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Characteristics of Cephalexin transport across isolated rabbit ileum

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Abstract

The purpose of this study was to examine the total transepithelial (transcellular, paracellular and carrier-mediated) transport of cephalexin (CFX) across isolated rabbit ileum. This epithelium was mounted in Grass diffusion cells to separate the mucosal and serosal compartments. The tissue was immersed on each side into a Ringer's solution or a PBS buffer solution at 37°C and oxygenated (O_2/CO_2 in 95/5, v/v). The concentration dependence of CFX transport was examined over a concentration range of 0.1–5 mM and showed a Michaelis–Menten kinetics. The inhibition of CFX transport was $97 \pm 1\%$ at 4°C and 30 and 61% with metabolic inhibitors such as sodium azide (1 mM) and 2.4 dinitrophenol (0.1 mM) respectively. The CFX transepithelial flux was greater at pH 6 (11.42 ± 2.24) than at pH 7–7.4 (5.0 ± 1.03) in nmol/h per cm^2. Without a pH gradient at pH 7.4 this effect was inhibited by FCCP and nigericin. At pH 7.4, amiloride did not affect CFX transport but ouabaine significantly affected the CFX flux with an inhibition of 90%. No paracellular diffusion was observed in the presence of D-glucose (25 mM). The mucosal to serosal flux of 0.1 mM of CFX was significantly inhibited by 1 mM of gly-pro. Similarly, cephradine (2 mM) inhibited 0.2 mM of CFX by 23% and amoxicilline (5 mM) reduced the transport by 56%. L-phenylalanine did not affect the transport of 0.5 mM of CFX. The transport of CFX through rabbit ileum followed Michaelis–Menten kinetics at lower concentrations, was pH-dependent, energy-dependent, Na^+-independent and occurred across the intestinal mucosa through a transcellular route via dipeptide carrier system(s). © 1997 Elsevier Science B.V.

Keywords: Cephalexin; Michaelis–Menten kinetic; Grass diffusion cell; Transcellular transport; Carrier-mediated transport

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1. Introduction

Aminocephalosporins are efficiently absorbed from the small intestinal lumen, although they are strongly ionized over the pH range in the gastrointestinal tract and have very low lipid solubility. The transport mechanism of these drugs have been therefore extensively investigated (Kimura et al., 1985; Iseki et al., 1988; Yoshikawa et al., 1989; Dantzig and Bergin, 1990; Sugawara et al., 1991; Gochoco et al., 1994). In situ perfusion, brush-border membrane vesicles and cultured intestinal epithelial cells studies have shown that absorption of aminocephalosporins is mainly carrier-mediated, saturable and concentration dependent (Nakashima et al., 1984a; Sanchez-Pico et al., 1989; Sinko and Amidon, 1989; Okano et al., 1986a; Inui et al., 1988). These experiments describe a passive diffusion that is non saturable at high concentrations and Michaelis–Menten kinetics saturable at low concentrations (kinetic order 0). This process of saturable absorption was described dy different authors who studied the β-lactam antibiotics in human and showed dose-dependence of bioavailability parameters (Aranciba et al., 1991; Faulkner et al., 1987; Larosa et al., 1982). Cephalexin transport is independent of sodium and is stimulated by an inward H^+-gradient. The pH-gradient required for the absorption of these molecules is generated by the combined action of a brush-border membrane Na^+/H^+ exchanger and basolateral membrane Na^+/K^+-ATP_{ase} (Ganapathy and Leibach, 1985).

Aminocephalosporins and peptides exerted a mutual inhibition on their absorption and shared a common transport system located in brush-border membrane of intestinal epithelial cells (Nakashima et al., 1984b; Dantzig and Bergin, 1990). Moreover, the discrepancies observed between in situ perfusion and brush-border membrane vesicles studies concerning the extent of transport inhibition of peptides seems to predict the existence of a paracellular way complementary to the transcellular one. Because of difficulties raised by the viability of culture cells and by in vivo experiments with animals, a model using rabbit ileum has been developed. Contrary to the brush-border membrane vesicles, this model provides information on the mechanisms, involved in cephalosporin transport across the apical and basolateral membranes of enterocytes.

The purpose of this study was to test a model based on the rabbit ileum membrane: the Grass diffusion cell.

This model allows the cephalosporins transepithelial transport to be determined (transcellular and paracellular) and the influence of pH, concentration, energy poisons and temperature on its absorption to be checked. Finally, the carrier specificity was characterized with different passage inhibitors.

2. Materials and methods

2.1. Chemicals

Cephalexin (CFX), cephradine (CFD), L-phenylalanine, glycyl-L-proline (gly-L-pro), sodium azide, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), 2,4-dinitrophenol, amiloride, ouabaïne, nigericin, D-glucose, trifluoro-acetic acid (TFA), were purchased from Sigma (St. Quentin Fallavier, France). Dulbecco's PBS buffer solution ('Phosphate Buffer Saline'), a 10X solution, was purchased from Gibco, SARL, France. CLHP grade acetonitrile was obtained from Prolabo, France. All other chemicals were of the highest grade.

2.2. Grass diffusion cell experiments and transport studies

Male New Zealand white rabbits were used. The animals, weighing 2.3–2.8 kg were fasted for 24 h and killed by an intravenous injection of sodium pentobarbital (Sanofi, France) given via the marginal ear vein at the dose of 100 mg/kg. A portion of distal ileum ending approximately 10 cm proximal to the ileoappendiceal attachment was removed and quickly prepared for the study by rinsing it free from intestinal contents, removal of the serosa, *muscularis propria* (Stripping), taking off Peyer's patches described previously (Donowitz et al., 1980a,b), and opened along the

mesenteric border. Four to six pieces of tissue from each animal were immediately mounted as flat sheets between two half Grass diffusion cells (Precision Instrument Design, Los Altos, CA) (Grass and Sweetana, 1988).

The exposed tissue surface area was 2.52 cm^2, attached to separate mucosal and serosal reservoirs and the fluid volume in each half was 7 ml of isotonic Ringer's solution (containing the following in mM: 140 Na^+, 5.2 K^+, 1.2 Ca^{2+}, 1.2 Mg^{2+}, 120 Cl^-, 25 HCO_3^-, 2.4 HPO_4^- and 0.4 $H_2PO_4^-$) or buffer Dulbecco's PBS solution. A Ringer's solution was used as control. The oxygenation of tissue and circulation were provided by means of bubbling with a mixture of 95% O_2 and 5% CO_2, and the Ringer's solution pH was adjusted to 7.4. The bubbling was adjusted to ensure a permanent blending of the solution in the two compartments. Except for the transport measurements at lower temperatures (4–8°C), all experiments were conducted at 37°C using a constant-temperature bath connection passing through a water jacket in the Grass cells. This temperature value (37°C) was used to simulate the physiological conditions in man. Before starting the experiment, 30 min were allowed for the tissue to stabilize (Donowitz et al., 1980a).

2.3. Transport measurements

For the concentration dependence experiment, the transport was examined over a concentration range of 0.1–5 mM. Beyond these concentrations, dissolution of cephalexin was not possible. Transepithelial fluxes were measured on adjacent pieces of tissue from the same animal. After a 30 min equilibrium period (stabilization of electrical parameters), cephalexin was added to the mucosal compartment at the study concentration and 0.5 ml aliquots were collected from mucosal and serosal tissues at 30, 60, 90, 120 min for HPLC analysis. The withdrawn volumes were replaced with buffer. Under the same conditions, the concentration dependence was again studied at 4°C, to determine the influence of temperature on transport of cephalexin, when the intestinal membrane was immersed in an ice Ringer's buffer at 4°C. The effect of pH on transport of 0.1 mM of antibiotic was studied in a Grass diffusion cell with a Phosphate Buffer Saline at different pH values ranging from 6 to 7.4.

Dulbecco's buffer solution, which is a Phosphate Buffer Saline (PBS) was prepared from a 10X solution (Gibco, SARL, France). After dilution, this buffer contained the following components, in mmol/l: 137 NaCl, 3 KCl, 8.1 Na_2HPO_4, 1.5 KH_2PO_4, 1 $CaCl_2$, 0.5 $MgCl_2$. The pH was adjusted to 6, 7, 7.4 with concentrated NaOH or HCl. For the studied paracellular transport, D-glucose was used as a solvent drag because it is likely probable that the main force for osmotic flow through the junctions is contributed by glucose (Pappenheimer, 1990). The transport of 1 and 5 mM cephalexin through the ileum was studied at pH 7.4 in a Ringer's solution containing 25 mM of D-glucose. The action of different energy poisons on the initial rate of 1 mM cephalexin transport was studied at pH 7.4 on isolated ileum rabbit. Within the metabolic inhibitors, 2.4-dinitrophenol (0.1 mM) and sodium azide (1 mM); nigericin was dissolved in ethanol in an amount of 3 mg/ml (10 μg/ml) and FCCP (10 μg/ml) as protonophores was added to both apical and basolateral reservoirs. The transport of 0.5 mM of cephalexin at pH 7.4 was also evaluated on ileum when the Na^+ pumps (antiport Na^+-H^+, Na^+-K^+-ATP_{ase}) were inhibited respectively by amiloride (1 mM) and ouabaïne (1 mM) on both sides of the tissue.

The effect of competing substrates on the transport of cephalexin was also studied on Grass diffusion cells at pH 7.4. Before starting experiments, the different competitors were added for 15 min in the mucosal compartment at a concentration ten times that of cephalexin to saturate the different sites of aminocephalosporins and peptide carriers. Glycyl-L-proline, L-phenylalanine, amoxicillin and cephradine were studied as competitors. Fresh solutions of the competitors were prepared for each experiment.

2.4. High-pressure liquid chromatography

Standard curves were obtained for each pH studied by integration of the peak area after injection of various amounts of Cephalexin (0.1–1 μg)

or (1–10 μg) for serosal and mucosal aliquots, respectively. Samples were filtered in a 0.45 μm pore size filter (Millipore, St. Quentin en Yvelines, France) and injected in a Shimadzu HPLC system equipped with a Kromasil C18 column, 5 μm (250 × 4.6 mm) (Touzart and Matignon), and an ultraviolet detector at 254 nm. The column was maintained at 40°C and the mobile phase, a mixture (13:87 v/v) of acetonitrile and purified water containing 1% TFA, was used at a flow rate of 1.5 ml/min. Under these conditions cephalexin was eluted in 7–10 min.

2.5. Calculations

The mucosal to serosal and serosal to mucosal fluxes, (Φ_{m-s}) and (Φ_{s-m}) respectively were calculated from:

$$\Phi = \frac{Q_n - Q_{n-1}}{A \times (t_n - t_{n-1})} = \frac{\mathrm{d}\,Q}{2.52 \times \mathrm{d}\,t} \text{ nmol/h per cm}^2$$

$\mathrm{d}\,Q/\mathrm{d}\,t$ is the transepithelial flux measured at each time point for samples in the serosal or mucosal compartment, Q is the cumulative amount (nmol) of drug and $A = 2.52$ cm^2 is the area of contact epithelium between the two parts (serosal and mucosal).

The rate of cephalexin absorption was measured at 30, 60, 90, and 120 min and the initial absorption rates were calculated by a linear fit over these points.

The percent inhibitions were calculated based on the absorption rate observed in the absence of inhibitor or competitor for each experiment.

2.6. Statistical analysis

The means $\pm$ S.E. of the results are reported with the number (n) of observations.

The difference observed between the means of the groups are compared by either Student's or paired t test.

3. Results

3.1. Transepithelial transport of cephalexin on grass diffusion cells

3.1.1. Transepithelial serosal to mucosal flux

The transepithelial serosal to mucosal transport of cephalexin (0.5 mM) was studied under in the same conditions at 37°C and the results showed a significantly different transport rate between this direction and mucosal to serosal. At 0.5 mM cephalexin, passes through the intestinal epithelium with a flux of 20.3 ± 1.2 and 0.78 ± 0.2, respectively, for the mucosal to serosal and serosal to mucosal directions. Between the two directions, the difference was 97% (***$p < 0.001$). Therefore, the transepithelial passage of cephalosporin in this direction is almost insignificant.

3.1.2. Concentration dependence of cephalexin fluxes on grass cells

The concentration dependence of cephalexin uptake and transport was examined over a concentration range of 0.1–5 mM. Fig. 1 shows that the transport of cephalexin is based on Michaelis–Menten kinetics, and that the value for the effective K_m of cephalexin accumulation from the apical side was 10.5 mM. The V_{max} value was 493 nmol/h per cm^2. These pharmacokineticals parameters were obtained through a mathematical computer sitting process extrapolating the saturable transport.

3.2. Effect of temperature and metabolic inhibitors

As indicated in Table 1, the effect of temperature on transport of cephalexin at different concentrations was very strong. At all concentrations the transport of cephalexin was significantly higher at 37°C than at 4°C ($p < 0.001$). For the different concentrations studied, the inhibition of cephalexin transport was 97 ± 1%. The same phenomenon was observed with metabolic inhibitors; the use of sodium azide (1 mM) and 2.4 dinitrophenol (0.1 mM) on both sides of the tissue, showed an inhibition of cephalexin fluxes of the order of 30 and 61%, respectively (Table 2).

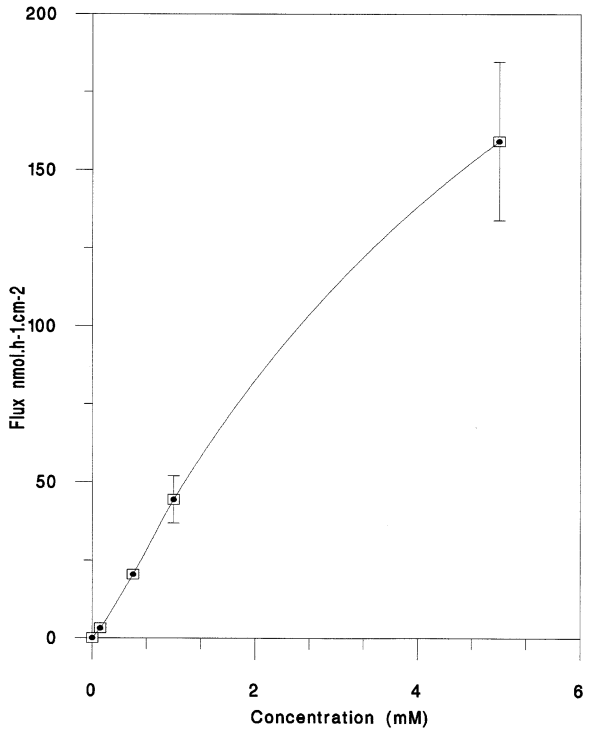

Fig. 1. Concentration dependence of cephalexin transport. Drug flux was calculated in nmol/h per cm^2. Transport of various concentrations was measured at 37°C for 120 min by immersed the tissue in Ringer's buffer (pH 7.4). Each value represents the average of 12 determinations (two animals).

Table 1
Effect of temperature on transport of cephalexin

Cephalexin (mM)	37°C	4°C
0.1	3.16 ± 0.82	n.d.
0.5	20.34 ± 1.22	0.56 ± 0.02***
1	44.40 ± 7.53	1.19 ± 0.30***
5	159.22 ± 25.41	3.10 ± 0.11***

Drug was added at different concentrations in the mucosal compartment.
The transport was studied until 120 min.
Transport is expressed as flux nmol/h per cm^2 (S.E.) and values represent the average of 12 determinations (two animals).

Table 2
Effect of energetic inhibitors on transport of 1 mM of cephalexin

	Flux nmol/h per cm^2	% inhibition
Control (Cephalexin, 1 mM)	37.6 ± 3.43	100
2.4 dinitrophenol (0.1 mM)	14.63 ± 1.58	61
Sodium azide (1 mM)	26.241 ± 2.01	30

The two inhibitors were solubilized in the medium in both side of tissue 15 min before starting experiment.
Means $\pm$S.E. of 18 determinations (three rabbits).

3.3. Effect of pH and ionophores on cephalexin absorption

It has been reported that the uptake of both cephradine, ceftibuten and cephalexin in brush-border membrane vesicles from the small intestine is mediated by the inward H^+ gradient-dependent

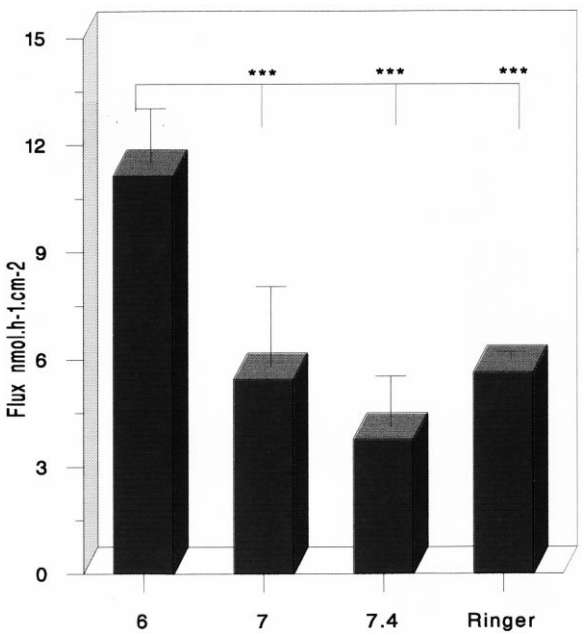

Fig. 2. pH-dependence of transepithelial transport of cephalexin (0.1 mM) through isolated rabbit ileum. Each point represents the means $\pm$S.E. of 18 determinations (three animals) (***$p < 0.001$, significant difference from pH 6 flux using student's t test).

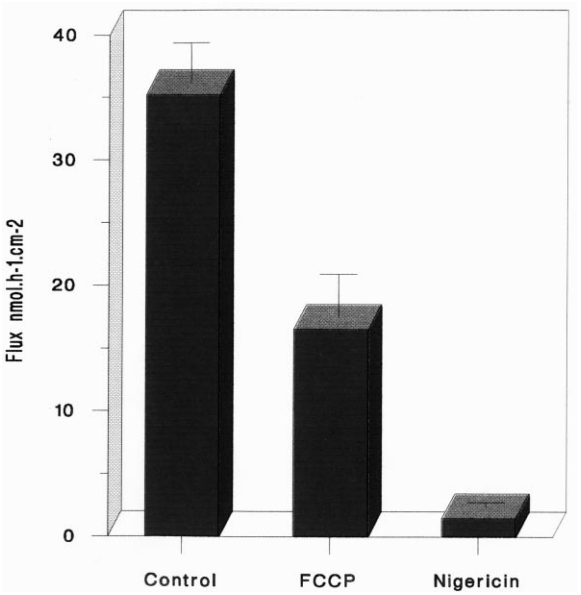

Fig. 3. Effect of FCCP and nigericin ($1.5 \cdot 10^{-2}$ μmol/l) on cephalexin transport by isolated rabbit ileum. These compounds were added in the transport medium 10 min before the experiment start. The mean cephalexin flux in Ringer's buffer without protonophore (control) is represented. Each bar represents the mean $\pm$S.E. of 18 experiments (three animals).

dipeptide co-transport system (Dantzig and Bergin, 1990; Inui et al., 1988; Sugawara et al., 1991). Thus, the effect of medium pH on transport of cephalexin was studied on isolated ileum. Fig. 2 shows the effect of varying mucosal pH on transepithelial fluxes (Φ_{m-s}) of cephalexin on Grass cells at 37°C. The mucosal pH ranged from 6 to 7.4 (PBS) in comparison with a Ringer's solution (pH 7.4), and the maximum transport rate was obtained when the pH was 6. The data in Fig. 2 demonstrate that cephalexin transport in the mucosal-serosal direction is two times greater at pH 6 (11.42 ± 2.24) than at pH 7–7.4 (5.0 ± 1.03) when expressed in nmol/h per cm^2. The transport of cephalexin was markedly stimulated through acidifying the mucosal medium to pH 6. Without a pH gradient, at pH 7.4, this simultaneous effect was inhibited by protonophore FCCP and an ionophore such as Nigericin (Fig. 3). The effect of Nigericin (10 μg/ml) was more pronounced than FCCP, and the inhibitions were 96 and 55%, respectively. These results suggested that the cephalexin was transported across the isolated ileum via an H^+ gradient-dependent transport system, which is in agreement with the literature (Ganapathy and Leibach, 1985; Gochoco et al., 1994). As shown in Fig. 4, at pH 7.4, amiloride does not affect cephalexin transport, but adding 1 mM ouabaine on the Ringer's solution, significantly affects the cephalexin flux with an inhibition of 90%.

3.4. Effect of D-glucose on the cephalexin transport rate in the ileal mucosa

D-glucose does not affect the transport of cephalexin (mucosal–serosal) through the isolated ileal mucosa. With 25 mM of D-glucose the cephalexin fluxes remain unchanged (Fig. 5). At 1 mM, cephalexin passes through the ileum membrane with a flux of 38.75 ± 5.23 in Ringer's solution and 35.9 ± 6.45 in Ringer added with 25 mM of D-glucose. Also, no significant difference was observed for 5 mM.

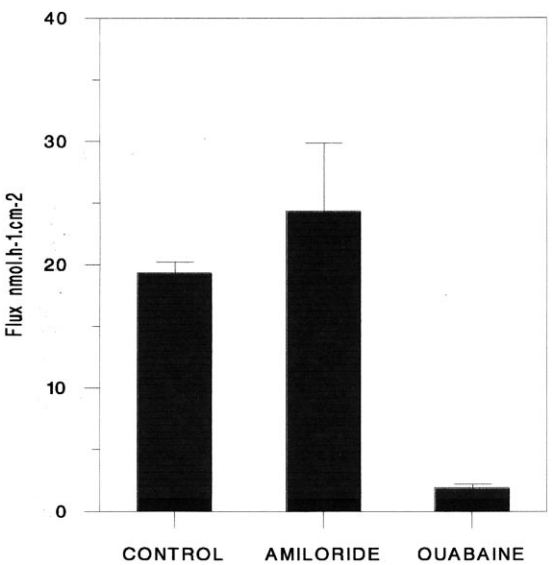

Fig. 4. Effect of amiloride and ouabaine on 0.5 mM cephalexin transport. The tissue was immersed in Ringer's buffer for control or in the presence of amiloride or ouabaine in 1 mM. Each bar represents the mean $\pm$S.E. of 12 experiments (two animals).

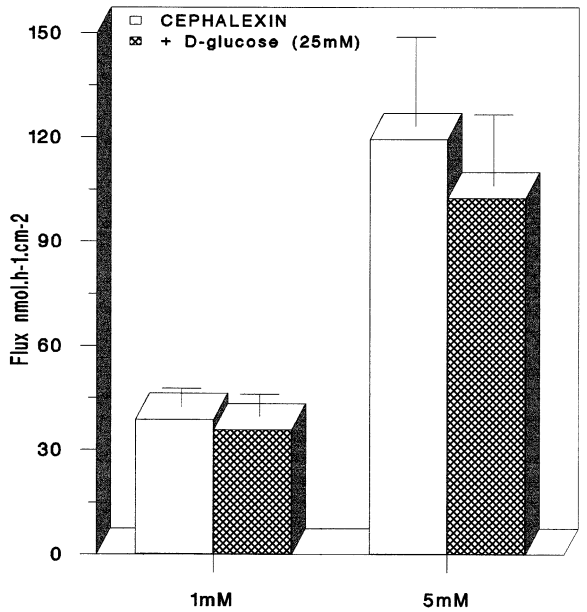

Fig. 5. Effect of D-glucose on cephalexin transport in isolated rabbit ileum. At t_0 cephalexin was added at 1 or 5 mM in the mucosal side of a stripped rabbit ileum mounted in Grass diffusion cell (exposed area: 2.52 cm^2), in Ringer's buffer for control or in the presence of 25 mM D-glucose.

3.5. Competition for cephalexin transport by gly-L-pro, L-phenylalanine, amoxicilline and cephradine

Cephalexin transport is mediated by a dipeptide carrier located in the intestine (Nakashima et al., 1984b). To further understand the transport mechanism of cephalexin, a selected dipeptide, amino acid, cephalosporin and aminopenicillin were tested for their potential as inhibitors of cephalexin absorption on isolated tissue. Table 3 summarizes the effects of such competitors on the transport of different concentrations of cephalexin. The mucosal to serosal flux of 0.1 mM of cephalexin was significantly inhibited by 1 mM of gly-pro. Similarly, cephradine (2 mM) inhibits 0.2 mM of cephalexin by 23% and amoxicilline (5 mM) reduces the transport by 56%. In contrast, an amino acid such as L-phenylalanine did not affect the transport of 0.5 mM of cephalexin.

4. Discussion

The purpose of this study has been to test model based on the rabbit ileum membrane: Grass diffusion cell. This model allow to determine the main characteristics of cephalexin transepithelial transport. At 1 mM, cephalexin was transported through the ileum mucosa and the concentration increased during the experiment until 120 min without equilibrium being reached (Benkhelifa et al., 1993), which was indicative of a non saturable transport because the cephalexin concentration added in the mucosal compartment was lower (1 mM) to saturating a different site of the carrier.

At higher concentrations between 1 and 5 mM, the transport assumes a non linear kinetic behaviour (kinetic order 0) (Fig. 1) and has a tendency to confirm a Michaelis–Menten Kinetics behaviour. These results were obtained at lower concentrations, without saturation and are in agreement with literature (Iseki et al., 1984; Nakashima et al., 1984a; Sinko and Amidon, 1989), as different authors also reported a mixed kinetics. Aminocephalosporins with α-amino groups such as cefadroxil perfused in situ in rats (Sanchez-Pico et al., 1989) and cephradine in brush-border membrane vesicles (Okano et al., 1986a) are transported with the same kinetics.

Nevertheless, Tsuji et al., 1987a; showed this phenomenon for cefixime (cephalosporin without an α-amino group), when non saturable passive diffusion occurs at high concentrations and saturable Michaelis–Menten kinetics occur at lower concentrations. The higher concentrations were not tested for a confirmation of the passive diffusion failure, because the cephalexin is no longer soluble above 5 mM. The effect of temperature on transport of cephalexin at different concentrations was very strong. At 4°C, the transport of cephalexin was significantly decreased and reached a 97% diminution. At this temperature, all mechanisms involved in the energetic metabolism were stopped and there only remains a non saturable transport such as passive diffusion. With such metabolic inhibitors as 2.4 dinitrophenol and sodium azide, the same results were obtained with an experiment at 4°C. The two

Table 3
Inhibitory effect of Glycyl-L-Proline (gly-L-pro), Cephradine, Amoxicilline and L-Phenylalanine on Cephalexin transport in isolated ileum rabbit

[CFX] mM	Inhibitor	Control J_{m-s} (nmol/h per cm^2)	Addition J_{m-s} (nmol/h per cm^2)	% inhibition
0.1	gly-L-pro (1 mM)	5.03 ± 1.2	3.22 ± 0.5*	36 ± 2
0.2	Cephradine (2 mM)	12.54 ± 1.0	9.63 ± 1.2**	23 ± 2
0.5	Amoxicilline (5 mM)	17.24 ± 1.5	7.56 ± 1.6***	56 ± 7
0.5	L-Phenylalanine (5 mM)	17.24 ± 1.5	15.67 ± 2.7	NS

Results are expressed as mean $\pm$S.D. from nine to 18 observations (from two to three animals).
Data were analyzed by student's t test.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

previous experiments show that the transport of cephalexin is energy-dependent.

The dependency of cephalexin transport on a proton gradient was shown and demonstrated by two different series of experiments: the first was a comparison of cephalexin fluxes between different pH values (neutral pH and pH-gradient), and the second was the effect and the inhibition observed with FCCP and nigericin. The results obtained by varying the pH of the mucosal part (apical) confirm that the cephalexin transport was controlled by a proton gradient. The maximum absorption of cephalexin was observed at pH 6, which is in agreement with the pH-partition hypothesis (Crevoisier and Buri, 1976). At the pH values studied cephalexin was ionized with a maximum of zwitterionic forms at 98.34 and 50%, at pH 5 and 7.4 respectively. De Young et al., 1978 studied the profile of different ionic species of cephalexin, cephradine and cephaloglycin in situ in the rat and showed that the maximum absorption was between pH 5.1 and 6, and then decreased up to pH 9. This process can be explained by the amphoteric chemical properties of cephalexin. In addition, the presence of the antiport Na^+-H^+ on the apical membrane and its responsibility for maintaining an acidic microenvironment in this area justifies this pH dependence. These observations were obtained from different studies on vesicles membranes brush-border (Ganapathy and Leibach, 1985; Hori et al., 1988; Okano et al., 1986a). The transport of β-lactam antibiotics and dipeptides is rather due to proton gradients from outside to inside the cells (Hori et al., 1988). Hobgen et al., 1959 explained the kinetics of weak acid and basis transport through intestinal epithelia according to the 'pH-partition hypothesis' suggest the pH at the surface of the brush-border membrane is as acidic as that measured in the intestinal lumen when the middle was less buffered. Different methods using pH sensitive microelectrodes (Lucas et al., 1985; Shionoiri, 1993), confirm this hypothesis. When there is a microenvironment (pH 5.5–6) at the surface of the brush-border membrane the pH_i values remain near 7.4. This gradient thus created on both sides of the membrane causes the energetic transport of β-lactam given per os.

The active transport of cephalexin can be inhibited by ionophores such as FCCP (which quickly dissipates the H^+ gradient across biological and artificial lipid membranes (Kramer et al., 1990) and nigericin, and at pH 7.4, the cephalexin absorption was inhibited up to 96% (Fig. 3). The same results were obtained with cefixime on brush-border membrane vesicles from rat and rabbit small intestine (Inui et al., 1988; Tsuji et al., 1987b). These observations are contradictory. On the other hand, recent studies on the transport of different cephalosporins (with or without α-amino groups), in intestinal epithelial Caco-2 cells, confirm these observations and indicate that the FCCP had little effect on the uptake of ceftibuten in the basolateral membranes, but the addition of FCCP to the apical side medium

caused a quick efflux of drug from the monolayers. The authors demonstrate and conclude that cephradine (aminocephalosporin) and ceftibuten can be transported actively by the H^+/dipeptide cotransport system localized in the apical membrane of the Caco-2 cells (Matsumoto et al., 1994).

In the literature, it was shown that such cephalosporins as cephalexin, cephradine and cefaclor intended for per os administration were co-transported with H^+ across the intestinal brush-border membranes via the dipeptide transport system (Okano et al., 1986a,b).

In addition, on Caco-2 cells the transport of these drugs is Na^+-independent (Dantzig and Bergin, 1988; Dantzig et al., 1992). In a Grass diffusion cell, the cephalexin flux was unchanged with amiloride (an inhibitor of the Na^+/H^+ antiporter), which shows the acidic microclimat on the apical side of enterocytes was present, and this, physiologically without a proton gradient at pH 7.4. An acidic pH in this part of enterocytes can be ensured by the basal Na^+/K^+-ATP_{ase}. As a matter of fact, the cephalexin transport was significantly affected by ouabaine (an inhibitor of the Na^+/K^+-ATP_{ase}) with a flux inhibition of up to 90% (Fig. 4). These findings can be explained by the role of sodium in the aminocephalosporin transport, which are indirectly involved via the Na^+/H^+ antiporter. Nevertheless, the cephalexin and cefaclor transport was not affected by ouabain on culture cells (Caco-2) (Dantzig et al., 1992) and was significantly inhibited on everted sac intestine (Nakashima et al., 1984a,b; Sanchez-Pico et al., 1989). In the later case, the authors conclude that the β-lactam antibiotic transport was Na^+-dependent. Nevertheless, the absence of Na^+ in the transport medium does not affect the transport of aminocephalosporins (Dantzig et al., 1992; Dantzig and Bergin, 1990), so, that it can concluded that the transport is independent of sodium ions. Thus, to examine whether dipeptides and cephalexin share a common transport system in the mucosal ileum, we studied the effect of gly-pro, cephradine and amoxicillin on the transport of cephalexin. Table 3 indicates that the transport of cephalexin through the isolated ileum at pH 7.4 was inhibited by gly-pro, cephradine and amoxicillin. These inhibitions were not observed with amino-acid such as L-phenylalanine. With such information, we showed that the cephalexin was transported by a di-/tripeptide transporter. These results are in accordance with experimentations concerning cephradine absorption reported by Okano et al., 1986b; Inui et al., 1992. The same transport is described for cyclacillin which is inhibited by dipeptides (glycylglycine) (Iseki et al., 1984). Moreover, aminopenicillins such as amoxicillin possess a common carrier with dipeptides (Iseki et al., 1989; Sjovall et al., 1992).

On other models such as the human intestinal cell line, these indications were not certain, and a contradictory study showed that the protein molecule with a molecular weight of 127 kDa, isolated by Kramer et al., 1990 was not present in Caco-2 or HT-29 cells (Gochoco et al., 1994). For our study, this question was not raised because the Grass diffusion cells used as a model use the entire epithelium and the work conditions drawn and simulate the intestinal tract under physiological conditions.

With D-glucose, at 25 mM on both sides of the tissue the cephalexin transport was unchanged for the two used concentrations (1 and 5 mM). Therefore, at these lower concentrations cephalexin cannot efficiently pass through the ileum epithelia by through passive diffusion with expanded tight junctions. The passive diffusion observed in these experiments by paracellular way was very low. Nevertheless this passage cannot be interpreted as part of a passive way for the total cephalexin transport, but provides useful information on the trans- and paracellular passive diffusion.

Finally, the transport of cephalexin through rabbit ileum follows Michaelis–Menten kinetics at lower concentrations, is pH-dependent, energy dependent, Na^+-independent and occurs across the intestinal mucosa through a transcellular route via dipeptide carrier system(s). Further studies are needed to examine these mechanisms. No studies concerning intestinal transport of cephalosporins were conducted on this model. One clear advantage is that the epithelium remains viable throughout the transport studies. Also, the isolated epithelium in a Grass cell repre-

sents the best model to study different mechanisms for drug and xenobiotics intestinal transport because it combines all ways of transepithelial transports such as the transcellular and paracellular ones. In addition, when the transport is transcellular, the mechanisms occurring in the apical absorption and basolateral efflux of drugs were represented. Established Grass cell models using an isolated tissue offer an alternative model to study the drug intestinal absorption processes.

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