

CHARACTERIZATION OF AMINOCEPHALOSPORIN TRANSPORT ACROSS RAT SMALL INTESTINE

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The absorption mechanism of aminoccephalosporins was investigated in rat small intestine. Cephalosporins chosen in this study were cephalixin, cephradine, SCE-100, cefroxadin, cefatrizine, and cefadroxil. Absorption experiments were carried out by both *in situ* and *in vitro* techniques. *In situ* recirculation experiments through the whole small intestine demonstrated that the absorption rates of the antibiotics were saturable (except cephalixin) and inhibited by the short-term pretreatment of the intestinal mucosa with a low concentration of HgCl_2 , suggesting the participation of proteins and/or sulfhydryl groups within the brush border membrane. Among the tested antibiotics, cefadroxil showed the highest absorption, in spite of its lowest lipophilicity, and its absorption was inhibited by the simultaneous perfusion of other aminoccephalosporins and aminopenicillins. So, the carrier system for cefadroxil seems to be common, at least in part, to amino- β -lactam antibiotics. Also, comparison of the apparent directional permeabilities of the jejunum and ileum by using the everted sacs and noneverted ones *in vitro* indicated that this drug penetrates more rapidly from mucosa to serosa (M-to-S) than from serosa to mucosa (S-to-M) in both segments. The (M-to-S)/(S-to-M) ratios at the concentration of 0.1 mM were 2.26 and 4.82 in the jejunum and ileum, respectively. The M-to-S flux of the drug was saturable, Na^+ -dependent, and inhibited by 2,4-dinitrophenol. Thus, it is suggested that energy is required for M-to-S cefadroxil transport. The location of SH-groups of the brush border membrane essential for cefadroxil transport was discussed.

Keywords—intestinal absorption; aminoccephalosporin; cephradine; cefadroxil; carrier-mediated transport; sulfhydryl reagent; mutual inhibition

INTRODUCTION

Aminoccephalosporins are orally active and efficiently absorbed from the small intestine,¹⁻³⁾ although they are ionized and poorly lipophilic at the physiological pH (pH 6.5) of the small intestine. While some basic studies on the intestinal absorption of aminoccephalosporins have been carried out,⁴⁻¹²⁾ the mechanism by which these polar drugs not following pH-partition theory are absorbed remained unresolved.

The present study was undertaken to investigate whether the absorption of aminoccephalosporins was facilitated by the use of carrier mechanisms within the intestinal mucosal membrane.

MATERIALS AND METHODS

Materials—Cephalixin (Fujisawa Pharmaceutical Co., Osaka), cephradine (Sankyo Co., Tokyo), cefatrizine (Banyu Pharmaceutical Co., Tokyo), cefadroxil (Bristol Meyers Co., Tokyo), cefroxadin (Ciba-Geigy (Japan) Co., Tokyo), SCE-100 (Takeda Chemical Industries, Osaka), cyclacillin (Takeda Chemical Industries), and amoxicillin (Fujisawa Pharmaceutical Co.) were used as supplied. L-Phenylalanylglycine and glycylglycine were kindly supplied from Professor H. Yajima and his coworkers, Faculty of Pharmaceutical Sciences, Kyoto University. All other reagents used in these experiments were of

reagent grade and were used without further purification.

Preparation of Drug Solution — Drugs were dissolved in isotonic buffer solution of $\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$, pH 6.5.

Procedure of Absorption Experiments — Male Wistar rats weighing 160–200g were used in all experiments. The procedure of absorption experiments from rat small intestine *in situ* was similar to those of Schanker *et al.*¹³⁾ with slight modification. Rectal temperature was maintained at $37 \pm 1^\circ\text{C}$. The bile duct was ligated in all experiments. Forty ml of drug solution was perfused at the rate of 5 ml/min. After one hour, the perfused solution in the small intestine was withdrawn as completely as possible, and the intestine washed with pH 6.5 buffer solution. The washings were combined with the perfusate and brought up to 100 ml with the same buffer solution. The amount absorbed was calculated from the difference in amount of drugs between the initial perfused solution and the combined effluent.

Measurement of Apparent Directional Transfer Rate — Apparent directional transfer rates of cefadroxil in rat jejunum and ileum were measured *in vitro* according to the method of Yasuhara *et al.*¹⁴⁾ with slight modification. Briefly, the intestinal sacs (10 cm), everted and non-everted, were prepared for the jejunum, first 10 cm beyond the ligament of Treitz, and the ileum, 10 cm above the ileocecal junction. The sacs were filled with 1.5 ml of pH 6.5 isotonic buffer solution and were placed in 20 ml of the same buffer solution containing 0.1 mM cefadroxil. They were incubated with continuous shaking and aerating with 95% O_2 -5% CO_2 at 37°C . After an appropriate incubation period, the inner solution was collected and the concentration of transferred drug was determined. The transfer rate from mucosal side (M) to serosal side (S) was obtained from everted sac experiments and the reverse transfer rate from S to M was obtained from non-everted sac experiments.

Analytical Methods — The reversed phase HPLC method was used to determine the aminocephalosporins. A high pressure liquid

chromatograph TRI ROTAR (Japan Spectroscopic Co., Tokyo) equipped with an ultraviolet detector (UVIDEC 100-III, Japan Spectroscopic Co.) was used with a Cosmosil 5C₁₈ column (15 cm \times 4.6 mm I.D., Nakarai Chemical Co., Kyoto). Mobile phases of methanol-water containing 0.01 M ammonium acetate for the assay of cefatrizine, cephradine, cephalixin, SCE-100, cefroxadin, and cefadroxil were 25:75, 3:7, 3:7, 2:3, 25:75, and 4:96 (by volume), respectively, and the flow rate was maintained at 0.8 ml/min. In some experiments for cefadroxil, another mobile phase of methanol-water containing 0.02 M pH 7.5 sodium phosphate buffer and 5 mM tetra-*n*-butylammonium bromide (18:82 by volume) was applied. The wave length of the detector was 270, 262, 262, 262, 270, and 262 nm for the above antibiotics, respectively. An aliquot of the sample solution was filtered through 0.45 μm pore size triacetylcellulose membrane (Fuji Photo Film, Tokyo) and an appropriate volume of the filtrate was injected into the liquid chromatograph using a high precision micro-syringe (Precision Sampling, Baton Rouge, LA, U.S.A.). The drug concentration was calculated from the peak height using the calibration curve.

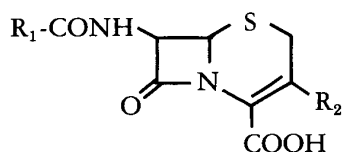
RESULTS AND DISCUSSION

Chart 1 summarizes the chemical structures of aminocephalosporins examined in this study. As is evident from the chart, these compounds have closely related structures. The differences among cephalixin, cephradine, and SCE-100 are the number of double bonds in R_1 . Cefroxadin was produced by the introduction of an oxygen atom to R_2 of cephradine. Dunn *et al.*¹⁵⁾ demonstrated that the benzene-ring hydroxylation in *para*-, but not *meta*-, position in R_1 significantly increased the absorption although the resultant compound may be more hydrophilic than the parent compound. Similarly, cefadroxil is a *para*-hydroxy derivative of cephalixin. All of the compounds are ionized, zwitterions being dominant ionic species, and poorly lipophilic at the physiological pH (pH 6.5) of the small intestine.

In order to investigate the contribution of car-

rier mechanisms to the intestinal absorption of aminocephalosporins, concentration dependency of the absorption was first examined by *in situ* recirculation experiments through the whole

General structure of cephalosporin



Aminocephalosporin	R ₁	R ₂
Cephalexin		-CH ₃
Cephradine		-CH ₃
SCE-100		-CH ₃
Cefroxadin		-OCH ₃
Cefadroxil		-CH ₃
Cefatrizine		

CHART 1. Structures of Aminocephalosporins Tested

small intestine. Results are shown in Table I. Since cefatrizine was unstable in aqueous solution, the values in the table were corrected ones, calculated from the rate of disappearance from the perfusate and the degradation rate constant. The absorption rates of the aminocephalosporins were saturable, except cephalexin. The concentration dependency of aminocephalosporin absorption was demonstrated for cephradine and cefadroxil by Tsuji *et al.*^{8,9} In the case of cephalexin, higher concentrations might be needed to saturate its absorption, as reported in rat upper small intestine.⁸⁾ The saturation of aminocephalosporin absorption suggests the possible contribution of carrier-mediated transport mechanisms in rat small intestine.

Table II shows the effect of pretreatment of the intestinal mucosa for 2 min with 2 mM HgCl₂ on the intestinal absorption of the antibiotics. Bihler and Cybulsky reported that a short-term pretreatment of the intestinal mucosa with a low concentration of HgCl₂ irreversibly modifies the sulfhydryl groups of the apical plasma membrane of intestinal epithelial cells.¹⁶⁾ As is evident from the table, the absorption of all the aminocephalosporins are significantly inhibited by the pretreatment with HgCl₂, suggesting the participation of proteins and/or sulfhydryl groups within the brush border membrane. Similar results were demonstrated for aminopenicillins¹⁷⁾ and *p*-aminobenzoic acid.¹⁴⁾

TABLE I. Absorption of Aminocephalosporins from Rat Small Intestine

Aminocephalosporin	% absorbed in one hour at the concentration of			
	0.01 mM	0.1 mM	1.0 mM	10.0 mM
Cefadroxil	37.0±1.3 (3)	38.5±2.1 (11)	37.3±1.6 (4)	19.8±1.5 (4)
Cephalexin	25.7±2.3 (3)	23.2±1.2 (4)	21.3±2.5 (3)	23.2±2.8 (4)
Cephadrine	32.8±0.8 (4)	33.8±2.1 (4)	34.5±2.0 (4)	20.0±0.8 (4)
SCE-100	19.4±0.6 (3)	20.1±2.5 (4)	20.8±2.3 (3)	11.6±1.3 (4)
Cefroxadin	28.2±0.7 (4)	24.9±0.4 (4)	25.4±0.9 (4)	23.2±2.4 (4) ^{a)}
Cefatrizine ^{b)}	19.5±2.0 (4)	20.7±1.3 (3)	19.8±1.8 (3)	14.2±1.2 (4)

a) 7.0 mM, b) Degradation in the perfusate was corrected.

Results are expressed as the mean ± SE with the number of experiments in parentheses.

Among the tested antibiotics, cefadroxil showed the highest absorption, in spite of its lowest lipophilicity, and its absorption (0.1 mM) was inhibited by the simultaneous perfusion of other aminocephalosporins (cephradine, cephalixin, and cefatrizine) and aminopenicillins (cyclacillin and amoxicillin) when the substrate: inhibitor concentration ratio was 1:10 (Table III).

TABLE II. *Effect of $HgCl_2$ -Pretreatment on Intestinal Absorption of Aminocephalosporins*

Aminocephalosporin	Absorption (% of control)
Cefadroxil	48.3 ± 3.1 (4)
Cephalexin	69.8 ± 2.6 (3)
Cephadrine	61.7 ± 4.0 (4)
SCE-100	51.7 ± 4.0 (4)
Cefroxadin	54.2 ± 4.8 (4)
Cefatrizine	56.5 ± 4.3 (4)

Concentration of drugs = 0.1 mM.

Results are expressed as % of control (mean \pm SE) with the number of experiments in parentheses.

Similarly the absorption of cephradine was inhibited by cefadroxil, cephalixin, cefroxadin, SCE-100, and cyclacillin (Table III). The mutual inhibition of the intestinal absorption between β -lactam antibiotics was first demonstrated in our previous paper.¹⁷⁾ Recently Miyazaki *et al.* reported the inhibition of the absorption of cephradine and cephalixin by aminopenicillins (amoxicillin and cyclacillin) and the mutual inhibition between cephradine and cephalixin.¹¹⁾ They postulated that cyclacillin shares a common carrier-mediated transport mechanism with cephradine, but not cephalixin, and that cephradine and cephalixin competitively inhibit the accumulation or uptake by the intestinal mucosa. Our results shown in Table III suggest that these amino- β -lactam antibiotics could be interacting each other in some permeation processes across the mucosal membrane although we cannot draw a conclusion on the site of interaction at present.

Table III also shows the effect of amino acids and dipeptides on the absorption of cefadroxil and cephradine. Of the amino acids, cycloleucine is a model amino acid behaving like valine and

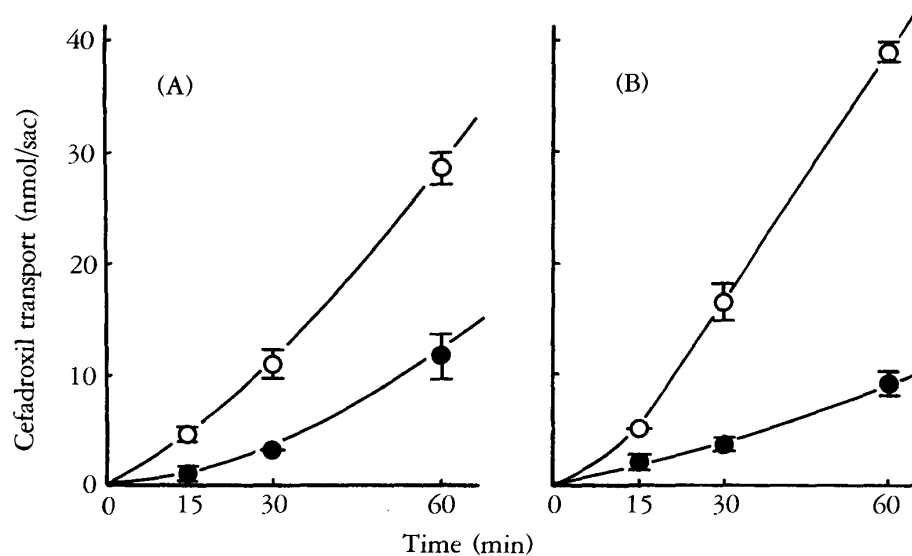


FIG. 1. *Forward Flux into Serosal Fluid and Back Flux into Mucosal Fluid for Cefadroxil in Rat Jejunum (A) and Ileum (B)*

Initial concentration in either the mucosal or serosal fluid was 0.1 mM. Results are expressed as the mean \pm SE; \circ , mucosal-to-serosal flux; \bullet , serosal-to-mucosal flux.

methionine.¹⁸⁾ Contrary to the results for cyc-lacillin,¹⁷⁾ both aminocephalosporins were not inhibited by the presence of 50 times higher concentration of dipeptides (glycylglycine and L-phenylalanylglycine). On the other hand, the absorption of cephradine, but not cefadroxil, was significantly inhibited by amino acids. Quay showed the transport interaction of glycine and L-phenylalanylglycine with cephalixin in rat jejunum.^{4,5)} Further work is needed to correlate the carrier system for the antibiotics with ones for nutrients.

The presence of an active transport mechanism of cephradine was indicated by Umeniwa *et al.*¹⁰⁾ while cephalixin was reported not to be transported against a concentration gradient.^{6,10)} The experiments on cefadroxil transport indicated

that cefadroxil can be transported against a concentration gradient; final serosal-to-mucosal concentration ratios were 1.06 ± 0.05 in the jejunum and 1.21 ± 0.04 in the ileum at the concentration of 0.01 mM after 30 min incubation of the everted sacs.

In order to clarify the transport mechanism of cefadroxil in more detail, apparent directional transfer rates were measured *in vitro*. As shown in Fig. 1, the mucosal-to-serosal (M-to-S) flux rate was significantly faster than serosal-to-mucosal (S-to-M) one in both jejunum and ileum. The flux ratios at the concentration of 0.1 mM were 2.26 and 4.82 in the jejunum and ileum, respectively. Furthermore, M-to-S flux was Na^+ -dependent and inhibited by the presence of 2,4-dinitrophenol (Fig. 2). The results in Fig. 2 also showed the saturation of the M-to-S fluxes. These findings indicate that cefadroxil can be transported from M to S by an active transport mechanism.

The results shown in Table II suggested that sulfhydryl groups within the brush border membrane are involved in the facilitation of aminocephalosporin absorption. Klip *et al.*¹⁹⁾ divided membrane SH-groups into four classes, based on their location and reactivity: (a) extracellular groups; (b) groups facing the cytoplasmic medium; (c) groups locating inside the bilayer of the membrane; and (d) 'buried' or 'cryptic' sites which cannot be reached unless the membrane is disrupted and the proteins denatured by strong surfactants. Our preliminary investigation on the distribution of SH-groups in rat intestinal brush border membrane, by using the method of Klip *et al.*,¹⁹⁾ showed that 54.4% of the total SH-groups are 'reactive' and that externally located groups are only 23.0%. These values are similar to those for rabbit intestinal brush border membrane.¹⁹⁾ Then, we investigated the effect of some other SH-reagents on the M-to-S cefadroxil flux. As shown in Table IV, *N*-ethylmaleimide and 4,4'-dithiodipyridine inhibited the flux in both jejunum and ileum, while *p*-chloromercuriphenylsulfonate (PCMBS) and 5,5'-dithiobis (2-nitrobenzoic acid) had no effect. The effective inhibitors in the table are

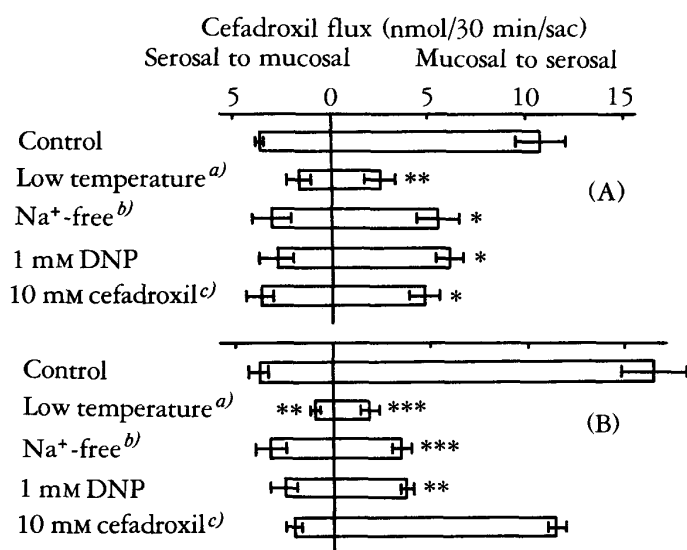


FIG. 2. Forward and Back Fluxes of Cefadroxil in Rat Jejunum (A) and Ileum (B) in Various Conditions

Initial concentration in either the mucosal or serosal fluid was 0.1 mM. DNP, 2,4-dinitrophenol. a) 19°C. b) Na^+ replaced with K^+ . c) Cefadroxil concentration was 10 mM. Fluxes are reduced to a scale of one-hundredth the real values. Results are expressed as the mean \pm SE of 3 experiments.

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$, compared with each control.

membrane permeable. Similarly, Klip *et al.* showed that D-glucose uptake by brush border membrane vesicles was inhibited by the treatment with membrane-permeating *p*-chloromer-

curibenzoate, but not with impermeant PCMBS.¹⁹⁾ In this study, PCMBS was applied at low concentration (0.1 mM), considering trace amounts of contaminating inorganic mercury in

TABLE III. *Intestinal Absorption of Cefadroxil and Cephadrine Perfused in Combination with Their Analogues, Amino Acids or Dipeptides*

Inhibitor	Concentration (mM)	% absorbed in one hour	
		Cefadroxil	Cephadrine
None	—	44.4±0.9 (16)	33.2±1.3 (14)
Cephadrine	1.0	36.6±3.0 (4) ^{b)}	—
Cefadroxil	1.0	—	27.6±1.4 (4) ^{c)}
Cephalexin	1.0	35.6±3.6 (4) ^{b)}	27.5±0.9 (4) ^{c)}
Cefatrizine	1.0	37.6±1.6 (4) ^{b)}	n.e.
Cephroxadin	1.0	n.e.	26.1±0.6 (3) ^{c)}
SCE-100	1.0	n.e.	24.7±2.0 (4) ^{b)}
Cyclacillin	1.0	32.7±1.8 (4) ^{a)}	18.9±2.5 (3) ^{a)}
Amoxicillin	1.0	30.5±1.4 (4) ^{a)}	28.3±1.4 (4)
Glycine	5.0	44.0±1.0 (4)	25.8±2.3 (3) ^{c)}
Cycloleucine	5.0	45.3±3.1 (4)	21.8±1.0 (4) ^{a)}
L-Phenylalanine	5.0	n.e.	22.5±2.6 (4) ^{b)}
Gly-Gly	5.0	43.3±1.4 (5)	38.3±1.1 (3)
L-Phe-Gly	5.0	41.8±1.9 (7)	33.0±1.6 (4)

Substrate concentration = 0.1 mM.

Results are expressed as the mean ± SE with the number of experiments in parentheses. a) $p < 0.001$; b) $p < 0.01$; c) $p < 0.05$, compared with each control. n.e., not examined.

TABLE IV. *Effect of sulfhydryl Reagents on Mucosal-to-Serosal Flux of Cefadroxil in Rat Jejunum and Ileum*

SH-reagent	Concentration (mM)	Cefadroxil flux (nmol/30 min/sac)	
		Jejunum	Ileum
None	—	11.8±0.6 (8)	17.7±1.8 (8)
PCMBS	0.1	12.2±0.9 (4)	19.4±1.8 (4)
DTNB	1.0	9.9±1.0 (8)	18.8±1.9 (6)
NEM	1.0	6.1±0.7 (4) ^{a)}	9.7±0.5 (4) ^{c)}
4-PDS	1.0	6.8±0.7 (4) ^{a)}	6.0±0.6 (4) ^{b)}

PCMBS, *p*-chloromercuriphenylsulfonate; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid); NEM, *N*-ethylmaleimide; 4-PDS, 4,4'-dithiodipyridine.

Concentration of cefadroxil = 0.1 mM.

Results are expressed as the mean ± SE with the number of experiments in parentheses. a) $p < 0.001$; b) $p < 0.01$; c) $p < 0.05$, compared with each control.

the commercial PCMBS.²⁰⁾ Furthermore, 0.1 mM PCMBS might be enough to modify, if it could reach the site, the transport carrier on the brush border membrane since methotrexate transport was inhibited by PCMBS at this concentration in everted rat jejunum.²¹⁾ Our findings are interpreted as indicating that the SH-groups essential for cefadroxil transport are not readily accessible on the outer surface of the brush border membrane but are located either within the bilayer or facing the cytoplasmic medium.

Additional studies are in progress further to characterize the carrier systems for the drug absorption by using brush border membrane vesicles.

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