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Research Paper

Intestinal enzymatic metabolism of drugs

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Abstract

Objectives The intestinal stability of perorally administered drugs has so far been determined using simulated intestinal fluid containing porcine pancreatin (SIF/P), as human gastrointestinal fluids are in most cases not available. In this study the metabolism of six low molecular mass drugs in SIF/P was compared with that in freshly collected porcine intestinal juice and on excised porcine intestinal mucosa.

Methods The drugs used were oseltamivir, atazanavir, diloxanide, diltiazem, cephalothin and cefoxitin. Metabolism studies were carried out by incubating each drug in the in-vitro models and by analysing the percentage of unmodified remaining drug at fixed time points.

Key findings Three drugs showed higher degradation on porcine mucosa compared with that in SIF/P and for five compounds a significantly higher metabolism in collected porcine intestinal juice versus SIF/P was observed. Metabolism of diloxanide furoate in collected intestinal juice, for example, was 40-fold higher compared with SIF/P. Moreover, the involvement of different metabolic pathways in porcine mucosa and intestinal juice was observed for cephalothin, being metabolized to desacetylcephalothin and thienylacetylglycine, whereas these metabolites were not found in SIF/P. In addition, diltiazem solution (0.25% m/v) was found to be significantly degraded in intestinal juice whereas its metabolism in SIF/P was negligible.

Conclusions These findings demonstrated that the use of SIF/P for evaluation of presystemic drug metabolism could be highly misleading. Incubation of drugs in freshly collected porcine intestinal juice will likely lead to the improvement of the mimicry of body conditions to evaluate presystemic drug metabolism.

Keywords enzymatic hydrolysis; esterases; pancreatin; proteases; simulated intestinal fluid

Introduction

The oral bioavailability of many small drugs, especially those with peptidic substructure, is limited by their rapid degradation/metabolism in the gastrointestinal tract. One of the major barriers encountered in the intestine is caused by proteolytic enzymes including luminally secreted proteases trypsin, chymotrypsin, elastase and carboxypeptidases A and B, and the brush-border membrane-bound aminopeptidases, carboxypeptidases, endo- and enteropeptidases. Some of these enzymes are produced by the pancreas and secreted in an aqueous bicarbonate solution into the duodenum where they are responsible for drug digestion and deconjugation followed by intestinal reabsorption or excretion.[1]

Any therapeutic small molecule or formulation destined for oral use should be tested in-vitro at an early stage of development regarding its stability in the intestinal environment. To do so, the scientific community has so far used a 1% (w/v) pancreatin suspension in phosphate buffer, the so-called simulated intestinal fluid containing pancreatin (SIF/P). This is a well-established model fluid, reported in each Pharmacopoeia. Although representative for a pH value of the intestinal fluid in fasted state (6.8–7.0), the use of SIF/P as test media for stability and dissolution studies does not faithfully reproduce the in-vivo condition of human intestinal fluid.[2] In fact in its composition, fundamental elements such as electrolytes, products of lipid digestion or cholesterol, important enzymatic co-factors or activating ions are not present or available in excess, as in the case of pancreatic proteins the amount of which is 5-fold higher in comparison with that in the intestinal lumen of humans.[3] These discrepancies may result in an overestimation or underestimation of the actual events *in vivo*.

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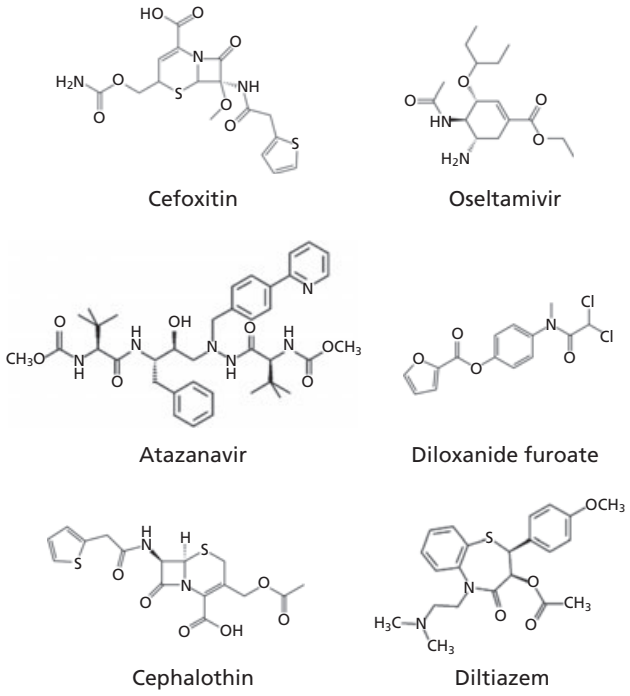

Figure 1 Chemical structure of the six drugs tested.

Freshly withdrawn human intestinal fluids, although representing the best source for analysis of presystemic intestinal drug degradation, are in most cases, however, not available and cell culture models such as human intestinal Caco-2 cells can only to a limited extent simulate the in-vivo conditions.[4] An alternative and more predictive model of intestinal fluid and mucosa is therefore required for in-vitro analysis of drug stability.

The aim of this study was therefore to establish such a model, by comparing drug degradation in a freshly collected porcine intestinal juice and on porcine intestinal mucosa with that in the SIF/P. It was, moreover, the aim to determine whether the collected porcine intestinal mucosa and juice maintained their metabolic capacity at concentrations of drugs which saturated the hydrolytic enzymes of the SIF/P. For this study, the drugs oseltamivir phosphate, atazanavir carbamate, diloxanide furoate, diltiazem hydrochloride, cephalothin sodium and cefoxitin sodium (Figure 1) were chosen for their susceptibility to enzymatic metabolism, which is facilitated by the presence of amide and/or ester groups on their structure and by low molecular mass.[5–7]

Porcine intestinal juice and porcine intestinal mucosa were expected to show different metabolic profiles in comparison with SIF/P, since their compositions are enriched by many elements missing in the artificial fluid. Finally, the two alternative models would be advantageous being easily available and inexpensive, thus suitable for routines analyses.

Materials and Methods

Materials

Oseltamivir phosphate (ethyl(3*R*,4*R*,5*S*)-4-deacetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate) and atazanavir ((methyl N-[(1*S*)-1-butanehydrazido]-1-phenylbutan-2-yl]carbamoyl}-2,2-dimethylpropyl] carbamate) were purchased from Sequoia Research Products Ltd. Diloxanide furoate (4-[(dichloroacetyl)(methyl) amino]phenyl furan-2-carboxylate), diltiazem hydrochloride ((2*S*,3*S*)-5-[2-(dimethyl-amino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate hydrochloride), cephalothin sodium ((6*R*,7*R*)-3-[(acetoxy) methyl]-8-oxo-7-[(2-thienylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate), cefoxitin sodium (6*S*,7*R*)-4-(carbamoyloxymethyl)-7-methoxy-8-oxo-7-[(2-thiophen-2-ylacetyl)amino]-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylate), 7-aminocephalosporanic acid, pancreatin and casein (from bovine milk) were purchased from Sigma-Aldrich (Vienna, Austria). Acetonitrile and 2-thiopheneacetic acid were purchased from ACROS, Sankt Augustin, Germany. All other reagents were of analytical grade and received from commercial sources.

For the synthesis of thienylacetyl glycine 0.1 g 2-thiopheneacetic acid was first dissolved in distilled water (0.1% m/v) and 0.2 g 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) was added to the solution to activate the carboxylic acid moieties of 2-thiopheneacetic acid. The pH of the mixture was adjusted to 5.5 and glycine was added to give a 2-thiopheneacetic acid/glycine weight ratio of 1 : 1. A pH of 5.5 for the reaction mixture was maintained over a 3-h incubation period, with constant stirring at room temperature. The resulting product was purified and stored at room temperature until use. Deacetylcephalothin was synthesized as following. In brief, 100 mg 7-aminocephalosporanic acid was hydrolysed in 100 ml NaOH aqueous solution (pH 8) under reflux for 2 h.[8] The obtained 7-amino-3-deacetoxycephalosporanic acid was subsequently added to a solution of 2-thiopheneacetic acid (1% m/v), previously activated as described above. The mixture was stirred for 90 min and the resulting desacetylcephalothin was purified and stored at 4°C until use.

Equipment and chromatographic conditions

Drugs and metabolites in samples were quantified via high performance liquid chromatography (HPLC) using a Merck-Hitachi Elite La Chrome Chromatograph, equipped with a Merck-Hitachi Elite La Chrome UV photodiode array detector.[5,9,10] The chromatographic separations were carried out using a LC-18 column (250 × 4.6 mm 5 μm, Supelcosil) in the case of oseltamivir phosphate and atazanavir carbamate, a 5C18 column (250 × 4 mm, ARC-Seibersdorf) for cefoxitin sodium, diloxanide furoate, cephalothin sodium, desacetylcephalothin and thienylacetyl glycine, while a 5C18 column (125 × 4.6 mm, Nucleosil Macherey-Nagel) was used for diltiazem hydrochloride.

The mobile phase for atazanavir and diloxanide furoate consisted of a mixture of phosphate buffer solution (PBS, 0.05 M pH 6) and acetonitrile at volume ratio 57/43 and 46/54, respectively, and being eluted at different flow rates (Table 1). Oseltamivir phosphate was eluted by a mixture of PBS (containing 1.0 ml Et_3N pH 3) and acetonitrile (70 : 30). Diltiazem hydrochloride and cephalothin sodium were analysed using a mobile phase consisting of ammonium acetate buffer (10 mM, pH 6.58) : 0.2%

Table 1 Mobile phases and chromatographic conditions

Drug	Mobile phase	Temperature	λ and flow rate	R_t
Atazanavir	PBS (0.05 M pH 6) + acetonitrile (57/43)	25°C	240 nm; 1.5 ml/min	4.0 min
Oseltamivir	PBS (+ Et_3N pH 3) + acetonitrile (70/30)	50°C	216 nm; 1.6 ml/min	3.1 min
Diltiazem	$CH_3CO_2^-NH_4^+$ + 0.2% diethylamine (pH 6.5) + acetonitrile (60/40)	25°C	240 nm; 1.0 ml/min	12 min
Diloxanide furoate	PBS (0.05 M pH 6) + acetonitrile (46/54)	25°C	258 nm; 1.0 ml/min	5.8 min
Cephalothin sodium	$CH_3CO_2^-Na^+$ (pH 5.9) + acetonitrile + EtOH (78/15/7)	40°C	254 nm; 1.0 ml/min	5.0 min
Cefoxitin sodium	H_2O + acetonitrile + CH_3COOH (83/16/1)	25°C	254 nm; 1.0 ml/min	7.4 min

PBS, phosphate buffer solution; R_t, retention time.

diethylamine : acetonitrile (60/40), and sodium acetate buffer (10 mM, pH 5.9) : acetonitrile : EtOH (78/15/7), respectively. Desacetylcephalothin and thienylacetyl glycine were analysed using the same column and chromatographic conditions as used for cephalothin sodium. The mobile phase for cefoxitin sodium consisted of water : acetonitrile : acetic acid (83/16/1).

For all analysis an isocratic elution was performed with a flow rate in the range of 1.0–1.6 ml/min and drugs were quantitatively and qualitatively detected at a wavelength ranging from 215 to 258 nm (Table 1). Standard solutions of drugs were prepared dissolving and diluting each compound in the corresponding mobile phase, with the exception of atazanavir and diloxanide furoate which were solubilized in MeOH because of their poor solubility in water. System suitability parameters such as retention factor and peak area were evaluated to determine the daily system performance using a 500 μg/ml solution of drugs in the according mobile phase. The reliability of the quantification method was determined by measuring precision, linearity, repeatability, accuracy and the detection limit of each drug with the given set up. To determine the linearity of the system a set of calibration standards ranging in concentrations from 10 to 1000 μg/ml were prepared and analysed.

Determination of enzymatic activity of SIF/P and intestinal juice

The enzymatic activity of proteases of freshly collected porcine intestinal juice was determined and compared with that of pancreatin used to prepare SIF/P. The proteases activity was measured according to USP specifications *Assay for Casein Digestive Power* (official monographs, United States Pharmacopeia 30, NF 25, 2007). Briefly, 1.25 g finely powdered casein was dissolved in 100 ml water, stirring the suspension for approximately 1 h and the pH was adjusted to 8 using 0.1 M NaOH. Pancreatin (0.1% w/v, 3 USP U/g) was suspended in PBS (0.05 M, pH 7.5 $\pm$ 0.2). Two batches of pancreatin samples were prepared and served as the standard. The first batch was prepared as following: 2, 1.5 and 1 ml PBS and 1, 1.5 and 2 ml pancreatin suspension were added in three tubes, respectively. Furthermore, 5 ml trichloroacetic acid (0.3 M) was added to each tube. The second batch of standards was prepared as described above, without trichloroacetic acid solution. Two series of samples containing collected intestinal juice were prepared according to the above mentioned procedure, replacing pancreatin suspension with the juice. All tubes were mixed by vortex, placed in a 40°C water bath and after equilibration 2 ml preheated casein solution was added. Sixty minutes later, 5 ml trichloroacetic acid solution was added to both the second batch of pancreatin samples and to intestinal juice samples to stop enzymatic reactions. After 10 min, the suspension in each tube was filtered through a filter paper previously washed with trichloroacetic acid solution. All filtrates were determined regarding their absorbance at 280 nm. The absorbance of samples initially prepared with trichloroacetic acid was corrected by subtracting absorbance of their corresponding acidic samples. Values were plotted against the volumes of the pancreatin suspension and from the obtained calibration curve, using corrected absorbance value of the intestinal juice, the protease activity, in USP U/l, was calculated and compared.

Enzymatic metabolism in simulated intestinal fluid (SIF/P)

The simulated intestinal fluid (SIF/P) was prepared according to USP specifications (Test Solutions, United States Pharmacopeia 30, NF 25, 2007). In brief, 6.8 g monobasic potassium phosphate was dissolved in 250 ml water. To this solution, 77 ml 0.2 M sodium hydroxide solution and 500 ml water were added and mixed along with 10 g pancreatin (from porcine pancreas, 3 USP units activity/g). The SIF/P suspension was adjusted to pH 6.8 $\pm$ 0.1 with either 0.2 M sodium hydroxide or 0.2 M hydrochloric acid and diluted with water to 1000 ml. Solutions of each drug, 0.5 mg/ml, were prepared in SIF/P in triplicate. In the case of atazanavir and diloxanide furoate, samples were prepared in DMSO containing SIF/P (10% v/v) to estimate the effect of drug solubility on its enzymatic metabolism. Samples (1 ml) of these solutions were placed in a 37°C shaking water bath. At the time points 0, 30 min, 1, 2, 3 and 4 h, incubated samples (100 μl) were removed and then diluted by adding 100 μl 99% (v/v) MeOH to precipitate the enzymes of the SIF/P. Afterwards samples were purified via a brief mixing by vortex, heated at 50°C for 1 min and then centrifuged at 13 400 rev/min for 30 min. The supernatant was then removed and analysed via HPLC.

Enzymatic metabolism on porcine mucosa

The stability of drugs to enzymatic hydrolysis was measured on porcine mucosa by means of Ussing chambers. In particular, freshly excised porcine small intestine was cut into strips and mounted in Ussing-type chambers (0.64 cm^2 surface area) on a parafilm strip to isolate the donor compartment from the receptor compartment, thus avoiding permeation of drug through the mucosa. A phosphate buffer solution (0.1 M, pH

6.8) was warmed to 37°C and used to dissolve 0.05% (w/v) oseltamivir phosphate, diltiazem hydrochloride and the two cephalosporins, in triplicate. Atazanavir and diloxanide furoate were suspended at a concentration of 0.05% (w/v) in 1 ml phosphate buffer. Final drug concentration was 0.05% (w/v). A 1-ml sample of each drug solution was added to the donor compartment of the Ussing chamber. After 0, 30 min, 1, 2, 3 and 4 h, 100-μl samples of the incubated solutions were taken out from the donor chambers and replaced by the same amount of fresh PBS. All samples were processed as mentioned above.

Enzymatic metabolism in collected porcine intestinal juice

To investigate the enzymatic activity in intestinal juice, a freshly excised porcine small intestine was used. In detail, before cutting the upper jejunum along its length it was rinsed free by pouring 10 ml pure water into the lumen to facilitate removal of particulate matter. The resulting fluid, comprising 10 ml water, was collected and added to the material scraped off from the mucosa with the aid of a spatula afterwards. This juice was used to determine the stability of test drugs. Before the analysis, the pH of the juice was adjusted to 6.8 using 0.1 M sodium hydroxide solution. The preparation was not centrifuged but used in its entirety as enzymes could be bound to particulate matter.[11] In brief, drugs were dissolved in 0.05% (w/v) collected intestinal juice in triplicate and then 1-ml samples were incubated at 37°C in a shaking water bath. From each sample a 100-μl portion was removed after 0, 30 min, 1, 2, 3 and 4 h, and 100 μl MeOH was added to purify the samples. All samples were processed as mentioned above.

Determination of enzymatic activity at increasing drug concentrations

To determine the digestive capacity of collected porcine intestinal juice and mucosa at concentrations of drugs which saturate enzymatic activity of SIF/P, diltiazem hydrochloride was chosen. Diltiazem solutions were prepared in phosphate buffer at increasing concentrations (0.5, 1.0, 1.5 and 2.5 mg/ml) and metabolism studies were conducted on porcine intestinal mucosa. Drug stability studies were performed in SIF/P and in freshly collected juice by dissolving the drug in the above described concentrations. All experiments were performed in triplicate following the same protocol as for other drugs.

Statistical data analyses

Statistical data analyses were performed using the Kruskal–Wallis test with $P < 0.05$ as the minimal level of significance. Afterwards the Dunn's post hoc test was performed to compare individual differences between the treatments.

Results and Discussion

Determination of enzymatic activity of intestinal juice

Although the contribution of the intestine to drug metabolism has been recognised, it is often overlooked or its importance is underestimated. Many enzymes involved in phase I and II reactions in the human liver, the major site of first-pass metabolism, have also been identified on and in intestinal epithelial cells.[12] In this study three in-vitro intestinal models were compared with each other regarding their metabolic capacity toward small drugs. The systems being compared were SIF/P, porcine intestinal mucosa mounted on Ussing chambers and freshly collected porcine intestinal juice. The hydrolytic capacity of proteases in intestinal juice was first determined to compare it with that of SIF/P. Pancreatin used as the standard for such comparison was the same used to prepare SIF/P, but at a concentration of 0.1% (w/v), as specified in the US Pharmacopeia. This substance is a mixture of amylases, lipases and proteases and had a casein digestive power equal to 3 USP U/g, which in a 0.1% (w/v) suspension was equivalent to 3 USP U/l, as declared by the supplier. The proteases activity of intestinal juice, calculated from the curve, was determined to be 3.48 USP U/l. This result showed that the capacity of porcine juice to hydrolyse casein was only a little bit higher than that of the pancreatin suspension. In fact, the absorbance given at 280 nm from peptides liberated by casein hydrolyses was comparable with that in tested fluids. However, considering the concentration of pancreatin suspension used herein (0.1% w/v) and that of pancreatin used for drug metabolism studies (1% w/v), the casein hydrolysing power of the intestinal juice preparation was found to be approximately 10-fold smaller than that of SIF/P. Nevertheless, degradation of drugs in intestinal juice was the highest among the three systems compared, likely due to a greater activity and variety amount of enzymes in the intestinal fluid. Moreover, the intestinal juice from animals and humans contains at least traces of nutrients, products of lipid digestion, mineral or fundamental enzymatic co-factors such as cationic Zn^{2+} and Mg^{2+}. All these components affect the stability of drugs in the intestine by acting as solubilizing agents, in particular in the case of poor water soluble drugs, and by influencing the enzyme turnover and activity.

Enzymatic metabolism in SIF/P, porcine mucosa and collected porcine intestinal juice

Drugs used in this study (Figure 1) had a molecular mass in the range of 300–700 Da and possessed amide and/or ester bonds in their structure, which made them susceptible to intestinal enzymatic metabolism. Oseltamivir phosphate and diltiazem hydrolchloride, for example, are recognised to be hydrolysed by pancreatic carboxylesterases or so-called carboxy ester lipases (EC.3.1.1.1.).[5,6,13]

Drug enzymatic metabolism in SIF/P was confirmed for all test compounds. However, only 10% of oseltamivir phosphate and of cefoxitin sodium was found to have been hydrolysed in SIF/P at the end of the experiment. In particular, cefoxitin sodium stability was the highest among the tested drugs, showing a degradation of 28% in the collected intestinal juice. However, differences between the metabolism of cefoxitin in the three systems were found to be statistically nonsignificant (Figure 2). The lack of any ester bond on the cefoxitin structure and therefore its higher resistance to hydrolysis by esterases, compared with other test compounds, might explain this stability. On the contrary, both side amide and acetyl groups of oseltamivir phosphate seemed to be responsible for

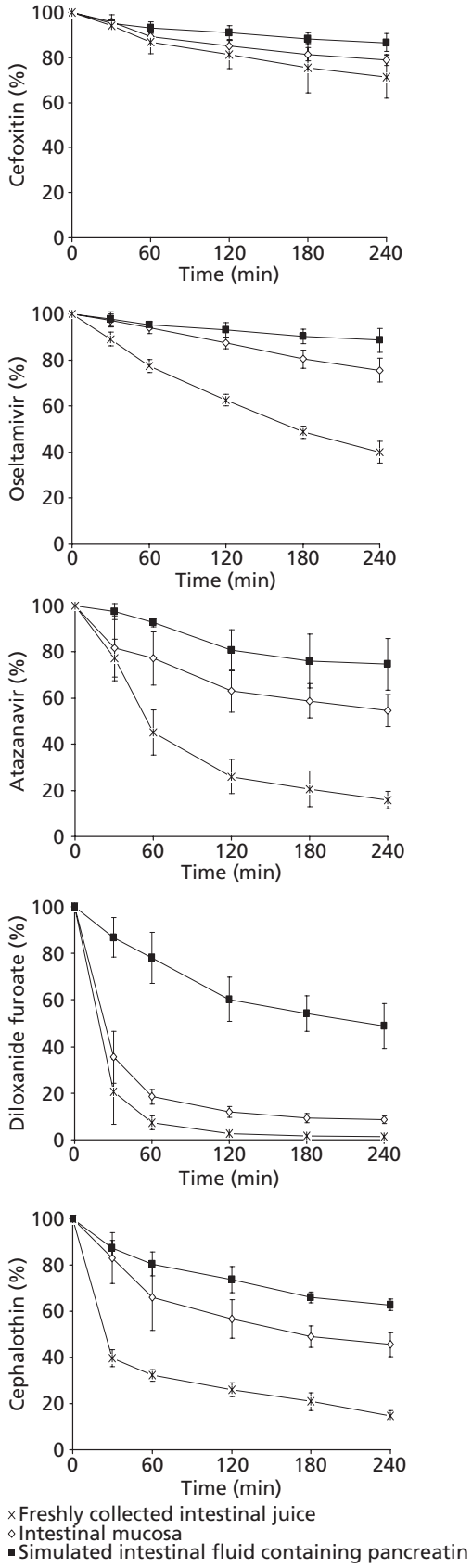

Figure 2 Percentage of remaining unmodified drugs.

its higher metabolism in intestinal juice, compared with cefoxitin (Figure 2). In particular, 60% of total oseltamivir incubated in the porcine juice was hydrolysed within the time experiment. This result proved the major capacity of intestinal juice versus SIF in the mimicry of human body conditions, being as approximately 90% oseltamivir is metabolised in the human intestinal tract.[14]

As depicted in Figure 2, the enzymatic hydrolysis of atazanavir and diloxanide furoate in collected intestinal juice was significantly higher compared with that in SIF/P. In particular, the percentage of unmodified (not metabolized) atazanavir and diloxanide furoate in SIF/P at the end of the experiment was 4.8- and 40-fold higher than that of unmodified drugs in the intestinal juice, respectively. Considering the low water solubility of the two drugs, the result might also be explained by the higher solubilizing capacity of the collected intestinal juice in comparison with that of SIF/P. Solubility of atazanavir and diloxanide furoate in SIF/P was measured to be approximately 0.39 and 0.40 mg/ml, respectively. In the freshly collected juice atazanavir and diloxanide solubility was 0.46 and 0.49 mg/ml, respectively. To determine how much drug solubilization in SIF affected these results, DMSO was added to SIF/P in a final concentration of 10% (v/v) and experiments were repeated. As shown in Figure 3, DMSO increased atazanavir and diloxanide furoate solubilization in SIF/P and consequently their metabolism. In freshly collected intestinal juice the effect given by DMSO in SIF/P was due to compounds such as natural detergents, whereas these were not present in the SIF/P. However, the increase of atazanavir and diloxanide furoate metabolism in DMSO/SIF/P at the end of the experiment was found to be only 0.6- and 0.8-fold compared with that in SIF/P, respectively. These findings proved that most of the observed higher metabolism in the collected juice was attributable to its enzymatic activity.

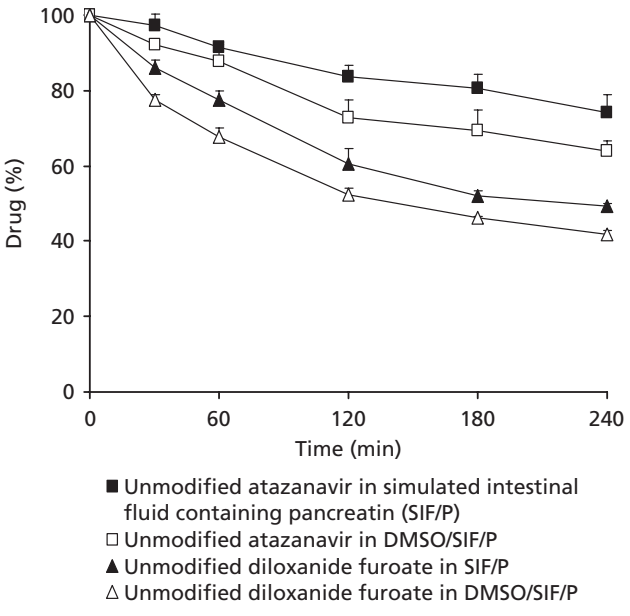

Figure 3 Percentage of remaining unmodified atazanavir or diloxanide furoate versus time.

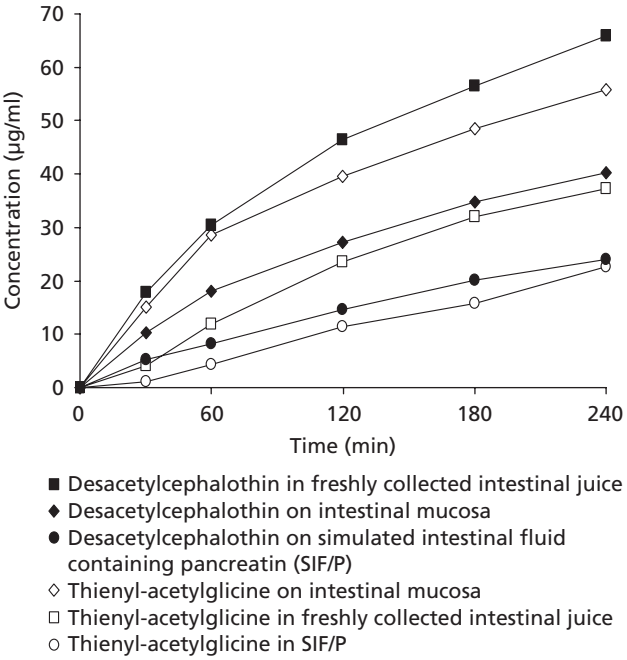

Figure 4 Percentage of cephalothin metabolites versus time profiles.

Diloxanide furoate was found to be the less stable tested drug, being hydrolysed by 99% in collected intestinal juice. This result was in agreement with findings of diloxanide furoate extensive metabolism in man.[15]

Concentration of unmodified cephalothin sodium in SIF/P after 4 h from the beginning of the experiment was observed to be 1.5- and 4.1-fold higher in comparison with that on intestinal mucosa and in fresh intestinal juice, respectively (Figure 2). Moreover, a progressive increase in the integration of two peaks was registered on the cephalothin chromatogram during the experiment. These peaks were attributed to cephalothin's metabolites, desacetylcephalothin and thienylacetyl glycine, which represent 50 and 32% of cephalothin's metabolites, respectively.[16] The formation of these compounds was caused by the hydrolysis of an ester bond in the case of desacetylcephalothin, and of the amide bond on the β-lactamic ring in the case of thienylacetyl glycine. This implied the activity of different enzymes such as carboxylesterase and the aminopeptidase hydrolysing cephalothin. Interestingly, it was noticed that the two metabolites were liberated differently on the mucosa and in the collected juice (Figure 4). The extent of formation of the main metabolite (desacetylcephalothin) in freshly collected intestinal juice was higher than that on the mucosa. On the other hand, the extent of the second metabolite, liberated by contact with the porcine mucosa, was higher than that in the intestinal juice. The findings suggested a difference in the localization of metabolic enzymes. Deacetylation of cephalothin sodium was catalysed by esterases, which were mainly located in the fluid of the intestinal lumen, whereas hydrolysis of the β-lactamic ring took place by the action of endopeptidases, which seemed to be predominant on the brush-border membrane.

Finally, the activity of enzymes derived from the 'sloughing off' or shedding of intestinal epithelial cells should not be underestimated. These represent another important source of metabolic enzymes in the lumen of the small intestine and keep exploiting their catalytic action, free or bound to particulate matter.[17] The more pronounced metabolic capacity of fresh intestinal juice in comparison with that on the mucosa might also depend on the contribution of these 'poured' enzymes.

Consideration should be given regarding the environment which the drugs are subjected to, even in the same portion of the intestinal tract. The surface of the enterocytic brush-border membrane is known to be significantly acidic compared with the bulk of luminal fluid. Measurement of this 'microclimate pH' in human revealed that the pH value of the membrane surface lies between 5.4 and 6.2.[18,19] This situation cannot be reproduced by the use of a fluid like SIF/P, which has a homogeneous composition and therefore pH value, whereas it might be simulated by using fresh intestinal mucosa. The pH value on the luminal surface of porcine intestinal mucosa, although not determined, might therefore mimic the differences with luminal fluid which have been observed in humans.

Determination of enzymatic activity at increasing drug concentrations

SIF/P, collected intestinal juice and intestinal mucosa were investigated regarding their drug metabolizing capacity using increasing concentrations of diltiazem hydrochloride. This further analysis was performed to demonstrate that at increasing drug concentrations the porcine intestinal juice was able to yield some metabolism. To this purpose 0.5, 1, 1.5 and 2.5 mg/ml drug solutions were used. As expected, metabolism of diltiazem hydrochloride tended to decrease in all in-vitro models as the concentration increased from 0.5 to 2.5 mg/ml (Figure 5a–c). In particular, it was found that a concentration of 2.5 mg diltiazem per ml provoked saturation of enzymes in the SIF/P. In fact, only 2% of 2.5 mg/ml drug solution was found to be metabolized in SIF/P within 4 h. On the contrary, 36% of drug still underwent enzymatic degradation in freshly collected intestinal juice within 4 h. These findings might be explained by the lower susceptibility of enzymes of the intestinal juice to undergo saturation at high drug concentrations, compared with that of the SIF/P. Therefore, the use of porcine intestinal juice enabled the unmasking of the fake stability of a drug in SIF/P. In fact, metabolism of a drug in SIF/P might not take place only due to the initial saturation by the drug of the digestive enzymes.

These results were confirmed further by the extent of the metabolite desacetyldiltiazem measured in the samples. In particular, the amount of desacetyldiltiazem liberated in collected juice from 2.5 mg/ml drug solution was found to be 7.5-fold higher in comparison with that produced in SIF/P.

Conclusions

The major determinants for the gastrointestinal fraction absorbed of a drug are permeability, solubility/dissolution, absorption time and stability.[4] The simulated intestinal fluid containing pancreatin (SIF/P) is a media used for testing intestinal dissolution and when it additionally contains 1% pancreatin for testing the stability of drugs and drug delivery

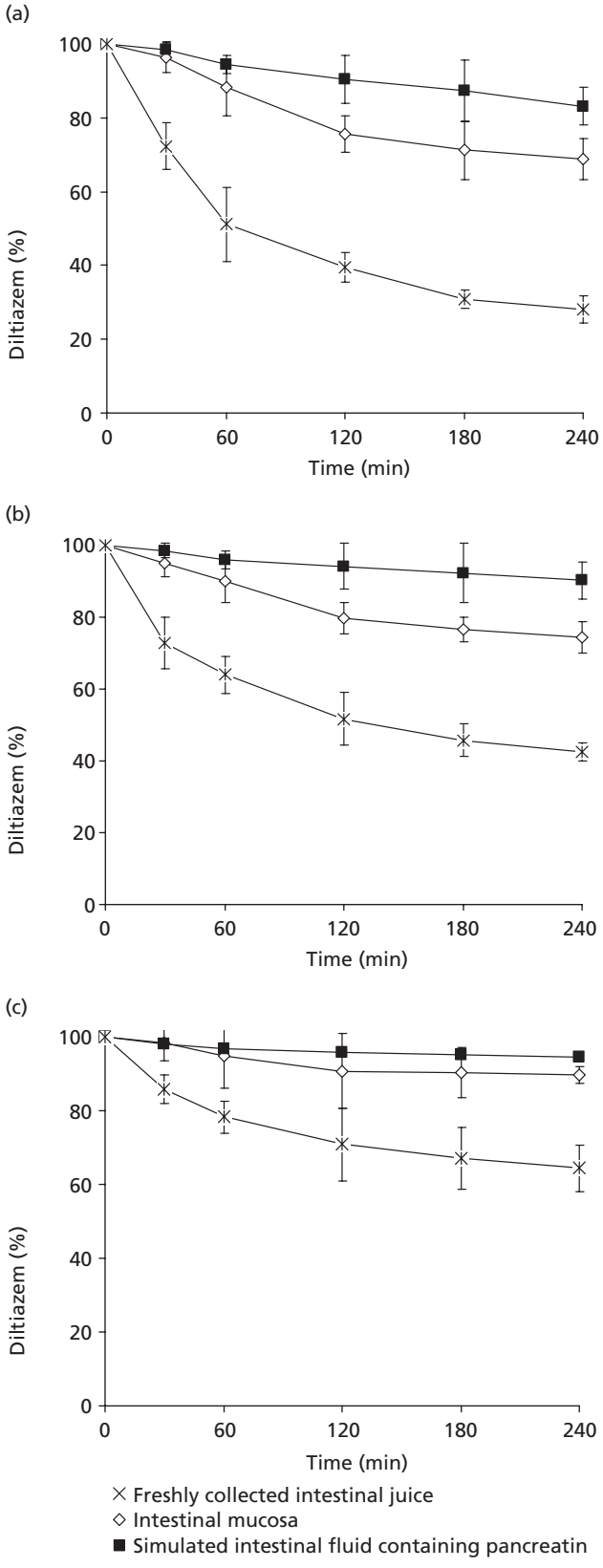

Figure 5 Percentage of remaining unmodified diltiazem hydrochloride. (a) 0.5 mg/ml, (b) 1.0 mg/ml, (c) 2.5 mg/ml.

systems, is well-accepted by the worldwide scientific community. Although SIF/P can be considered representative media of the intestinal pH in the fasted state and of its main enzymatic proteins, it is nevertheless frequently misleading. For example, a homogeneous suspension of enzymes cannot simulate the diverse enzymatic content in each segment of the intestine. Furthermore, SIF/P does not contain important components such as factors activating ions or products of food digestion. These compounds have been proven to interact with drugs, influencing their fate in the body. The chemical characteristics of human intestinal fluids not only vary between individuals but are also subjected to day to day changes which are crucial for the overall rate and extent of drug metabolism. The lack of constancy in fluid composition determines that any standardization of their composition is possible. On the other hand, the freshly aspirated juice from human intestine is of limited source. Although freshly collected porcine intestinal juice represents a simplification of the luminal environment, it has shown to better mimic the in-vivo metabolic fate of small molecular drugs with peptidic substructure, as compared with SIF/P. At the same time its chemical composition is closer to the human intestinal fluid than SIF/P, it is less susceptible to enzymatic saturation and is easily available. This media could therefore be considered a useful tool to determine the stability of small molecules in the screening of oral drug candidates.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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