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Intestinal transport of cefuroxime axetil in rats: absorption and hydrolysis processes

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Abstract

Studies were performed using three cefuroxime axetil solutions (11.8, 118 and 200 μM) in three selected intestinal segments and one cefuroxime axetil solution (118 μM) in colon of anaesthetized rats. First-order absorption rate pseudoconstants, k_{ap} and effective permeability coefficients, P_{eff}, were calculated in each set. Absorption of cefuroxime axetil can apparently be described as a carrier-mediated transport, which obeys Michaelis–Menten and first order kinetics in the proximal segment of the small intestine and a passive diffusion mechanism in the mean and distal segments. The absorption kinetic parameters for cefuroxime axetil were obtained: $V_m = 0.613$ (0.440) μM min^{-1}; $K_m = 31.49(28.31)$ μM and $k_a = 0.011(0.003)$ min^{-1}. Parameters characterizing degradation of the prodrug were obtained in each intestinal segment: proximal segment $k_{dp} = 0.0049(0.0003)$ min^{-1}, mean segment, $k_{dm} = 0.0131(0.0007)$ min^{-1} and distal segment $k_{dd} = 0.019(0.0009)$ min^{-1}. Therefore, in situ intestinal absorption of cefuroxime axetil in the proximal segment of the rat in the presence of variable concentrations of cefadroxil has been investigated in order to examine the inhibitory effect of cefadroxil on cefuroxime axetil transport. The data suggest that cefadroxil and cefuroxime axetil share the same intestinal carrier.

Keywords: Carrier-mediated; Cefadroxil; Cefuroxime axetil absorption; Competitive inhibition; Intestinal permeability

1. Introduction

Cefuroxime axetil is an oral prodrug form of the cephalosporine cefuroxime sodium. When administered orally to humans, the parent drug cefuroxime is not absorbed, whereas, the prodrug shows absolute bioavailabilities of 36 and 52% in the fasted and fed states, respectively (Finn et al., 1987). Following absorption from the gastrointestinal tract, cefuroxime axetil is rapidly hydrolyzed by non-specific mucosal esterases to cefuroxime and acetaldehyde. No cefuroxime axetil (intact ester) was detected in the blood (Powell et al., 1991).

In a recent paper (Ruíz-Balaguer et al., 1997), we demonstrated that the absorption process of

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the prodrug along the whole length of the small intestine is clearly saturable and can be described by Michaelis–Menten kinetics and this process is inhibited by sodium azide. Both characteristics correspond to a specialized absorption process, to which the variable and incomplete oral bioavailabilities of this prodrug could be attributed.

The aim of this paper is to study the absorption mechanism of cefuroxime axetil and understand better the factors that could modify bioavailability when the drug is administered by the oral route. Bearing this in mind, we focused our next study on the analysis of possible preferent absorption in one fraction of the small intestine of the rat in order to gain an insight into the oral absorption model of this prodrug. In this study, the absorption process of cefuroxime axetil is induced in selected rat intestinal segments by perfusion of cefuroxime axetil solutions at different concentrations. The results obtained were analyzed in order to: (1) quantify the hydrolysis of the cefuroxime axetil ester in each segment of the gastrointestinal tract; (2) characterize the influence of the intestinal segment in cefuroxime axetil absorption; and (3) corroborate the existence of nonlinearities in absorption as a function of prodrug concentration in the perfusion fluids.

2. Materials and methods

2.1. Absorption studies

All absorption tests were performed on male Wistar rats weighing 250–300 g, fasted for 20 h but with free access to water. Anesthesia was induced 1 h before surgery by an intraperitoneal injection of ethyl-urethane. All pharmacokinetic studies adhered to the 'Principles of Laboratory Animal Care'.

A rat gut in situ preparation (Doluisio et al., 1969), modified as previously reported (Merino et al., 1989; Sánchez-Picó et al., 1989), was used. The whole length of the small intestine (about 1 m) was divided into three segments of equal lengths ($L = 33$ cm). Static perfusion was performed in one of the segments or in the whole colon ($L = 15$ cm). After rinsing with physiological saline solution (25 ml) in order to eliminate faecal residues and debris, 5 ml of a buffered solution of cefuroxime axetil (11.8, 118 or 200 μM for small intestinal fractions, and 118 μM for colon) was perfused at 37 °C. The pH was adjusted to the mean value found for each intestinal segment, using physiological conditions, in preliminary 5-rat experiences (Merino et al., 1989): 6.7 for proximal, 7.6 for mean, 8.2 for distal, and 7.5 for colon. The remaining concentrations of the prodrug in the intestinal lumen were measured every 5 min, for a total time of 30 min, using 300 μl samples. In the proximal segment tests, the biliary duct was ligated. From six to eight animals per set were employed to study cefuroxime axetil absorption kinetics.

These aliquots (300 μl) were added to small tubes containing 90 μl 10% trichloroacetic acid and 90 μl acetonitrile. This procedure precipitated any proteins present, thereby deactivating the esterases present in the luminal liquid and stopping the hydrolysis of the cefuroxime axetil ester (Mosher Gerold et al., 1992).

Water reabsorption was evaluated separately for each animal. This process obeys apparent zero-order kinetics. In order to characterize the zero-order equation in each experiment, the volume remaining at 30 min was measured. Separating it from the intestinal fraction assessed the residual volume. The volume remaining at 0 min was evaluated as previously reported (Merino et al., 1989; Ruíz-Balaguer et al., 1997).

From the values of the mean remaining volume at 0 min ($n = 13$) and the individual volume at 30 min, a straight line was used. Thus, the theoretical volumes at each sampling time can be known and the remaining cefuroxime axetil and cefuroxime concentrations properly corrected.

The above mentioned in situ technique was also used to evaluate the interaction between cefuroxime axetil and cefadroxil in their absorption process. In this case, two solutions were perfused in the proximal intestinal segment, each containing cefuroxime axetil 118 μM in the presence of cefadroxil 0.98 or 27 mM. Six animals per set

were used for the inhibition assays. These solutions were also used to evaluate luminal disappearance of cefadroxil in the presence of the prodrug.

2.2. Analytical procedures

Intestinal samples were assayed for cefuroxime and the two diastereomers of cefuroxime axetil content by high-performance liquid chromatography (HPLC), which provided an excellent separation and quantification technique. The method used was described in detail in a previous paper (Ruíz-Balaguer et al., 1997)

Calibration curves covering the entire range of cefuroxime, cefuroxime axetil and cefadroxil concentrations in luminal samples were estimated in triplicate. Excellent linear plots relating the peak area and solute concentration were found, and the intercept did not significantly differ from zero. Due to the simplicity of the procedure, no internal standard was needed.

The accuracy and precision of the method were validated. The criteria were assessed using four concentrations for each initial perfusion solution, covering the entire calibration range of the analytical method. Accuracy was evaluated by calculating the relative error, which was always less than 8 %. Precision was evaluated by calculating the coefficient of variation, which was in all the cases lower than 5 %. These results were considered satisfactory (Karnes and March, 1993).

2.3. Fittings of models to data and statistical procedures

2.3.1. Cefuroxime axetil first order absorption rate measurements

Cefuroxime axetil is hydrolyzed in the lumen to cefuroxime, which has a negligible level of gastrointestinal absorption. Therefore, the cefuroxime concentration (CF) had to be added individually to the cefuroxime axetil concentration (CFA) in order to measure the total concentration of residual nonabsorbed cefuroxime axetil at each sampling time (CFA_T). As a preliminary step, cefuroxime axetil intestinal absorption was quantified using the apparent first-order rate constants, in the usual way, according to the expression:

$$CFA_T = CFA_{T0} \cdot e^{-k_{ap}t} \quad (1)$$

where CFA_T values are the concentrations of cefuroxime axetil remaining in the luminal content—already corrected for water reabsorption—at the sampling times, t; k_{ap} is the apparent absorption rate constant and CFA_{T0} is the initial concentration of the cefuroxime axetil, which is always lower than the actual concentration perfused because of membrane adsorption (Doluisio et al., 1970). In order to overcome these drawbacks, only the samples obtained between 5 and 30 min were used for calculations, i.e. the zero time samples was not used for regression (Doluisio et al., 1969; Sánchez-Picó et al., 1989; Ruíz-Balaguer et al., 1997). Absorption rate pseudoconstants, k_{ap}, were then determined for each intestinal segment and each initial cefuroxime axetil concentration data set ($n = 6–8$ animals).

Both parameters (CFA_{T0} and k_{ap}) were calculated for each animal according to a non-linear regression least-squares procedure using the ADAPT II program (D'Argenio and Schumitzky, 1990). Moreover, the effective permeability coefficient of cefuroxime axetil were determined in each set of data by the equation (Amidon et al., 1995):

$$P_{eff} = k_{eff}\frac{R}{2} \quad (2)$$

where P_{eff} is the effective permeability and R is the radius of the intestinal segment, assuming $R = 0.22$ cm in the segments of the small intestine and $R = 0.32$ cm in the colon.

In order to assess the influence of the intestinal segment in cefuroxime axetil absorption the k_{ap} and P_{eff} average values found at the same initial concentration in different intestinal segments were compared using a one-way ANOVA test followed by a multiple-range posthoc test. The same method was used to detect nonlinearities in drug absorption, through the comparison of the k_{ap} and P_{eff} values found in a particular segment at the three different initial concentrations used.

2.3.2. Global joint kinetics of absorption and degradation of cefuroxime axetil

When the k_{ap} decreases significantly, as the initial solute concentration in the perfusion fluid increases, the existence of a carrier mediated transport can be suspected. Since, non-linearities in the cefuroxime axetil absorption process were detected when the drug was perfused in the initial segment of the small intestine (Table 2); global fits to data of cefuroxime axetil and cefuroxime were performed in order to characterize and quantify the absorption and degradation processes of cefuroxime axetil in each segment of the small intestine. Therefore, a six compartment model was used for each level of starting cefuroxime axetil concentration (Fig. 1). Compartments 1, 2 and 3 represents the cefuroxime axetil concentration in proximal, mean and distal segments, respectively, and compartments 4, 5 and 6 represents the cefuroxime concentrations in proximal, mean and distal segments, respectively. A total of 18 differential equations that include both processes were fitted to available experimental data of remaining concentrations in the intestinal lumen of cefuroxime axetil and cefuroxime (Fig. 2). Thus, nine groups were available for both kinds of data (cefuroxime axetil and cefuroxime concentrations), it is 18 functions output. A stepwise forward inclusion search was performed to select the models. A new parameter was included if it improves the objective function by at least 3.84 (χ^2-value for $\alpha = 0.05$ and $df = 1$).

Finally, absorption and degradation kinetic parameters were obtained using in the proximal segment for absorption process combined Michaelis–Menten and first order differential equation and for degradation of cefuroxime axetil process first order equation. In mean and distal segment first order equation were used for absorption and degradation processes. The selected final model was obtained using the following equations.

Proximal segment: compartments 1 and 4.

$$\frac{dCFA}{dt} = -\frac{V_m \cdot CFA}{K_m + CFA} - k_a \cdot CFA - k_{dp} \cdot CFA \quad (3)$$

$$\frac{dCF}{dt} = k_{dp} \cdot CFA \quad (4)$$

Mean segment: compartments 2 and 5.

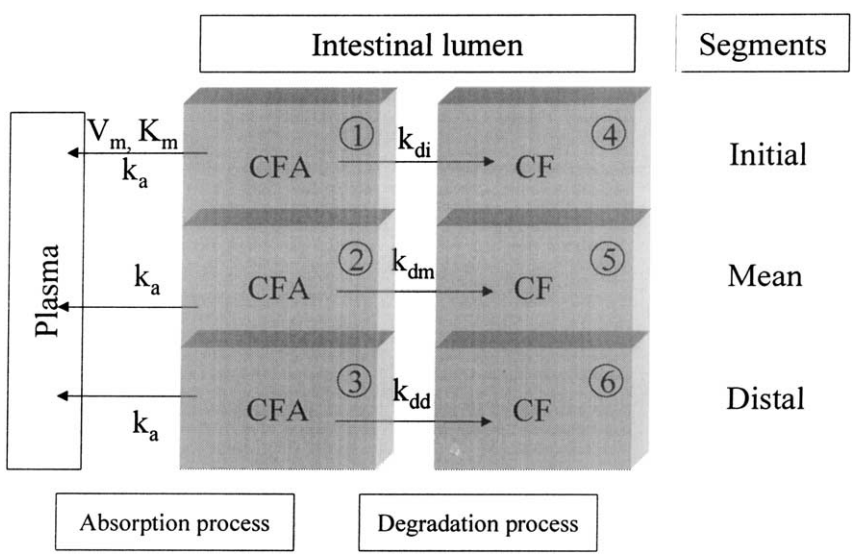

Fig. 1. Six compartment model of absorption and degradation of cefuroxime axetil in the intestinal lumen used in the fits. The absorption and degradation of cefuroxime axetil in the small intestine are illustrated. CFA and CF represent the remaining concentrations of cefuroxime axetil and cefuroxime, respectively, in each intestinal segment. Absorption and degradation kinetic parameters of cefuroxime axetil are also shown.

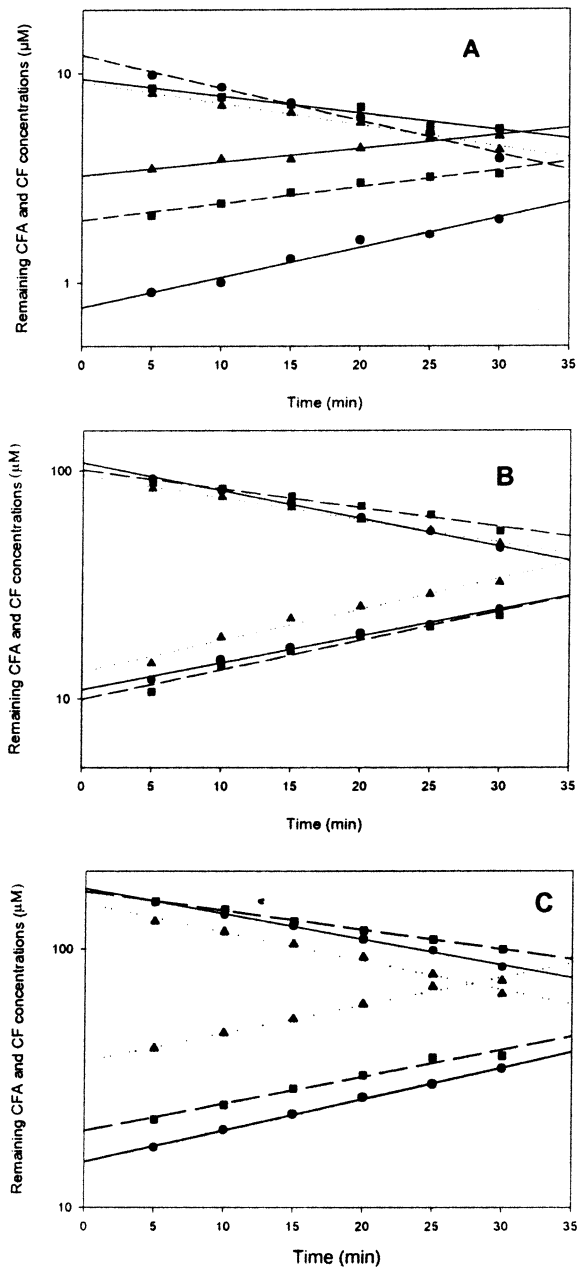

Fig. 2. Luminal concentrations of cefuroxime axetil and cefuroxime. Disappearance of cefuroxime axetil (negative slopes) and appearance of cefuroxime (μM) (positive slopes) in small intestine (●) initial segment, (■) mean segment and (▲) distal segment after perfusing (A) 11.8; (B) 118; and (C) 200 μM initial concentration of cefuroxime axetil, respectively.

$$\frac{\mathrm{dCFA}}{\mathrm{d}t} = -k_{\mathrm{a}}\cdot\mathrm{CFA} - k_{\mathrm{dm}}\cdot\mathrm{CFA} \quad (5)$$

$$\frac{\mathrm{dCF}}{\mathrm{d}t} = k_{\mathrm{dm}}\cdot\mathrm{CFA} \quad (6)$$

Distal segment: compartments 3 and 6.

$$\frac{\mathrm{dCFA}}{\mathrm{d}t} = -k_{a}\cdot\mathrm{CFA} - k_{\mathrm{dd}}\cdot\mathrm{CFA} \quad (7)$$

$$\frac{\mathrm{dCF}}{\mathrm{d}t} = k_{\mathrm{dd}}\cdot\mathrm{CFA} \quad (8)$$

Here, dCFA/dt is the concentration change rate of cefuroxime axetil, V_{m} the maximal absorption rate, K_{m} the Michaelis–Menten constant for cefuroxime axetil, k_{a} the first-order absorption rate constant, CFA the remaining cefuroxime axetil concentration in the corresponding intestinal segment; dCF/dt is the concentration change rate of cefuroxime, k_{dp}, k_{dm}, and k_{dd} are the first order degradation rate constant and CF is the cefuroxime remaining concentration in the proximal, mean or distal segment.

As experimental data, the remaining cefuroxime axetil and cefuroxime concentrations at each sampling time were used (Fig. 2). Calculations were performed globally for all the remaining cefuroxime axetil and cefuroxime concentrations in free solution, that is, for the three sets of data found in each intestinal segments 18 functions outputs, by using all individual values at each time (naive pooled data). These fits were performed using the ADAPT II (D'Argenio and Schumitzky, 1990) program (Simplex algorithm; weighting factor = $1/y^2$). The model files of absorption and degradation kinetics of cefuroxime axetil were written in FORTRAN and compiled and linked with the ID program. A set of six differential equations for each starting concentration was used. Weighted sum of squares of residuals, W.S.S. and standard errors of the parameters, S.E., were used to assess the goodness of the fits.

2.4. Inhibitory studies

Inhibitory effects on cefuroxime axetil absorption resulting from the inhibition of possible carriers were characterized by statistical comparison

between the k_{ap} values found in the presence and in the absence of the inhibitor (cefadroxil).

The influence of cefuroxime axetil on the cefadroxil absorption pathway was characterized by statistical comparison between the k_{ap} values found in the presence and in the absence of the prodrug.

3. Results

The average concentrations of cefuroxime axetil and cefuroxime remaining in the intestinal lumen samples after static perfusion of the prodrug solutions (11.8, 118 and 200 μM) and corrected for water reabsorption are given in Fig. 2. Only one solution of cefuroxime axetil (118 μM) was perfused in colon segment. The average apparent first-order absorption rate constant, k_{ap} (S.D.), and membrane permeability, P_{eff} (S.D.), for each set of data, at each intestinal segment and colon, are show in Table 1. Results of the one-way ANOVA tests are also given.

Table 2 gives the kinetic parameter values of absorption and degradation and statistical figures obtained after fitting for absorption process the differential form Michaelis–Menten and first-order equation to the experimental data found in the proximal segment, and differential form first order equation to the experimental data found in mean and distal segments. The hydrolysis parameters were obtained after fitting the differential form first order equation to the experimental data found in the three segments of the small intestine. The sum of weighted squares of residuals, W.S.S., standard error, S.E. and variation coefficient, CV%, are also shown.

Table 3 reports the average total concentrations of cefuroxime axetil (CFA_T) remaining in the intestinal samples after perfusion in the proximal segment of the 118 μM cefuroxime axetil solution added 0.98 and 27 mM cefadroxil, as well as, the remaining cefadroxil concentrations found in the same perfusion solutions. The average apparent first-order absorption rate constant, k_{ap} (S.D.), and membrane permeability, P_{eff} (S.D.), values for each set of data, is also shown.

A one-way ANOVA test was used to detect the significant differences between the k_{ap} values of cefuroxime axetil obtained in the absence and in the presence of variable concentrations of cefadroxil ($P < 10^{-4}$).

A one-way ANOVA test revealed no significant differences between the k_{ap} values of the cefadroxil 27 mM tested solution, obtained in the absence and in the presence of cefuroxime axetil 118 μM ($P = 0.4874$). The same test showed no significant differences between the k_{ap} values of the cefadroxil 0.98 mM tested solution, obtained in the absence and in the presence of cefuroxime axetil 118 μM ($P = 0.4523$).

Table 1
Absorption rate constants and effective permeability coefficients of cefuroxime

	Intestinal segment	Cefuroxime axetil perfusion concentrations (A_i, μM)			P
		11.8	118	200	
k_{ap} (S.D.) (h^{-1})	Proximal	1.64 (0.3)	1.02 (0.2)	0.75 (0.1)	$<10^{-4}$
	Mean	0.55 (0.1)	0.52 (0.1)	0.52 (0.1)	N.S.
	Distal	0.40 (0.1)	0.48 (0.1)	0.39 (0.0)	N.S.
	Colon		0.22 (0.1)		
	P	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	
P_{eff} (S.D.) ($\times 10^{-4}$ cm s^{-1})	Proximal	0.50 (0.09)	0.31 (0.06)	0.23 (0.03)	$<10^{-4}$
	Mean	0.17 (0.03)	0.16 (0.03)	0.16 (0.03)	N.S.
	Distal	0.12 (0.03)	0.15 (0.03)	0.12 (0.00)	N.S.
	Colon		0.12 (0.03)		
	P	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	

Absorption rate constants (k_a (S.D.)) and permeability coefficients (P_{eff} (S.D.)) of cefuroxime axetil, relative to initial concentration perfused of 11.8, 118 and 200 μM for each intestinal segment tested.

Table 2
Cefuroxime axetil absorption and degradation kinetics

Segment	Absorption parameters		Hydrolysis parameters	
Proximal	V_m (μM min^{-1})	0.613(0.440) 71.76%	k_{dp}(min^{-1})	0.0049(0.0003) 6.36%
	K_m (μM)	31.49(28.31) 89.91%		
	k_a (min^{-1})	0.011(0.003) 26.07%		
Mean	k_a (min^{-1})	0.011(0.003) 26.07%	k_{dm} (min^{-1})	0.0131(0.0007) 8.31%
Distal	k_a (min^{-1})	0.011(0.003) 26.07%	k_{dd} (min^{-1})	0.0190(0.0009) 6.77%
W.S.S.	91.74			

Absorption parameter values (standard errors) and variation coefficients obtained after fitting the combined Michaelis–Menten and first-order differential equation to the data obtained in proximal segment of the small intestine, and first order differential equation to the data obtained in mean and distal segments of the small intestine. Degradation parameter values (standard errors) and variation coefficients obtained after fitting the first order differential equation to the data obtained in proximal, mean and distal segments of the small intestine, weighted sum of squares, W.S.S., are also given.

4. Discussion

4.1. Experimental absorption technique

In order to demonstrate a carrier-mediated transport, several factors can be studied: concentration dependence, inhibition by competitive substrates or other types of inhibitors, temperature dependence, and directional (apical to basolateral and vice versa) transport, etc. Using the in situ perfusion method previously described, the latter two tests would be difficult to perform, but the first two tests are critical for proof of concept.

Intestinal single-pass or recirculation methods in situ have often been used to characterize the specialized absorption processes of drugs in small animals instead of static perfusion methods like the one employed in this study. It has been demonstrated that the intrinsic permeability constants obtained by both types of methods, when normalized for perfused volumes and intestinal lengths, are virtually identical provided that suitable corrections for water reabsorption are made. Since the latter procedures allow absorption rates several-times greater (Plá-Delfina and Moreno, 1981), nearer to in vivo values (Doluisio et al., 1969) and much more suitable for kinetic calculations (Plá-Delfina and Moreno, 1981; Merino et al., 1989; Sánchez-Picó et al., 1989), we chose the static in situ perfusion method as routine working procedure, in order to better characterize the possible nonlinearities and to fit the absorption model more easily to experimental data.

4.2. Influence of the intestinal segment in cefuroxime axetil absorption

A one-way ANOVA test was used to assess significant differences between the k_{ap} and P_{eff} values for cefuroxime axetil in free solutions in the three intestinal segments of the small intestine and colon at each starting solution perfused. A subsequent multiple range posthoc tests allow to compare the behavior of a particular intestinal segment respect to any other.

When 11.8 and 200 μM of cefuroxime axetil solutions were perfused the one-way ANOVA test revealed significant differences among the values obtained in each intestinal segment ($P < 10^{-4}$) (Table 1). The Scheffé test indicated that the k_{ap} and P_{eff} values found in proximal segment were significantly different from k_{ap} and P_{eff} values, respectively, obtained in mean and distal segments at 0.05 α level.

When the tested solution was cefuroxime axetil 118 μM, the one-way ANOVA test revealed significant differences among the values obtained in each intestinal segment ($P < 10^{-4}$). A multiple-range analysis test showed that in the proximal fraction, the k_{ap} values of the cefuroxime axetil was greater than in mean and distal segments, and at the same time these values are greater than colon k_{ap} value. The P_{eff} value obtained in the proximal fraction is statistically different from the values obtained in others intestinal segments ($P < 10^{-4}$). Nevertheless, when the P_{eff} values are used

as absorption representative parameter, the value obtained in the colon is the same than the one obtained in the mean and distal intestinal segments.

This may indicate that the proximal fraction has a greater absorption capacity for the prodrug, that is, there is an absorption window in this intestinal segment.

4.3. Influence of the initial perfused concentration in cefuroxime axetil absorption

To detect non-linearities in the cefuroxime axetil absorption process, three concentrations (11.8, 118 and 200 μM) were selected according to the hydrosolubility of the prodrug (Ruíz-Balaguer et al., 1997). The kinetics of the absorption process can be first-order, Michaelis–Menten or combined Michaelis–Menten and first order. In the first case, the k_a is concentration-independent, while in the second and third cases, it is possible to calculate k_{ap} which is a pseudoconstant that decreases as the concentration of the drug in the solution increases. Since the apparent first order kinetic fits data well during the 30 min interval of the experiment along the entire range of concentrations used in the situ absorption tests, the changes k_{ap} undergoes depending on the cefuroxime axetil concentration constitute an excellent reference point for determining whether there is (if k_{ap} remains constant) or is not (if k_{ap} decreases as the cefuroxime axetil concentration increases) linearity. In the latter case the Michaelis–Menten or combined Michaelis–Menten and first order kinetics should be considered highly probable. Table 2 shows the apparent k_{ap} and P_{eff} values obtained along the concentration range used.

Statistical analysis of the k_{ap} and P_{eff} found as a function of the cefuroxime axetil concentration in the perfusion fluids in the same intestinal segment clearly shows that there is some nonlinearity. A one-way ANOVA test shows significant differences between k_{ap} values obtained in the proximal segment of the small intestine ($P < 10^{-4}$). The same result is obtained when the P_{eff} values are compared ($P < 10^{-4}$). In mean and distal segments not differences in both, k_{ap} and P_{eff} values, were found ($P = 0.75$ and $P = 0.31$) (Table 1). A subsequent multiple range analysis shows that in the proximal fraction, the k_{ap} value of the cefuroxime axetil 11.8 μM was significantly different from the k_{ap} values obtained for the

Table 3
Inhibition assays of cefuroxime axetil

Time (min)	Remaining concentration of			
	Cefuroxime axetil 118 μM with added Cefadroxil 0.98 mM	Cefadroxil 0.98 mM with added Cefuroxime axetil 118 μM	Cefuroxime axetil 118 μM with added Cefadroxil 27 mM	Cefadroxil 27 mM with added Cefuroxime axetil 118 μM
5	98.9(5.5)	0.76(0.07)	105.0(11.8)	20.3(1.3)
10	93.7(5.5)	0.65(0.05)	101.8(10.7)	19.1(1.1)
15	89.1(5.6)	0.55(0.05)	99.8(11.3)	17.6(0.9)
20	84.8(4.5)	0.47(0.04)	98.5(11.4)	16.2(1.4)
25	81.1(3.8)	0.42(0.04)	96.5(11.2)	14.9(1.3)
30	77.3(4.7)	0.34(0.04)	94.4(11.4)	13.9(1.5)
$k_{ap}(h^{-1})$	0.59(0.1)	1.90(0.20)	0.25(0.04)	0.93(0.23)
P_{eff} ($\times 10^{-4}$ cm s^{-1})	0.18(0.03)	0.58(0.06)	0.76(0.01)	0.28(0.07)
r	0.999	0.999	0.993	0.998

k_{ap} of cefadroxil (0.98 mM) = 1.987 h^{-1}, P_{eff} ($\times 10^{-4}$ cm s^{-1}) = 0.61; k_{ap} of cefadroxil (27 mM) = 1.044 h^{-1}, P_{eff} ($\times 10^{-4}$ cm s^{-1}) = 0.32 (Sánchez-Picó et al., 1989). Average concentrations of cefuroxime axetil remaining in the intestinal samples after perfusion, in proximal segment, of the 118 μM cefuroxime axetil solution supplemented with 0.98 and 27 mM cefadroxil. The remaining cefadroxil concentrations found in the same perfusion solutions are also given. The average apparent first-order absorption rate constant (S.D.) for each set of data and correlation coefficient are also shown.

cefuroxime axetil 118 and 200 μM, respectively. These results show that the absorption process of the prodrug in the proximal fraction is not linear, whereas in the mean and distal segments, the k_{ap} values are not significantly different for the three initial perfusion solutions. This suggests that the absorption process of the cefuroxime axetil in these segments is linear for the range of perfused concentrations.

4.4. Absorption and degradation models

In view of the results reported here, we focused the subsequent studies on each intestinal fraction in order to calculate the absorption and degradation cefuroxime axetil parameters.

According the weighted sum of squares values, and the variation coefficients of the parameters the best result was obtained when the absorption process was combined Michaelis–Menten and first order for proximal segment and first order for mean and distal segments, and hydrolysis process was first order for all segments assayed. Passive diffusion was constant along the gastrointestinal tract, in other words, the absorption rate constant, k_a is the same in proximal, mean and distal segments. On the other hand, the cefuroxime axetil hydrolysis process is well described by first order kinetic, but is different in each intestinal segment, therefore, three hydrolysis rate constants were obtained, k_{dp}, k_{dm} and k_{dd} for proximal, mean and distal segments, respectively. Thus, the zero value is not included in a 95% confidence interval of the differences between k_{dp} and k_{dm}, and k_{dp} and k_{dd}

Absorption and degradation parameter values obtained indicate that the absorption rate is higher than degradation rate in the proximal segment. However, in mean and distal segments the cefuroxime axetil degradation rate is higher than the absorption rate. Results obtained here tend to indicate that poor extent bioavailability of cefuroxime axetil could be obtained when an important fraction of dose administered arrive unabsorbed to mean or distal fractions of small intestine, i.e. when a high intestinal motility exists.

The value of apparent K_m for absorption of cefuroxime axetil is 31.49 (28.31) μM. This value indicates high affinity by the transporter, higher than others β-lactam antibiotics (K_m ranging from 500 to 14 000 μM). These differences could be attributed to methods employed for transport assays and experimental conditions used in each laboratory.

4.5. Influence of the cefadroxil in cefuroxime axetil absorption

This assay was performed only in the proximal segment of small intestine because a saturable kinetics was detected. Many studies in various animal species, including humans and with different tissue preparations have shown that certain hydrophilic β-lactam antibiotics can be transported by oligopeptide transporters in the small intestine (Tsuji and Tamai, 1996; Walter et al., 1996; Graul and Sadée, 1997).

In the present study we have used cefadroxil, a β-lactam antibiotic, as a potential inhibitor of cefuroxime axetil absorption. The choice of this compound was based on data from the literature. According to some authors, a carrier-mediated pH-dependent peptide transporter (PepT1) transports this antibiotic (Tsuji and Tamai, 1996; Bretschneider et al., 1999).

In order to gain an insight into the intestinal absorption pathway of cefuroxime axetil, perfusions were carried out with a 118 μM isotonic solution of cefuroxime axetil in the presence of increasing concentrations of cefadroxil (0.983 and 27 mM) in the perfusion fluid (Table 3). The results show that cefadroxil acts as an inhibitor of the cefuroxime axetil absorption process, generating 42.5 and 76.0% inhibition, respectively. The k_{ap} value decreases to a limiting value of 0.25 h^{-1} ($\pm$ 0.04) as the cefadroxil concentration is increased, as shown in Table 3.

This behavior could be due to the fact that the highest cefadroxil concentration in the mixture solution does not completely abolish the cefuroxime axetil absorption because a passive diffusion coexists.

In light of this result, we can conclude that cefuroxime axetil absorption in proximal segment of small intestine is mediated by a transport system shared by cefadroxil.

4.6. Biopharmaceutical implications

The saturable and selective absorption observed is not due to saturation of the luminal metabolism of cefuroxime axetil. It is true that when the cefuroxime axetil concentration increases in the perfusion fluid, an increase in the hydrolysis of the prodrug is observed (Fig. 2). A two-way ANOVA test revealed significant differences among prodrug hydrolysis percentages after 30 min of perfusion in the three intestinal segments of the small intestine ($P < 0.0034$), while no significant differences were found at all the tested concentrations ($P < 0.7081$).

According to these results, the luminal degradation of the prodrug is probably not responsible for the nonlinear absorption, although it is one of the causes of its poor bioavailability. This effect is very important in the mean and distal segments. On the other hand, the variability in the bioavailability obtained can be explained by the existence of interindividual variability in the enzymatic activity of the intestinal esterase responsible for the hydrolysis of the prodrug, as the greater the hydrolyzed fraction is, the smaller the absorbed fraction would be.

The improvement in bioavailability after food ingestion could be explained, on one hand, by the fact that the esterase is now hydrolyzing not only the prodrug but also the food and on the other hand, by the slowing of the gastric emptying. The prodrug arrives at its absorption site (proximal segment) slowly and so, in the case of a carrier-mediated transport, the carriers are not saturated.

In proximal segment where the cefuroxime axetil absorption is higher, pH is lower than mean and distal segments. Data from literature shows that the influence of pH on the bioavailability of cefuroxime axetil is substantial. The bioavailability is significantly enhanced in children by the concomitant ingestion of prodrug and infant formula or whole milk (Ginsburg et al., 1985). This behavior could be due to the fact that lactic acid (pK_a 3.86) largely exists as a dissociated form at the pH of intestinal luminal solution, and increases the proton concentration (Ginsburg et al., 1985; Walter et al., 1996). The opposite effect can be observed in the pretreatment with ranitidine and sodium bicarbonate; it results in a lower bioavailability for cefuroxime axetil than in the fasting state and also tends to cancel the enhanced postpandrial absorption (De Sommers et al., 1984).

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