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Intestinal brush-border transport of the oral cephalosporin antibiotic, cefdinir, mediated by dipeptide and monocarboxylic acid transport systems in rabbits

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Abstract—Intestinal absorption of the orally active cephalosporin, cefdinir, was investigated using brush-border membrane vesicles prepared from rabbit small intestine. The initial uptake of cefdinir was pH-dependent, with increased uptake at acidic pH, and was not influenced by either sodium gradient or membrane potential difference. Cefdinir uptake was saturable with an apparent Michaelis constant of 8·1 mM. Initial uptake of cefdinir was inhibited by dipeptides (glycyl-L-proline and glycylsarcosine), β-lactam antibiotics (cephradine, cefixime and penicillin V), and monocarboxylic acids (acetic acid and L-lactic acid), whereas the uptake of cephradine and cefixime was not inhibited by monocarboxylic acids. Cefdinir significantly inhibited the initial uptake of cephradine, cefixime and [^{3}H]acetic acid. From these results, it was suggested that cefdinir was transported across brush-border membranes by both dipeptide and monocarboxylic acid carriers.

Amino-β-lactam antibiotics such as cephalexin and cephradine (Nakashima et al 1984; Okano et al 1986; Kramer et al 1988; Sinko & Amidon 1989) which have an α-amino group on the side chain, and dicarboxylic acid derivatives such as cefixime (Tsuji et al 1987a,c; Inui et al 1988) and ceftibuten (Muranushi et al 1989) are absorbed via the dipeptide transport system across the intestinal brush-border membranes. We have previously suggested that β-lactam antibiotics have affinities for the intestinal dipeptide carrier (Tsuji et al 1987b). However, the structural requirement for this transport system is not fully established.

The recently developed cephalosporin antibiotic, cefdinir (Fig. 1), which is a monocarboxylic acid cephalosporin, is effective after oral administration. Cefdinir has a structural similarity to cefixime; however, intestinal absorption of these two derivatives have different features including animal species differences and intestinal regional specificities (Sakamoto et al 1985; Mine et al 1987). These observations suggest a certain specialized transport function in cefdinir absorption in the intestine which is distinct from cefixime transport.

We have recently found transport systems specific for monocarboxylic acids in the small intestine (Simanjuntak et al 1990; Tsuji et al 1990) and also have suggested that some of the monocarboxylic acid β-lactam antibiotics have affinities for these transport systems (Simanjuntak et al 1991).

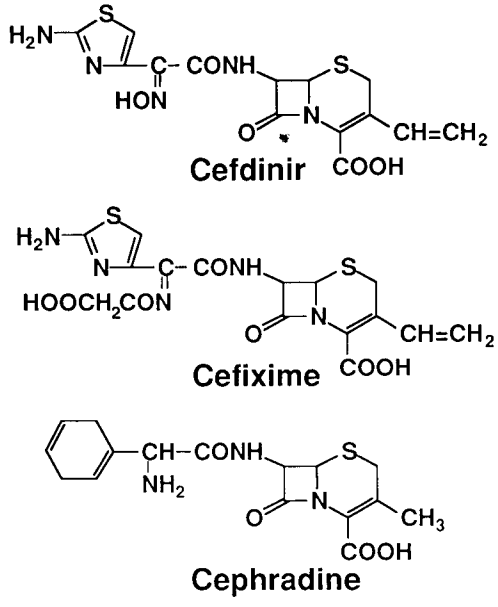

FIG. 1. Chemical structures of cefdinir, cefixime and cephradine.

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Materials and methods

Membrane preparation and uptake experiments. Brush-border membrane vesicles were prepared from the small intestine of male rabbits weighing ~1·5 kg (Sankyo Laboratories & Co., Toyama, Japan) according to the calcium precipitation method (Tsuji et al 1987c). The membrane vesicles were suspended in 10 mM HEPES/Tris buffer, pH 7·5, containing 100 mM mannitol and 100 mM KCl and used immediately. Uptake experiments were performed by a rapid filtration technique using a Millipore filter (HAWP, 0·45 μm, Nihon Millipore Ltd, Tokyo, Japan) as described previously (Tsuji et al 1987c).

Analytical procedures. Cefdinir, cefixime (Fujisawa Pharmaceutical Co., Osaka, Japan) or cephradine (Shionogi & Co., Osaka) trapped on the Millipore filter was extracted with 500 μL 0·01 M HCl by shaking for 20 min in a glass tube and the resultant extract was assayed by HPLC using a reversed-phase column (15 cm × 4·6 mm, TSK-gel, ODS 80-TM, Tosoh, Tokyo) with a guard column (Guard pac, Waters, Milford, MA, USA). The mobile phase, 12% acetonitrile·88% aqueous citric acid (7 g L^{-1}), pH 3·0, was used at a flow rate of 1 mL min^{-1}. The eluent was monitored at 290 nm for cefdinir and cefixime and at 260 nm for cephradine. In the case of [^{3}H]acetic acid (3·3 Ci $mmol^{-1}$, New England Nuclear, Boston, MA, USA), the filter was dried and dissolved in the scintillation fluid and the radioactivity was determined by liquid scintillation counting (model LSC-700, Aloka Co., Tokyo). Protein concentration was measured by the method of Bradford (1976) using a Bio-Rad protein assay kit.

Results and discussion

The time courses for the initial uptake of cefdinir (0·3 mM) by the intestinal brush-border membrane vesicles were measured in the presence (pH_{out} 6·0) and in the absence of a pH gradient (pH_{out} 7·5) at a constant intravesicular pH (pH_{in} 7·5). The results are shown in Fig. 2. At an acidic extravesicular pH, uptake of cefdinir at 10 and 30 s increased slightly, but significantly, when compared with those at pH 7·5 ($P < 0·05$). In separate experiments, the effects of a sodium gradient and a membrane potential across the brush-border membrane on the initial uptake of cefdinir were studied. Membrane potential difference was tested by inducing an inside-negative potassium diffusion potential by valinomycin. However, neither the sodium nor membrane potential had a significant effect on cefdinir transport (data not shown).

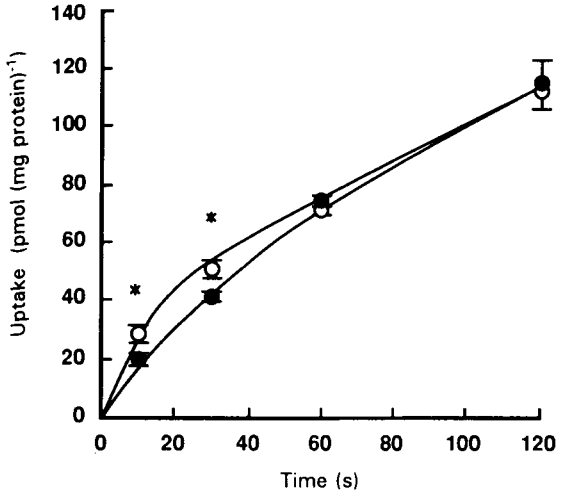

FIG. 2. Time course for the initial uptake of cefdinir. The membrane vesicles were preloaded with 10 mM HEPES/Tris buffer, pH 7·5 containing 100 mM mannitol and 100 mM KCl. Uptake of cefdinir at 0·3 mM was measured at 37 C by incubating the membrane vesicles in 10 mM 2-(*N*-morpholino)ethanesulphonic acid (Mes)/Tris buffer, pH 6·0 (○) or 10 mM HEPES/Tris buffer, pH 7·5 (control, ●), each containing 100 mM mannitol and 100 mM KCl. Each point represents the mean ± s.e. of four to five experiments. $*P < 0·05$, significantly different from the control uptake P as assessed by Student's t-test.

The initial uptake rate of cefdinir at acidic extravesicular pH (6·0) showed saturation with increasing concentration of cefdinir (data not shown). The apparent Michaelis constant and the maximum uptake rate were 8·1 mM and 1·068 nmol min $^{-1}$ (mg protein) $^{-1}$, respectively. The apparently nonsaturable uptake rate constant was 0·422 nmol min $^{-1}$ (mg protein) $^{-1}$. The pH-dependent and saturable uptake suggests that the uptake of cefdinir is mediated by a pH- or pH-gradient-dependent and carrier-mediated process.

To characterize the transporter responsible for the cefdinir transport, inhibitory effects of several compounds which are known to be taken up via carrier-mediated processes were studied. The initial uptake of cefdinir was measured at pH 6·0 for 30 s and the results are shown in Table 1. Dipeptides, glycyl-L-proline and glycylsarcosine at a concentration of 50 mM, significantly reduced the uptake of cefdinir ($P < 0·05$). The β-lactam antibiotics, cephradine and cefixime, which have already been shown to be transported by a dipeptide carrier, also inhibited cefdinir uptake. The uptake of cephradine and cefixime, was significantly inhibited by cefdinir ($P < 0·05$). These results suggest that cefdinir is transported, at least partly, via the carrier-mediated system for dipeptides.

As is shown in Table 1, L-lactic acid and acetic acid also inhibited cefdinir uptake, but none of the monocarboxylic acids including L-lactic acid, acetic acid or nicotinic acid reduced the uptake of cefixime and cephradine. Transport of L-lactic acid (Balkovetz et al 1988), acetic acid (Tsuji et al 1990; Simanjuntak et al 1991) and nicotinic acid (Simanjuntak et al 1990) have already been shown to be mediated by specific carriers which are dependent on the pH gradient. Thus, the results shown in Table 1 suggests that the transporter for monocarboxylic acids is independent of that for dipeptides, and that cefdinir is taken up by both dipeptide and monocarboxylic acid transporters.

To determine whether monocarboxylic acid transport systems participate in cefdinir uptake, the inhibitory effects of various compounds on a pH-gradient-dependent [^{3}H]acetic acid transport were studied. The results are shown in Table 2.

The inhibition observed by cefdinir strongly suggests that the monocarboxylic acid carrier as well as the dipeptide carrier can transport cefdinir. Significant inhibitory effects of cephradine and cefixime on the transport of [^{3}H]acetic acid suggest that the two derivatives have binding affinities for the monocarboxylic acid carriers but are not transported by the carrier, since their uptake was not reduced by monocarboxylic acids (Table 1). Considering the structural similarity of dicloxacillin, propicillin and penicillin V to monocarboxylic acids and the significant inhibitory effects of them on the uptake of [^{3}H]acetic acid, they may also share the same carriers.

Table 1. Inhibitory effects of various compounds on the uptake of cefdinir, cefixime and cephradine.

	Uptake rate (% of control)		
Inhibitors	Cefdinir	Cefixime	Cephradine
Acetic acid	73·1 ± 3·09*[a]	94·6 ± 3·25[a]	100 ± 7·40[a]
L-Lactic acid	73·4 ± 7·66*[a]	93·0 ± 6·24[a]	97·1 ± 4·28[a]
Nicotinic acid	—	104 ± 1·91[a]	112 ± 11·7*[a]
Glycyl-L-proline	67·7 ± 4·05*[c]	—	—
Glycylsarcosine	83·1 ± 1·81*[c]	58·2 ± 4·60*[b]	72·1 ± 3·73*[b]
Penicillin V	53·0 ± 0·578*[b]	—	—
Cefdinir	—	66·5 ± 7·42*[b]	82·5 ± 7·06*[b]
Cefixime	80·8 ± 4·28*[a]	—	—
Cephradine	73·0 ± 5·63*[b]	—	—
D-Glucose	—	98·8 ± 7·65[a]	90·1 ± 5·11[a]

The preloaded conditions of the membrane vesicles were the same as described in the legend for Fig. 2. Uptake of β-lactam antibiotics at a concentration of 2 mM was measured at 37°C for 30 s by incubating membrane vesicles in 10 mM Mes/HEPES buffer, pH 6·0 for cefdinir and cephradine, or pH 5·0 for cefixime, each containing 100 mM NaCl and 100 mM mannitol. In the presence of inhibitors, mannitol concentration was reduced to adjust the osmolarity to that of the control study. Each value represents the mean ± s.e. of three to six experiments. $*P < 0·05$, significantly different from the control uptake studied in the absence of inhibitors as assessed by Student's t-test. Concentrations of inhibitors were [a] 10, [b] 20 or [c] 50 mM.

Table 2. Inhibitory effects of various compounds on the uptake of [^{3}H]acetic acid.

Inhibitors	Acetic acid uptake (% of control)
Acetic acid	34·4 ± 5·91*
Glycyl-L-proline	101 ± 4·81
Dicloxacillin	33·6 ± 2·96*
Propicillin	61·7 ± 5·91*
Penicillin V	75·8 ± 0·390*
Cefdinir	22·2 ± 9·50*
Cefixime	72·5 ± 5·75*
Cephradine	60·6 ± 6·15*
D-Glucose	103 ± 15·3

The preloaded conditions of the membrane vesicles were the same as described in the legend for Fig. 2. Uptake of [^{3}H]acetic acid at a concentration of 2 μM was measured at 27°C for 10 s by incubating membrane vesicles in 10 mM Mes/HEPES buffer, pH 6·0 containing 100 mM NaCl, 100 mM mannitol and 2 μM valinomycin. In the presence of inhibitors, concentration of mannitol was reduced to adjust the osmolarity to the control study. Each value represents the mean ± s.e. of three to eight experiments. $*P < 0·05$, significantly different from the control uptake in the absence of inhibitors, as assessed by Student's t-test. Concentrations of inhibitors used were 10 mM.

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The effect of a nutritional model of chronic liver injury on the hepatic glucuronidation of morphine in rats

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Abstract—The effect of a choline-deficient diet on the hepatic glucuronidation of morphine was investigated using a rat perfused liver system. Rats fed a choline-deficient diet developed a fatty liver with minimal necrosis. Despite the morphological changes, neither hepatic extraction ratio ($0{\cdot}51 \pm 0{\cdot}02$ in control; $0{\cdot}45 \pm 0{\cdot}04$ in the choline-deficient rats) nor intrinsic clearance ($0{\cdot}85 \pm 0{\cdot}05$ in control; $0{\cdot}77 \pm 0{\cdot}09$ in choline-deficient rats) were affected by this injury model. This finding suggests that glucuronidation is relatively resistant to this chronic liver injury.

The elimination of a number of drugs is impaired in patients with chronic liver injury (Wilkinson & Schenker 1975). The two most important factors responsible for this impairment are decreased hepatic metabolizing enzyme activity and alterations in hepatic circulation.

Several models of chronic liver injury in experimental animals have been established. The most frequently used model involves co-administration of CCl_4 and phenobarbitone to rats (McLean et al 1969). Another model of acute and chronic liver injury involves feeding rats a choline-deficient diet (Murray et al 1986). Both the CCl_4 (Villeneuve et al 1978) and nutritional (Murray et al 1986) models are associated with decreased content of total cytochrome P450 and reductions in activity of several P450 isozymes. The effect of either model of chronic liver injury on the glucuronidation pathway has received less attention.

The purpose of this study was to examine the effect of a chronic choline-deficient diet on the hepatic elimination of morphine, the major metabolic pathway of which is the formation of 3-*O*-glucuronide.

Materials and methods

The choline-deficient model of hepatic injury was based on published methods (Murray et al 1986). Rats, initially weighing 150 g, had free access to either a choline-deficient or choline-supplemented diet for 28 weeks. The diet consisted of 12% protein, 58% sucrose, 26% oils and fats, 4% salts and minerals and a vitamin supplement.

A rat non-recirculating isolated perfused liver system was used (Bartosek et al 1973). The perfusion medium was composed of 90% (v/v) Krebs–Henseleit buffer and 10% (v/v) washed human erythrocytes. Bovine serum albumin (10 g L^{-1}), glucose (3 g L^{-1}) and sodium taurocholate (75 mg L^{-1}) were dissolved in the medium. The viability of the preparation was assessed by the lactate/pyruvate ratio, perfusion back-pressure and bile flow. Each preparation was studied over four 30-min periods using perfusion rates of 7, 10 and 12 mL min^{-1} in control, and 10, 12 and 15 mL min^{-1} in choline-deficient preparations. Morphine was added to the perfusate to give an inflow concentration of

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