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Transport Characteristics of Cephalosporin Antibiotics Across Intestinal Brush-border Membrane in Man, Rat and Rabbit

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Abstract—The uptake of orally active cephalosporins, ceftibuten and cephradine, by intestinal brush-border membrane vesicles isolated from man, rat and rabbit was studied. In the presence of an inward H^+ gradient, ceftibuten but not cephradine was taken up into intestinal brush-border membrane vesicles of man and rat against the concentration gradient (overshoot phenomenon). In rabbit jejunal brush-border membrane vesicles, the uptake of both cephalosporins in the presence of an inward H^+ gradient exhibited the overshoot phenomenon. In human and rat vesicles, the initial uptake of ceftibuten was strongly inhibited by compound V, an analogue of ceftibuten, but the uptake of cephradine was not affected by any of the cephalosporins tested, whereas in the rabbit brush-border membrane vesicles, initial uptake of both ceftibuten and cephradine were markedly inhibited by all cephalosporins and dipeptides used. These results suggest that the transport characteristics of human and rat intestinal brush-border membrane for cephalosporins are comparable, and that rabbit is an inadequate animal for investigating the transport characteristics of β-lactam antibiotics.

Although there have been many studies concerning transport mechanisms of orally active cephalosporins using rat or rabbit intestinal brush-border membrane vesicles (Iseki et al 1985, 1988, 1989; Kimura et al 1985; Okano et al 1986a, b; Tsuji et al 1987; Inui et al 1988; Muranushi et al 1989; Yoshikawa et al 1989; Sugawara et al 1990, 1991a, b), there are few reports comparing the transport characteristics of human intestinal preparations with those of rat and rabbit. Moreover, the detailed permeation characteristics across small intestinal brush-border membranes remain unclear.

Recently, we studied the uptake of cephalosporins by human jejunal brush-border membrane vesicles (Sugawara et al 1991b), and suggested that the effects of an inward H^+ gradient on the uptake of cephalosporins differ between cephalosporins in agreement with our previous results in rat intestinal brush-border membrane vesicles (Iseki et al 1989; Sugawara et al 1990, 1991a).

In this study, we compared the effect of an inward H^+ gradient and the inhibitory effect of several cephalosporins and dipeptides on the transport of ceftibuten and cephradine across intestinal brush-border membranes prepared from man, rat and rabbit.

Materials and Methods

Chemicals

Ceftibuten, cephalexin, compound V, S-1006 (Shionogi Co., Osaka, Japan), cefixime (Fujisawa Pharmaceutical Co., Osaka, Japan), cephradine (Sankyo Co., Tokyo, Japan) and cefadroxil (Banyu Co., Tokyo, Japan) were kindly donated (for structures see Fig. 1). Glycylglycine, L-alanyl-L-alanine and L-phenylalanylglycine were purchased from Sigma Chemical Co. (St Louis, MO, USA) and glycylsarcosine and L-carnosine (β-alanyl-L-histidine) were from Fluka Chemie AG (Buchs, Switzerland). All other chemicals were of the highest grade available.

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Preparation of intestinal brush-border membrane vesicles for transport studies

Brush-border membrane vesicles were isolated from human jejunum, rat whole intestine and rabbit jejunum, respectively, by $CaCl_2$ precipitation (Kessler et al 1978) as described previously for rat (Iseki et al 1989) and man (Sugawara et al 1991b). Brush-border membranes were suspended in a HEPES/Tris buffer used for the transport studies. This buffer contained (mM): D-mannitol 100, KCl 100, and HEPES/Tris 20, pH 7·5.

Uptake experiments

The uptake of substrates was measured by a rapid filtration

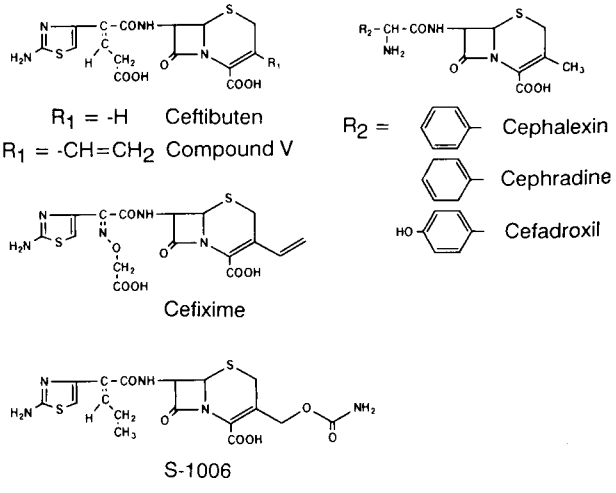

FIG. 1. Structures of cephalosporin antibiotics tested.

technique (Sugawara et al 1990). The reaction was initiated by the addition of 100 μL of 20 mM Mes/Tris buffer, pH 5·5, containing (mM): D-mannitol 100, KCl 100 and cephalosporin 1·2, to 20 μL of membrane vesicle suspension at 25°C. The cephalosporin trapped on the Millipore filter (HAWP, 0·45 μm, 25 mm) was extracted with 300 μL distilled water and was measured by HPLC.

Analytical methods

The concentrations of ceftibuten, compound V, cephalexin and cephradine were determined by HPLC (Hitachi L-6000, Hitachi Ltd, Tokyo, Japan) equipped with an L-4000 UV detector (Hitachi Ltd, Tokyo, Japan) at 262 nm for ceftibuten, cephalexin and cephradine, or 280 nm for compound V. Separation was achieved on a reversed phase column (ODS, Hitachi 3053, 5 μm, 4 mm i.d. 250 mm) using a mobile phase consisting of methanol:0·05 M citrate buffer, pH 2·5 (1:9, for ceftibuten; 15:85 for compound V) or methanol:0·1 M acetate, pH 6·0 (17:83 for cephalexin and cephradine) at a flow rate of 0·7 mL min^{-1}. The limit of detection was 2 pmol for ceftibuten and compound V, 5 pmol for cephalexin and cephradine. Protein was measured by the method of Lowry et al (1951) with bovine serum albumin as the standard.

Results

Time course of uptake of cephalosporins by intestinal brush-border membrane vesicles

The equilibrium uptake values of cephradine after 30 min incubation with human, rat and rabbit brush-border membrane vesicles were examined and those uptake values were 651·1 ± 83·4, 869·1 ± 57·5, 908·5 ± 18·8 pmol (mg protein)$^{-1}$ (mean ± s.e.m., n = 3–6), respectively. The function of Na^+-dependent [^{14}C]-glucose uptake was maintained in all vesicles (data not shown).

Fig. 2 shows the time course of uptake of cephalosporins by human jejunal brush-border membrane vesicles in the presence of an inward H^+ gradient. In human intestinal brush-border membrane vesicles, uptake of ceftibuten exhibited a clear overshoot phenomenon. Such an effect was not observed for cephradine. Fig. 3A shows the results from rat intestinal brush-border membrane vesicles. These are similar to the results of human brush-border membrane vesicles, but the uptake of ceftibuten showed lesser overshoot phenomenon. On the other hand, as shown in Fig. 3B for rabbit brush-border membrane vesicles, a clear overshoot was observed for both ceftibuten and cephradine. Moreover, the degree of initial uptake was nearly the same as that of ceftibuten by rat brush-border membrane vesicles.

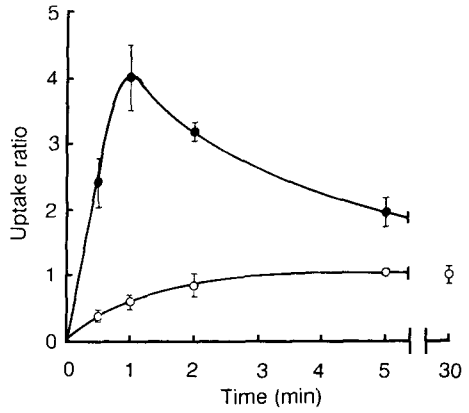

FIG. 2. Time course of uptake of cephradine (○) and ceftibuten (●) in human jejunal brush-border membrane vesicles. Membrane vesicles were preincubated in a 20 mM HEPES/Tris buffer containing 100 mM D-mannitol, 100 mM KCl, pH 7·5, at 25°C for 2 min. The vesicles (20 μL) were incubated with 100 μL of 20 mM Mes/Tris buffer, pH 5·5, containing 100 mM D-mannitol, 100 mM KCl and 1·2 mM cephalosporin at 25°C. Each point represents the mean ± s.e.m. of three measurements.

Effect of cephalosporins and dipeptides on the uptake of cephalosporins

Table 1 shows the effect of several cephalosporins and dipeptides on the initial (30 s) uptake of ceftibuten, com-

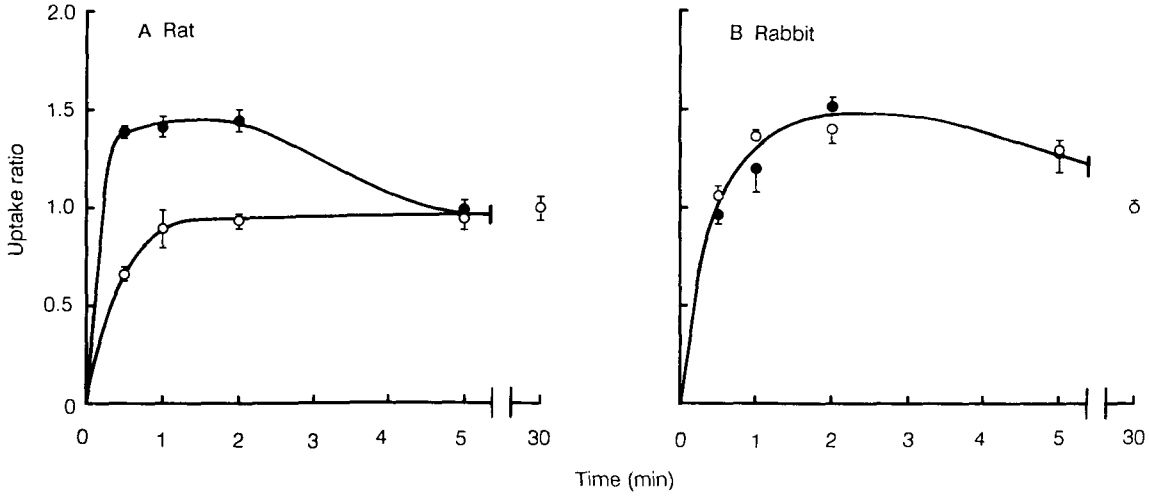

FIG. 3. Time course of uptake of cephradine (○) and ceftibuten (●) by rat (A) and rabbit (B) intestinal brush-border membrane vesicles. Membrane vesicles were preincubated in a 20 mM HEPES/Tris buffer containing 100 mM D-mannitol, 100 mM KCl, pH 7·5, at 25°C for 2 min. The vesicles (20 μL) were incubated with 100 μL of 20 mM Mes/Tris buffer, pH 5·5, containing 100 mM D-mannitol, 100 mM KCl and 1·2 mM cephalosporin at 25°C. Each point represents the mean ± s.e.m. of 3–6 measurements.

Table 1. Effect of cephalosporins and dipeptides on uptake of ceftibuten, compound V, cephalexin, and cephradine in rat intestinal brush-border membrane vesicles.

Inhibitor	Uptake (% of control)				
	Ceftibuten		Compound V	Cephalexin	Cephradine
	5 mM	10 mM	10 mM	10 mM	10 mM
Control	100·0 ± 7·9	100·0 ± 2·4	100·0 ± 3·0	100·0 ± 13·0	100·0 ± 7·0
Ceftibuten			36·4 ± 2·3†††	77·4 ± 21·6	84·1 ± 7·8
Compound V	32·1 ± 5·4††	34·4 ± 7·3††			
Cefixime	81·7 ± 3·2	80·3 ± 2·6**	83·2 ± 6·9	95·9 ± 24·7	99·8 ± 8·5
Cephalexin	98·8 ± 7·3	95·7 ± 3·8	92·7 ± 10·4		76·2 ± 14·8
Cefadroxil				95·3 ± 7·3	
S-1006	88·3 ± 6·8	107·2 ± 16·4			
L-Carnosine	83·0 ± 2·3	99·4 ± 3·3			100·6 ± 10·0
Glycylsarcosine	107·3 ± 4·5	91·2 ± 12·3	107·2 ± 11·2	138·5 ± 20·2	
L-Alanyl-L-alanine		42·3 ± 2·4†††	47·8 ± 2·5†††	44·3 ± 10·1†	52·2 ± 7·3†
L-Phenylalanylglycine		64·4 ± 3·8††	53·3 ± 8·9**		
Glycylglycine		73·4 ± 8·7*			

Membrane vesicles were preincubated in a 20 mM HEPES/Tris buffer, pH 7·5, containing 100 mM D-mannitol, 100 mM KCl at 25°C for 2 min. The vesicles (20 μL) were incubated for 30 s with 100 μL of 20 mM Mes/Tris buffer, pH 5·5, containing 100 mM D-mannitol, 100 mM KCl, 1·2 mM cephalosporin and various compounds (final 5 or 10 mM). Each value represents the mean ± s.e.m. of 3–7 measurements. *$P < 0.05$, **$P < 0.01$, †$P < 0.005$, ††$P < 0.002$, †††$P < 0.001$ compared with control.

pound V, cephalexin and cephradine by rat intestinal brush-border membrane vesicles in the presence of an inward H^+ gradient. The uptake of ceftibuten was strongly inhibited by compound V at 5 and 10 mM. On the other hand, cefixime exhibited only a slight inhibitive effect, and cephalexin and S-1006 had no effect whatsoever on the uptake of ceftibuten. The uptake of compound V was significantly inhibited by ceftibuten, suggesting the presence of a common transport mechanism of ceftibuten and compound V. Moreover, as for ceftibuten, the uptake of compound V was not inhibited by cephalexin. On the other hand, initial uptake of aminocephalosporins (cephalexin and cephradine) was not inhibited by either dianionic cephalosporins (ceftibuten and cefixime) or zwitterionic cephalosporins (cephalexin, cefadroxil and cephradine). The inhibitory effect of dipeptides on the uptake of dianionic and zwitterionic cephalosporins were similar, in that, the initial uptake of all cephalosporins was strongly inhibited by L-alanyl-L-alanine (10 mM), but was not inhibited by glycylsarcosine and L-carnosine. Interestingly, glycylsarcosine, which is known to be transported depending upon the H^+ gradient in rabbit intestinal or renal brush-border membrane (Ganapathy et al 1984; Miyamoto et al 1985), did not inhibit the uptake of ceftibuten, compound V or cephalexin.

The results using human jejunal brush-border membrane vesicles are shown in Table 2. The uptake of ceftibuten was inhibited by compound V (10 mM). In contrast, the uptake of cephradine was not inhibited by any of the cephalosporins tested with the exception of cefixime which caused weak inhibition. These results suggest that the transport mechanism of aminocephalosporins is different from that of ceftibuten and compound V in human intestinal brush-border membrane. However, as for rat brush-border membrane, there was a noted difference in the inhibitory effect among dipeptides, i.e. the uptake of both ceftibuten and cephradine was inhibited by L-alanyl-L-alanine, but not by L-carnosine.

Table 3 shows the results of inhibition studies with rabbit jejunal brush-border membrane vesicles. In contrast to the

Table 2. Effect of cephalosporins and dipeptides on uptake of ceftibuten and cephradine in human jejunal brush-border membrane vesicles.

Inhibitor	Uptake (% of control)	
	Ceftibuten 10 mM	Cephradine 10 mM
Control	100·0 ± 5·6	100·0 ± 7·3
Ceftibuten		104·7 ± 5·9
Compound V	3·0 ± 2·8†	
Cefixime	80·0 ± 3·2*	58·9 ± 10·1**
Cephalexin	88·0 ± 14·6	93·8 ± 9·1
L-Carnosine	88·6 ± 9·8	106·5 ± 25·1
L-Alanyl-L-alanine	38·5 ± 2·9†	36·4 ± 4·4†

Membrane vesicles were preincubated in a 20 mM HEPES/Tris buffer, pH 7·5, containing 100 mM D-mannitol, 100 mM KCl at 25°C for 2 min. The vesicles (20 μL) were incubated for 30 s with 100 μL of 20 mM Mes/Tris buffer, pH 5·5, containing 100 mM D-mannitol, 100 mM KCl, 1·2 mM cephalosporin and various compounds (final 10 mM). Each value represents the mean ± s.e.m. of 3–6 measurements. *$P < 0.05$, **$P < 0.02$, †$P < 0.001$ compared with control.

results of man and rat, the uptake of ceftibuten and of cephradine was inhibited to varying degrees by all cephalosporins and dipeptides. These data indicate that the transport characteristics of orally active cephalosporins in rabbit intestinal brush-border membrane differ from those of human and rat intestinal brush-border membranes.

Discussion

This is the first study of its kind to compare the transport characteristics of orally active cephalosporins by brush-border membrane vesicles isolated from human intestine with those from other animals (rat and rabbit). There have been conflicting reports concerning transport mechanisms of cephalosporin antibiotics across brush-border membranes. Okano et al (1986a) observed an uphill transport of cephradine in rabbit intestinal brush-border membrane vesicles. On the other hand, in their rat intestinal preparation, stimulation of transport by an inward H^+ gradient was much lower

Table 3. Effect of cephalosporins and dipeptides on uptake of ceftibuten and cephradine in rabbit jejunal brush-border membrane vesicles.

Inhibitor	Uptake (% of control)	
	Ceftibuten 10 mM	Cephradine 10 mM
Control	100·0 ± 5·7	100·0 ± 12·8
Ceftibuten		30·8 ± 7·5††
Compound V	35·2 ± 6·6††	22·1 ± 14·8††
Cefixime	68·7 ± 10·1†	55·3 ± 7·6††
Cephalexin		38·6 ± 23·4**
Cephradine	39·7 ± 3·3††	
Cefadroxil		29·4 ± 9·5††
L-Carnosine	61·4 ± 8·0††	67·1 ± 35·8**
Glycylsarcosine	59·4 ± 5·3††	54·1 ± 5·7††
L-Alanyl-L-alanine	20·1 ± 3·3††	18·1 ± 13·3††
Glycylglycine	51·4 ± 5·7††	54·3 ± 17·3*

Membrane vesicles were preincubated in a 20 mM HEPES/Tris buffer, pH 7·5, containing 100 mM D-mannitol, 100 mM KCl at 25°C for 2 min. The vesicles (20 μL) were incubated for 30 s with 100 μL of 20 mM Mes/Tris buffer, pH 5·5, containing 100 mM D-mannitol, 100 mM KCl, 1·2 mM cephalosporin and various compounds (final 10 mM). Each value represents the mean ± s.e.m. of four measurements. *$P < 0.01$, **$P < 0.005$, †$P < 0.002$, ††$P < 0.001$ compared with control.

than that in the rabbit and moreover no overshoot was observed. Using rat intestinal brush-border membrane vesicles, we have previously reported that only ceftibuten and its analogues showed an overshoot in uptake in the presence of an inward H^+ gradient among β-lactam antibiotics tested (ampicillin, amoxicillin, cephalexin, cephradine, cefadroxil, cefazolin, cefixime, ceftibuten and analogues of ceftibuten) (Iseki et al 1989; Sugawara et al 1990, 1991a). Yoshikawa et al (1989) and Muranushi et al (1989) reported that in the presence of a H^+ gradient, ceftibuten was transported into rat brush-border membrane and indicated that its transport was different from aminocephalosporins, cephalexin and cephaclor, because of the existence of no counter-transport effect.

In the present study, we investigated the uptake of ceftibuten and cephradine in the presence of an inward H^+ gradient by intestinal brush-border membrane vesicles isolated from man, rat and rabbit. The uptake of ceftibuten exhibited clear overshoot phenomenon with all vesicles isolated from man, rat and rabbit (Figs 2, 3). Although direct comparison of uptake values are difficult because of the difference of equilibrium uptake (intravesicular volume), when comparing the uptake ratio with the equilibrium value, the order of stimulation of initial uptake by H^+ gradient was man > rat > rabbit. In contrast, overshoot was observed with cephradine only in rabbit brush-border membrane vesicles. We reported previously that the initial uptake of ceftibuten and its analogues by rat intestinal brush-border membrane vesicles increased against the concentration gradient in the presence of an inward H^+ gradient, but the uptake of cefixime and S-1006 exhibit no overshoot (Sugawara et al 1991a). Moreover, in human jejunal brush-border membrane vesicles ceftibuten, but not cefixime or cephalexin, exhibited the overshoot phenomenon (Sugawara et al 1991b). These previous results are in agreement with the present studies of the inhibitory effects of several cephalosporins with respect to structural specificity, i.e. significant mutual inhibition was observed between ceftibuten and compound V (Table 1), inhibition was not observed between aminocephalosporins and ceftibuten or compund V (Tables 1, 2), mutual inhibition was not observed between aminocephalosporins (Tables 1, 2), and results of inhibition studies by human intestinal brush-border membrane vesicles were similar to those of the rat intestinal preparation (Tables 1, 2). From these previous observations and our present results, we consider that the different transport characteristics among animal species is one of the reasons for disagreements among the reported transport mechanisms of orally active cephalosporins.

Tsuji et al (1987) reported that cefixime was transported by a H^+ gradient-dependent peptide carrier system using rat intestinal brush-border membrane. In our previous study, however, H^+ gradient-dependent overshoot of cefixime was not observed in man and rat, and our data suggested that the pH of the medium rather than H^+ gradient affected the uptake of cefixime (Sugawara et al 1991a). Moreover, as shown in man (Table 2) and rat (Table 1), the degree of inhibition by cefixime on the uptake of ceftibuten and compound V was slight. Understanding the details concerning the transport mechanism of cefixime requires further examination.

Additionally, we examined the effect of dipeptides on the uptake of cephalosporins. In man and rat, L-alanyl-L-alanine strongly inhibited uptake in all cases, but L-carnosine exhibited no effect. These data suggest the possibility that the mechanism of inhibition of dipeptides is different from those of cephalosporins. On the other hand, in the case of rabbit brush-border membrane, both uptake of ceftibuten and cephradine were markedly inhibited by all cephalosporins and dipeptides used. We consider that the transport characteristics of cephalosporins across rabbit intestinal brush-border membrane differ from those of man and rat not only in their dependency on the inward H^+ gradient, but also in the manner of their inhibition by drugs and dipeptides.

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