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Research paper

Effects of absorption-modifying excipients on jejunal drug absorption in simulated fasted and fed luminal conditions

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A B S T R A C T

Oral administration of drug products is the preferred administration route. In recent decades there has been an increase in drug candidates with low solubility and/or low permeability. To increase the possibility of oral administration for the poorly permeating drugs, the use of absorption modifying excipients (AMEs) has been proposed. These types of AMEs may also affect the regulatory assessment of a novel drug delivery system if they affect the absorption of a drug from any of the four BCS classes. The effects of AMEs have previously been investigated in various animal models, including the single-pass intestinal perfusion (SPIP) in rats. To further improve the biorelevance and the *in vivo* predictiveness of the SPIP model, four compounds (atenolol, enalaprilat, ketoprofen, metoprolol) were perfused in fasted or fed state simulated intestinal fluid (FaSSIF or FeSSIF) together with the AMEs *N*-acetyl-cysteine, caprate, or sodium dodecyl sulfate. For the highly soluble and poorly permeating compounds enalaprilat and atenolol (BCS class III), the flux was increased the most by the addition of SDS in both FaSSIF and FeSSIF. For ketoprofen (BCS class II), the flux decreased in the presence of all AMEs in at least one of the perfusion media. The flux of metoprolol (BCS class I) was not affected by any of the excipients in none of simulated prandial states. The changes in magnitude in the absorption of the compounds were in general smaller in FeSSIF than in FaSSIF. This may be explained by a reduced free concentration AMEs in FeSSIF. Further, the results in FeSSIF were similar to those from intrajejunal bolus administration in rat in a previous study. This suggests that the biorelevance of the SPIP method may be increased when investigating the effects of AMEs, by the addition of intraluminal constituents representative to fasted and/or fed state to the inlet perfusate.

1. Introduction

Active pharmaceutical ingredients (APIs) with a low molecular mass (approximately smaller than 500 Da) are preferably given orally to patients for convenience and compliance [1]. However, there are an increasing numbers of APIs with less than optimal biopharmaceutical properties, extending beyond Lipinskis “rule-of-5” [2–4]. A large proportion of these molecules have low solubility and/or low permeability and belong to classes II-IV of the biopharmaceutics classification system (BCS) [5,6]. There are few possible biopharmaceutical strategies for drugs with low and variable intestinal permeation. The most common approach includes changing the molecular structure, for instance by masking polar properties, which may also change the pharmacokinetics and the pharmacological potency of the drug. Clearly, it would be more desirable to increase the potential for intestinal permeation of the API. Intestinal permeability may be increased by using pharmaceutical excipients that increase the transport over the enterocytes [7]. These excipients are usually labelled absorption modifying excipients (AMEs) or permeation enhancers (PEs) [7,8]. The two most common mechanisms of action for AMEs are either altering the fluidity/integrity of the apical membrane of the enterocytes, which facilitates passive transcellular diffusion, or increasing the space of the tight junctions, thus increasing the paracellular diffusion [9]. The main concerns with AMEs are safety, because a reduced barrier integrity may facilitate bacterial and/or viral translocation, and potentially increase the unwanted absorption of xenobiotics, pathogens and dietary antigens [9,10]. A dysregulation of the intestinal epithelial cell barrier may also contribute to pathogenesis, such as inflammatory bowel disease [11].

We have previously investigated the effect of AMEs on jejunal absorption of a set of APIs (Table 1) with different physicochemical properties in buffer using the rat single-pass intestinal perfusion (SPIP) model [12–14]. The SPIP model has been shown to predict human intestinal permeability and fraction dose absorbed with a high accuracy [15–17]. The AMEs and their concentrations in the perfusion media were selected based on clinical and pre-clinical experiences 9, 14, 18. The appearance drug flux (J_{app}) in plasma for the poorly permeating compounds investigated was substantially increased by the AMEs’ effects on the mucosal barrier, with a concentration-dependent enhancement of the transcellular routes. It was also found that the SPIP model was less predictive of the *in vivo* effect of AMEs from bolus dosing

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Table 1
Physicochemical properties of the four study drugs [16].

Substance (BCS class)	MW (g/mol)	pK_a	PSA	Log P	Log $D_{6.5}$
Atenolol (III)	266	9.6[b]	88.1	0.18	< −2.0
Enalaprilat (III)	348	3.2[b]/7.8[a]	102	−0.13	−1.0
Metoprolol (I)	267	9.7[b]	75.2	2.1	−0.5
Ketoprofen (II)	254	3.9[a]	54.2	3.37	0.8

BCS = Biopharmaceutics Classification System; Log $D_{6.5}$ = n-octanol–water coefficient at pH 6.5; Log P = n-octanol–water coefficient; MW = molecular weight; pKa = dissociation constant; PSA = polar surface area.
[a] Acid.
[b] Base.

into the proximal small intestine in both rat and dog [12]. These findings emphasize the need to improve the knowledge of the gastrointestinal (GI) factors influencing the effect of the AMEs on the intestinal absorption *in vivo* [12].

To further improve *in vivo* predictions from non-clinical models, the use of simulated intestinal fluids (SIF) has been proposed [19]. Fasted or fed state simulated intestinal fluids (FaSSIF and FeSSIF), containing bile acids and lecithin which form colloidal structures (CS), and have an osmolality and pH similar to that seen in human aspirated intestinal fluids, have been extensively investigated in dissolution testing [20–22]. These systems have been little used in *in vivo* relevant non-clinical intestinal absorption models, such as SPIP model, due to their complexity and concerns with viability of the intestinal membrane barrier [23,24]. However, it has recently been shown that the rat jejunum tolerated FaSSIF and FeSSIF without an increase in the blood-to-lumen clearance of the intestinal barrier integrity marker ^{51}Cr-EDTA [25]. In both FaSSIF and FeSSIF the luminal presence of CS alter the apparent solubility and free concentration of APIs [26]. The presence of CS may increase the rate and extent of intestinal absorption, for low solubility API monomers that are partitioning into and/or interacting with CS, with a subsequent increased transport across the aqueous boundary layer (ABL) [27,28]. However, for high solubility API monomers partitioning into and/or interacting with CS is high, this may result in a decreased absorption, and/or absorption rate [29,30].

The primary objective of this study was to investigate the effect of three AMEs (caprate, *N*-acetyl cysteine, and sodium dodecyl sulfate) on the intestinal absorption rate of four selected model drugs (atenolol, metoprolol, ketoprofen, and enalaprilat) using FaSSIF and FeSSIF and the single-pass jejunal perfusion in the rat. The secondary objective was to determine the effect on the clearance of an intestinal integrity marker (^{51}Cr-EDTA) from the blood to the intestinal lumen in the two prandial states.

2. Methods

2.1. Drugs and chemicals

Atenolol and metoprolol tartrate were provided by AstraZeneca (Gothenburg, Sweden). Ketoprofen, enalaprilat, maleic acid, sodium chloride, *N*-acetylcystein, sodium caprate, sodium dodecyl sulfate, and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, US). Freeze-dried FaSSIF-V2 and FeSSIF-V2 powder were purchased from Biorelevant.com (Croydon, UK). Water was purified using an ELGA Purelab Flex 2 (Marlow, UK). The reference standards and the internal standards were purchased from the following suppliers: ketoprofen, metoprolol tartrate, enalaprilat, atenolol, the internal standards ketoprofen-d3, metoprolol-d7 tartrate and atenolol-d7 were all purchased from Sigma-Aldrich, Darmstadt, Germany. The internal standard enalaprilat-d5 was bought from Toronto Research Chemicals, North York, Ontario, Canada.

Table 2
The compositions of fasted and fed state simulated intestinal fluids (FaSSIF and FeSSIF).

FaSSIF		FeSSIF	
Component	Concentration (mM)	Component	Concentration (mM)
Taurocholate	3	Taurocholate	10
Phospholipids	0.2	Phospholipids	2
Sodium	106	Sodium	218
Chloride	69	Chloride	125
Maleic acid	19	Maleic acid	55
		Oleate	0.8
		Glycerol monoleate	5

2.2. Preparation of study compositions

Pre-FaSSIF (FaSSIF before the final addition of bile acids and lecithin) was created by dissolving 2.23 g maleic acid, 4.01 g sodium chloride, and 1.39 g sodium hydroxide in 1000 ml purified water. Pre-FeSSIF (FeSSIF before the addition of bile acids and lecithin) was created by dissolving 6.39 g maleic acid, 7.33 g sodium chloride, and 3.27 g sodium hydroxide in 1000 ml purified water. 255.7, 328.7, 244.1, and 369.0 mg of atenolol, metoprolol tartrate, ketoprofen, and enalaprilat, respectively, were dissolved in either pre-FaSSIF or pre-FeSSIF. The final concentration of atenolol, metoprolol, ketoprofen, and enalaprilat in this stock solution were 2 mM. The solutions were dispensed into aliquots to prevent excessive freeze-thaw cycles, and stored at −20 °C until the day of use. The composition of FaSSIF and FeSSIF has been summarized in Table 2.

A fresh batch of FaSSIF or FeSSIF was produced each day by mixing 20 ml thawed stock solution of atenolol, metoprolol, ketoprofen, and enalaprilate with 180 ml pre-FaSSIF/FeSSIF. 0.36/1.95 g of FaSSIF/FeSSIF powder (containing bile acids, lecithin, and in the case of FeSSIF also glycerol monoleate and oleate) was added to the solution, and mixed using a magnetic stirrer until everything was dissolved. The resulting final FaSSIF/FeSSIF was allowed to equilibrate for 1 h at room temperature without mixing. After equilibration, the mixture was dispensed into two flasks, the control and the study solution. 0.1 or 0.5 g of either *N*-acetylcystein (NAC), sodium caprate, or sodium dodecyl sulfate (SDS) was added to the study solution to produce either 0.1% or 0.5% w/w solution. This corresponds to 6.1 or 31 mM for NAC, 5.2 or 26 mM for sodium caprate, and 3.5 or 17 mM for SDS. The pH was adjusted with 1 M NaOH to 5.8 for FeSSIF and 6.5 for FaSSIF, for the composition labelled “0.5% NAC pH adj”. The buffer strength for FaSSIF and FeSSIF was 10 and 25 mM/ΔpH.

2.3. Animals and study design

The study was approved by the local ethics committee for animal research in Uppsala, Sweden (number C64/16). Six male Wistar Han rats (strain 273, Charles River, Germany), weighing 250–350 g, were included in each group. There were seven groups using FaSSIF and seven groups using FeSSIF, meaning the total number of groups investigated were 14, and the total number of animals used were 84. All animals arrived at the animal housing facility at the Biomedical Center (BMC) in Uppsala, Sweden at least one week prior to the experiment. They were kept with water and food ad libitum, on a 12:12 h light:dark cycle, at 21–22 °C temperature. All surgeries and experiments were performed at the BMC by well-trained persons. The surgery follows that of several previously published studies [14,28,31,32]. The animals were anaesthetized by an intraperitoneal injection of Inactin© (10% w/w solution, 160 mg/kg body weight). Each animal was placed on a heating pad, under an infrared lamp. Body temperature was monitored by a rectal probe, and was kept at 37.5 °C throughout the entire experiment. To facilitate breathing, a tracheotomy was performed using a

poly-ethylene tube (I.D. 1.4 mm, O.D. 1.9 mm). The right femoral artery and vein were cannulated with poly-ethylene catheters (Becton Dickinson, Franklin Lakes, NJ). The arterial catheter was filled with 20 IU/ml heparin saline solution, and was connected to a transducer operating a PowerLab system (AD Instruments, Hastings, UK), monitoring the blood pressure throughout the entire experiment. A midline incision of the abdomen of approximately 5 cm was made and a ~10 cm segment of the jejunum was cannulated with silicone tubing (Silastic, 1.0 mm I.D., Corning, Midland, MI). The jejunal segment was carefully rinsed (4 ml/min) with 37 °C saline solution until a clear perfusate was attained, to ensure removal any excess mucus and undigested foodstuff. The bile duct was cannulated 2–3 mm before connecting to the intestine, to prevent build up of pancreatobiliary secretions in front of the cannulated intestinal segment and to reduce the risk of endogenous bile acids entering the segment. Immediately after the completion of the surgery, a bolus injection of ^{51}Cr-EDTA (75 μCi, 4 ml) was given through the catheter in the right femoral vein, which was followed by a constant infusion of a solution of ^{51}Cr-EDTA (50 μCi/h, 1 ml/h) for the remainder of the experiment. At the same time, an intrajejunal perfusion of 37 °C pre-FaSSIF was commenced (0.2 ml/min) to allow for stabilization of the jejunal segment and for physiological functions to normalize. This step also allowed for a stable plasma concentration of ^{51}Cr-EDTA to be established. After 30 min, a perfusion rate of 4 ml/min of atenolol, ketoprofen, enalaprilat, and metoprolol dissolved in either FaSSIF or FeSSIF was commenced to fill the segment, followed by a single-pass perfusion (0.2 ml/min) of the same media (37 °C). The first 75 min were labelled as the control period. After 75 min the perfusate media was changed to an identical media, with the addition of either 0.1% or 0.5% of AME (caprate, NAC, or SDS). The study period media was first perfused at 4 ml/min to fill the segment, and then single-passed perfused at 0.2 ml/min for 75 min. The total experiment time was 150 min. Perfusate was quantitatively collected in 15 min fractions. Blood samples of 300 μL were collected in Li-Heparin tubes (Sarstedt, Nümbrecht, Germany) before the start of the test period, and every 15 min during the experiment. The blood samples were centrifuged (4 °C, 3 min, 5000*g*) immediately after being taken. The plasma was transferred to Eppendorf tubes and stored at −20 °C, until analysis. In total 11 blood samples per rat were taken. Equivalent volumes of a 7% bovine serum albumin in saline solution was given to prevent any negative effects of a reduction in blood volume.

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

The ^{51}Cr-activity (cpm, counts per minute) in all perfusate samples and the blood plasma samples at time 0 and 150 min were determined on a gamma counter (1282 Compugamma CS, Pharmacia AB, Uppsala, Sweden). A linear regression was made from the ^{51}Cr-EDTA activity of the plasma sample at time 0 to the ^{51}Cr-EDTA activity of the plasma sample at time 150 min, to obtain a time-corresponding blood-value to the perfusate sample. The blood-to-lumen ^{51}Cr-EDTA clearance ($CL_{Cr\text{-}EDTA}$) was calculated according to Eq. (1):

$$CL_{Cr-EDTA} = \frac{C_{perfusate} \times Q_{in}}{C_{plasma} \times tissue\ \ weight} \times 100 \quad (1)$$

where $C_{perfusate}$ is the ^{51}Cr-EDTA activity (cpm/ml) in the perfusate, Q_{in} is the perfusion rate of the perfusate, C_{plasma} is the ^{51}Cr-EDTA activity in the blood plasma from the linear regression (cpm/ml), and tissue weight is the weight of the perfused jejunal segment, measured *ex vivo*.

2.5. Bioanalytical method

The quantitative analyses of ketoprofen, metoprolol, enalaprilat, and atenolol in plasma were carried out using ultra-high performance liquid chromatography coupled to tandem quadrupole mass spectrometry (UHPLC-MS/MS) at the National Veterinary Institute (SVA) in Uppsala, Sweden.

A portion of 20 μL plasma (calibrator, QC or study sample) was mixed with 100 μL of the internal standard solution containing a mixture of ketoprofen-d3 (170 nM), metoprolol-d7 (2.4 nM), enalaprilat-d5 (14.9 nM) and atenolol-d7 (22 nM) in methanol in 96 well plates. The plates were vortex-mixed for 10 min, prior centrifugation for 10 min at 10 000*g*. The supernatants were injected into a UHPLC-MS/MS system consisting of an Acquity UPLC coupled to a TQS micro tandem quadrupole mass spectrometer (Waters Corporation, Milford, MA). The ionization mode was positive electrospray and the injection volume was 5 μL. The analytical separation was carried out using an Acquity Ethylene Bridged Hybrid C18 column (length 50 mm, I.D. 2.1 mm, particle size 1.7 μm) kept at 40 °C at a flow-rate of 600 μL/min. The mobile phase was composed of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The gradient was as follows: initially at 2.0% B for 0.30 min, increase to 90% B over 1.7 min, increase to 95% B over 0.10 min, constant at 95% B for 0.40 min, decrease to 2.0% B over 0.10 min, and finally held constant at 2.0% B for 0.40 min. The total run time was 3.0 mins.

The four analytes were quantified simultaneously in the same chromatographic run using a positive capillary voltage of 1.5 kV. The desolvation and source block temperatures were 350 °C and 150 °C, respectively, and desolvation gas flow was 650 L/h. The quantifications were performed in the selected reaction monitoring (SRM) mode with the collision cell filled with argon gas at a pressure of 1.3×10^{-5} mBar. The mass transitions used in SRM were m/z 255 → 105 for ketoprofen (collision energy 6 eV), m/z 258 → 105 for ketoprofen-d3 (collision energy 10 eV), m/z 268 → 116 for metoprolol (collision energy 16 eV), m/z 275 → 123 for metoprolol-d7 (18 eV), 349 → 206 for enalaprilat (collision energy 16 eV), 354 → 211 for enalaprilat-d5 (collision energy 16 eV), 267 → 145 for atenolol (collision energy 26 eV) and 274 → 190 for atenolol-d7 (collision energy 18 eV). The dwell time was 0.039 sec. The calibration range for ketoprofen was 0.15–65.7 μM, the precision (relative standard deviation) range was 3.5–5.0%, and the accuracy range was 102–109%. The calibration range for metoprolol was 0.20–102 nM, the precision range was 4.2–8.2%, and the accuracy range was 103–107%. The calibration range for enalaprilat was 1.0–181 nM, the precision range was 4.0–5.1%, and the accuracy range was 99–103%. The calibration range for atenolol was 3.0–243 nM, the precision range was 4.1–11%, and the accuracy range was 96–100%.

2.6. Small intestinal in vivo flux calculations (J_{app})

The flux of drug from the jejunum into the system circulation was calculated based on the blood plasma concentrations. The process has been described in detail elsewhere [33]. Briefly, a deconvolution method was used to calculate an input rate from the plasma concentration-time profiles in this study, and by using the intravenous disposition PK parameters from Dahlgren et al in 2017 as unit impulse response [14]. The absorption flux (J_{app}) was calculated using Eq. (2):

$$J_{app} = \frac{input\ \ rate}{2\pi rL} \quad (2)$$

where L is the length of the individual perfused segment (measured *in vivo*) and r is the radius of the jejunum (0.2 cm). For the control period J_{app} was calculated from the first plasma concentration above limit of quantification (typically 15 min for ketoprofen, enalaprilat and atenolol, and 30 min for metoprolol) until 75 min. For the study period J_{app} was calculated between 90 and 150 min.

2.7. Statistical analysis

Differences in ratios between study period and control period for both ^{51}Cr-EDTA CL and absorption flux (J_{app}) were evaluated with unpaired t-tests, with Holm- Šídák multiple comparisons adjustment. Differences in both the ^{51}Cr-EDTA CL ratio and absorption flux (J_{app})

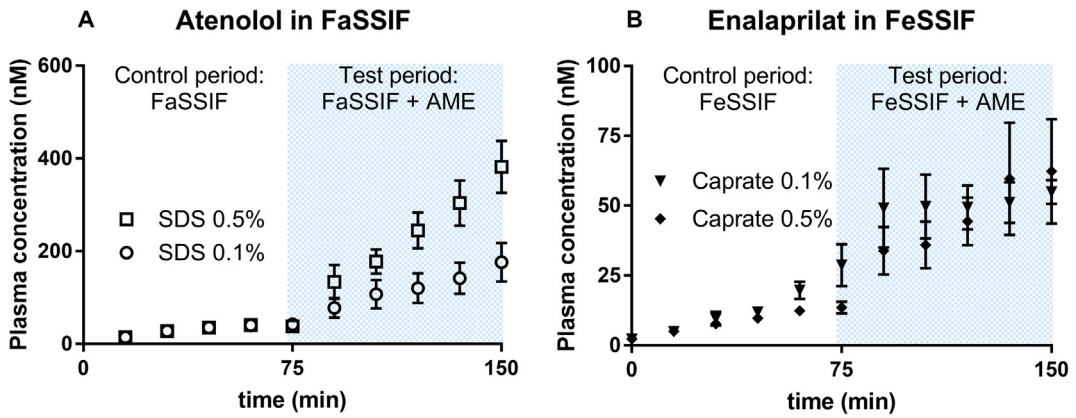

Fig. 1. (a) Representative mean (± SEM) plasma concentration-time profiles for atenolol when perfused with fasted state simulated intestinal fluid (FaSSIF), and the absorption-modifying excipient (AME), sodium dodecyl sulfate (SDS). (b) Representative plasma concentration-time profiles for enalaprilat when perfused with fed state simulated intestinal fluid (FeSSIF), and the AME, sodium caprate. The white area is the control period and the shaded area is the study period where the AMEs where added at 0.1 and 0.5% w/w. Values are shown as mean ± SEM (n = 6).

ratio between the same permeation enhancer and concentration in the two different media (FaSSIF and FeSSIF) were evaluated with unpaired *t*-tests, with Holm-Sidak multiple comparisons adjustment. Difference in the summarized control period J_{app} between FaSSIF and FeSSIF for the four compounds were determined with unpaired t-tests, with Holm-Sidak multiple comparisons adjustment. Differences were considered statistically significant if the p-value was less than 0.05.

3. Results

3.1. Small intestinal in vivo drug flux

Representative mean (± SEM) plasma concentration-time profiles for atenolol when perfused with FaSSIF and SDS, and enalaprilat when perfused with FeSSIF and caprate is illustrated in Fig. 1a and b, respectively. The mean (± SEM) intestinal absorption flux (J_{app}) for all four drugs from all control period experiments in FaSSIF and FeSSIF (i.e. without any AME added) are shown in Fig. 2, which predicts the potential food-drug interaction within the intestinal lumen. J_{app} was significantly higher in FaSSIF compared to FeSSIF for all four compounds. The ratios between J_{app} in FaSSIF to FeSSIF in the control periods were 1.9 for atenolol, 1.5 for enalaprilat, 1.4 for ketoprofen, and 13 for metoprolol.

The effects of the different AMEs in the two prandial states on drug absorption are shown in Fig. 3a–d. The flux ratios between the study and control periods are reported in Table 3. The J_{app} for atenolol increased 3.1- and 7.6-fold with the addition of 0.1% SDS and 0.5% SDS, respectively in FaSSIF (Table 3, Fig. 3a). The corresponding increasing data in FeSSIF were 3.5- and 5.6-fold, respectively (Table 3, Fig. 3a). The J_{app} for enalaprilat was increased with the addition of 0.5% SDS in FaSSIF (89-fold increase) and with the addition of 0.1% and 0.5% SDS in FeSSIF (6.6- and 21-fold increases respectively) (Table 3, Fig. 3b). There were no significant differences between the control and the study periods for metoprolol in any of the prandial states (Table 3, Fig. 3c). For ketoprofen there was a significant decrease after addition of all excipients except for 0.5% NAC with pH adjustment to 6.5 in FaSSIF, while in FeSSIF, there was a decrease for all compositions containing NAC (0.1%, 0.5%, and 0.5% with pH adjustment to 5.8) and 0.1% caprate (Table 3, Fig. 3d). The only significant difference between the study period:control period ratios of J_{app} between FaSSIF and FeSSIF for any of the compounds was for enalaprilat, where the addition of 0.5% SDS increased the flux ratio in FaSSIF compared to FeSSIF (4.2-fold difference) (Table 3, Fig. 3b).

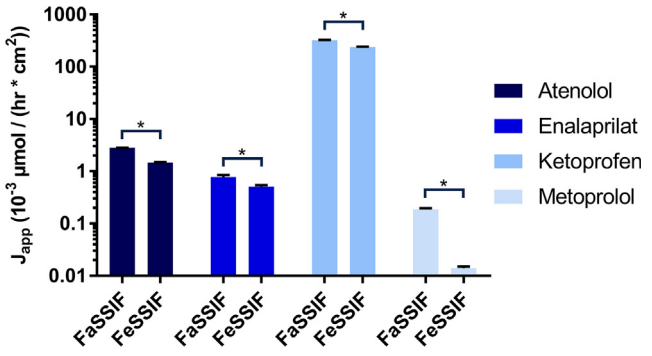

Fig. 2. Jejunal absorption flux (J_{app}) values (mean ± SEM) from the control period in all experiments of atenolol, enalaprilat, metoprolol, and ketoprofen. n = 42 for enalaprilat and ketoprofen in FaSSIF and FeSSIF, and for atenolol in FaSSIF. n = 41 for atenolol in FeSSIF. n = 28 and 32 for metoprolol in FaSSIF and FeSSIF, respectively. Asterisks * indicate statistical significance (p-value < 0.05).

3.2. Clearance of ^{51}Cr-EDTA from blood to lumen

The mean (± SEM) clearance values of ^{51}Cr-EDTA ($CL_{Cr\text{-}EDTA}$) from the blood to the intestinal lumen for the control periods and the study periods are shown in Fig. 4a and b. There was a significant increase in $CL_{Cr\text{-}EDTA}$ in FaSSIF for 0.5% caprate, 0.1% SDS, and 0.5% SDS compared to control, where the increase was 3.3, 2.5, and 7.4-fold, respectively (Fig. 4a). In FeSSIF there was a significant increase for 0.5% NAC and 0.5% SDS, with increases of 3.4 and 2.9 times, respectively. The $CL_{Cr\text{-}EDTA}$ increase was higher in FaSSIF than in FeSSIF for 0.5% SDS (7.4 vs 2.9) but lower in 0.5% NAC (1.5 vs 3.4) (Fig. 4b).

4. Discussion

This study examined the effect of three absorption-modifying excipients (AMEs) on the small intestinal absorption of four drug molecules with low molecular mass (< 350 Da) when single-passed perfused with simulated intestinal fluids. The AMEs investigated are all in clinical use or are being evaluated in pharmaceutical development of new oral drug products [9,14,18]. SDS is a synthetic anionic surfactant that has been shown to increase the transcellular transport of drug due interaction with and the disrupting the apical membrane of the enterocytes [34,35]. Caprate is an anionic fatty acid salt (C_{10}) that is considered a safe food additive [9]. Its mechanism(s) of action is not fully understood, but the current hypothesis is that it acts on both the paracellular and the transcellular pathways by interacting with tight junctions and by destabilizing the apical membrane of the enterocytes

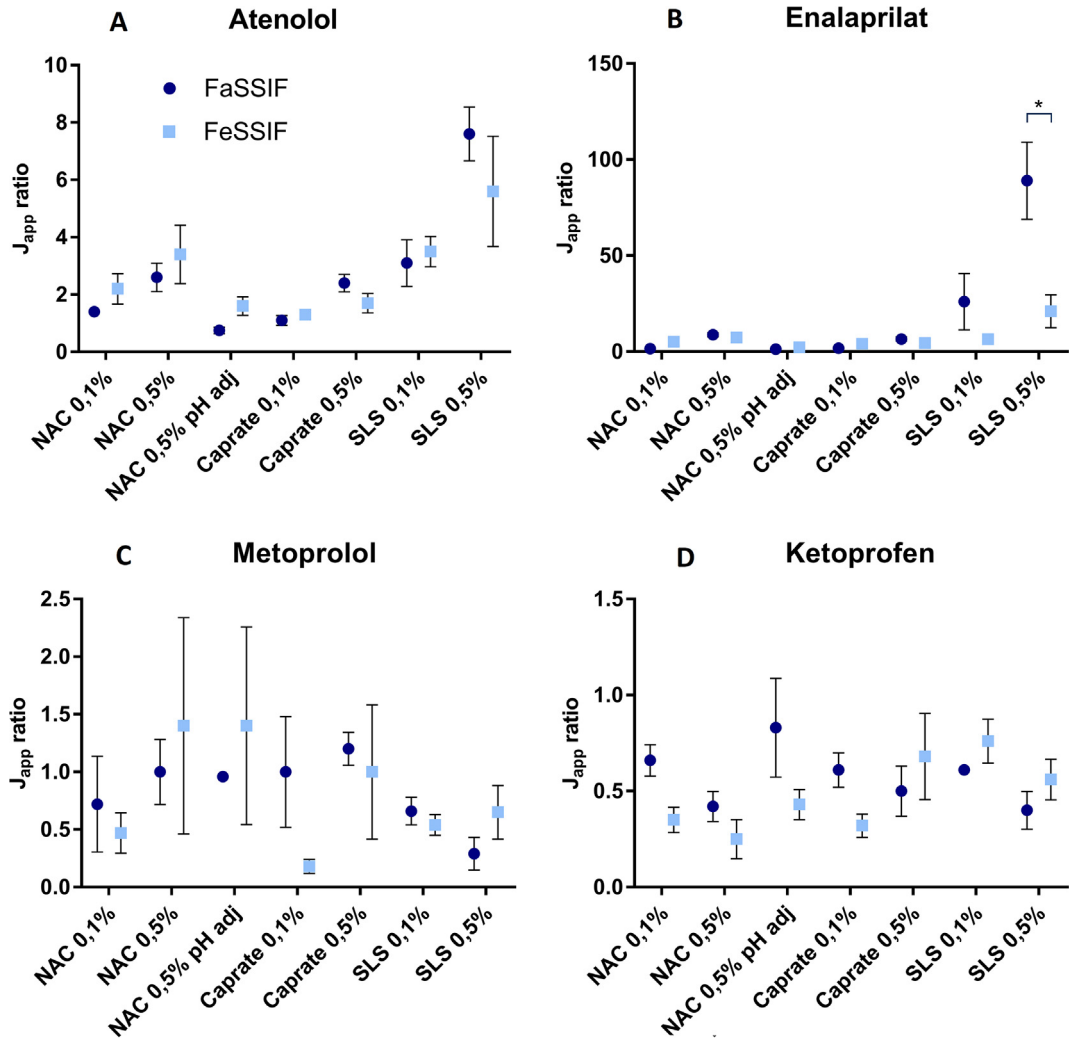

Fig. 3. Ratios between study and control periods jejunal absorption flux (J_{app}) for (a) atenolol, (b) enalaprilat, (c) metoprolol, and (d) ketoprofen in FaSSIF and FeSSIF. Values are shown as mean ± SEM. n = 42 for enalaprilat and ketoprofen in FaSSIF and FeSSIF, and for atenolol in FaSSIF. n = 41 for atenolol in FeSSIF. n = 28 and 32 for metoprolol in FaSSIF and FeSSIF, respectively. Asterisks * indicate statistical significance (p-value < 0.05).

[10]. *N*-acetyl-cysteine (NAC) is a mucolytic agent, which among other things is used to treat patients with cystic fibrosis. NAC breaks the disulfide bonds between mucins, and thereby reduces the thickness of the mucus layer [36]. The addition of 0.5% NAC reduced the pH of the perfusion media from 5.8 ± 0.0 to 3.7 ± 0.1 for FeSSIF and from 6.5 ± 0.0 to 3.3 ± 0.1 for FaSSIF. To be able to distinguish the luminal pH effect from the mucolytic effect, the perfusion media in the two groups had the pH adjusted with 1 M NaOH to 5.8 and 6.5 for FeSSIF and FaSSIF, respectively. The four drug molecules used in this study have previously been investigated simultaneously in a number of studies and have been shown to not influence each other's intestinal absorption or flux [12].

Table 3
Flux ratios (i.e. increase ratios) between the study period and the control period for the different APIs in the different compositions. Values are given as mean ± SD. n = 6 unless otherwise stated.

	Flux ratios							
	FaSSIF				**FeSSIF**			
	Atenolol	**Enalaprilat**	**Ketoprofen**	**Metoprolol**	**Atenolol**	**Enalaprilat**	**Ketoprofen**	**Metoprolol**
AME								
NAC 0.1%	1.4 ± 0.38	1.6 ± 0.76	0.66 ± 0.20*	0.72 ± 0.72[c]	2.2 ± 1.3	5.2 ± 5.3	0.35 ± 0.16*	0.47 ± 0.35[b]
NAC 0.5%	2.6 ± 1.1	8.8 ± 2.2[a]	0.42 ± 0.19*	1.0 ± 0.63[a]	3.4 ± 2.5	7.4 ± 6.3	0.25 ± 0.25*	1.4 ± 2.1[a]
NAC 0.5% ph adj	0.75 ± 0.27	1.3 ± 1.0	0.83 ± 0.63	0,96[e]	1.6 ± 0.79	2.3 ± 1.4	0.43 ± 0.19*	1.4 ± 2.1
Caprate 0.1%	1.1 ± 0.41[a]	1.8 ± 1.1	0.61 ± 0.22*	1.0 ± 0.83[c]	1.3 ± 0.40	4.1 ± 5.0	0.32 ± 0.15*	0.18 ± 0.15
Caprate 0.5%	2.4 ± 0.75	6.6 ± 2.6	0.50 ± 0.32*	1.2 ± 0.35	1.7 ± 0.83	4.5 ± 3.8	0.68 ± 0.55	1.0 ± 1.3[a]
SDS 0.1%	3.1 ± 2.0*	26 ± 36	0.61 ± 0.059*	0.66 ± 0.24[b]	3.5 ± 1.3*	6.6 ± 3.2*	0.76 ± 0.28	0.54 ± 0.20[a]
SDS 0.5%	7.6 ± 2.3[a*]	89 ± 49*	0.40 ± 0.24*	0.29 ± 0.20[d]	5.6 ± 4.7*	21 ± 21*	0.56 ± 0.26	0.65 ± 0.57

sAME – absorption modifying excipient; FaSSIF – Fasted state simulated intestinal fluid; FeSSIF – Fed state simulated intestinal fluid; NAC – *N*-acetyl-cysteine; [a] – 5 animals in group, [b] – 4 animals, [c] – 3 animals, [d] – 2 animals, [e] – 1 animal. Asterisks * indicate statistical significance (p-value < 0.05).

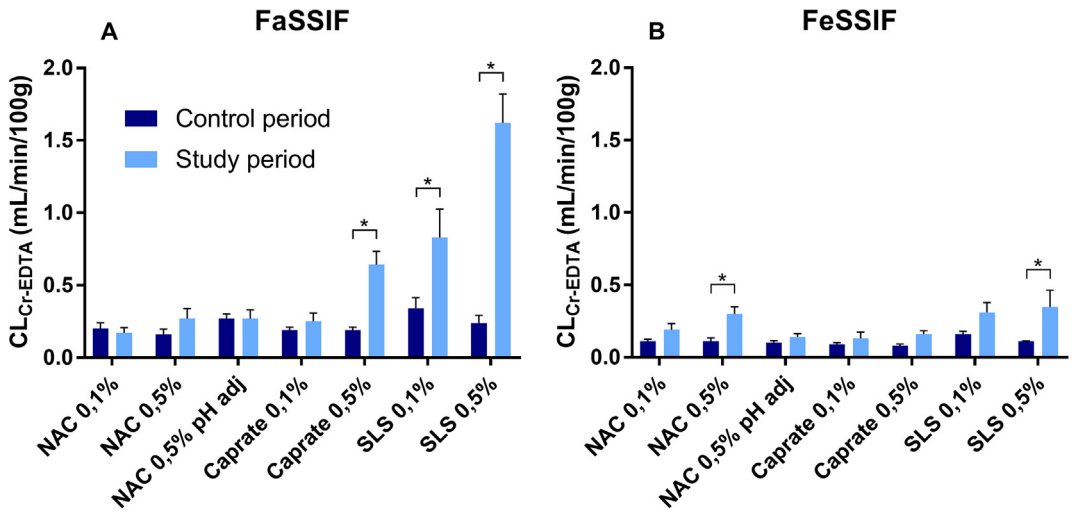

Fig. 4. ^{51}Cr-EDTA blood-to-lumen clearance ($CL_{Cr\text{-}EDTA}$) values determined during single-pass jejunal perfusion in rats using (a) FaSSIF and (b) FeSSIF as the perfusion media. Values are shown as mean ± SEM (n = 6). Asterisks * indicate a significant difference between the control period and the study period (p-value < 0.05).

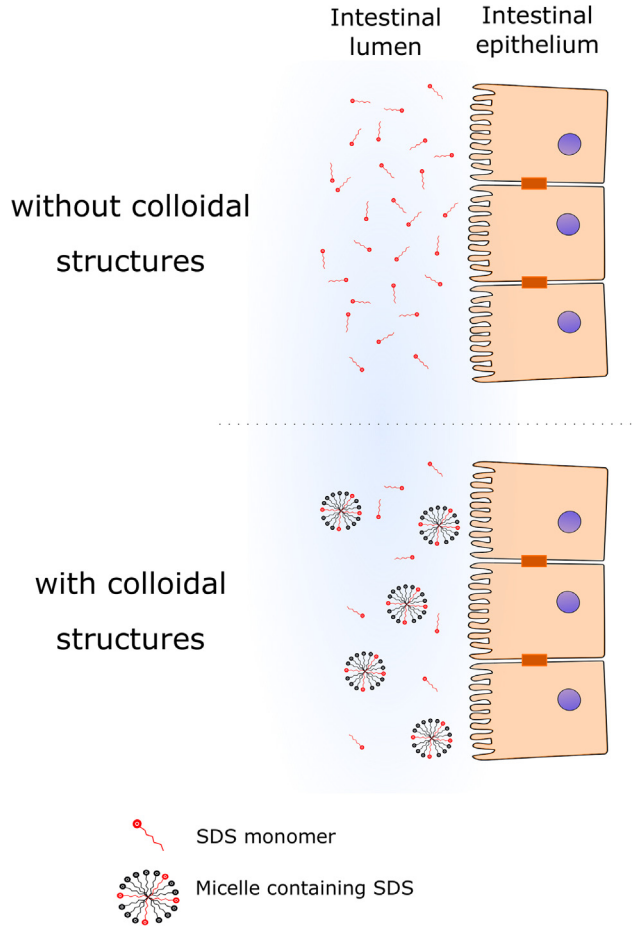

Fig. 5. A graphical illustration of the entrapment of SDS into colloidal structures (CS). The total number of SDS molecules is the same in both scenarios, but in the lower panel the presence of CS is limiting the free concentration of SDS available for acting on the membrane of the intestinal epithelium.

A key difference between the perfusion media is the size and abundance of colloidal structures (CS). CS are less abundant in FaSSIF than in FeSSIF, but larger in size (around 40–100 nm size range in FaSSIF and 10–20 nm in FeSSIF), which has been shown previously using both DLS and cryo-TEM [22,25]. All investigated drug compounds showed a higher J_{app} in FaSSIF than in FeSSIF, which suggests the possibility of a food-drug interaction in the intestinal lumen. For atenolol, enalaprilat, and ketoprofen, the effect was limited, while there was a substantial reduction in FeSSIF for metoprolol. This pattern might be explained by an increased partitioning or interaction of API into the CS in fed state due their higher capacity, which prevents APIs from crossing the apical membrane of the enterocytes. Partitioning or interaction with CS has previously been shown to increase or decrease the intestinal absorption of drug molecules, depending on the biopharmaceutical properties and the concentration of API in the intestinal lumen [28,37,38].

The extent and type of interaction between any of the investigated drugs with the colloidal structures or other constituents cannot readily be explained by the physicochemical properties of the four drugs. The drugs were all ionized (> 99%) at pH 5.8 (FeSSIF), and there are no reports indicating substantial ion-pair partitioning mediated membrane transport of any of the investigated small drug molecules. There were a large difference in partitioning/interaction between the two cationic drugs, atenolol and metoprolol, regardless of their physicochemical similarity. This food-drug interaction observed in perfusion studies might have resulted from high-affinity association to CS in the intestinal lumen as reported for β-adreno antagonists in various models [29,39,40]. The difficulty in experimentally investigate the type of interactions in complex media indicate that advanced molecular dynamics simulations would need to be used to fully elucidate what type of interaction that is taken place [41].

Partitioning into and/or interaction with CS may partially explain the differences between the absorption promoting effect of AMEs observed when single-passed perfused (SPIP) in buffer in the rat jejunum and the effects observed when the same drugs were bolus-dosed in the rat proximal small intestine [12,14]. AME effects in SPIP, during perfusion with a buffer solution, were in general greater than in *in vivo* bolus-dosing into proximal small intestine, possibly due to the lack of biorelevant CS present in the SPIP experiment [9,14,18]. The key issue determining the magnitude of the AME's absorption-promoting effect is probably the free concentration of AME available adjacent to the epithelium. An AME may partition into and/or interact with endogenous CS, like bile acid and phospholipid micelles, reducing its free concentration without affecting its total concentration (see Fig. 5). This phenomenon has previously been observed for maltosides, in both Caco-2 cells and *in vivo* perfusion of rats [42]. The results from SPIP with FaSSIF and FeSSIF in this study, agreed better with the flux ratios

for the same drugs determined following a single bolus dose in the rat jejunum [12]. The increase seen for J_{app} in the bolus-dosing with 0.5% SDS for enalaprilat and atenolol was 15- and 3.3-fold, respectively [12]. The corresponding flux ratios for the two drugs in the SPIP using buffer, FaSSIF, and FeSSIF were; 34 and 9.6; 89 and 7.5; and 21 and 5.6. As previously mentioned, SDS is a surfactant that interacts with the phospholipid membrane of the enterocytes, thereby altering the structure and/or fluidity of the membrane and subsequently increases the transcellular flux of other low permeability molecules (e.g. drug molecules) across the barrier [9,34,43]. The SDS monomers might form ion pair with any of the study drugs and subsequently partition and/or interact with both the enterocyte membrane and/or CS. This suggests that SPIP experiments using simulated and biorelevant intestinal fluid media may improve the accuracy of the prediction of the effect of AMEs on intestinal permeability, absorption, and bioavailability. This is clearly demonstrated by the good agreement with the intra-intestinal bolus study, in which the rats had free access to food prior to anesthesia, and also maintained their normal continuous pancreatobiliary secretion of bile acids [12,14]. Ideally, mechanistic studies should be performed using SPIP as the luminal conditions are highly controlled, but need to be accompanied with *in vivo* experiments, e.g. intraluminal bolus-dosing, to gain comprehensive mechanistic insights and high *in vivo* predictive values, for complex oral drug delivery systems.

Enalaprilat has the lowest intestinal permeability of the four compounds investigated here, and accordingly it has the highest potential of increasing the flux across the membrane (human P_{eff} $0.08–0.2 \cdot 10^{-4}$ cm/s, human f_a 8–25%) [44,45]. In this study, SDS showed a very strong positive effect on the intestinal flux of enalaprilat, even though the 26-fold increase observed in FaSSIF for 0.1% SDS was not statistically significant. A pH-dependent increase in the clearance of the blood-to-lumen integrity marker ^{51}Cr-EDTA has previously been shown in the rat jejunum and duodenum, when 1–100 mM hydrochloric acid was perfused [46]. This effect may be attributed to a damage on the mucosa and subsequent leakage of interstitial fluid [46]. Similar trends were seen for atenolol as well as ^{51}Cr-EDTA in this study, providing compelling evidence that the effect seen for NAC in fact is a pH effect, not a mucolytic effect.

Atenolol has an intestinal permeability that is higher than enalaprilat (human P_{eff} $0.2 \cdot 10^{-4}$ cm/s, human f_a 50–60%) [44,45]. The smaller effect on atenolol, compared to enalaprilat, agreed with previous results, and might be a consequence of its higher baseline permeability in comparison with enalaprilat [12,14]. It has been suggested that, like enalaprilat, atenolol is absorbed primarily paracellularly [47,48]. However, a recent study reported that the possible paracellular flux of atenolol could not fully explain the fraction dose absorbed (around 50% in an oral dose range of 12.5–400 mg), based on theoretical approximations of the pore size and the anatomy of the gastrointestinal tract [44,49]. Several other reports have instead proposed that atenolol partitions into the lipid bilayer and subsequently is transported primarily by the transcellular route [45,50]. The fact that atenolol's bioavailability remains linear across a wide dose range (12.5–400 mg) is further support for the idea that passive lipoidal transcellular diffusion may be its major mechanism of transport across enterocytes [49,51].

For ketoprofen and metoprolol, no increases of small intestinal absorption were observed for any of the AMEs investigated, which agrees with earlier studies [14,16]. The reduction of J_{app} observed for ketoprofen in both FaSSIF (and FeSSIF for some of the AMEs) has also been observed previously. However, the mechanism is not possible to elucidate in this study, and will need to be further investigated. In summary, the results from these two highly permeable compounds (ketoprofen human P_{eff} $8.4 \cdot 10^{-4}$ cm/s, metoprolol human P_{eff} $1.3 \cdot 10^{-4}$ cm/s) suggest that no additional benefit is gained from combining BCS class I and II compounds with AMEs if the aim is to increase drug permeation [45]. Nonetheless, an AME may potentially affect the absorption rate of also high permeation compounds, which will have an impact on bioequivalence assessment.

The clearance of ^{51}Cr-EDTA ($CL_{Cr\text{-}EDTA}$), a well-established intestinal mucosal damage marker, from the blood to the lumen and the J_{app} of the drug molecules during the control period showed no increase over time (0–75 min) [52]. This demonstrates that the rat jejunal SPIP model well tolerates both FaSSIF and FeSSIF, which has been shown previously [25,28]. There was a trend for larger increases in $CL_{Cr\text{-}EDTA}$ combined with SDS in FaSSIF, than with SDS in FeSSIF, suggesting that $CL_{Cr\text{-}EDTA}$ was more affected by AMEs in FaSSIF than in FeSSIF. This further supports that these AMEs may be more effective in the fasted state compared to the fed state, possibly due to a higher free concentration of AME. As suggested previously, the significant increase in $CL_{Cr\text{-}EDTA}$ in FaSSIF for 0.5% NAC can be attributed to a luminal pH effect on the membrane rather than to any mucolytic effects. Overall, the effect of the AMEs on $CL_{Cr\text{-}EDTA}$ corresponds well to the effect on J_{app} for the poorly permeating APIs, which is in line with previous publications [13,14]. Finally, it is important to remember that the increases seen in J_{app} are highly dependent on the compounds investigated. For this reason it is encouraged to include well-established markers for effect on the barrier functions, ideally one blood-to-lumen (such as ^{51}Cr-EDTA) and at least one lumen-to blood.

Extensive research has been performed on the effect of different AMEs in less complex *in vitro* models, like Caco-2 cells and excised intestinal tissue in Ussing chambers. In general, AMEs tested in Caco-2 models show greater increases in absorption than the increases seen in this study. For instance, the addition of 0.005–0.025% SDS increased the flux of mannitol by 20- to 142-fold, while also reducing the transepithelial electrical resistance (TEER) in two different studies using Caco-2 cell lines [53,54]. These results demonstrate the high inter-experimental variability associated with Caco-2 experiments, which has been shown previously [55]. In contrast, when giving 0.1–2% SDS by *in vivo* methods, e.g. intestinal perfusions and instillations in jejunum, the flux of marker compounds increased in the range of zero to 6-fold, in accordance with the values from this study for enalaprilat and atenolol [56–59]. The large increase observed in *in vitro* systems are most likely attributable to the lower physiological relevance of the system, including the absence of a blood supply, the absence of hormonal and neural feed-back mechanisms to handle stress or trauma, and the lack of supportive tissue [30,56]. Negative stress and damaging effects on membrane fluidity or integrity may therefore increase in these *in vitro* models, and an overprediction of effect on drug absorption is common [16,60]. Further, the steady-state perfusion of the same intestinal segment is similar to a cell based in vitro systems for evaluating AMEs, as the same mucosal segment is exposed during the whole perfusion. This maximal tissue exposure may overpredict the effects of AME on intestinal absorption. Long exposure times and large volumes are commonly applied, which is shown to have a low correlation to the effect following the physiologically relevant intraintestinal bolus [61,62]. Nonetheless, the use of cell-based models and SPIP, once proper refinements are made, could be of great service for mechanistic investigations.

5. Conclusions

This single-pass intestinal perfusion study showed that SDS, a surfactant AME, had a less pronounced absorption promoting effect in FeSSIF, than in FaSSIF and buffer, which was also observed for the blood-to-lumen clearance of ^{51}Cr-EDTA. This is possibly due to a higher partitioning and/or interaction of SDS with colloidal structures in the fed state, which limits its free luminal concentration adjacent to the apical membrane of the enterocyte. Further, the effect seen for SDS in FeSSIF was similar to the effect seen in an *in vivo* bolus dosing study in rats. Finally, this study demonstrated that the use of simulated intestinal fluid in SPIP is well tolerated by the rat intestine and that the use of complex biorelevant media may increases the biorelevance of the SPIP model.

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