi rL$) was calculated using the intestinal radius and the length of the segment. P_{eff}, which is calculated from the disappearance of the drug from the jejunal segment, is a direct measured parameter of intestinal transport that can be used regardless of the mechanisms involved in the drug transport across the human epithelium.[4,13−15]

Statistical Analysis. The effect of fexofenadine on the absorption variables for furosemide was evaluated using Student's t test for paired data (GraphPad Prism version 3.0, GraphPad software, San Diego, CA). Differences between mean values were considered significant at $P < 0.05$. Throughout the paper, the data are expressed as mean values ± standard deviation (mean ± SD) or as standard error of the mean (±SEM) as stated in the text.

Determination of Sample Size. The inclusion of 7 volunteers in each group provided an 80% power to detect a 2-fold change in the mean effective jejunal permeability (P_{eff}) between the groups, assuming that the common SD is not higher than 75% of the respective means.

Results

Imaging Investigations (Study Parts I and II). The results from the imaging experiments with the two different techniques are illustrated in Figures 2−6. In the first part of the study six subjects had their jejunal segment between the two inflated balloons filled with barium contrast (Mixobar), which is displayed in Figures 2 and 3. The inflated balloons make an impression into the contrast medium (arrows) (Figure 3). The radius and cylinder surface area were calculated with plain abdominal X-ray to be 1.61 ± 0.05 cm and 101 ± 1.17 cm^2, respectively (Table 1). The volume was estimated to be 81.4 ± 1.0 cm^3 (Table 1). Figures 4−6 are based on the multislice computed spiral tomography (MCST) and together with air as a contrast medium a three-dimensional view of the positioning of the closed intestinal segment in the abdominal cavity is obtained. These virtual endoscope figures illustrate the size of the small intestine.

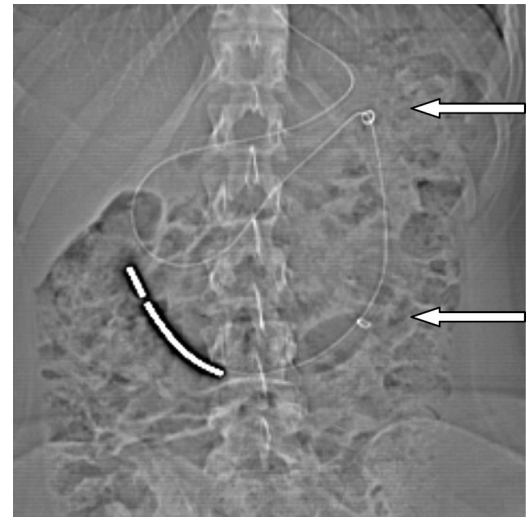

Figure 2. Radio opaque markers delineate the LOC-I-GUT segment (arrows) and reveal the positioning of the tube in the proximal part of jejunum. The outer diameter of the LOC-I-GUT tube is 5.3 mm.

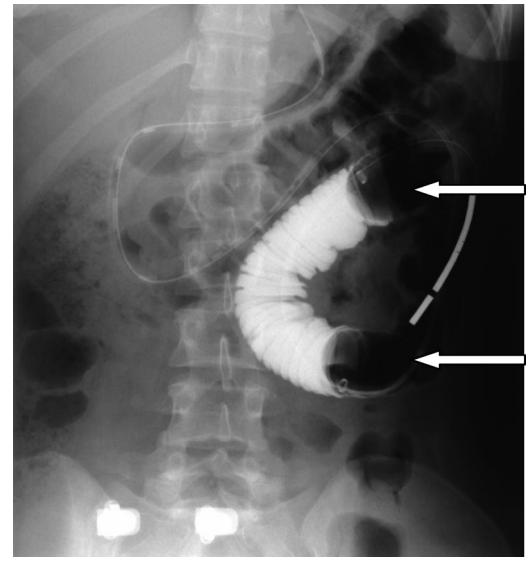

Figure 3. In the first set of experiments (n = 6) the closed jejunal segment was filled with barium contrast (Mixobar). The inflated balloons make an impression into the contrast medium (arrows).

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Table 1. Mean (± SE) of the Radius and Cylinder Mass Transfer Surface Calculated with Plain Abdominal X-ray and Multislice Computed Spiral Tomography (MCST)

radius (plain X-ray)	(cm)	1.61 ± 0.05
V (plain X-ray)	(cm^3)	81.4 ± 1.0
area (plain X-ray)	(cm^2)	101 ± 1.17
radius (MCST)	(cm)	1.93 ± 0.11
V (MCST)	(cm^3)	117 ± 3.02
area (MCST)	(cm^2)	121.3 ± 3.5

Maximal dilatation of the closed segment with room air as contrast and using MSCT revealed a mantel area of 121.3 ± 3.5 cm^2. The radius and volume of the segment were found to be 1.93 ± 0.11 cm and 117 ± 3.02 cm^3, respectively (Table 1). All individual values for radius and surface area based on plain abdominal X-ray and multislice computed spiral tomography, respectively, are given in Table 1.

The Effect of Fexofenadine on the Jejunal Transport of Furosemide. P_{eff} and f_{abs} from a perfused jejunal segment in vivo of furosemide were determined under steady-state conditions at pH 6.5 between 60 and 100 and 160 and 200 min in the control (T1) and treatment period (T2), respectively. The jejunal P_{eff} of furosemide was low, 0.17 ± 0.07 and 0.12 ± 0.09 × 10^{-4} cm/s in T1 and T2, respectively (Table 2 and Figure 7). Even if substantial interindividual differences were observed, P_{eff} remained low in every subject (<0.4 × 10^{-4} cm/s) in both periods. The mean value of f_{abs} during the perfusion experiment for furosemide was 5 ± 2 and 4 ± 3% in the control and treatment periods, respectively (Table 2). The jejunal P_{eff} of antipyrine was 3.96 ± 1.33 and 3.14 ± 1.96 × 10^{-4} cm/s in T1 and T2, respectively. The recovery of the nonabsorbable volume marker was 96 ± 5 and 96 ± 3% in these two periods, respectively (Table 2).

Discussion

Equations published earlier to calculate the effective and/or apparent intestinal permeability (P_{eff} and/or P_{app}) from in situ or in vivo studies and to predict fraction of dose absorbed (fa) after oral administration is based on quantitative disappearance from the perfused lumen. It is based on several physiologic and pharmacokinetic observations and assumptions; for example, (a) the intestine is behaving like a well-stirred model according to a residence time distribution analysis; (b) the binding of the drug to the tube material is examined and corrected for; (c) there is no chemical and/or enzymatic degradation of the drug; (d) there is no accumulation of drug in the gut wall or tissue; (e) a smooth cylinder surface area available for absorption; and (f) there is a well-defined luminal concentration profile.[16] One of the aims of this study was to evaluate accuracy of values used for the intestinal radius and intestinal mantle area of this single-pass perfused intestinal segment. The small intestine in humans and animals has been suggested to be somewhat flat in the fasting state,[17] but when food or drugs (taken together with fluid) are given, the intestine reacts with segmental distention as part of the normal propulsion mechanism. In a large number of single-pass perfusion investigations we have used 2 mL/min as a the perfusion flow since the normal flow in the proximal small intestine in humans has been reported to range between 0.6 and 4.2 mL/min including both fasted and fed states.[18,19]

Virtual endoscopy is a rapidly evolving technique in which data from computed tomography (CT) are used to generate both two-dimensional and three-dimensional displays of the small and large intestine as well as other abdominal organs. In many regions of the body, MSCT imaging has changed from a purely morphologic imaging technique to one that combines functional and morphologic imaging.

We have earlier been using the radius of 1.75 cm based on measurement of the inflated balloons on the LOC-I-GUT tube when filled with the 25 mL of air at 37 °C (Figure 1).[3] We have also made an earlier estimate of the radius with X-ray that has been reported to be around 1.75 cm.[20] In this study we have used two different imaging techniques, and the radius was found to be in this range (1.61−1.93), which validates and confirms the accuracy of the value used in a large number of previous studies.[1−3,11−15] The average volume of the perfusion solution within the perfused intestinal segment has previously been approximated to be between 46−75 mL on average at a flow rate of 3 mL/min.[3] The somewhat larger volume measured with the imaging techniques in this in vivo study suggests that the jejunal segment between the two balloons is not completely filled during a single-pass perfusion. However, the contact between drug solution and the epithelial surface area is most likely high since the effective permeability predicts both rate and extent of absorption accurately.[1,2,15,16,21−23] For instance, these data have been shown to predict both rate and extent of absorption and to provide high accuracy in the parameter

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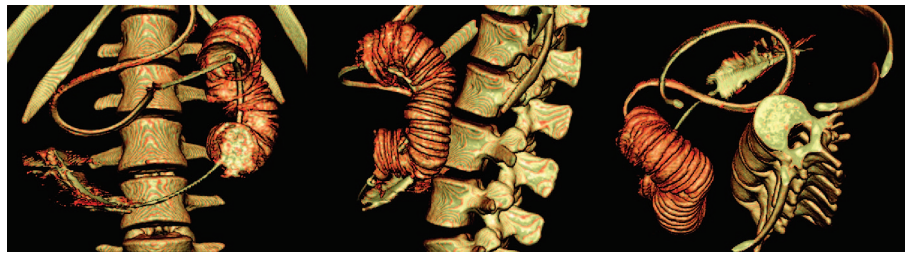

Figure 4. With the use of MSCT and air as a contrast medium a three-dimensional view of the positioning of the closed intestinal segment in the abdominal cavity is obtained. All other organs except spine and ribs have been omitted using the software included in the technique.

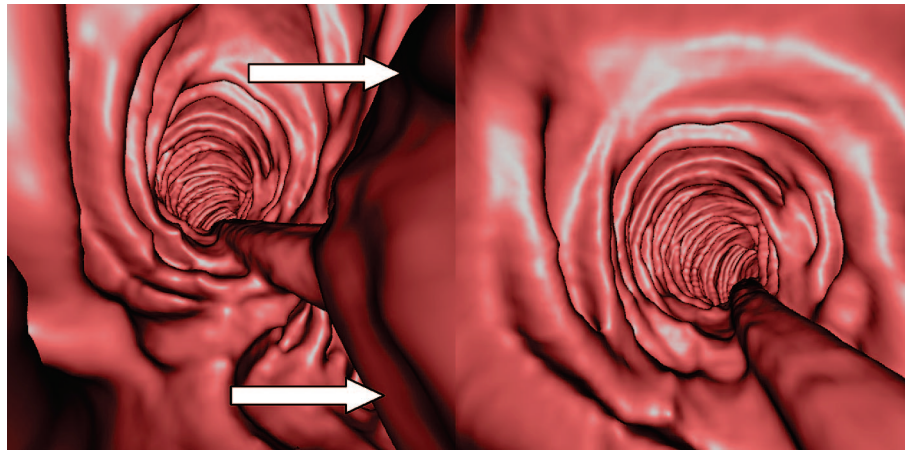

Figure 5. With the use of "virtual endoscopy" you are given the feeling of being inside the small intestine and in fact performing a real enteroscopy. On the left you enter the jejunal segment with the proximal balloon clearly visible (arrows). To the right you are in the middle of the LOC-I-GUT segment with the snakelike tube entering from the lower right corner.

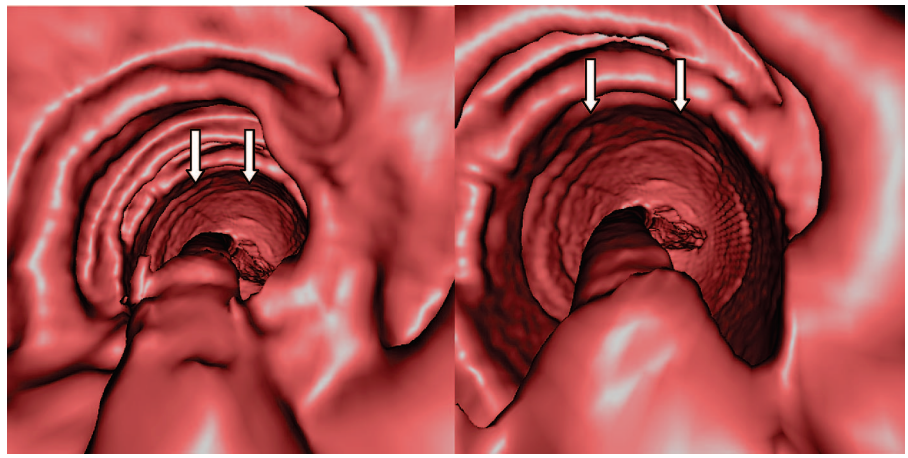

Figure 6. At the distal end of the intestinal segment the other occluding balloon meets the spectator as an impregnable wall (arrows).

sensitivity analysis performed in the pharmacokinetic prediction computer programs such as Gastroplus and Simcyp.[1,24] In addition, these virtual endoscopic imagines are important in our development of novel single-pass perfusions where we aim to investigate the permeability of drugs in distal ileum in humans.

The future application of the BCS is most likely increasingly important when the present framework gains increased recognition, which will probably be the case if the BCS

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Table 2. Mean (±SD) Absorption Variables of Furosemide and Antipyrine as Well as the Other Perfusion Parameters after the Perfusion Experiments in Which Furosemide Was Administrated Alone (Control) or with 50 mg/L Fexofenadine (+ Fexofenadine) ($N = 7$)[a]

		treatment	
		control	+ fexofenadine
$P_{eff\ furosemide}$	($\times 10^{-4}$ cm/s)	0.17 ± 0.07	0.12 ± 0.09
$f_{abs\ furosemide}$	(%)	5 ± 2	4 ± 3
$P_{eff\ antipyrine}$	($\times 10^{-4}$ cm/s)	3.96 ± 1.33	3.14 ± 1.96
$f_{abs\ antipyrine}$	(%)	53 ± 10	44 ± 13
PEG 4000_{rec}	(%)	96 ± 5	96 ± 3
NWF	(mL/h × cm)	2.06 ± 0.84	1.98 ± 0.38

[a] P_{eff}, effective jejunal permeability; f_{abs}, the fraction of drug being absorbed in the segment; PEG 4000_{rec}, recovery of ^{14}C labeled PEG 4000; NWF, net water flux.

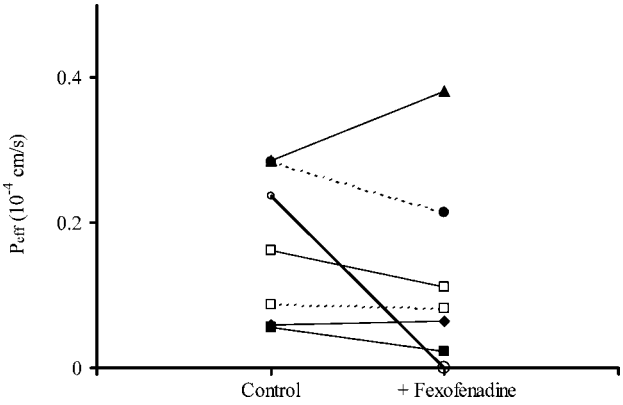

Figure 7. Individual values of the human jejunal effective permeability (P_{eff}) of fexofenadine at a luminal concentration of 100 mg/L (186 μM) administered alone (Control, 0−100 min), and with the addition of 50 mg/L (97 μM) fexofenadine (+ Fexofenadine, 100−200 min).

borders for certain class II and III drugs are extended. The future revision of the BCS guidelines by the regulatory agencies in communication with academic and industrial scientists is exciting and will hopefully result in an increased applicability in drug development. Finally, we emphasize the great use of the BCS as a simple tool in early drug development to determine the rate-limiting step in the oral absorption process, which has facilitated the information between different experts involved in the overall drug development process.[25−27] This increased awareness of a proper biopharmaceutical characterization of new drugs has resulted in drug molecules with a sufficiently high permeability. Instead the solubility and dissolution rate are issues that require further investigations especially in this in vivo perfusion model. This has been proven to provide very useful data for improvements of in vitro models and correlation to nonclinical models.[1,28−31]

In this study it was shown that the jejunal permeability of furosemide was not affected by fexofenadine. The working hypothesis was that these two low permeability drugs with acid properties might to some extent share the same carrier systems in the small intestine. This was based on the fact that both furosemide and fexofenadine had been reported to be secreted more rapidly than absorbed in vitro [32−34] although the transporter(s) responsible for the secretion of furosemide remains to be identified.[35] Moreover, fexofenadine has been shown to be transported by the uptake transporter OATP-B in vitro and furosemide has recently been suggested to at least inhibit OATP-B mediated transport.[36,37] An inhibitory effect of fexofenadine on any carrier mediated uptake of furosemide would have resulted

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in decreased permeability of furosemide. This was indeed observed in five out of seven subjects; however, the effect was not statistically significant. This lack of effect may be due to the fact that these two drugs do not share the same transport proteins or that the concentration of fexofenadine within the jejunal epithelium was not sufficiently high (due to low permeability), despite the concentration was high in the lumen. The permeability of the two markers compounds, antipyrine and PEG 4000, was high and low and agreed with previous data and support that the current perfusion experiment was valid ([3,11,38]). The jejunal permeability data of furosemide in this study agreed with previous published data.[34] The data also suggest that the small intestinal permeability of furosemide (log $D_{6.5}$ −0.5, PSA 124 Å^2, HBD 4) is low as a consequence of its polar nature. Interestingly, after oral administration the absorption and bioavailability of furosemide are highly variable (35−75%).[15] There are several hypotheses for this high variability such as active intestinal secretion, low passive diffusion, highly pH-dependent dissolution and permeability. A correlation between human jejunal P_{eff} and physicochemical descriptors suggests that drugs with octanol/buffer partitioning coefficients higher than 0 and a PSA <100 Å^2 will be highly permeable ($P_{eff} > \approx 1.0 \times 10^{-4}$ cm/s and fa > 90%) across the human jejunum. This suggests that pH will strongly influence the passive intestinal permeability in vivo. At pH 7.4, 6.5 and 5.5 the experimentally determined partitioning coefficients for furosemide at different pH were −0.9, −0.5 and 0.4 respectively.[1,2,15,25,26] In addition, uncharged furosemide (an acid with pK_a at 3.3/10.1) has an experimentally determined partition coefficient (i.e., log *P*-value) of 2.53 ± 0.01.[25,26] A strong pH-dependent permeability may also explain the reported high inter- and intraindividual pharmacokinetic variability.[39] In addition, it supports the suggestion that passive transcellular diffusion is the main transport mechanism for furosemide.[40,41]

Finally, this study confirmed that the in vivo and physiologically relevant radius for the intestinal in vivo drug absorption process from the perfused small intestinal segment is about 1.61−1.93 cm based on two different imaging techniques. The validity of these human in vivo permeability data has been confirmed by many direct and indirect results including this study. This concludes the validity of the human permeability database and supports the future application of these human permeability data for various physiological based pharmacokinetic modeling for both pharmacological and toxicological assessments. These virtual endoscopy techniques are important for us in the development of novel single-pass perfusion tubes where our aim is to investigate the transport and excretion of drugs and metabolites in distal ileum in humans. The last part of the study also showed that fexofenadine would have no effect on the intestinal absorption of furosemide. It also suggests that fexofenadine does not share the same small intestinal transporters as furosemide and that furosemide is mainly transported by passive diffusion.

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