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Article

Effect of Common Excipients on Intestinal Drug Absorption in Wistar Rats

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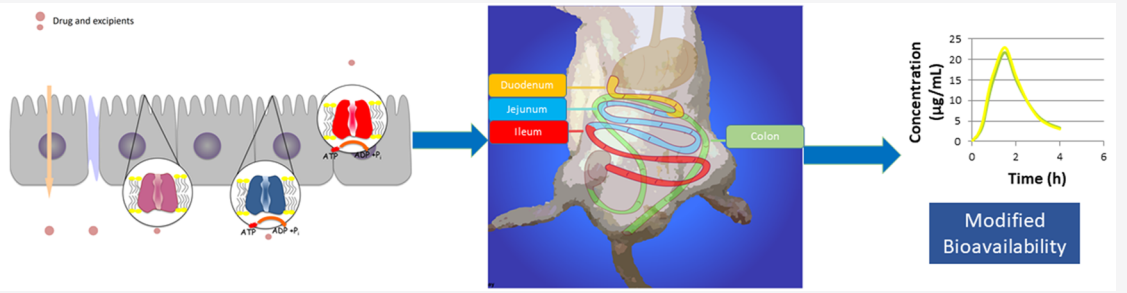

ABSTRACT: The aim of the present paper is to study the effect of common excipients on the permeability of atenolol (as drug absorbed mainly by passive diffusion) and rhodamine (as P-glycoprotein substrate). The apparent permeability was measured by an in situ perfusion method in Wistar rats using the closed loop Doluisio's method. Permeability values were characterized in the absence and presence of 18 commonly used excipients. Excipient concentrations were selected based on the amounts in oral immediate release dosage forms, which failed the test during the human bioequivalence studies. Atenolol was studied with and without excipients in the whole small intestine, whereas rhodamine was tested in three different intestinal segments to account for the differential expression of P-glycoprotein, and it was further on tested in the ileum, in the presence of excipients. Atenolol presented higher permeability values when it was administered with colloidal silica, croscarmellose, hydroxypropyl methylcellulose (HPMC), magnesium stearate, $MgCO_3$, poly(ethylene glycol) 400, poly(vinylpyrrolidone), sorbitol, starch, and TiO_2 rhodamine showed higher permeability values when it was administered with croscarmellose and HPMC. On the one hand, the mechanisms of action were not discernible with the proposed experiments. On the other hand, commercial formulations do not present a single excipient but several, which can counteract their effects. The in situ perfusion technique can be useful for a preliminary screening and risk analysis, while the in vivo pharmacokinetic results would be needed to define conclusive effects.

KEYWORDS: *permeability, excipient, passive diffusion, P-glycoprotein*

1. INTRODUCTION

There are several routes of drug administration, but the oral one is the most used and preferred by patients because of its many advantages such as ease of ingestion, low production cost, safety, and good patient compliance.[1−4] Oral pharmaceutical products are composed of active pharmaceutical ingredients (API) and a set of excipients. These excipients have different objectives, from facilitating their production to improving their stability. Although pharmaceutical excipients are considered inert from a pharmacological point of view and safe substances, they could affect drug bioavailability and, eventually, drug effects. Nevertheless, these effects on rate and extent of absorption and their underlying mechanism are poorly characterized.[5−13]

The main parameters that determine rate and extent of absorption are drug intestinal permeability, drug solubility in combination with dissolution rate, and intestinal transit time. Permeabiliy and solubility were the parameters selected by Amidon et al. to define the Biopharmaceutic Classification System (BCS).[14,15] The intestinal permeability or effective permeability (P_{eff}) of a molecule reflects its velocity of diffusion across the intestinal barrier; consequently, it is one of the determinants of drug absorbability.[16,17] Excipients can affect not only permeability but also apparent solubility, dissolution rate, and gastrointestinal transit times.[7]

In this paper, we are focusing on the effects over membrane permeability by means of an in situ perfusion method in Wistar rats. Doluisio's method[18] is a closed loop in situ perfusion technique in rat that has been demonstrated to be a reliable

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method in measuring the drug permeability in the small intestine and in different intestinal segments.[19] The method presented an excellent correlation with single-pass perfusion method[19−22] and a good predictive performance of oral fraction absorbed in humans.[23,24]

The passage through the intestinal membrane can be done by passive diffusion or by carrier-mediated mechanism (either active or facilitated diffusion).[25] It has been observed that some excipients can cause changes in the intestinal membrane and/or affect the specific transporters of the gastrointestinal tract by several mechanisms and, consequently, modify the permeability.[11,26,27] Mechanisms involved in the change of permeability include (but are not limited to): changes in membrane fluidity,[10,28,29] alterations of tight junctions,[12,13] and reduction in the resistance of the stagnant water layer. These effects in general increase permeability, while the inclusion in carriers as cyclodextrins or surfactants over their critical micelle concentration (CMC) reduce the free fraction available for diffusion and reduce the effective or apparent permeability.[12,13,30−36]

Osmotic effects, and alteration of a carrier-mediated mechanism, have also been proposed for some excipients.[10,37] For instance, a study by Schulze et al. demonstrated a concentration-dependent effect of poly(ethylene glycol) (PEG) 400 on the absorption of ranitidine in humans. At low concentration the excipient modulates drug permeation and enhances ranitidine transport, while at high concentrations the osmotic activity causes a reduction of intestinal transit time, which is reflected in a reduced fraction absorbed.[38]

Surfactants can alter the fluidity of the intestinal membrane and, as a consequence, affect carrier functionality either by impairing substrate recognition or adenosine triphosphate (ATP) binding.[39,40] The inhibition of efflux proteins leading to increase in the rate and extent of absorption has been characterized in in vitro and in vivo animal models or even in human clinical studies.[41−49]

The objective of this work was to explore the effect of common excipients in the permeability of atenolol, as a model of drugs absorbed mainly by passive diffusion, and rhodamine as P-glycoprotein (P-gp) substrate and to evaluate the potential consequences in the absorbed human oral fraction. The mechanisms behind the interactions cannot be elucidated, but we aim to detect those excipients with a relevant magnitude of the effect.

2. MATERIALS AND METHODS

2.1. Compounds. Atenolol, rhodamine, and all excipients were purchased from Sigma-Aldrich. Methanol, acetonitrile, and water were of high-performance liquid chromatography (HPLC) grade. All other chemicals were of analytical reagent grade.

The concentrations of drug assayed were 0.026 mg/mL (100 μM) for atenolol and 0.0021 mg/mL(5.5 μM) for rhodamine. The atenolol concentration was high enough to ensure its detection with the validated HPLC analytical method and adequately below its solubility to avoid precipitation on the intestinal samples. Previous results of rhodamine (not shown) indicated that, at this concentration, the efflux transporter was below the saturation concentration.

To select the concentrations of excipients to be tested, we took the data from our previous publications[50−54] in which we evaluated pharmaceutical formulations from bioequivalence studies with some in vivo failures. The composition of tests (Bioequivalent, BE or failing BE tests, i.e., Non-BE) and reference products were compared, and those excipients from the Non-BE formulations that were not contained in the reference or BE ones were selected (Table 1). The selected amount corresponds to the amount contained in those Non-BE formulations. Some excipients were tested at very low concentrations, as they correspond to elements for the coating of the oral tablets. In principle, they were not expected to have any effect, but we wanted to have the experimental demonstration. The amounts were divided by 250 mL as the standard administration volume. The selected amount was dissolved or dispersed in the perfusion buffer to obtain that apparent concentration.

Table 1. Concentration of Excipients Used in the In Situ Perfusion Tests[a]

excipient	CAS No.	molecular weight (g/mol)	concentration[b] (mg/mL)
colloidal silica	7631-86-9	60.08	0.012
Cremophor EL	61791-12-6	172.01	0.1 (CMC= 0.1)[55]
Croscarmellose sodium	74811-65-7	982.44	2
HPMC	9004-65-3	1261.4	0.2
lactose	63-42-3	342.3	0.5
magnesium stearate	557-04-0	591.24	0.1
MgCO3	39409-82-0	84.31	0.12
MgO	1309-48-4	40.30	0.06
microcrystalline cellulose	9004-34-6	342.3	0.5
PEG 400	25322-68-3	380−420	0.246
PEG 6000	25322-68-3	5000−7000	0.24
PVP40	9003-39-8	∼40 000	0.026
SLS	151-21-3	288.38	0.1 (CMC = 0.29)[56]
sorbitol	50-70-4	182.17	74.5
starch	9005-84-9	342.3	2.32
talc	14807-96-6	379.27	0.02
TiO_2	1317-70-0	79.87	0.032
Tween 80	9005-65-6	604.8	56.7 (CMC = 0.022)[56]

CMC= critical micelle concentration

[a]These concentrations are those commonly used in oral immediate release dosage forms. [b]CMC experimentally determined in the perfusion vehicle.

2.2. Rat Permeability Studies. This study involves animal experiments using Doluisio perfusion technique. Consequently, it required the approval of the Ethical and Scientific Committee of the Miguel Hernandez University. The study received the committee approval, as it showed that all the animal and surgical procedures fulfill the Spanish and European laws governing the use of animals in research, in particular, the EC Directive 86/609, of the Council of the Europe Convention ETS 123.

The absorption rate coefficients and the permeability values of the drugs studied were determined in complete small intestine (atenolol) and duodenum, jejunum, and ileum (rhodamine) (n = 6−7) using Doluisio's in situ "closed loop" perfusion[18] The technique was adapted to each intestinal segment.[19−22] The perfusion experiments were performed in male Wistar rats (body weight, 250−300 g). Anesthesia was induced with pentobarbital (40 mg/kg). The segment length for perfusion was as follows:

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

Table 2. HPLC Methods of Atenolol and Rhodamine

high-performance liquid chromatography[a]										
compound	nm	mobile phase	flow	retention time (min)	sample volume	linearity	range (mg/mL)	accuracy (%error)	precision (cv%)	LoQ (mg/mL)
atenolol	231 UV	5:90:5 (v/v) methanol/acidic water/acetonitrile	1 mL/min	3.5	40 μL	r^2 = 0.999	0.027−0.0008	1.07	<2	0.0013
rhodamine	485 ex: 546 em (fluorescence)	60:40 (v/v) acidic water/acetonitrile	1 mL/min	2.3	20 μL	r^2 = 0.999	(0.0021_ 6.28) × 10^{-05}	0.77	<2	7.44 × 10^{-05}

[a]Column used: Nova Pak Waters (C18 3.9 × 150 mm; 4.0 μm) *C-18 Agilent Eclipse XDB (4.6 150 mm; 3.5 m).

- duodenum: 10 cm
- jejunum: 45 cm
- ileum: 45 cm
- complete intestine: 100 cm

Before the experiment each intestinal segment was cleaned of any solid debris by flushing them with a physiologic isotonic solution (1% Sörensen phosphate buffer (v/v), 37 °C). During the perfusion experiment it is necessary to avoid water evaporation and heat losses; thus, the rat abdomen was covered with a cotton wool pad. Sampling was done every 5 min up to 30 min. Drug solutions were prepared with Sörensen phosphate buffer (66.6 mM) isotonized with NaCl.

Samples were centrifuged for 5 min at 5000 rpm in Eppendorf Centrifuge 5424 (rotor FA-45-24-11). Concentrations were obtained by HPLC (Alliance-Waters 2695) using a Nova-Pak C18 column (4 μM, 3.9 × 150 mm) and UV detector (Waters 2487) as described in Table 2. The methods were previously validated with adequate linearity, precision, and accuracy (R > 0.99 and coefficient of variation <5%).

During the perfusion experiment water is reabsorbed; consequently, the experimental sample concentration must be corrected as described in ref 57. The water reabsorption zero-order constant (k_o) was estimated by a direct measurement of the final fluid volume on the rat intestine (V_{end}) in comparison to the volume at the beginning of the experiment (V_0: 10 mL to complete the small intestine, 2 mL for the duodenum, and 4 mL for the jejunum and ileum). An individual value of k_o was estimated for each animal as

$$k_o = (V_0 - V_{end})/t_{end} \quad (1)$$

Finally, the experimental analyzed drug concentrations (C_e) were corrected at each time point to obtain the actual C_t by the following equation

$$C_t = C_e(V_t/V_0) \quad (2)$$

where (V_t) is the remaining water volume in the different segments at each time point. The C_t values (corrected concentrations) were used to calculate the actual absorption rate coefficients (k_a) with eq 3.

$$C_t = C_0 e^{-k_a t} \quad (3)$$

where C_0 represents the intercept at time zero. Permeabiliy values were estimated from k_a values with eq 4.

$$P_{eff} = k_a R/2 \quad (4)$$

where R is the effective radius of the intestinal segment. The R value was calculated for each segment with the next equation

$$\text{volume} = \pi R^2 L \quad (5)$$

in which L is the length of the segment (100 cm for the complete small intestine, 10 cm for the duodenum, and 45 cm for the jejunum and ileum), and "volume" corresponds to the perfused volume (10 mL for the incomplete small intestine, 2 mL for the duodenum, and 4 mL for the jejunum and ileum).

2.3. Data Analysis. The mean drug permeabilities were calculated in each experimental group (in the absence or presence of the excipient). Effective permeabilities in the presence of excipients were divided by the effective permeability of the API in the absence of excipients to estimate the ratios,[58] using the following equation

$$r = \frac{\overline{P_{eff}\text{excipient}}}{P_{eff}\text{API}} \quad (6)$$

where P_{eff}excipient is the permeability in the presence of the excipient, and P_{eff}API is the permeability of the API without additives.

The calculation of the standard deviation (SD) of the ratio, r, was obtained using the Delta method[59] with the following equation

$$\text{SD} = \left(\lambda \cdot \frac{\overline{P_{eff}\text{excipient}}}{P_{eff}\text{API}}\right)^2 \quad (7)$$

where the coefficient λ was calculated using eq 8

$$\lambda = \left(\frac{\text{SD}P_{eff}\text{excipient}}{\overline{P_{eff}\text{excipient}}}\right)^2 + \left(\frac{\text{SD}P_{eff}\text{API}}{\overline{P_{eff}\text{API}}}\right)^2 \quad (8)$$

in which SDP_{eff} excipient and SDP_{eff}API are the standard deviations of the permeabilities of the excipient and the API means, respectively. Ratios were compared to 1 at a 0.05 significance level. In addition, the ratios were also compared with chosen limits of 2 and 0.5 to consider relevant the effect size. Comparison was done by student's t test (at 0.05 confidence level) of the obtained ratio versus 1, 0.5, and 2 and/or by constructing the 90% confidence interval (CI90%) around the ratio and checking if the CI90% includes 1, 0.5, or 2.

3. RESULTS AND DISCUSSION

All pharmaceutical products contain excipients, which could be inert components but could also affect drug rate and the extent of absorption and, consequently, therapeutic activity.[5,7,8,60]

Unfortunately, excipient effects cannot be generalized, not even within a particular BCS class. Excipient effects in nature (positive or negative over absorption) and magnitude are dose-dependent, drug-dependent, and subject-dependent (since small amounts can affect absorption in a subpopulation of patients);[8] in consequence, predicting a potential excipient effect is not straighforward.

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

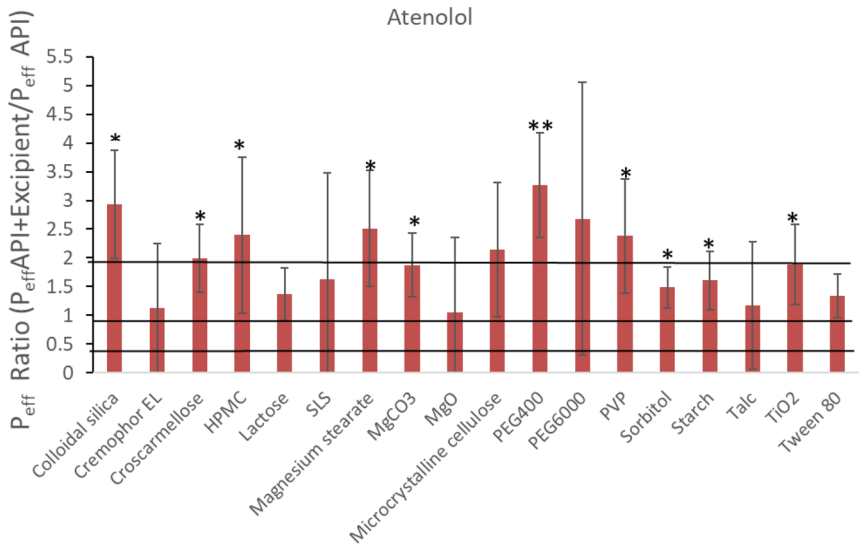

Figure 1. Values of ratio (atenolol with excipient P_{eff}/ atenolol P_{eff}) (mean ± SD). *Ratio significantly different from 1. ** Ratio significantly higher than 2.

The in situ perfusion method in rat was selected, because it is considered that, in general, in situ animal procedures reproduce with more reliability the absorption processes that take place in vivo, while the in vitro models can be too sensitive for excipient effects.[34,61,62] Nevertheless, in some cases, the effects observed in animals were not observed in Caco-2 cells. On the one hand, for instance, in ref 34, the authors used an SLS concentration of 0.04 mg/mL (which we acknowledge was lower than the concentration used in this study), and they did not observe any effect on atenolol permeability. On the other hand, they observed an increase in permeability of all the other model compounds, which they attributed to a marked effect on the tight junctions, so the lack of effect on atenolol in that study was difficult to explain. In this example, the discrepancy between both experimental models could be, in part, explained by the different tested concentrations. Parr et al.[63] compared the effects of several excipients in similar concentration ranges in rat perfusion model and Caco-2 cells and found no relevant differences in both experimental systems with the exception of SLS, which at concentrations higher than 1 mg/mL showed toxic effects on the cell monolayers.

The solutions used in the in situ test were isotonic, with an osmolarity of 300 milliosmoles. This aspect is important, as perfusion fluid osmolarity has shown in the animal model to have a clear effect on the apparent permeability.[37]

Figure 1 shows that atenolol present higher permeability values and statistical differences when it is administered with colloidal silica, croscarmellose, hydroxypropyl methylcellulose (HPMC), magnesium stearate, $MgCO_3$, PEG400, poly-(vinylpyrrolidone) (PVP), sorbitol, starch, and TiO_2.

The membrane permeability of 5(6)-carboxyfluorescein in the jejunum was significantly increased by the administration of croscarmellose at concentrations of both 0.02% and 0.2% (w/v). The mechanism seems to be related to paracellular route.[26] Ginski et al.[63] studied the impact of croscarmellose on the permeability of ranitidine and demonstrated that it binds to Ca^{2+} cations and compromises tight junction integrity while increasing ranitidine permeability.[64] On the one hand, a similar mechanism could be the reason for the observed change on atenolol permeability by croscarmellose in our study. On the other hand, Vaithianathan et al.[65] concluded that croscarmellose sodium did not impact oral absorption of two BCS III drugs (cimetidine and acyclovir) in a clinical study in humans. Thus, as the caco-2 system is considered to be more sensitive to excipients than the rat model, it might be that preclinical models in general are more sensitive than the human gastrointestinal (GI) system; thus, any observed effect needs to be confirmed in the clinical setting.

The Food & Drug Administration (FDA) considers magnesium stearate as an inactive excipient in a concentration of 400.75 mg/tablet,[65] corresponding to a concentration of ∼1.5 mg/mL, which is higher than the one used in this study. Other studies proposed that magnesium stearate decreased absorption by means of a delayed dissolution due to overlubrication. Overlubrication consists of the coating of drug particles by the hydrophobic excipient (as magnesium stearate) caused by the excessive shearing in extended mixing processes.[66] This second mechanism could explain the apparent lack of effect of magnesium stearate in the global absorption process in such a way that as it increases permeability it could also delay dissolution, so both effects are compensated. As in our experiment, the effect on permeability could be observed when the drug was in a dissolution state. The potential mechanisms would need further research.

In the case of PEG400, some studies developed in mice intestine and artificial membrane indicated that, at high concentrations (50%), PEG400 could act as a chemical permeation enhancer, which reversibly promotes the paracellular drug delivery.[67,68] Other authors in human studies indicated that low concentrations (0.25 to 0.5%) of PEG 400 could enhance the absorption of a BCS class III drug (ranitidine), while at high concentration, it has a detrimental effect due to its effect on GI transit time.[38] A similar concentration to that used in this study was assayed in rat male intestinal tissue (Figure 2) in using chambers demonstrating an enhancing effect over ranitidine permeability, but the proposed mechanism was related to p-glycoprotein inhibition (29530563). As PEG 400 is absorbed via a paracellular route, it might also affect tight junction structures altering atenolol permeability, as at the same concentration in this

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

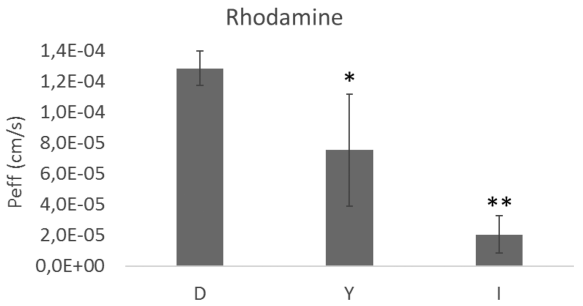

Figure 2. P_{eff} of rhodamine in Duodenum (D), Jejunum (Y), and Ileum (I) was obtained in in situ tests based on the Doluisio method in Wistar rat. (*) Significant difference between jejunum and ileum ($p < 0.05$). (**) Significant difference between duodenum and ileum ($p < 0.05$).

study it did not show any effect on rhodamine transport. To support this hypothesis it is interesting to notice that PEG6000 (which due to its longer chain length and molecular weight has a negligible permeation (PMID 9572915) did not impact atenolol P_{eff}.

Colloidal silica and PVP also demonstrated a significant increase in atenolol permeability, but there has been no previous study showing this effect, and the potential mechanism is not clear and warrants further research.

Sorbitol has been demonstrated to reduce Cmax and even the AUC of other BCS class III model drugs.[7,66,69] However, no effect on atenolol was observed in this study. Our hypothesis is that the referenced effects for other BCS class III drugs are mainly due to the effect of sorbitol on GI transit times more than a direct effect on permeability.

Regarding the lack of effect of both surfactants, cremophor EL and SLS, on atenolol permeability, for which some effect was expected, it is noticeable that the observed variability with these two excipients was very high. According to Dahlgren et al. 2017,[70] the authors observed a concentration-dependent effect of SLS on atenolol permeability in a rat perfusion model working at 0.1% and 0.5% p/V, that is, 1 and 5 mg/mL. According to Dahlgren et al. (2018),[71] the authors used SLS at 2.3 or 11.4 mg/mL in rat duodenal bolus experiments and observed an effect on atenolol permeability at the highest concentration. The SLS concentration used in this study was 10 times lower. This could be responsible for the discrepancy as well as the high variability observed in our sample that precludes the evidence of statistical significance, even if the observed ratio was higher than 1. Parr et al.[63] in a rat perfusion model and at SLS concentration of 0.17 mg/mL (slightly higher that the used in the present study) did not observe any significant increase in atenolol permeability and also attributed the lack of effect of the higher experimental variability for the low-permeability compounds. In summary, for these two excipients their effects are clearly related to their concentrations in luminal fluids.

HPMC was tested in Caco-2 cells and in the rat perfusion model at concentrations ranging from 0.012 to 0.06 mg/mL, but it did not produce any change in the permeability of five BCS III drugs (atenolol being among them). We used a concentration more than twice that level; thus, its effect could be concentration-dependent.[63]

Regarding the effect observed for $MgCO_3$, there is not a clear explanation for the observed effect. A plausible explanation already proposed for atenolol is ion-pair formation (30578978). We have not found any previous reference about a potential effect of TiO_2 on membrane permeability, which surprisingly caused a statistically significant increase in atenolol permeation and could have a similar mechanism as that of $MgCO_3$. The null effect of MgO could then be explained by its lower concentration and lower ionization degree. Chelation with magnesium was also proposed as the mechanism to enhance Dicumarol bioavailability in dogs.[72]

In the case of starch, its role in the disintegration and dissolution is widely acknowledged, but few reports about its influence on drug permeability have been published. It was demonstrated to cause a decrease in dicumarol absorption in dogs,[72] while its effect on atenolol in the present study is a slight increase in permeability. The different effect might be related with the distinctive biopharmaceutical properties of Dicumarol (lipophilic) versus atenolol (hydrophilic).

To evaluate if the observed increase in permeability could have any impact on the oral fraction absorbed, we used a

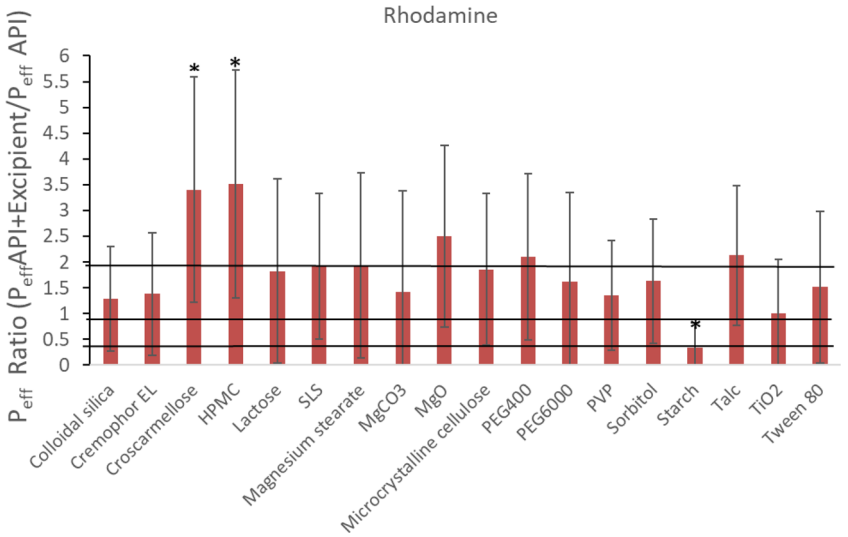

Figure 3. Values of ratio (rhodamine with excipient P_{eff}/rhodamine P_{eff}) (mean ± SD). (*) Ratio significantly different from 1. (**) Ratio significantly higher than 2.

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

previously developed correlation between apparent permeability (obtained from Doluisio's method) and human fraction absorbed by Ruiz-Picazo et al.[24] On the basis of the initial slope of the sigmoidal correlation, that is, from zero to rat permeability up to 3.00×10^{-05} cm/s (value corresponding to 80% fraction absorbed), an increase of 1.00×10^{-05} cm/s corresponds to an increase of 25% in the human fraction absorbed. The atenolol apparent permeability in the absence of excipients was 1.00×10^{-05} cm/s, so the significant increase in the ratio of permeability higher than 2 could be relevant in vivo. In our study, only PEG400 was able to produce an increase in permeability ratio higher than 2.

Rhodamine experiments were performed in ileum, as its permeability was the lowest in this segment. This fact is in accordance with the higher reported expression level of P-gp in the distal intestinal segment of the rat.[73−77]

Figure 3 shows that rhodamine presents higher permeability values and statistical differences when it is administered with croscarmellose and HPMC.

The mechanism by which croscarmellose and HPMC affect the secretion pump is unknown, and these excipient effects on efflux pumps have not been previously reported.

In this study, cremophor EL had no impact on rhodamine permeability, although a half maximal inhibitory concentration (IC_{50}) of 11 μM (0.015 mg/mL) has been estimated in transfected MDCK-MDR1 cells for cremophor EL, inhibiting digoxin carrier-mediated transport.[78] The concentration used in the present study of cremophor EL was clearly higher, but the different expression level of the transporter in both models (rat vs MDCK cells) could be the reason for the discrepancy.

Likewise, Tween 80 showed no great influence on rhodamine permeability, even though this surfactant inhibited Pg-p in transfected MDCK-MDR1 cells[78] and presumably also in Caco-2 cells.[33] In this case, the lack of effect of Tween80 in our study could be related either to the concentration of the surfactant in this study (which was close to the lower range used on those in vitro models) or to the differences in expression levels of the transporters besides the lower sensitivity of the animal model.

As previously mentioned for atenolol, no effect was observed for SLS on rhodamine permeability despite the permeability ratio being higher than 1, but the observed variability did not allow us to statistically evaluate the difference.

The negative effect of starch on rhodamine P_{eff} could be the adsorption of the dye to the carbohydrate molecules and particles.[79]

A limitation for the interpretation of the present results is that the excipients were studied separately, and in any formulation, several excipients are present simultaneously, so their overall counteracting effects could result in the absence of effect on the permeability. The mechanisms of action were not discernible with the proposed experiments. To determine the mechanism by which excipients alter absorption, it would be necessary to perform tests at different doses of excipients and in the presence of several markers of the different transport mechanism and of the functionality of intestinal membrane components.

Another aspect not addressed in this study is the potential variability on excipient characteristics and the impact of that variability on their biopharmaceutical behavior as pointed out recently by Zarmpi et al.[80] The excipients and the amounts selected in this study were those corresponding to some products used in human bioequivalence studies, but we probably did not use exactly batches of products with the same critical properties.

In addition, the excipients could also exert effects on the dissolution process and/or the gastrointestinal transit, which can nullify any potential effect on permeability. In conclusion, even if the perfusion method could be an interesting technique for assessing excipient effects and performing a risk evaluation, its combination with other in vivo animal methods is advisible.[71]

In spite of the highlighted limitations, this study adds relevant information to the formulation developers, as they might try to find a balanced mix of excipients without including, for instance, several ones with a positive or negative effect on permeability in order to avoid any change in comparison with the API.

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Notes

The authors declare no competing financial interest.

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