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Research paper

Evaluation of drug permeability calculation based on luminal disappearance and plasma appearance in the rat single-pass intestinal perfusion model

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ABSTRACT

The rat single-pass intestinal perfusion (SPIP) model is commonly used to investigate gastrointestinal physiology and membrane drug transport. The SPIP model can be used with the intestinal segment inside or outside the abdomen. The rats can also be treated with parecoxib, a selective cycloxygenase-2 inhibitor that has been shown to affect some intestinal functions following abdominal surgery, such as motility, epithelial permeability, fluid flux and ion transport. However, the impact of extra-abdominal placement of the intestinal segment in combination with parecoxib on intestinal drug transport has not been investigated. There is also uncertainty how well intestinal permeability determinations based on luminal drug disappearance and plasma appearance correlate in the rat SPIP model. The main objective of this rat *in vivo* study was to investigate the effect of intra- vs. extra-abdominal SPIP, with and without, pretreatment with parecoxib. The effect was evaluated by determining the difference in blood-to-lumen ^{51}Cr-EDTA clearance, lumen-to-blood permeability of a cassette-dose of four model compounds (atenolol, enalaprilat, ketoprofen, and metoprolol), and water flux. The second objective was to compare the jejunal permeability values of the model drugs when determined based on luminal disappearance or plasma appearance. The study showed that the placement of the perfused jejunal segment, or the treatment with parecoxib, had minimal effects on membrane permeability and water flux. It was also shown that intestinal permeability of low permeability compounds should be determined on the basis of data from plasma appearance rather than luminal disappearance. If permeability is calculated on the basis of luminal disappearance, it should preferably include negative values to increase the accuracy in the determinations.

1. Introduction

The rat single-pass intestinal perfusion (SPIP) model is commonly used to study gastrointestinal (GI) physiology, as a way to elucidate the underlying mechanisms and regulation of intestinal motility, fluid transport, and epithelial membrane permeability [1,2]. The SPIP model is also frequently used in pharmaceutical science to investigate membrane drug transport and to aid decision making in drug product development [3,4].

To increase the relevance of the SPIP model for these investigations, rats can be treated with a cyclooxygenase (COX) inhibitor after abdominal surgery [5]. This leads to a reduction in surgery-induced postoperative ileus, a condition in which normal peristalsis and other physiological functions are compromised. Intestinal functions can then be evaluated that would otherwise be substantially affected by the abdominal surgery. For instance, the ability of the intestinal mucosa to respond to luminal hypotonicity in the rat SPIP model is only intact if the rats are pretreated with parecoxib, a selective COX-2 inhibitor; untreated rats display absent, or delayed, normalization of luminal tonicity [6]. Parecoxib also enables investigation of the interplay between gut hormones and intestinal motility, as it restores physiologic enteric nerve activity [7]. For example, the decrease in duodenal motility, induced by vasoactive intestinal polypeptide, is only possible to investigate in the SPIP model following parecoxib treatment [8].

The investigations of GI physiology and drug transport are typically performed with the perfused intestinal segment placed back into the

Abbreviations: SPIP, single-pass intestinal perfusion; J_{water}, water flux; $CL_{Cr\text{-}EDTA}$, blood-to-lumen ^{51}Cr-EDTA clearance; $P_{eff,\ app}$, permeability determined based on plasma drug appearance; $P_{eff,\ dis}$, permeability calculated from luminal disappearance

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abdominal cavity, but there are advantages with leaving the intestinal segment outside. As the extra-abdominal segment is visible, intestinal folding can be avoided. Folding leads to an uneven perfusate flow and build-up of luminal pressure, both of which may affect drug transport, as well as intestinal physiology and viability [9]. Intestinal peristalsis is also possible to monitor visually with extra-abdominal perfusions. Although peristalsis is typically measured with a luminally placed manometer or balloon, visual observation is more suitable for detecting weaker luminal contractions in rat models.

Drug permeability using the SPIP model can be determined in two ways: drug disappearance in the intestinal lumen, or drug appearance in plasma [3,10]. One advantage with determinations based on disappearance is that the drug is readily quantified in the perfusate, while plasma concentrations of the same drug may be very low. However, for drugs with a low permeability, it is substantially easier to determine plasma appearance data, because the difference in perfusate concentrations may be too small to be accurately quantified.

The impact of extra-abdominal SPIP on drug transport studies has not been investigated, and neither has the ability of parecoxib for restoring normal intestinal physiology under this experimental condition. The correlation between permeability determinations from luminal drug disappearance and plasma appearance is also uncertain in the rat SPIP model. A systematic evaluation of the effect of extra-abdominal SPIP experiments would allow for this type of perfusion to be routinely performed and become an established biopharmaceutical tool. The benefit of these experiments is that they allow continuous controlled perfusate flow rate and luminal pressure. A validation of the two methods (luminal disappearance and plasma appearance) would increase the likelihood for accurate determination of the permeability value of drugs with low membrane permeability. This would improve *in vivo* evaluation of oral modified-release products, for which regional differences in permeability are important. It would also facilitate investigation of compounds with very low membrane permeability, such as peptides.

One objective of this study was to compare the effect of intra- vs. extra-abdominal positioning of the intestinal segment in the rat SPIP model, with and without pretreatment of the rats with parecoxib (a selective COX-2 inhibitor). We compared the difference in jejunal: (i) lumen-to-blood drug permeability of two high-permeability model compounds (metoprolol and ketoprofen), and two low-permeability ones (atenolol and enalaprilat); (ii) blood-to-lumen clearance of the clinical marker for mucosal integrity ^{51}Cr-EDTA; and (iii) water flux. Another objective was to compare the jejunal permeability values derived from luminal drug disappearance with those from plasma drug appearance.

2. Methods

2.1. Active pharmaceutical ingredients and other chemicals

Four model compounds were selected: atenolol, enalaprilat, ketoprofen, and metoprolol. These belong to classes I, II and III according to the biopharmaceutics classification system (BCS) [11]. BCS class and physicochemical properties are summarized in Table 1. Atenolol and metoprolol tartrate were provided by AstraZeneca AB (Mölndal, Sweden). Enalaprilat, ketoprofen, bovine albumin, and inactin (thiobutabarbital) were purchased from Sigma-Aldrich (St. Louis, MO, US). Sodium phosphate dibasic dihydrate ($Na_2HPO_4{\cdot}2H_2O$), potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide (NaOH), and sodium chloride (NaCl) were purchased from Merck KGaA (Darmstadt, Germany). ^{51}Chromium-labeled ethylenediaminetetraacetate (^{51}Cr-EDTA) was purchased from PerkinElmer Life Sciences (Boston, MA). Parecoxib (dynastat) was obtained from Apoteket, Uppsala, Sweden.

2.2. Study solutions

An isotonic (290 mOsm) phosphate-buffered (6 mM) test solution, at pH 6.5, containing 5 μM atenolol, 228 μM enalaprilat, 40 μM ketoprofen, and 24 μM metoprolol, was single-pass perfused in rat jejunum in the four experiments evaluating the effect of intra- vs. extra-abdominal perfusion, with and without parecoxib. The preparation procedure of the perfusion solution (100 mL) is described by Dahlgren et al. 2017.[10]No incompatibility, no degradation, or no apparent binding to glass/plastic of the study compounds in solution (pH 6.5, 37 °C) was observed during 4 h. Osmolarity was determined after addition of all test solutions constituents (e.g., salt, water) by freezing point depression using a Micro Osmometer (Model 3MO; Advanced Instruments, Needham Heights, MA, USA)

2.3. Animals and surgery

The surgical procedure and experimental setup for the rat SPIP was performed as described previously [10]. The study was approved by the local ethics committee for animal research (no: C64/16) in Uppsala, Sweden. Male Wistar Han rats (strain 273) from Charles River (Germany) weighing 319–591 g, were used. On the study day, the rats were allowed water and pelleted food *ad lib* until they were anesthetized with an intraperitoneal injection of a 5% (w/v) inactin solution (180 mg/kg). Inactin was used as it has minimal effect on intestinal functions [12,13] Body temperature was maintained and monitored at 37.5 ± 0.5 °C with a rectal thermometer connected to a heating pad, and the systemic arterial blood pressure was continuously recorded. The abdomen was opened along the midline and the bile/pancreatic duct was cannulated next to the intestine to avoid pancreaticobiliary secretion into the duodenum. A jejunal segment of 8–11 cm was cannulated and thereafter placed either: (i) *inside* the abdomen, which was closed with sutures, or (ii) *on top of* the abdomen, and covered with polyethylene wrap.

After completion of surgery (30–40 min), 75 μCi (0.4 mL) ^{51}Cr-EDTA was administered intravenously as a bolus, followed by a continuous infusion at a rate of 50 μCi per hour (1 mL/h) for the duration of the experiment (130 min: 30 min resting period and 100 min experiment). After the ^{51}Cr-EDTA bolus administration, each intestinal segment was single-passed perfused at 0.2 mL/min (peristaltic pump,

Table 1
Physicochemical properties, Biopharmaceutics Classification System (BCS) classification, and human jejunal effective permeability (P_{eff}) as historically determined with the single-pass jejunal perfusion model [26,31].

Compounds (BCS class)	MM (g/mol)	pK_a	PSA	HBA/HBD	Log P	Log $D_{7.4}$	Log $D_{6.5}$	Human P_{eff} ($\times 10^{-4}$ cm/s)
Atenolol (III)	266	9.6[b]	88.1	4/4	0.18	−2.0	< -2.0	0.2
Enalaprilat (III)	348	3.17[b]/7.84[a]	102.1	6/3	−0.13	−1.0	−1.0	0.2
Metoprolol (I)	267	9.6[b]	57.8	4/2	2.07	0.0	−0.5	1.3
Ketoprofen (II)	254	3.89[a]	54.2	3/1	3.37	0.1	0.8	8.7

HBA/HBD – hydrogen bond acceptor/donor, Log $D_{7.4/6.5}$ - n-octanol – water partition coefficient at pH 7.4/6.5, Log P - n-octanol – water coefficient, MM – molar mass, pKa – dissociation constant, PSA – polar surface area.

[a] Acid.

[b] Base.

Gilson Minipuls 3, Le Bel, France) for 30 min with phosphate buffered saline (6 mM, pH 6.5, 37 °C). This was a resting period to allow cardiovascular, respiratory, and small intestinal functions to stabilize, and to achieve ^{51}Cr-EDTA equilibrium activity in the blood plasma.

In the investigation of intra- vs. extra-abdominal placement of the perfused intestinal segment, each rat was single-pass perfused during 100 min. The effect of intestinal placement was investigated on intestinal drug absorption, ^{51}Cr-EDTA clearance, and water transport. Twenty-four rats were included in the study, with half of these being perfused with the intestines inside the abdomen, and the other half with the intestines outside. Half of the rats (n = 6) in each group (intra/extra) were administered an intravenous bolus dose of 10 mg/kg parecoxib before the start of the resting period; the other half (n = 6) of each group did not receive any parecoxib. Parecoxib is a selective COX-2 inhibitor which reduces surgery-induced paralysis of the intestine [7]. The rats received the inhibitor to evaluate its effect on membrane jejunal permeability and water transport in the two groups (intra/extra). All perfusate leaving the jejunal segment was collected and quantified at 15-min intervals, starting from 40 min; this yielded four samples during the 60-min perfusion period. The first 40 min served as an equilibration period for drug absorption and ^{51}Cr-EDTA clearance.

The length of each jejunal segment was measured after the cannulation, and weighed after the end of the experiment. All experiments started with a rapid filling (< 30 sec) of the whole segment with the perfusion solution (≈1.5 mL for a 10-cm segment). The jejunal segment and perfusion solutions were kept at 37 °C. The weight and pH of all outgoing perfusate samples were determined. Water flux (J_{water}, mL/cm/h) was calculated by comparing the ingoing (Q_{in}) and outgoing (Q_{out}) water weight (g) to the length of the perfused segment (L) using Eq. (1).

$$J_{water} = \frac{Q_{out} - Q_{in}}{L} \quad (1)$$

Blood samples of < 0.3 mL were collected from the femoral artery for a total maximal volume of 2 mL during each SPIP experiment. All collected blood volumes were replaced by an equivalent volume of saline solution (0.9% NaCl) with 70 mg/mL bovine serum albumin. Blood was sampled immediately before administration and four times at 15-min intervals during 60 min, starting at 40 min (total of 5 blood samples). The first 40 min served as an equilibrium period for drug absorption and ^{51}Cr-EDTA clearance. The blood samples were put on ice and centrifuged (5000 × *g*, 3 min at 4 °C) within 10 min. Fifty microliters of the plasma was transferred to a 1-mL 96-well plate (Thermo Scientific) and stored at −20 °C until analysis. The analytical method for the SPIP experiment has been described previously, are there were no interaction or suppression between the drug compounds [10]. Model drug concentrations were determined in both the perfusates and in the plasma.

2.4. Determinations of jejunal blood-to-lumen ^{51}Cr-EDTA clearance

All luminal perfusates and the blood plasma at 0 and 100 min were analyzed for ^{51}Cr activity (cpm) in a gamma counter (1282 Compugamma CS, Pharmacia AB, Sweden). A linear regression analysis of the plasma samples was made to obtain a corresponding plasma value for each perfusate sample leaving the jejunal segment. The blood-to-lumen ^{51}Cr-EDTA clearance ($CL_{Cr\text{-}EDTA}$) was calculated using Eq. (2) [14].

$$CL_{Cr\text{-}EDTA} = \frac{C_{perfusate} \times Q_{in}}{C_{plasma} \times tissue\ weight} \times 100 \quad (2)$$

where $C_{perfusate}$ and C_{plasma} are the activity in the perfusate and plasma (cpm/mL), respectively, and Q_{in} is the flow rate (mL/min) through the jejunal segment. Jejunal $CL_{Cr\text{-}EDTA}$ is expressed as mL/min/100 g wet tissue weight. $CL_{Cr\text{-}EDTA}$ was determined during the last 60 min of each perfusion, allowing the first 40 min for equilibration. The mean jejunal $CL_{Cr\text{-}EDTA}$ value was regarded as representative for each individual rat.

2.5. Calculations of jejunal lumen-to-blood permeability

The jejunal permeability values based on luminal disappearance ($P_{eff,dis}$) were determined using Eq. (3).

$$P_{eff,dis} = Q_{in} \times \left(-\ln\left(\frac{C_{out}}{C_{in}}\right)\right) \times \frac{1}{A} \quad (3)$$

where Q_{in} is the inlet flow rate, C_{out} is the drug concentration in the outgoing perfusate, C_{in} is the drug concentration in the ingoing perfusate, and A is the area of the exposed intestinal segment described as a smooth cylinder with a radius of 0.2 cm. The concentrations leaving the jejunal segment were corrected for J_{water}. The $P_{eff,dis}$ was determined during the last 60 min of each perfusion, allowing the first 40 min for equilibration. $P_{eff,dis}$ determinations were performed with three different calculations, i.e., with negative $P_{eff,dis}$ values excluded or included, or by negative values set to zero. The mean jejunal $P_{eff,dis}$ value was regarded as representative for each individual rat.

The jejunal permeability values based on plasma appearance ($P_{eff,app}$) used a modification of the method described by Sjögren et al., 2015, which has been successfully implemented in human, dog and rat studies [4,10,15–17]. In short, an input rate was acquired by deconvolution of the plasma concentration–time profiles following the intestinal perfusion. The intravenous PK data from a separate, two-compartment PK analysis was used as impulse response in the deconvolution [10]. Intestinal absorption rate was then calculated by compensating for the first-pass extraction ($F_{firstpass}$) of each compound in the rat intestine and liver. The $F_{firstpass}$ values for atenolol (1.0), enalaprilat (0.99), ketoprofen (0.99), and metoprolol (0.22), were based on: (i) literature values of the fraction of the model compound metabolized in the rat liver; (ii) plasma CL values derived from the two-compartment analysis of intravenous plasma data from our laboratory; and (iii) an assumed rat liver blood flow of 47 mL/min/kg [18–22]. The jejunal $P_{eff,app}$ (cm/s) was then calculated by relating the absorption rate to the intestinal luminal area, and to the luminal concentration, using eq (4).

$$P_{eff,app} = \frac{absorption\ rate}{A \times C} \quad (4)$$

where A is the area of the exposed intestinal segment described as a smooth cylinder with a radius of 0.2 cm, and C is the average luminal concentration ($C_{in} + C_{out}/2$). The $P_{eff,app}$ was determined during the last 60 min of each perfusion, allowing the first 40 min for equilibration. The mean $P_{eff,app}$ value was regarded as representative for each individual rat.

2.6. Calculations of theoretical jejunal permeability values

To be able to discuss the minimum P_{eff} values that can be determined using luminal drug disappearance from the perfusate, theoretical luminal drug disappearances permeability ($P_{eff,dis}$) values have been calculated. The theoretical $P_{eff,dis}$ values were calculated with Eq. (3) and using standard SPIP model set-up parameters: perfusion rate of 0.2 mL/min, segment length of 10 cm, and a jejunal radii of 0.2 cm. Luminal drug disappearance from the perfusate between 0 and 99% was used to obtain a range of theoretical $P_{eff,dis}$ values. However, variations on the standard set-up can be found in literature. Consequently, theoretical $P_{eff,dis}$ values were calculated using the reported lowest and highest SPIP model set-up parameters: 0.05 mL/min flow rate and 10 cm segment length (low), or 1.0 mL/min flow rate and 15 segment length (high) [23,24].

2.7. Statistical analysis

The sample size in each study group (intra/extra and with/without

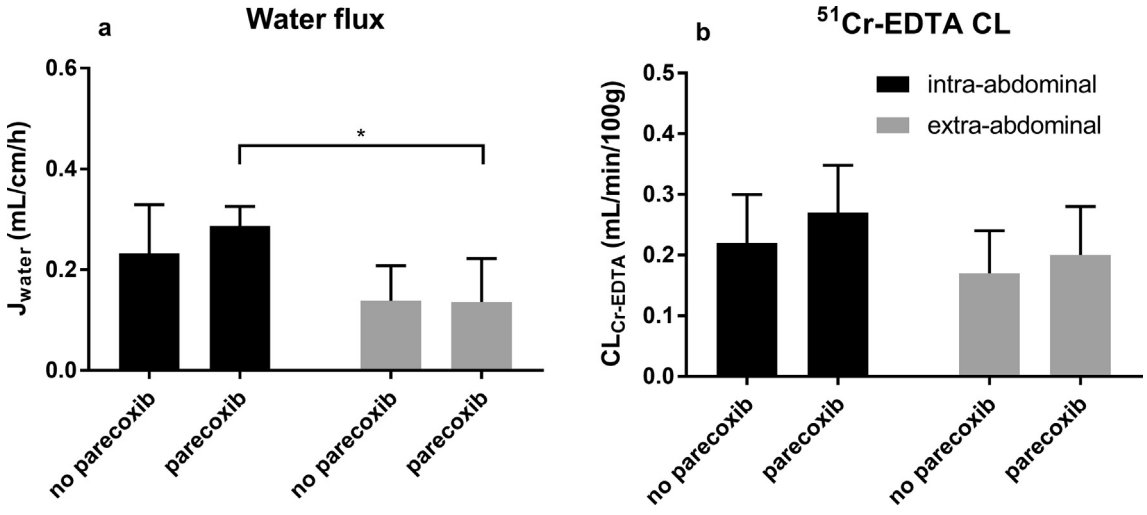

Fig. 1. The mean ± SD jejunal rat (n = 6) absorptive (a) water flux (J_{water}) and (b) ^{51}Cr-EDTA secretion ($CL_{Cr-EDTA}$) during intestinal perfusion with the intestinal segment placed inside or outside the abdomen, and with or without pretreatment with parecoxib. The * represents a significant ($p < 0.05$) difference in J_{water} and $CL_{Cr-EDTA}$ between the different groups—intra/extra-abdomen and parecoxib/no parecoxib—using Tukey's post hoc two-way ANOVA.

parecoxib) was six rats, on the basis of a previous perfusion study [10]. The $CL_{Cr-EDTA}$, model compound $P_{eff,dis}/P_{eff,app}$, and J_{water} are presented as the mean ± SD or SEM for the test period (40–100 min). The effect of the four experimental conditions (intra/extra and with/without parecoxib) on the $CL_{Cr-EDTA}$, J_{water}, and $P_{eff,dis}/P_{eff,app}$ of each model compound, were compared using a two-way ANOVA with a post-hoc Tukeýs multiple comparisons test.

3. Results

The effect of both position of the intestine and of selective COX-2 inhibition (parecoxib), on epithelial water flux, jejunal clearance of ^{51}Cr-EDTA, and jejunal drug permeability were investigated.

3.1. Epithelial water flux

The mean ± SD absorptive J_{water} (40–100 min) for the intra-abdominally placed intestine with parecoxib and without parecoxib were 0.287 ± 0.039 mL/cm/h and 0.232 ± 0.097 mL/cm/h, respectively (Fig. 1a). J_{water} was lower ($p < 0.05$) for the extra-abdominally placed intestine when treated with parecoxib 0.136 ± 0.086 mL/cm/h, but not without parecoxib treatment (0.138 ± 0.070 mL/cm/h).

3.2. Blood-to-lumen jejunal clearance of ^{51}Cr-EDTA ($CL_{Cr-EDTA}$)

The mean ± SD $CL_{Cr-EDTA}$ (40–100 min) values for intestine placed inside the abdomen were 0.24 ± 0.08 mL/cm/h (parecoxib) and 0.20 ± 0.07 mL/cm/h (no parecoxib) (Fig. 1b). The corresponding values for extra-abdominally placed intestine were 0.19 ± 0.07 mL/cm/h (parecoxib) and 0.16 ± 0.06 mL/cm/h (no parecoxib). There was no effect on $CL_{Cr-EDTA}$ from placement of the intestine or treatment with parecoxib.

3.3. Jejunal lumen-to-blood permeability ($P_{eff,dis}/P_{eff,app}$) for the model compounds

Jejunal permeability values, based on luminal disappearance ($P_{eff,dis}$), are presented in Table 2 for atenolol, enalaprilat, ketoprofen, and metoprolol. $P_{eff,dis}$ were determined three ways: with and without inclusion of negative values, and by setting negative values to zero. The $P_{eff,dis}$ values were higher when the negative values were excluded or set to zero for the two low-permeability compounds, atenolol and enalaprilat (Table 2). There were no differences in $P_{eff,dis}$ values for the high permeability compounds, ketoprofen and metoprolol, when comparing the different numerical approaches.

The permeability values based on plasma appearance ($P_{eff,app}$), are presented in Table 3. The $P_{eff,dis}$ values were in general several-fold higher than the $P_{eff,app}$ ones for enalaprilat, at all experimental conditions, and for atenolol at one experimental condition. This relationship is presented as the $P_{eff,dis}/P_{eff,app}$ ratio in Fig. 2. These differences in $P_{eff,dis}/P_{eff,app}$ ratio were even more pronounced when the negative values were excluded or set to zero. There were no differences between $P_{eff,dis}$ and $P_{eff,app}$ for the high permeability drugs.

There was no effect on the $P_{eff,dis}$ or $P_{eff,app}$ of any model compound regardless of abdominal placement (intra/extra) or treatment/non-treatment with parecoxib.

3.4. Theoretical $P_{eff,dis}$ values at different luminal disappearances

The theoretical $P_{eff,dis}$ values calculated at luminal disappearances between 0 and 99% are presented in Fig. 3. Fig. 3 also illustrates the luminal disappearance at which permeability is ideally determined from plasma appearance rather than luminal disappearance at standardized experimental conditions (perfusion rate of 0.2 mL/min, segment length of 10 cm): A value of 10% absorbed equates with a permeability of 0.28×10^{-4} cm/s. The corresponding $P_{eff,dis}$ values at lowest (0.05 mL/min, 10 cm) and highest (1.0 mL/min, 15 cm) reported experimental conditions were 0.07 and 0.9×10^{-4} cm/s, respectively [23,24].

4. Discussion

One objective of this rat *in vivo* study was to investigate the effect of intra- vs. extra-abdominal single-pass intestinal perfusion (SPIP), and with or without pretreatment by parecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor. The effect was evaluated by determining the difference in blood-to-lumen ^{51}Cr-EDTA clearance ($CL_{Cr-EDTA}$), lumen-to-blood jejunal permeability of a cassette-dose of four model compounds (atenolol, enalaprilat, ketoprofen, and metoprolol), and water flux (J_{water}). Another objective was to compare the jejunal values of the model drugs when determined by luminal disappearance ($P_{eff,dis}$) or plasma appearance ($P_{eff,app}$).

The theoretical rat jejunal $P_{eff,dis}$ values (Fig. 3) concludes that a $P_{eff,dis}$ of 0.28×10^{-4} cm/s corresponds to a 10% difference in the concentration leaving (C_{out}) vs. entering (C_{in}) calculated for a standard SPIP model set-up (a jejunal segment of 10 cm, radii of 0.2 cm,

Table 2
The mean ± SD effective permeability (P_{eff}) values for the four model compounds. Values were determined by luminal disappearance during single-pass jejunal perfusion under four experimental conditions: with the intestinal segment placed inside or outside the abdominal cavity, and with (+) or without (−) pretreatment with parecoxib. Individual negative $P_{eff,dis}$ values were calculated three ways: included in the mean value, excluded, or set to zero.

Conditions	Luminal disappearance $P_{eff,dis}$ ($\times 10^{-4}$ cm/s)			
	Atenolol	Enalaprilat	Ketoprofen	Metoprolol
Negative values included				
Intra-abdominal; + parecoxib	0.30 ± 0.7	0.21 ± 0.12	4.1 ± 0.56	1.3 ± 0.2
Intra-abdominal; − parecoxib	0.13 ± 0.61	−0.13 ± 0.37	3.3 ± 2.5	0.66 ± 0.51
Extra-abdominal; + parecoxib	0.13 ± 0.23	0.36 ± 0.4	1.9 ± 0.86	0.8 ± 0.27
Extra-abdominal; − parecoxib	0.54 ± 0.88	0.6 ± 0.79	2.5 ± 1	1.2 ± 0.65
Negative values excluded				
Intra-abdominal; + parecoxib	0.94 ± 0.91	0.45 ± 0.36	4.1 ± 0.56	1.3 ± 0.2
Intra-abdominal; − parecoxib	0.8 ± 0.43	0.27 ± 0.24	3.3 ± 2.5	0.87 ± 0.45
Extra-abdominal; + parecoxib	0.64 ± 0.37	0.76 ± 0.39	1.9 ± 0.86	0.87 ± 0.4
Extra-abdominal; − parecoxib	1 ± 0.72	0.87 ± 0.73	2 ± 0.99	1 ± 0.68
Negative values set to zero				
Intra-abdominal; + parecoxib	0.45 ± 0.49	0.21 ± 0.12	4.1 ± 0.56	1.3 ± 0.2
Intra-abdominal; − parecoxib	0.3 ± 0.45	0.095 ± 0.13	3.3 ± 2.5	0.66 ± 0.51
Extra-abdominal; + parecoxib	0.16 ± 0.18	0.38 ± 0.37	1.9 ± 0.86	0.8 ± 0.27
Extra-abdominal; − parecoxib	0.68 ± 0.65	0.66 ± 0.7	2.5 ± 1	1.2 ± 0.65

Table 3
The mean ± SD rat appearance permeability ($P_{eff,app}$) values for the four model compounds. Values were determined by plasma drug appearance under four experimental conditions: with the intestinal segment placed inside or outside the abdominal cavity, and with (+) or without (−) pretreatment with parecoxib.

Conditions	Plasma appearance $P_{eff,app}$ ($\times 10^{-4}$ cm/s)			
	Atenolol	Enalaprilat	Ketoprofen	Metoprolol
intra-abdominal; + parecoxib	0.15 ± 0.06	0.015 ± 0.004	3.1 ± 1.1	1.2 ± 0.56
intra-abdominal; − parecoxib	0.19 ± 0.11	0.012 ± 0.003	3.4 ± 1.6	0.91 ± 0.43
extra-abdominal; + parecoxib	0.13 ± 0.12	0.011 ± 0.007	1.6 ± 1.5	0.8 ± 0.64
extra-abdominal; − parecoxib	0.06 ± 0.03	0.010 ± 0.005	2.3 ± 0.9	0.43 ± 0.27

perfusion rate 0.2 mL/min). This is a limitation, as the quantification of drug in any matrix is associated with an uncertainty that is usually greater than ± 10%; an error of ± 15% is allowed in the FDA guidelines for bioanalytical method validation [25]. Consequently, accurate $P_{eff,dis}$ determinations are difficult for compounds with a low membrane permeability in the rat SPIP model (i.e. based on too small difference between entering and leaving concertation). Some individual measurements end up with a C_{in} that is lower than the C_{out} value, even after volume correction due to fluid flux, as was evident for one enalaprilat group in this paper (Table 2). This issue is often managed by ignoring the negative values in the mean $P_{eff,dis}$ calculations, or by setting them to zero. However, this results in a bias towards higher $P_{eff,dis}$ values for the low permeation compounds, which was also seen in this study. For the two low permeability compounds, atenolol and enalaprilat, the $P_{eff,dis}$ values were 2–3 times higher when the negative values were excluded, and 1–3 times higher when negative values were set to zero at all experimental conditions (Table 2). Further, the $P_{eff,dis}$ values of these two compounds could not be differentiated from the high permeation compound, metoprolol, when negative values were excluded or set to zero. There was no difference in $P_{eff,dis}$ for the high permeation compounds, metoprolol and ketoprofen, regardless of how the negative values were handled. In conclusion, it was not possible to differentiate between the low and high permeation compounds when negative values were excluded or set to zero in the calculations. Therefore, we recommend that negative values should be included in the calculation of $P_{eff,dis}$ based on luminal disappearance for the SPIP model.

The accuracy of the permeability determination for low permeation compounds can be improved by using the plasma drug appearance ($P_{eff,app}$) with an intravenous reference dose, rather than the luminal disappearance. A $P_{eff,app}$ value is calculated from a deconvoluted intestinal absorption rate by using the luminal concentration, and a correction for gut-wall and liver first-pass extraction with the intravenous dose [15]. This method requires a higher luminal drug concentration, but can determine the permeability of low permeability compounds with an improved accuracy compared to luminal disappearance data [15]. For example, the difference between a C_{out} of 99% or 98% of C_{in} is difficult to quantify accurately. However, this small difference corresponds to a 100% difference in plasma exposure, which is readily quantified. Accordingly, the $P_{eff,dis}$ values (negative values included) were 0.7–9.0 times higher for atenolol, and 14–59 times higher for enalaprilat, than their $P_{eff,app}$ values (Fig. 2). As a comparison, the corresponding human $P_{eff,dis}$ and $P_{eff,app}$ values of atenolol were 0.2 and 0.45×10^{-4} cm/s, which also suggests that variability in the disappearance estimate needs to be carefully considered [17,26]. The $P_{eff,app}$ values in this SPIP study were also in agreement with those previously determined following an intraintestinal bolus administration to rat [27]. Further, the variability was substantially lower for the $P_{eff,app}$ values (CV between 23% and 95%), than for the $P_{eff,dis}$ values (58% and 468%). For the high permeation compounds, metoprolol and ketoprofen, there were only small differences in the permeability values based on luminal disappearance and plasma appearance. This observation is supported by the corresponding comparison between disappearance and appearance made for these two high permeability drugs in humans [17,26]. These results strongly suggest that determinations of permeability values of low permeation compounds in the SPIP model should be performed on the basis of plasma appearance (Fig. 3). For high permeation compounds, both methods are valid. Therefore, the rest of this study used the values determined on plasma appearance.

Single-pass intestinal perfusion (SPIP) experiments in rat are typically performed with the intestinal segment placed back into the abdominal cavity. The rationale for this approach is that it reflects the physiological condition. However, it may also cause intestinal folding that prevents a continuous and steady perfusate flow. This leads to an increased intraluminal hydrostatic pressure with potentially deleterious effects on membrane permeability and intestinal physiology. Therefore, our group has shifted to performing SPIP experiments with the intestinal segment placed on the abdomen, heated to 37 °C with a lamp

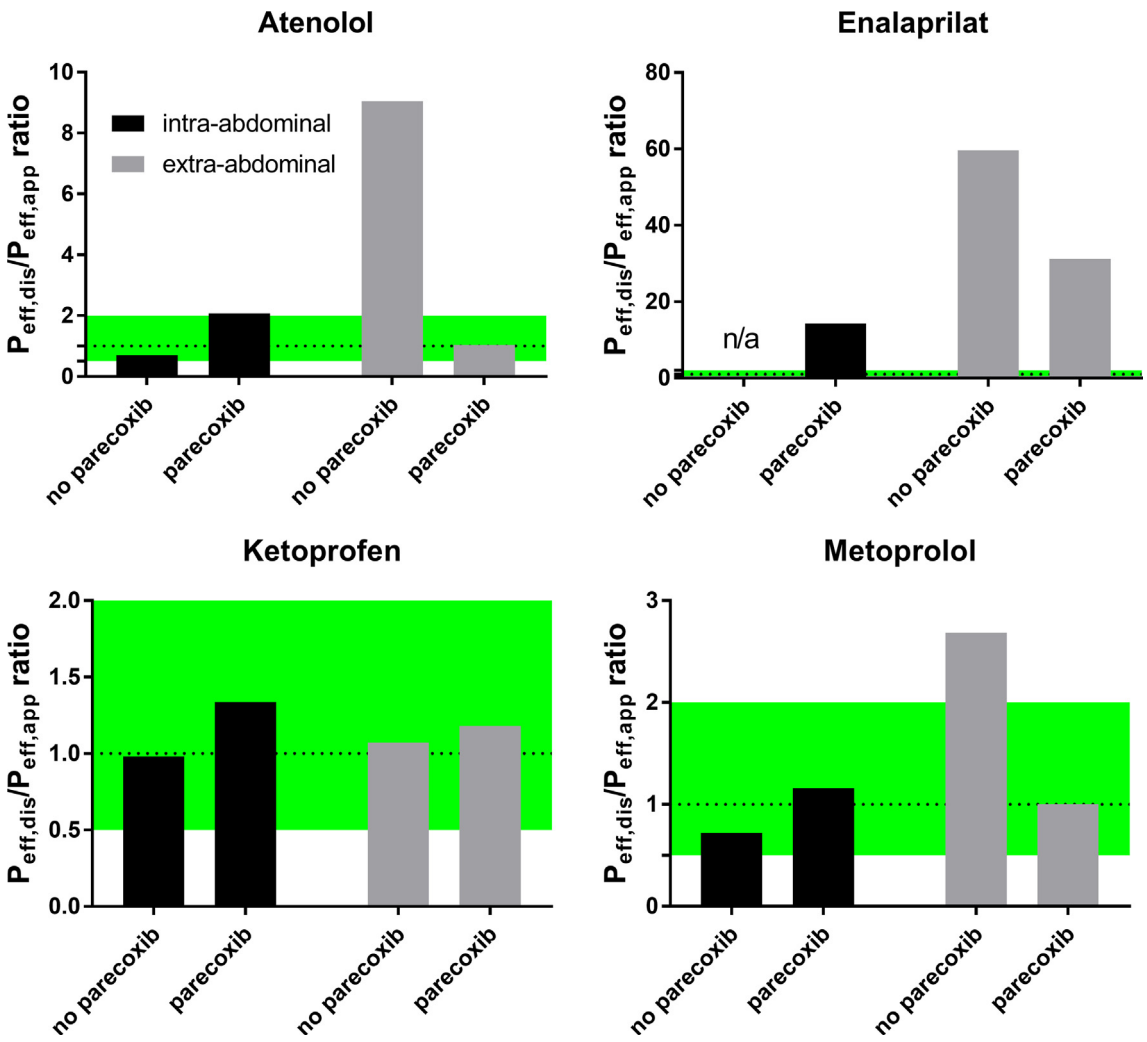

Fig. 2. The ratio between the rat single-pass jejunal permeability of the four model compounds, as determined from luminal disappearance ($P_{eff,dis}$) with negative values included in the calculation, and plasma appearance ($P_{eff,app}$) (Tables 2 and 3). The dashed line illustrates that there was no difference in permeability for the two methods. The green zone illustrates a twofold error in permeability ratio. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and heating pad, and covered in saran wrap to prevent drying. Previously, we have looked at the SPIP data generated for the absorptive flux of the high permeation model drug, aprepitant (as a 200 μM nanosuspension in buffer). For this drug, there was greater variability between animals when perfusions were performed extra-abdominally (CV% = 55), compared to intra-abdominally (CV% = 10) [4,28]. However, this observation was not repeatable in this study, as there was no difference in $CL_{Cr\text{-}EDTA}$ or model drug $P_{eff,app}$. Consequently, the greater variability observed for aprepitant does not seem to be related to any effects on the epithelial membrane from placing the intestinal segment on the abdomen. Instead, the potential interaction of the nanoparticles with the mucus layer may contribute to the inter-animal variability [28]. It is also possible that it relates to altered hydrodynamics induced by the slightly higher water absorption observed when the intestinal segment was placed inside the abdomen [29]. The potential effect of mucus and water flux on the absorption of nanosized aprepitant in the rat SPIP model therefore remains to be investigated.

Extra-abdominal SPIP allows visual monitoring of the intestinal motility. This could speed up and simplify analysis of the intestinal motility by biochemical factors in the perfusate, and systemically and/or locally injected hormones (such as GLP-1) [30]. It also enables the evaluation of minor bowel movements, which are not readily detected with conventional motility apparatus, such as pressure transducers placed in the perfused segment. However, any type of evaluation of intestinal motility following open abdominal surgery requires treatment in order to reverse the surgery-induced postoperative ileus. COX-inhibitors have been shown to be effective to abolish this condition, as surgery induce endogenous production of prostaglandins via an upregulation of COX [5]. These prostaglandins inhibit enteric nerve activity in the intestines, which is a condition called postoperative ileus. To circumvent this, rats can be treated with a COX-2 inhibitor following the surgery. Parecoxib is a commonly used drug for this purpose, and its administration restores normal bowel motility and mucosal response to hypotonicity in the rat SPIP model [7].

However, the direct effect of parecoxib on mucosal membrane permeability during intra- and extra-abdominal surgery has not been previously investigated until this study. We found that parecoxib had no effect on either membrane permeability or water flux, regardless of intestinal placement. This indicates that extra-abdominal SPIP in conjunction with parecoxib is a viable method for investigating intestinal motor function, with minimal effects on the membrane barrier. We are currently evaluating motor functions determined with extra-abdominal continuous monitoring, and an intra-abdominal pressure inducer. These studies have been initiated to further validate methods of intestinal motility determinations in our laboratory.

In conclusion, this study clearly showed that disappearance of drugs

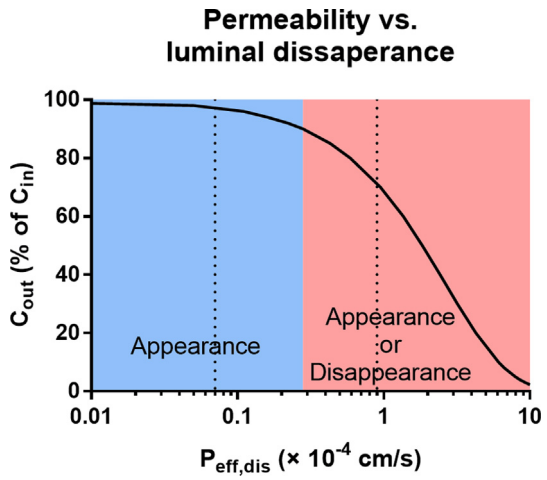

Fig. 3. Theoretical jejunal permeability ($P_{eff,dis}$) of a compound in the rat SPIP model, as determined by the difference in drug concentration of the solution entering (C_{in}) and leaving (C_{out}) the intestinal segment at standardized experimental conditions (segment length 10 cm, luminal radii 0.2 cm, and perfusion rate 0.2 mL/min). A drug with a $P_{eff,dis}$ value below approximately 0.28×10^{-4} cm/s (10% drug absorbed) should ideally be determined on the basis of its plasma appearance (blue). If the drug has a $P_{eff,dis}$ value above this cut-off, it can be equally well determined by either luminal disappearance or plasma appearance (red). The corresponding $P_{eff,dis}$ values at the lowest (0.05 mL/min, 10 cm) and highest (1.0 mL/min, 15 cm) reported experimental conditions were 0.07 and 0.9×10^{-4} cm/s, respectively [23,24]. These values are indicated by dashed lines in the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with a high jejunal membrane permeability from a perfused segment correlated well with the appearance of the drug in plasma. For low permeability compounds, permeability data calculated from plasma appearance were superior to those based on luminal disappearance. To improve accuracy of the permeability values based on luminal disappearance, they should include negative values. It was also shown that the position of the perfused segment inside or outside the abdominal cavity, or the treatment of post-surgery ileus with parecoxib, had minimal effects on membrane permeability of water flux. In future SPIP studies, both plasma and luminal drug data should be used to increase the accuracy of the model. Treatment with parecoxib and positioning of the intestinal segment outside the abdominal cavity should also be implemented.

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