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Research paper

Intestinal absorption-modifying excipients: A current update on preclinical in vivo evaluations

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A B S T R A C T

Pharmaceutical excipients in drug products are defined as pharmacologically inactive and are integral constituents of all types of oral dosage forms. However, some excipients may increase drug absorption by interacting with the mucosal membrane. If the strategy is to use an excipient with a *potential* to affect the processes determining the rate and/or extent of the intestinal drug absorption, it is defined as an absorption-modifying excipients (AME). These pharmaceutical excipients may act as AMEs, depending on the amounts applied, and accordingly influence bioequivalence assessment of innovative and generic drug products, as well as enable oral delivery of peptides and oligonucleotides. This review discusses the mechanisms by which AMEs increase drug absorption, and especially permeation step. The focus is on the most recent data regarding how AMEs can be evaluated in preclinical models, with an emphasis on in situ and in vivo intestinal absorption models. The in vivo predictive value of these models is reviewed for five factors of clinical relevance for the intestinal absorption performance: (a) effect and response rate of AMEs, (b) mucosal exposure time and intestinal transit of AMEs, (c) intraluminal AME dilution and prandial state, (d) mucosal recovery and safety, and (e) variability in the effects of the AMEs. We argue that any preclinical investigations of AMEs that fail to consider these processes will ultimately be of limited clinical value and add little to our understanding of how excipients affect intestinal drug absorption.

1. Introduction/Background

Pharmaceutical excipients are integral constituents of most of oral dosage forms and approved oral pharmaceutical products. They are necessary to ensure a consistent and reproducible manufacturing process, and to optimize the physicochemical stability and various biopharmaceutical properties of the drug product. In traditional oral drug formulations, they are classified as pharmacologically and physiologically inactive. The amounts included in these formulations are expected to result in luminal concentrations and epithelial exposures that only affect the drug product dissolution and API solubility. Most oral formulations are designed *not to* directly interact with the gastrointestinal (GI) epithelial tissue or its physiological regulation, because epithelial permeability, pancreatic secretion, and GI transit may affect the fraction dose absorbed (f_{abs}) [1–3]. A few representative examples of pharmaceutical excipients and their functions are presented in Table 1.

Bioavailability (F) is the key pharmacokinetic parameter describing the fraction of an orally administered drug that reaches systemic circulation (Eq. (1)):

$$F = f_{abs} \times (1 - E_G) \times (1 - E_H) \quad (1)$$

where E_G and E_H are the fractions extracted in the gut wall and liver, respectively. If an excipient has the *potential* to affect the processes determining the rate and extent of the intestinal F (i.e both f_{abs} and E_G), it is called a absorption-modifying excipient (AME). As f_{abs} is influenced by the dissolution, solubility, intestinal permeability and/or intestinal transit time of the drug compound, AMEs can be sub-classified as permeation-modifying excipients and/or transit-modifying excipients, respectively. In addition, post-absorption processes, such as gut-wall metabolism (i.e., E_G) of the API, might also be affected by the excipient [4]. It should be emphasized that pharmaceutical excipients may also be formulated *intentionally* to increase the intestinal f_{abs} and bioavailability for low permeation compounds; they are then commonly referred to as permeation or absorption enhancers.

The gastrointestinal transit time of an API can be reduced with osmotically active excipients. The excipients can be used at luminal concentrations that increase the intestinal fluid volume to levels that stimulate the GI motility [5]. For instance, sorbitol and mannitol (at oral doses between 2.2 and 2.5 g) are two sugar alcohols with low membrane permeability that significantly reduce the bioavailability of the BCS class III drugs, ranitidine and cimetidine, by 25% and 31%, respectively [6,7]. In contrary, the intestinal transit time of an API may also be increased by an excipient that has transit prolonging properties (e.g. mucoadhesive or motility inhibitor) [8,9].

Gut-wall metabolism (first-pass extraction, E_G) of the API may be affected if an excipient interacts with any of the phase I (CYP450) and/or phase II (transferases) metabolic enzymes in the enterocytes [4]. For instance, the intrinsic clearance (rat liver and intestinal microsomes) of

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Table 1
Representative examples of categories of pharmaceutical excipients used in solid oral drug products and their function(s) in the oral formulations [90].

Categories	Function(s)	Specific examples
Filler, binder, diluent	Improve handling and mechanical strength, and secure dosing uniformity	lactose, starch, mannitol microcrystalline cellulose
Preservatives	Improve shelf life by securing API and drug product stability	antioxidants such as butylated hydroxytoluene
Glidants and lubricants	Reduce particle adhesion, cohesion, and friction	magnesium stearate, talc
Disintegrants	Promote disintegration of a tablet/granule into smaller particles.	starch, crospovidone
Wetting and solubilizing agents	Prevent particle aggregation and increase particle dissolution rate	sodium dodecyl sulphate (SDS) and cyclodextrin
Tablet coatings	Improve stability and appearance, mask bad taste, facilitate swallowing, and control tablet and granule disintegration (e.g., with enteric coatings)	hydroxypropyl methylcellulose, cellulose acetate

the CYP3A4 substrate, midazolam, is reduced, and consequently the plasma exposure is increased, by a number of excipients (e.g. Cremophor, Tween, and HPMC) [10]. The mechanism(s) of the reduced intrinsic clearance and first-pass extraction are the altered enzyme regulation and/or the inhibition of enzymatic activity [10]. Less common mechanisms are: excipient-induced induction of CYP activity, inhibition of phase II enzymes, and interaction of the excipient with hepatic metabolism [4,11]. At present, the in vivo relevance of excipients interfering with intestinal metabolism is uncertain, as it has scarcely been investigated. To us, this scarcity of reports suggests that interactions between excipients and gut wall enzymes are of limited clinical importance and animal findings as above is due to very high amounts of excipients applied.

Intestinal permeability is a key biopharmaceutical variable (together with solubility, dissolution rate, dose, and GI transit) that determine the in vivo rate and extent of intestinal drug absorption [12–16]. The permeation of a drug across the intestinal epithelium depends on one, or several, of multiple, parallel transport processes that include passive transcellular/paracellular diffusion, carrier-mediated transport, and carrier-mediated efflux (Fig. 1) [17]. The net intestinal permeability of a drug molecule is determined by its chemical structure and physicochemical properties, and by the physiological and biochemical conditions along the GI tract [18–21]. The physiologically active site of an AME determine its classification: **transmucus**, **transcellular,** and/or **paracellular** (Fig. 2a–d) [1]. These three classes of permeation-enhancing excipients are reviewed later in this article.

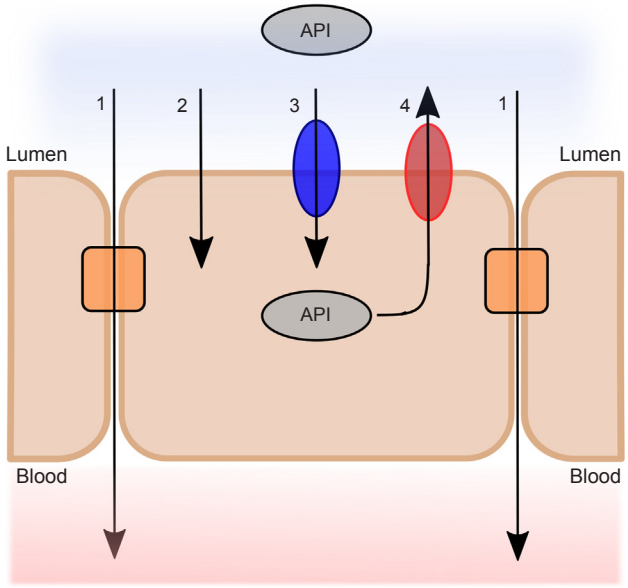

Fig. 1. The transport mechanisms across the intestinal epithelial barrier that determine the net permeability of a luminally dissolved compound. (1) Passive paracellular diffusion, (2) passive transcellular diffusion, (3) absorptive carrier-mediated transport, and (4) efflux carrier-mediated transport.

The major objective of this update is to review the recent data of how AMEs affect passive transcellular and/or paracellular diffusion of drugs in the small intestine. The implications for bioequivalence are discussed with respect to its effects on assessment of innovative and generic drug products, and briefly for oral delivery of new modalities such as peptides and oligonucleotides. The focus is on AMEs in preclinical models, with an emphasis on the ones for in situ and in vivo intestinal absorption. The in vivo predictive value of these models is reviewed for five factors of clinical relevance in the intestines: (a) effect and response rate of AMEs, (b) mucosal exposure time and intestinal transit of AMEs, (c) intraluminal AME dilution and prandial state, (d) mucosal recovery and safety, and (e) variability in the effects of the AMEs.

2. Effects of AMEs on the intestinal barrier

An AME, such as the mucolytic agent, n-acetylcysteine (NAC), may increase **transmucus** drug transport by reducing the thickness, viscosity, and/or porosity of the intestinal epithelial mucus layer (Fig. 2a). A reduction of the mucus barrier may result in an overall increase in drug permeability, in those cases where the diffusion across the mucus and aqueous boundary layer is the rate-limiting permeation step [22]. A number of in vivo studies in humans and other animals have shown that these transport processes are not rate-limiting even for drugs that have high membrane permeability. For instance, the permeability of ketoprofen is very high in both the jejunum and colon of humans ($> 3.4 \times 10^{-4}$ cm/s) and rats ($> 1.5 \times 10^{-4}$ cm/s), despite that the colonic mucus is substantially thicker (human 200 vs. 15 μm; rat 800 vs. 200 μm) and more strongly adherent than the mucus in the jejunum [15,23–25]. Further, co-administration of NAC, has no effect on the absorption rate of ketoprofen in the rat single-pass intestinal perfusion (SPIP) model SPIP model [1]. Nevertheless, mucus may affect the permeation of compounds that interact with it physically (i.e. hydrophobic or ionic interactions), or sterically. These interactions between the mucus and smaller solutes are generally insignificant, but an increased drug lipophilicity correlates slightly in vitro with a reduction in transmucus diffusion rate [24,26]. Complete steric interaction has been seen for larger peptides/proteins (> 12 kDa) and solid particles (about 200 nm in diameter, depending on shape and surface properties) [27]. Consequently, NAC has the potential to increase the transmucus diffusion rate of particles, and of peptides/proteins with a MW from about 2000 g/mol [27,28]. However, the use of mucolytic agents for increasing drug permeation is still only an experimental strategy that will most likely require combination with a transcellular or paracellular permeation enhancer to be effective

An AME may increase **transcellular** drug diffusional transport by increasing the fluidity and/or by reducing the integrity of the membrane lipid bilayer of the epithelial cells (Fig. 2b) [29]. This leads to an increased partitioning into, and/or higher diffusion rate across, the lipoidal membrane. One common property for these AMEs are that they

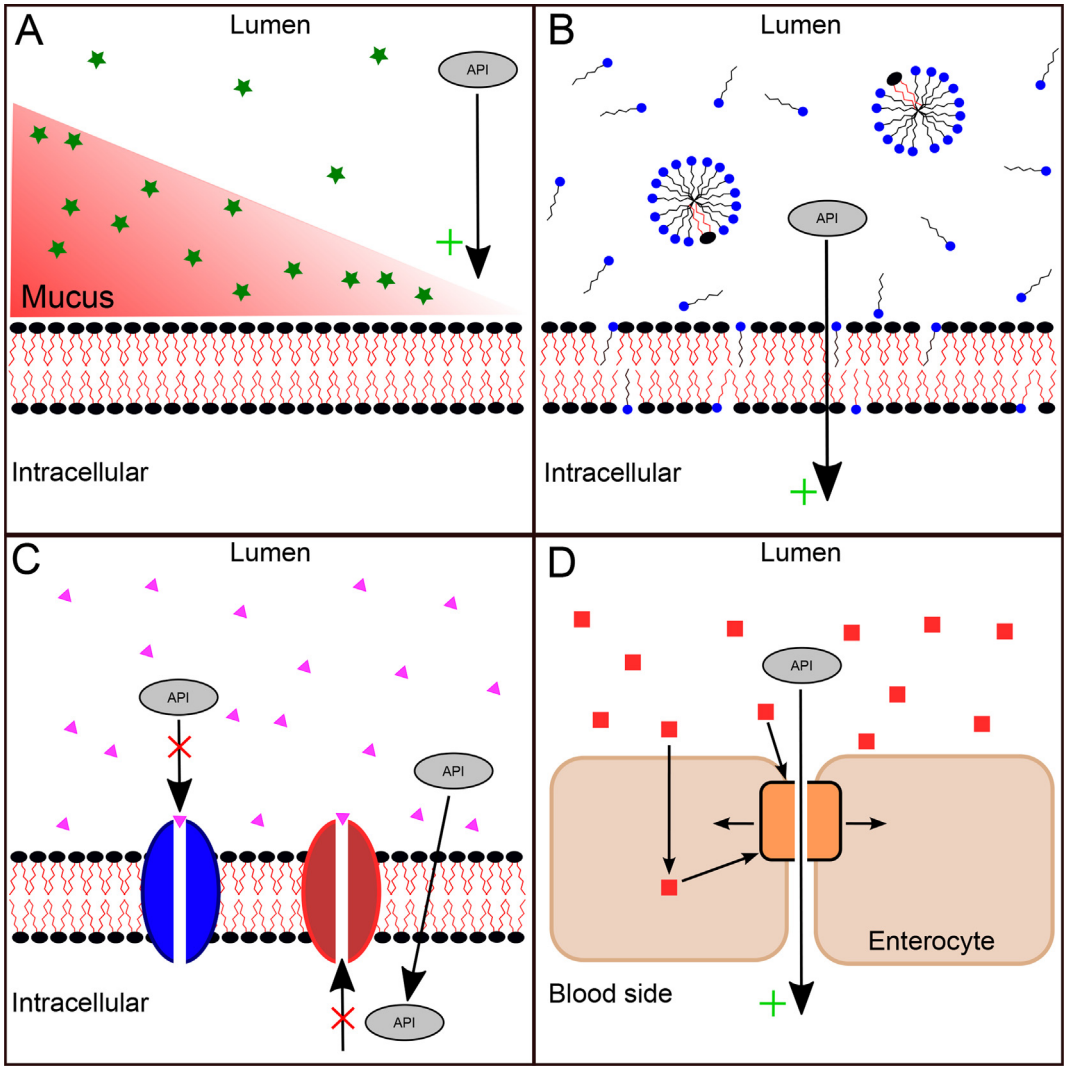

Fig. 2. The mechanisms by which an absorption-modifying excipient (AME) can affect the intestinal permeability of an active pharmaceutical ingredient (API). (A) An AME reducing the thickness, viscosity, and/or porosity of the intestinal mucus layer lining the epithelial barrier may increase the rate at which a drug is presented to the epithelial membrane. (B) An amphiphilic AME (e.g., surfactant) can be inserted into the lipoidal epithelial cell membrane and thereby increase its fluidity/porosity, and potentially dissolve phospholipids in the epithelial cell membrane. (C) An AME inhibitor of a membrane protein transporter may increase, or reduce, drug intestinal permeability, depending on if it is an influx (blue) or efflux (red) transporter. (D) An AME interacting, directly or indirectly, with tight junction protein components may increase the paracellular diffusion of an API.

are amphiphiles (e.g., bile acids, fatty acids, alkyl sulphates). Consequently, they can insert into the plasma membrane, and potentially solubilize constituents of the membrane, thereby reducing its integrity [30]. Generally, the permeation enhancing effect of a surfactant correlates with its ability to interact with the membrane (where large hydrophobic regions favor interaction), and to its critical micelle concentration (CMC) [31]. A high CMC results in a higher concentration of the surfactant in its free monomeric form, which explains why anionic surfactants are usually more potent than non-ionic ones. (the changed headgroups of the anionic surfactants repel each other, resulting in a higher CMC [30].) Luminal concentrations above the CMC may cause primarily hydrophobic drugs (or drugs that have been hydrophobised through interaction with the surfactant) to partition into the micelles. This reduces the free drug concentration, leading to a reduced rate and extent of intestinal absorption [32]. Finally, the transcellular permeability may also be affected if an excipient inhibits a drug transporter protein (Fig. 2c) [4]. How such an interaction affects the membrane permeation depends if the drug is a substrate for an efflux and/or influx transporter. For instance, it has been proposed that polysorbate 20 increases the rat intestinal absorption and consequently the oral bioavailability of the P-gp substrate, etoposide, by reducing its P-gp mediated efflux [33]. However, the contribution from other unspecific surfactant mechanisms cannot be excluded, such as an increased membrane fluidity, and/or reduced membrane integrity.

An AME may increase **paracellular** drug transport by interacting, directly or indirectly, with tight junction protein components, such as claudins and occludin (Fig. 2d) [34]. These AMEs are classified as first or second generation, which is determined by the site of action—first generation AMEs such as sodium caprate affect intracellular signalling pathways, and second ones by interacting directly with extracellular components of the tight junctions [31]. Caprate has been proposed to affect the intracellular signalling pathways mediated by phospholipase C, a protein that increases intracellular Ca^{2+} and diacylglycerol [35,36]. These signalling pathways control the contraction of actin and myosin II, and removes tricellulin from the tricellular tight junctions, which opens the paracellular tight junctions [35]. It is also important to consider that caprate has amphiphilic properties, and may act as a transcellular permeation enhancer [36]. Many of the intra/inter-cellular pathways affected by first-generation paracellular AMEs are physiologically regulated. For instance, it is well-established that

luminal hypotonicity and glucose regulate paracellular ion diffusion and convection, as well as the permeation of low permeability low molecular mass marker compounds [37,38]. These physiological regulations of paracellular permeability are generally regarded to be of little quantitative importance for in vivo drug absorption, as the processes are fast and transient. However, they may still have an impact on results in preclinical models [39,40]. The second-generation paracellular AMEs, whereof many are experimental peptides, interact directly with extracellular motifs of tight junction proteins, such as occludin and claudin [41,42].

3. AMEs – relevance for bioequivalence and drug delivery

As outlined above, AMEs incorporated into oral drug formulations may affect the intestinal permeability of any API, regardless of the BCS classification of the API. This has implications for regulatory bioequivalence assessments when pharmaceutical excipients are used at amounts that affects the rate and/or extent of intestinal absorption of the drug. In addition, an AME may have permeability enhancement as a key effect in a drug formulation if intentionally used at higher doses and luminal concentration, and/or at a special local condition (for instance, a drug delivery system with muco-adhesion properties). The implications of AMEs for both bioequivalence assessment and oral delivery of drugs are discussed below.

3.1. Bioequivalence assessment

Two drug products are defined as bioequivalent if the test and reference drug products display the same rate and extent of F, and this is demonstrated in vivo in healthy subjects by plasma PK (AUC, C_{max}, T_{max}) [43,44]. Bioequivalence may also be established on the basis of in vitro dissolution tests—this is called a BCS-based biowaiver [45]. A BCS-based biowaiver can only be used for immediate-release drug products, and only if the drug compound has a high solubility, and high or low permeability (BCS I and III, respectively). The FDA and EMA also require that the test products contain known excipients, at doses documented as safe, that do not affect intestinal transit and/or permeability [44,45]. Further, only small differences (± 10%) in excipient doses between the test and reference drug products are allowed. As the exact amounts of excipients are undisclosed for an approved product, this consequently results in many unnecessary clinical bioequivalence studies being performed, as the risk for bio*in*equivalence is minor for BCS class I drugs. However, in an evaluation of excipient effects there are claims that AMEs may affect the bioequivalence of BCS class I drugs, based on undisclosed human bioequivalence data from a drug product application of 1 mg risperidone formulated with 3.7 mg SDS in the reference product; reductions in AUC and C_{max} were observed, but there were no effects on the dissolution profiles [46]. This led to a claim that an excipient effect cannot be generalised, but rather that it is both dose and/or drug dependent [47]. Such a claim would inevitably lead to a situation where in vivo bioequivalence assessment in human healthy subjects is routinely performed on all oral formulations containing either new AME concentrations, or known AMEs in combination with new APIs. On the basis of our long experimental experience with animal and human in vivo studies, we consider that such a conservative approach is exaggerated. This review therefore discusses what properties and concentrations of AMEs that may result in bioequivalence issues for different drug compounds and products, and the possibility to evaluate this in preclinical models. However, a clear definitions of a safe space for the use of excipients and critical excipients (AMEs) formulation require an international collaborative project involving academia, regulatory agencies and to some extent pharmaceutical industry.

3.2. New modalities – peptides and oligonucleotides

Pharmaceutical peptides and oligonucleotides–i.e. new modalities–are generally not orally administered because of their low intestinal stability, permeability and/or extensive first-pass metabolism [48]. Nonetheless, by incorporating permeation-enhancing excipients in a drug formulation, or using mucus/cell penetrating particles, advocates of oral peptide delivery are revisiting this drug delivery issue, hoping to enable oral administration of these compounds [49–51]. However, this experimental strategy has already been investigated during several decades, with limited success, and there are currently no approved oral drug products using pharmaceutical excipients to increase intestinal drug permeation of any type of compound [52–54]. This has been attributed to the weak, and highly variable, effects of AMEs in vivo, as well as to safety issues [54,55]. For permeation-enhancing drug delivery technologies to reach a broad clinical breakthrough, these issues must be resolved. The evaluation of AMEs and their impact on oral drug delivery and safety in different preclinical models are therefore discussed in this review.

4. Preclinical models used to evaluate AMEs

There are many preclinical models in pharmaceutical science to investigate membrane permeation and predict human intestinal drug absorption [56,57]. These range from simple in vitro tools to complex in vivo animal models. One commonly used in vitro system is the diffusion chamber, in which the drug flux is determined across a biological membrane separating two compartments. This barrier can be composed of a monolayer of cells, such as Caco-2 or MDCK cells, or an excised tissue, such as intestinal segments derived from animals or humans [58,59]. An example of a more in vivo relevant tool is the single-pass intestinal perfusion (SPIP) model, in which a drug solution is perfused through a defined intestinal segment, and the luminal drug disappearance is evaluated, and/or appearance in plasma, per intestinal surface area [1]. Intestinal perfusions are most often performed in rat, mouse, dog and pig, but extensive experiments have been performed in human as well [14,20,60,61]. The most clinically relevant preclinical approach is to evaluate GI drug absorption by assessing plasma PK following intraintestinal, intragastric, or oral, administration to animals, such as rat and dog [62]. The biopharmaceutical evaluation of AMEs in all the above models can be performed in a variety of ways, depending on the question being investigated. The typical study design is based on an evaluation of changes in, for example, drug absorption/flux or mucosal injury/recovery, induced by an AME compared to a control period/group.

4.1. Potential of the different models

The cell/tissue-based diffusion-chamber model is suitable for high throughput because multiple AMEs can be investigated at a range of concentrations and exposure times [49,63]. It is also suitable for a mechanistic evaluation of AMEs, as some experimental conditions can be controlled. Changes can be visualized, for instance, in cytoskeletal and tight junction protein conformation, as well as epithelial membrane damage [29,63–65]. The disadvantage of the diffusion-chamber models is the absence of neural and hormonal feedback mechanisms, because these are considered to affect epithelial functions. The SPIP model allows for controlled conditions in the lumen of the intestinal segment and visualization of tissue following AME exposure, but it is associated with a lower throughput and higher cost than the in vitro systems [66,67]. The major advantage is that the perfusions are performed in vivo, with a dynamic intestinal blood flow and feedback mechanisms, which increases the robustness and in vivo relevance of the model. The most relevant in vivo preclinical model is the evaluation of the plasma PK of a drug in an animal following oral/intraintestinal administration of the drug together with an AME formulated as, for instance, a solution, immediate-release or multiple-unit enteric coated dosage form [52,62]. The effect of the AME in the PK models is expected to be affected by, among other things: intraintestinal spreading and dilution of

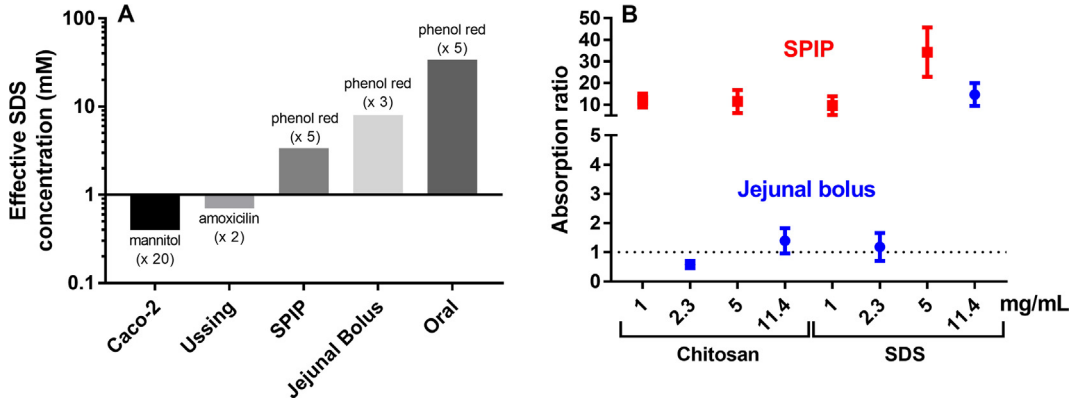

Fig. 3. (A) The lowest reported concentration at which sodium dodecyl sulfate (SDS) increases the permeability of low-permeability small model compounds (mannitol, phenol red, and amoxicillin). The exposure time was 20 min in the Caco-2 model, 210 min in the rat jejunal Ussing model, and 75 min in the rat single-pass intestinal perfusion (SPIP) model [1,91,92]. The administered volume in the rat intraintestinal bolus model was 0.5 mL (5.7 mg SDS), and 2 mL (20 mg SDS) in the rat oral bolus study [62,93]. (B) Reported mean (± SEM) increase in jejunal absorption ratio of the low-permeability drug enalaprilat, induced by SDS and chitosan in the rat SPIP (red) and intraintestinal bolus (blue) models, at different SDS concentrations (mg/mL). The intestinal exposure time was 75 min in the rat SPIP model, and the administered volume in the rat intraintestinal bolus model was 0.5 mL (5.7 mg SDS) [1,62]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the AME; precipitation; complex binding with colloidal structures; absorption of the AME; and intestinal transit of the AME/drug. This multitude of factors renders the PK model less suitable for mechanistic evaluations— even if the model is the most in vivo relevant—as it can be challenging to differentiate the individual contribution of the different factors.

4.2. Effect of AMEs in different preclinical models

It is a well-recognized pattern that the luminal (donor) concentration at which an AME is effective needs to be increased the more in vivo-like the applied model is (e.g., the effect of caprate: Caco-2 > Ussing > SPIP > oral bolus) [36]. The higher sensitivity (i.e. lower AME exposure required) in in vitro models can be attributed to the factors outlined in the previous section, whereas the more in vivo-like models have intact blood flow and mucosal repair mechanisms. In addition, the oral/intraintestinal bolus models are influenced by intestinal AME dissolution/release, dilution, absorption, and transit. This is exemplified by the AME concentration needed to increase the permeability of low-permeability model compounds in different preclinical models (Fig. 3a and b). The exposure times and administered volumes vary in the different models, but there is a clear trend that higher epithelial exposure (concentrations or doses) is needed in the more in vivo relevant models.

5. Ideal biopharmaceutical properties of an AME formulation intended for an absorption enhancing effect

If the purpose of an AME incorporated into an oral drug product is to increase the absorption of an API with low intestinal permeation, some ideal properties can be postulated for it (conversely, these properties should be avoided if the pharmaceutical excipients are not to enhance intestinal absorption.) The AME needs to be released from the formulation sufficiently fast and synchronized with the API release, as the API needs to be present at the intestinal mucosal barrier that has been compromised. The onset-time for the AME effect should be short to obtain increased intestinal drug absorption. If the local effect is slow, the API might be transported away from the targeted intestinal site prior the AME has exerted its sufficient effect on the intestinal mucosa [68]. The maximum duration during which an AME exerts its effect(s) is also reduced if the AME itself is absorbed, which is the case for caprate and SDS [64,65]. Luminal water volumes and contents, which vary substantially between intestinal segments and prandial states, also have a strong impact on the local concentration–time profiles along the intestines, and consequently on both intra- and interindividual variability. Large GI fluid volumes reduce the likelihood of effective luminal AME concentrations due to dilution and rapid GI transit, whereas fatty chyme and endogenous bile salts/acids may reduce the free monomeric concentrations of AME surfactants acting on the membrane [69–71]. For safety reasons, the intestinal mucosa should have a rapid recovery following an AME exposure to avoid translocation of harmful luminal contents, which could initiate intestinal inflammation [55,72]. The AME itself should have a good local and systemic safety profile, which is especially important if the AME itself is absorbed and causes a systemic exposure. Consequently, advanced formulation designs of complicated AME-containing products require the use of relevant in vivo predictive absorption models. The above mentioned physiological and biopharmaceutical processes determine the clinical effect of an AME. These processes—and how they can be investigated in preclinical models—are discussed in the following sections.

5.1. Response rate, mucosal exposure time, and intestinal transit

If the response rate of an AME on the intestinal mucosal membrane is slower (including delayed onset time) than the intestinal transit, the AME is predicted not to be able to increase the intestinal absorption of the API to clinically effective plasma levels [73]. Therefore, any model (in vitro or in vivo) using long AME exposure times may therefore generate data that is not clinically relevant. This is illustrated by the rat SPIP model, where a mucosal AME exposure time of 60-min generated an increase in model compound absorptive fluxes that were substantially higher than those observed in the rat intraintestinal bolus model [1,62]. However, when a more in vivo relevant exposure time of 15-min was used in the same SPIP model, the data was predictive of the rat bolus data, but only if both maximum absorptive flux, and time to maximum absorptive flux, were considered [68]. Consequently, only a high AME induced flux (Fig. 4a) was not predictive of the effect in the bolus model, and neither was only a rapid time to maximum flux (Fig. 4b). This combination of both a rapid onset and a high effect on the intestinal mucosa was only observed for SDS at a high dose in the 15-min SPIP model, and SDS at a high dose was the only AME that was effective at increasing drug absorption in the rat intraintestinal bolus model [1,68].

These three studies - the 60- and 15-min SPIP studies, and the rat intraintestinal bolus study - altogether indicate that the increase in absorptive flux (or permeability) observed in preclinical models, such

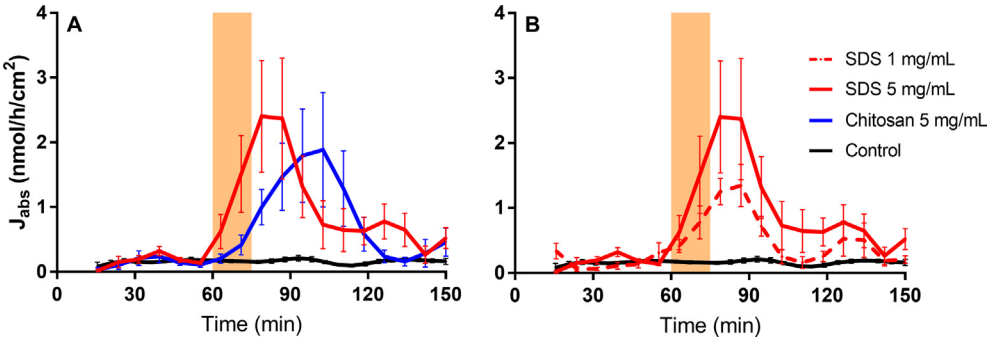

Fig. 4. Reported mean (± SEM) acyclovir absorptive flux (J_{abs}) during a 150-min rat single-pass intestinal perfusion (SPIP) [68]. Sodium dodecyl sulfate (SDS) and chitosan were only added to the perfusate in the 15-min test period (brown); the periods before and after were control periods in which the base-line value and recovery time were evaluated, respectively. (A) Comparison of SDS and chitosan at 5 mg/mL. (B) Comparison of SDS at 1 and 5 mg/mL.

as Caco-2 and SPIP, has limited in vivo relevance, unless time–dependent parameters, such as response rate and intestinal transit, are considered [1,62,68]. However, it should be stressed that time–independent models, such as evaluation of absorption ratio in the Ussing and SPIP models, may still be useful screening tools for new AMEs. They may also be useful in the investigation of the mechanisms of action of an AME, and for investigation of the *potential* effect of an AME in vivo [1]. Readers with a broad and focused interest in AME response rate and intestinal transit, and their impact on oral drug delivery, are referred to a recent review by Maher et al., 2019 [74].

5.2. Intraluminal effects

The primary determinant of an AME effect on the mucosal membrane is the free concentration adjacent to the epithelial barrier. This concentration can be reduced by different in vivo factors, the most obvious one being dilution in the intestinal fluid. Dilution may be even more pronounced if the formulation is dissolved and diluted already in the stomach, and only gradually released into the small intestine, which is usually the targeted intestinal region for AME effects and drug absorption [69]. These gastric dilution effects can be resolved by enteric coating of a multiple unit formulation [54]. Gastric emptying, as well as random intestinal motility, may spread the AME/drug over a larger section of the intestines. This may result in a lower total dose at any local mucosal site, and reduce the effect of the AME, even if the free AME concentration is unchanged. The risk is higher if the AME itself is absorbed. A reservoir of undissolved AME is then necessary to replenish any absorbed AME to maintain a constant free concentration adjacent to the membrane. Preliminary results from our laboratory demonstrated this effect—a saturated solution of caprate (5 mg/mL) at pH 7.4 was insufficient to increase the permeability of the mucosal barrier marker, ^{51}Cr-EDTA, in the rat SPIP model. There must be an excess of undissolved caprate (i.e., 10 and 20 mg/mL) in the perfusate (Fig. 5).

Prandial state and diet are expected to have a strong effect on the outcome of an AME on intestinal absorption, which is thoroughly discussed by Maher et al, 2019 [74]. For instance, ion concentration affects the CMC of ionic surfactants, (e.g., SDS has a CMC between 2 and 8 mM depending on NaCl concentration) [75,76]. High ion concentrations shield the repulsion between ionic head groups, reducing the CMC and hence free monomer concentration, which is the primary determinant of their effect on the mucosa [31]. Ion content may also affect the physicochemical properties of charged AME polymers, such as chitosan, by de-hydrating the polymer and by reduced charge repulsion [77]. However, changing the NaCl concentration in a rat SPIP study did not affect the SDS-and-chitosan-induced increase in membrane permeability [40]. In contrast, membrane permeability increased when the ion concentration was reduced with caprate, another AME with surfactant properties. The mechanism behind this is unclear, but it is most likely not related to alterations of the CMC of caprate (between 25 and 140 mM in saline, depending on reference), as the CMC is higher than the concentrations used in the perfusion study (5–25 mM) [36,40]. The free concentration of amphiphilic AMEs can also be reduced if they

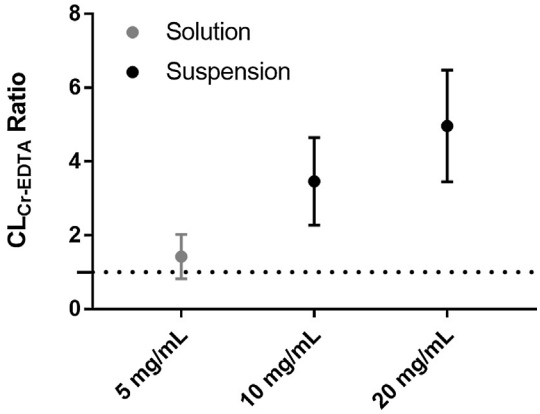

Fig. 5. The increase in jejunal rat ^{51}Cr-EDTA clearance ($CL_{Cr\text{-}EDTA}$) ratio of a test formulation compared to a control solution perfused in the same animal. The control solution contained buffer at pH 7.4 with no caprate, while the test formulation contained buffer at pH 7.4 with caprate at 5, 10, and 20 mg/mL. Caprate at 5 mg/mL was at the saturation concentration at pH 7.4, while caprate at 10 and 20 mg/mL was above its solubility (suspension).

form mixed micelles with endogenous surfactants (e.g., bile salts) or food components (e.g., phospholipids). Such effects have been observed in the Caco-2 and rat closed-loop models, in which surface active alkylmaltosides were co-administered with mixed micelles [78]. Preliminary results from our laboratory also found this for SDS in the rat SPIP model; there was a substantial reduction in the permeation enhancing effect of SDS at 1 and 5 mg/mL when co-administered with FeSSIF, compared to FaSSIF, which has a lower content of fatty acids, phospholipids, and bile salts (Fig. 6). The effects of ion concentration and food components clearly illustrate the importance of considering relevant in vivo luminal conditions when evaluating AMEs [78].

5.3. Gastrointestinal mucosal repair processes and safety

The effect and safety of an AME are directly related to the function of the GI mucosa to uphold homeostasis. The risk for safety issues is highest for potent AMEs such as claudin inhibitors, or AMEs administered at high doses (and high local concentrations), where the ambition is to affect the mucosal barrier [74]. High doses may not only increase drug permeation, but also translocate bacteria, viruses, and proteins. Such events may initiate intestinal inflammation, as well as dysregulate hormonal and neural processes involved in mucosal homeostasis. The risk for these effects depends on the frequency and duration of the oral drug treatment, as harmful epithelial effects may only show following long-term exposure.

Simpler absorption predictive systems, such as Caco-2 and the Ussing chamber, lack many fundamental physiological, neural, and hormonal processes involved in the homeostasis and repair of the epithelial barrier [79–81]. The intact blood supply in the in vivo models (i.e., SPIP- and PK-models), effectively oxygenates and brings nutrients

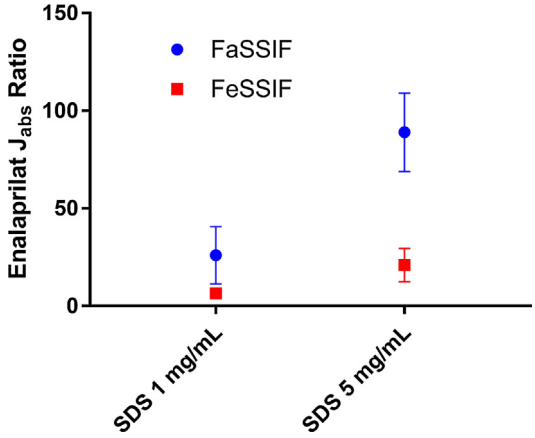

Fig. 6. Preliminary results showing the increase in mean (± SEM) enalaprilat absorption flux (J_{abs}) ratio of a test formulation compared to a control solution perfused in the same rat jejunum. The control solution contained FaSSIF or FeSSIF with no sodium dodecyl sulfate (SDS), while the test formulation contained FaSSIF or FeSSIF with SDS at 1 or 5 mg/mL.

to the intestinal mucosa. It also removes any absorbed AME and cellular waste, which ultimately results in a lower degree of their local accumulation. The hormonal and neural regulation of the supportive tissue in the different models strongly influence the response of the epithelial barrier to damage and AME exposure. For instance, enteric neurons may induce contraction of smooth muscle fibers extending into the villus core, which reduces the height of the villus as well as the exposed villus surface area [82]. This defence mechanism mitigates epithelial damage by reducing the surface area of the epithelial defect [83]. The impact of this phenomenon on drug absorption was possibly observed in a recent rat SPIP study with SDS and chitosan at 5 mg/mL. The increased absorptive flux of the model compounds induced by SDS and chitosan was temporarily reduced during a 60-min perfusion [68].

Arguably, the most important epithelial mechanism for preventing/resisting AME effects is its ability to repair itself. Every 72 h the epithelial barrier is renewed, and every day billions of cells are shed without affecting the mucosal barrier [55]. These cells are regenerated by stem cells situated in the crypt region of the intestinal epithelium, a process that is accelerated by epithelial damage [84]. Barrier defects are also rapidly healed by a process called restitution, in which adjacent healthy epithelial cells are mobilized [83]. Restitution is followed by a final process whereby the paracellular space between the cells is sealed tight. This process is proposed to be the most important process in barrier recovery [83,85].

The effect on AMEs by the different GI mucosal repair mechanisms has not been systematically investigated in detail in the different absorption models. However, two studies compared a 15-min exposure of SDS to Caco-2 cells (0.1 mg/mL) and in the rat SPIP model (1 and 5 mg/mL). Complete recovery of marker compounds to their baseline permeability value was 250 min in Caco-2, and 30–60 min in the rat SPIP study [64,68]. It is uncertain which of the physiological mechanisms (blood supply, villus contraction, restitution, or sealing of tight junctions) is primarily responsible for the faster GI mucosal recovery in the SPIP model - or if it was related to a greater membrane damage in the in vitro model - but the results are a good illustration of the important differences between the two intestinal absorption models.

However, there is a limit to the repair of the GI mucosa in the in vivo models. Following a 60-min intestinal mucosal exposure of SDS and chitosan (5 mg/mL each) in the rat SPIP model, there was only a 50% recovery of the model compounds absorptive flux during a 60-min recovery period [68]. The same experiment also showed that the blood-to-lumen transport of the clinical intestinal barrier marker, ^{51}Cr-EDTA, was elevated, and unaffected, during the whole 60-min recovery period. A reduction in drug flux does not necessarily equate with a healthy mucosa, and safety evaluations should preferably be coupled to histological examinations [63,71]. Nevertheless, the most difficult task is to categorize the risk of long-term treatment with an AME. Such studies need repetitive treatment—using in vivo relevant models—to monitor mucosal inflammation and the general health of the intestines and the animal. Complete evaluation of these long-term effects and their implication for different patient populations is difficult to assess, and the final risk-benefit analysis has to be evaluated in large, clinical studies, if pre-clinical toxicity studies allow human studies.

5.4. *Variability in absorption-modifying excipient effects*

A major hurdle for oral peptide and nucleotide oral delivery is the notoriously low and highly variable rate and extent of GI absorption [48]. Incorporation of an AME in an oral drug delivery system might resolve both these issues. For instance, oral administration of an oligonucleotide (ISIS 104838) together with 660 mg caprate in fasted humans resulted in an oral bioavailability of 12.0 ± 8.78%, without resolving variability (F ranged between 2.0 and 27.5%) [86]. In another example, the bioavailability of semaglutide (MW 4113 g/mol, HBD/A 57/63, cLogP −5.8) following oral single-dose administration in fasted dogs was 0.29% together with 600 mg SNAC, but with a coefficient of variance close to 200% (data from patent: wo2012080471). It is not easy to identify the source of this interanimal variability of ISIS 104,838 and semaglutide, It is most likely a combination of biopharmaceutical and physiological factors, such as failure to release drug and AME at the same place, fast intestinal transit, luminal AME dilution, low intestinal permeability and luminal drug degradation.

Another factor worth noting is the surprisingly large variation in intestinal mucosal response upon AME exposure–consistently there are a few animals not responding. This is exemplified by the plasma concentration of enalaprilat, when perfused together with SDS at 5 mg/mL during 75 min in the rat SPIP model, and following an 0.5 mL intraintestinal bolus together with SDS at 11.4 mg/mL (Fig. 7a and b) [1].

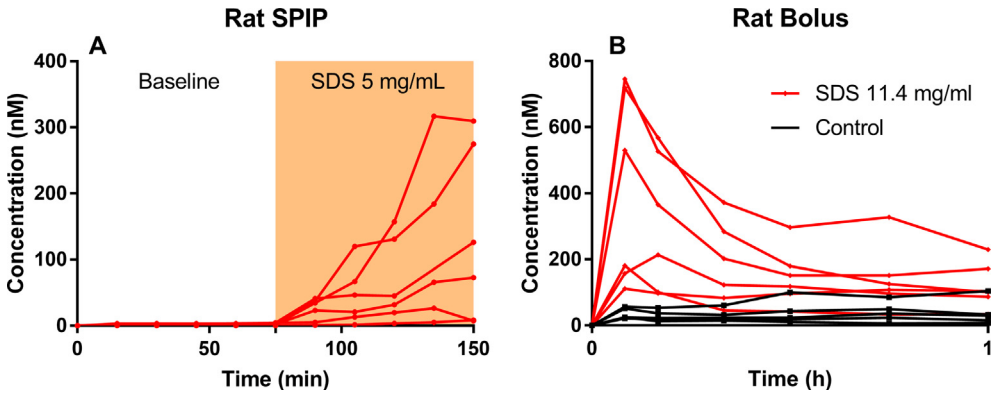

Fig. 7. (A) Reported individual plasma concentration–time profiles of enalaprilat, perfused during 150 min in the rat SPIP model [1]. The AME, sodium dodecyl sulfate (SDS) at 5 mg/mL, was added to the perfusate solution between 75 and 150 min. (B) Reported individual rat plasma concentration–time profiles of enalaprilat following an intrajejunal bolus administration of enalaprilat alone (control), and together with SDS at 11.4 mg/mL [62].

Our group has observed that it was always at least one animal in each study group for which there was no increase in absorptive flux. {Dahlgren, 2018 #515;Dahlgren, 2018 #559;Dahlgren, 2018 #585;Dahlgren, 2017 #445} These studies covered different AMEs, AME concentrations and intestinal exposure times, and two models (rat SPIP and intraintestinal bolus) [1,40,62,68]. These non-responders occurred even in spite of the controlled luminal conditions in the SPIP model, in which the same mucosal segment is exposed to a defined concentration. On the basis of these studies and published data from clinical studies evaluating AMEs, we conclude that variability between and within animals is a major hurdle in designing a robust AME-based oral formulation strategy for new modalities, such as peptides and oligonucleotides.

The knowledge of factors that explain the variability between and within individuals needs to be improved. Alternatively, the API must be designed with pharmacokinetic/pharmacodynamic properties for which a large day-to-day variability in intestinal absorption does not affect the clinical outcome. For instance, variability issues are circumvented for the peptide drug, desmopressin, because it has a flat concentration–effect curve, where a maximum effect is achieved already at oral doses of 250 ng [87]. Semaglutide, a peptide being explored for oral delivery together with an AME (SNAC), is chemically modified to have a terminal half-life of nearly a week [88,89]. This makes the steady-state plasma concentration less sensitive to changes in day-to-day variability in intestinal absorption. However, it does not resolve the issue with inter- and intraindividual variability, as low, or high, absorption of semaglutide several days in a row will result in a new steady-state plasma concentration. For any such drug design to be regulatory acceptable, it must be shown that these extremes in f_{abs} do not affect the clinical efficacy or safety of the drug. It is also important to consider that the absorption modifying drug delivery system do not affect the absorption of concomitant administered drugs.

6. Concluding remarks

AMEs incorporated into oral drug products may increase the rate and extent of intestinal drug absorption by increasing the membrane permeability of low intestinal permeability drugs (traditional low molecular mass drugs and new modalities)API. These excipients are therefore interesting from both regulatory and drug delivery perspectives, as they may affect bioequivalence. They may also be used intentionally to increase drug absorption of low-permeability compounds from different classes (traditional small drugs and new modalities). Currently, there are controversies regarding how to investigate and characterize the biopharmaceutical properties of AMEs for affecting in vivo intestinal drug absorption. These issues must be resolved to facilitate the drug approval process of test products (e.g., generics), and to enable oral administration of new modalities by using AMEs. This requires that the in vivo processes influencing the effect of an AME be better understood, and that mechanistic investigations of AMEs in preclinical models consider these processes. Key factors to focus on are gastrointestinal mucosal response rate of an AME, intestinal transit, luminal AME effects, intestinal epithelial repair, and variability in AME effects. Any preclinical investigations of AMEs that fail to consider these processes will ultimately be of limited clinical value and add little to our understanding of how excipients affect drug absorption.

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