Transport Characteristics of S-1090, A New Oral Cephem, in Rat Intestinal Brush-Border Membrane Vesicles

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Purpose. Elucidating the transport characteristics of S-1090, a new orally active cephalosporin in rat small intestinal brush-border membranes.

Methods. A rapid filtration technique.

Results. The uptake of S-1090 was stimulated by an inwardly directed H^+-gradient, but did not show overshooting uptake. To investigate the transport system, the inhibitory and countertransport effects of various compounds on S-1090 uptake were examined. Although the dipeptides and tripeptides composed of amino acids with aliphatic side chains did not inhibit the uptake of S-1090, those having histidine, proline or tryptophan as the N-terminal amino acid showed an inhibitory effect. Among the oral cephems tested, ceftibuten showed marked inhibition, while cefaclor and cephalexin had no inhibitory effect. Countertransport effects on S-1090 uptake were observed only when the vesicles were preloaded with histidyl peptides such as His-Gly or His-Ala, while other compounds which exhibited inhibition had no countertransport effect.

Conclusions. Based on the above results, there seems to be heterogeneity (multiplicity) in the oligopeptide transport system which may depend on the structure of the N-terminal amino acid. S-1090 may be dominantly transported via a system that recognizes peptides having histidine as the N-terminal amino acid.

KEY WORDS: S-1090; transport; brush-border membrane; oligopeptide; histidine; oral cephem.

INTRODUCTION

Several drugs are sufficiently absorbed from the intestine in spite of their hydrophilicity, and some of them are considered to be absorbed via specific transport systems for nutrients. It is natural to suppose that they are recognized as nutrients because they have chemical structures similar to corresponding nutrients. Oral β-lactam antibiotics are structural analogues of tripeptide [1] and much work has been done on their absorption mechanisms [1–7].

We reported that the uptake of ceftibuten showed a typical overshooting (up-hill) transport in the presence of H^+ gradient in rat intestinal brush-border membrane vesicles [6], while only a slightly stimulated uptake was observed in the case of cefaclor [7]. The new orally active cephalosporin, S-1090, (6R,7R)-7-[(Z)-2-(2-amino-4-thiazol)-2-hydroxyiminoacetamido]-8-oxo-3-(1,2,3-triazol-5-yl)thiomethylthio-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, has a broad antibacterial spectrum against gram-positive and -negative bacteria [8] and is well absorbed in animals [9].

S-1090 does not possess an α-amino group or a carboxyl group in the side chain at the 7-position of cephem skeleton, which is present in α-amino-β-lactam antibiotics or ceftibuten, but possesses a hydroxyimide instead (Figure 1). In this study, the inhibitory and countertransport effects of amino acids, oligopeptides and other oral cephems on the uptake of S-1090 were evaluated in rat small intestinal brush-border membrane vesicles to elucidate the transport characteristics of S-1090.

MATERIALS AND METHODS

Materials

S-1090, ceftibuten and latamoxef were used as obtained from Shionogi Research Laboratories. Cefaclor and cephalexin were supplied from Eli Lilly (Indianapolis, Indiana). Cephradine and cefadroxil were purchased from Sigma Chemical Co. (St. Louis, Missouri), cyclacillin from Takeda Chemical Industries (Osaka, Japan), cefazolin and ceftizoxime from Fujisawa Pharmaceutical Co. (Osaka, Japan). Histidyl-glycyl-glycine and γ-L-glutamyl-L-alanyl-L-alanine were obtained from Bachem (Bubendorf, Switzerland). All other peptides used were obtained from Sigma Chemical Co.

Preparation of Brush-Border Membrane Vesicles

Brush-border membrane vesicles were isolated from rat small intestine (Jcl SD males, 200–250 g) as described previously [6].

Transport Studies

Uptake studies were carried out at 25°C by the rapid filtration technique as reported previously [6, 7] except that the stop solution and the washing solution contained 1.5% (W/V) β-cyclodextrin to avoid the adsorption of S-1090 onto the filters. The S-1090 trapped on the filter was extracted with 200 μl of 50 mM NaH_2PO_4 (adjusted to pH 2.5 with phosphoric acid).

Analytical Method

S-1090 was measured with a high-performance liquid chromatograph LC-6A (Shimadzu Co., Kyoto, Japan). The conditions were as follows: column, Nucleosil $_5C_{18}$ 4.6 mm × 15 cm (Macherey-Nagel, Germany); mobile phase, 0.05% phosphoric acid/acetonitrile = 86/14; flow rate, 1.0 ml/min; wavelength, 262 nm. In the presence of antibiotics that interfere with S-1090 assay, the following conditions were used: column, YMC-Pack ODS-AM 4.6 mm × 15 cm (Yanako, Japan); mobile phase, 50 mM NaH_2PO_4 (pH 2.5)/methanol/acetonitrile = 80/15/5; flow rate, 1.0 ml/min; wavelength, 290 nm. Protein was assayed by the Bio-Rad Protein Assay Kit (Bio-Rad, Richmond, California) with bovine γ-globulin as a standard.

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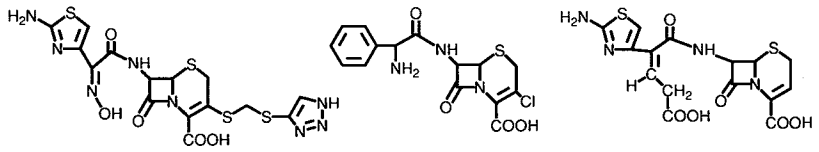

S-1090 Cefaclor Ceftibuten

Fig. 1. Chemical structures of S-1090, ceftibuten and cefaclor.

Ethics in Animal Investigation

This research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

RESULTS

Driving Force in the Uptake of S-1090

In the absorptive cells in the small intestine, Na^+ gradient and H^+ gradient are maintained across the brush-border membrane, and these two gradients are known to be driving forces for the absorption of nutrients. This led us to compare the uptakes of S-1090 in the presence or absence of both gradients. As shown in Figure 2, no overshooting uptake was observed in the presence of both gradients but the initial uptake was stimulated by the presence of both gradients. This uptake pattern is similar to that observed for cefaclor [7]. To clarify which gradient was dominant in the uptake, the initial (15 sec.) uptake data were compared under various gradient conditions. The uptake of S-1090 in the presence and absence of both gradients were 0.41 ± 0.06 and 0.12 ± 0.08 (nmol/mg protein/15 sec, mean ± S.D.), respective. When the pH was 5.5 both inside and outside, the uptake (0.14 ± 0.01, NaCl in both side) was almost the same as when both pH values were 7.5. The stimulated uptake was only observed by H^+ gradient (0.36 ± 0.07, KCl in both side) and not by Na^+ gradient (0.14 ± 0.02, pHs in both side were 7.5). This suggests a participation of carrier-mediated transport.

Kinetic Analysis of S-1090 Uptake

To confirm the existence of carrier-mediated transport, the concentration dependency of the uptake was examined. The concentration-dependent uptake was observed, and the kinetic parameters estimated as described previously (6) to fit the data to Equation 1 were as follows: Km = 0.37 mM; Vmax = 2.31 nmol/mg protein/min; Kdif = 0.40 nmol/mg protein/min/mM.

$$\text{Eq. 1} \qquad V = Vmax \cdot C/(Km+C) + Kdif \cdot C$$

where V is the initial uptake rate, C is the initial concentration, Vmax is the maximum uptake rate by the carrier mediated process, Km is the Michaelis constant, and Kdif is the coefficient of simple diffusion.

Inhibitory Effect of Oligopeptides

From the view point of similarity of the chemical structure and the driving force, dipeptide transport systems are considered to participate in S-1090 transport. Thus, the inhibitory effects of oligopeptides were examined. Oligopeptides composed of aliphatic amino acids had no inhibitory effects (Figure 3), although they significantly inhibited the uptake of ceftibuten [7]. On the other hand, the inhibitory effect was observed with oligopeptides possessing a cyclic amino acid at the N-terminal except for Phe-Gly.

Inhibitory Effect of Amino Acids, Antibiotics and Carboxylic Acids

Since some types of dipeptides inhibited S-1090 uptake, the inhibitory effects of their consisting amino acids were examined (Figure 4A). Although amino acids with aliphatic side chain showed no or little inhibition, cyclic amino acids significantly inhibited the uptake of S-1090. Tryptophan (and Trp-Ala), in particular, almost completely inhibited the carrier-mediated transport part of the uptake, when the simple diffusion was taken into consideration. These were N-terminal amino acids of the dipeptides which exhibited a strong inhibitory effect.

The effect of antibiotics involving those for parenteral use on S-1090 uptake was studied. The inhibitory effects varied among antibiotics with ceftibuten exhibiting the strongest inhibition (Figure 4 B). However, cefaclor and

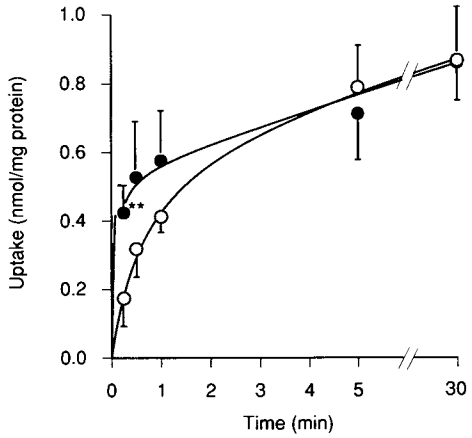

Fig. 2. Time course of S-1090 (0.5 mM) uptake into rat intestinal brush border membrane vesicles. The freshly isolated vesicles were resuspended with 10 mM Hepes buffer (pH 7.5) containing 100 mM mannitol and 100 mM KCl. The vesicle suspension (20 μl) was added to 200 μl of reaction mixture containing 0.5 mM S-1090, 100 mM mannitol, 100 mM NaCl and 10 mM Mes (pH 5.5) or 0.5 mM S-1090, pH 7.5 KCl buffer solution. ●: in the presence of Na^+ and H^+ gradients ○: in the absence of both gradients. Data represent the mean ± S.D. (n = 3–4) **,$P<0.01$ compared to control.

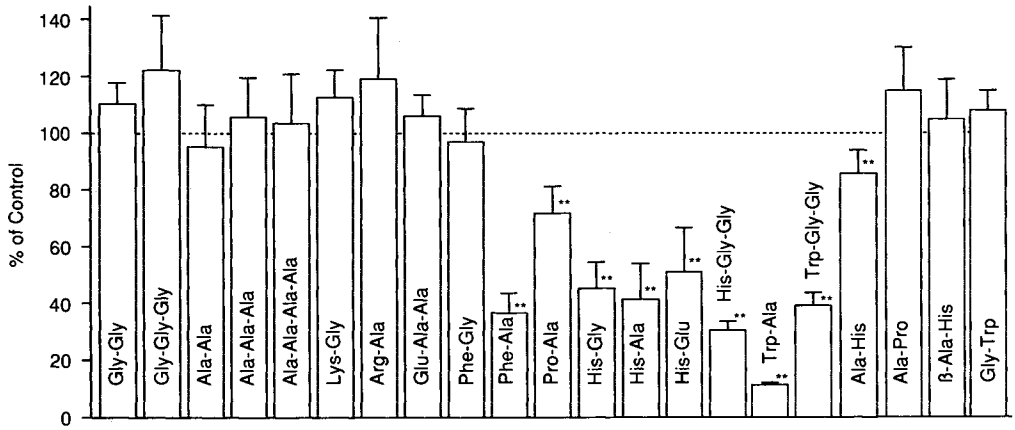

Fig. 3. Inhibitory effects of oligopeptides on S-1090 uptake. The inhibitory effect on S-1090 uptake was examined by the addition of various compounds in the reaction solution to a concentration of 10 mM. Control value of the initial uptake is 0.40 ± 0.03 nmol/mg protein/15 sec. Data represent the mean ± S.D. (n = 4–6) **,P<0.01 compared with control.

cephalexin did not show an inhibitory effect in spite of their efficient intestinal absorbability.

Tsuji et al. reported that monocarboxylic acid transporter as well as dipeptide transporter participated in the transport of cefdinir, a hydroxyimide-type antibiotic, in rabbit [10]. When we examined the effect of carboxylic acids (acetic acid, propionic acid, nicotinic acid, lactic acid, biotin and folic acid) on S-1090 uptake, we found none with an inhibitory effect. These results indicate that the transport system of S-1090 is different from that of carboxylic acids in rat.

Countertransport Effects of Amino Acids and Oligopeptides

In order to confirm that S-1090 is transported by an oligopeptide carrier, the countertransport effects of amino acids and oligopeptides were compared (Figure 5). Stimulated uptake of S-1090 was observed when the vesicles were preloaded with histidyl peptides, whereas tryptophan and its peptides showed inhibition. However, it can not be denied that tryptophanyl peptides share a common transport system because they exhibited severe *cis*-inhibition. The above findings suggest that S-1090 is transported by a carrier system which transports histidyl peptides, and this may be distinguished from carrier systems that transport other types of oligopeptides.

DISCUSSION

We tried to establish whether oral cephems have a structure similar to tripeptides. Figure 6(A) shows the stereostructure of α-amino-β-lactam antibiotic and Ala-Ala-Ala in β-structure. Stereochemically, the functional groups of α-amino-β-lactam-antibiotic are located at almost the same position as the tripeptide. Many β-lactam antibiotics lacking α-amino group are parenterally used in spite of their peptide-like β-lactam skeleton. Further, the transports of ceftibuten and cephalexin in the small intestinal brush border membrane are sterospecific depending on the isomerism of the side chain at the 7 position, that is, ceftibuten and cephalexin are a *cis*-isomer and a D-isomer, respectively; their *trans*-isomer or L-isomer was not taken up [6,11]. These demonstrate that cephem molecules are discriminated by the membrane protein and the structure of the side chain is very important for oral antibiotics to be recognized as tripeptide.

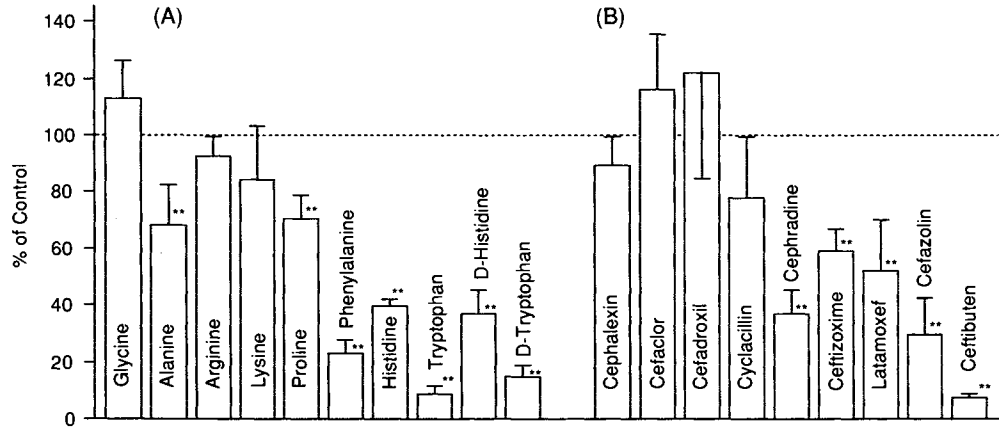

Fig. 4. Inhibitory effects of amino acids (A) and antibiotics (B) on S-1090 uptake. Data represent the mean ± S.D. (n=4–6). **,P<0.01 compared to control.

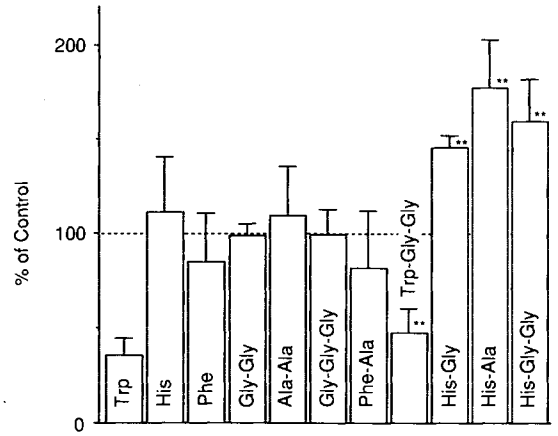

Fig. 5. Coutertransport effects of amino acids and oligopeptides on S-1090 uptake. To observe the countertransport effect, the membrane vesicles were resuspended with pH 5.5 buffer solution and a portion of the vesicle suspension (10 μl) was preloaded with 10 μl of pH 5.5 buffer solution containing various compounds (20 mM) for 5 min at 25°C, then the uptake study was carried out. Control value of S-1090 uptake is 0.23 ± 0.03 nmol/mg protein/15 sec. Data represent the mean ± S.D. (n = 4–5) **,P<0.01 compared to control.

Figure 6(B) compares the stereostructures of the side chains at the 7 position of oral antibiotics and the N-terminal of the corresponding peptides. Ceftibuten has a structure similar to the side chain of glutamic acid and the nitrogen of thiazole is located at the position of amino group of glutamic acid. The side chain of α-amino-β-lactam antibiotic is D-phenylglycine type. Interestingly, the aromatic ring of cefaclor is oriented in the same direction when L-Phe has the structures as shown, and D-phenylglycine may be taken for L-Phe. The present study and stereochemical considerations suggested that S-1090 might be mistaken for histidyl peptide.

We found common structures in the compounds that exhibit the inhibitory effect on S-1090 uptake including amino acids, peptides and antibiotics. They can be classified into two types: a carboxylic acid with a 5-membered heterocycle with nitrogen (ex. His, Trp and their peptides) and a compound with an aromatic ring and a carboxylic group attached to a chiral carbon (ex. Phe-Ala and Trp-Ala). This indicated that a heterocycle with nitrogen and the carboxyl group might play an important role in having the carrier protein recognize S-1090.

Although the transport systems of amino acids are known to be different from those of dipeptides, some amino acids inhibited S-1090 uptake and even D-tryptophan showed inhibition similar to L-tryptophan. As L-cephalexin, which was not transported, bound to the membrane protein (11), the inhibitions by amino acids may be due to the mere binding to the carrier protein and not to the competition in the transport.

S-1090 did not show an overshooting uptake, although it has the same substitute, an aminothiazole group, in the 7-position of cephem skeleton as ceftibuten. Ala-Ala or Glu-Ala-Ala that showed strong inhibition of cephalexin and ceftibuten uptake [4, 7] had no inhibitory effect on S-1090 uptake (Figure 3). S-1090 has similar uptake characteristics to cefaclor [7] in the presence of H^+ gradient, nevertheless, cefaclor and cephalexin did not inhibit S-1090 uptake (Figure 4). Thus, S-1090 may be transported by a different type of carrier system from ceftibuten, cefaclor or cephalexin.

Histidyl peptides showed *cis*-inhibition and *trans*-stimulation of S-1090 uptake. When histidine was at the C-terminal position of the dipeptide, the peptide did not have the inhibitory effect (Figure 3). Recent reports suggest heterogeneity (multiplicity) in the transport of dipeptides [12] and oral cephems [13, 14]. Therefore, the N-terminal amino acid of peptide is considered to be important in being recognized as substrate, and the above heterogeneity may depend on the N-terminal chemical structure of the molecules because the cephems have almost the same cephem skeleton.

From the viewpoint of the uptake of oral cephems and inhibition of their uptake, there seems to be at least three carrier-mediated transport systems for dipeptides, and the heterogeneity may depend on the structure of the N-terminal amino acid. The first is the concentrative transport system that transports an aliphatic (relatively hydrophilic) peptide such as Gly-Gly or Glu-Ala-Ala. The second is the transport system that transports aromatic (hydrophobic) peptides such as Phe-Ala-Ala. And the third is the transport system for peptides with heterocyclic amino acid (His or Trp) at the N-terminal. This classification is intriguing. Both the transporter and the substrate are peptides, and general interactions occur between peptides. Besides hydrogen bonds between the peptide backbones, further interactions may occur between the side chains, that is, aliphatic chain and aromatic ring at side chains are considered to interact with aliphatic chain and aromatic ring, respectively, and a heterocycle with nitrogen may form a hydrogen bond with His, Tyr etc. [15].

We hypothesize that ceftibuten is transported by the first system, while cefaclor is mainly transported by the second one and only partly by the first one as suggested previously [7]. Our present findings suggested that S-1090 is mainly absorbed through the oligopeptide transport which recognizes peptide having histidine as the N-terminal amino acid.

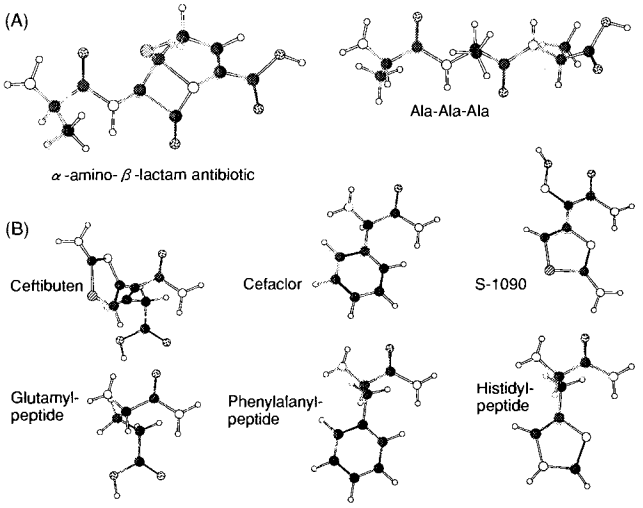

Fig. 6. (A) Comparison of stereostructures of α-amino-β-lactam-antibiotic and tripeptide in β-structure. (B) Stereostructures of the side chains at 7 position of ceftibuten, cefaclor and S-1090 compared with those of Glu-, Phe- and His-peptide.

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