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CARRIER-MEDIATED TRANSPORT SYSTEMS FOR AMINOPENICILLINS IN RAT SMALL INTESTINE

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The absorption of aminopenicillins from rat small intestine was investigated by using the *in situ* perfusion technique. Carrier-mediated transport systems were demonstrated for amoxicillin and cyclacillin. The absorption of amoxicillin and cyclacillin was saturable and inhibited by the short term pretreatment of the intestine with a low concentration of $HgCl_2$. The two penicillins inhibited intestinal absorption to each other. The simultaneous perfusion of dipeptides, L-phenylalanylglycine and glycylglycine, inhibited the absorption of cyclacillin but not of amoxicillin. Furthermore, only cyclacillin was shown to concentrate against a gradient. These data are consistent with the presence of two carrier systems for the active transport of cyclacillin in the intestinal mucosa: one which can be blocked by amoxicillin, and the other by dipeptides. Amoxicillin may be transported by the facilitated diffusion mechanism, the carrier of which seems to be common to one of the carrier systems for cyclacillin transport. No evidence of carrier-mediated transport of ampicillin could be found.

Keywords——aminopenicillins; cyclacillin; amoxicillin; intestinal absorption; carrier-mediated transport; mutual inhibition

INTRODUCTION

Although intestinal absorption of most weak organic acids and bases can be explained in terms of simple diffusion of uncharged molecules across a lipid-like boundary,[1] the mechanism by which organic ions are absorbed remains unresolved. We have investigated the mechanism of the intestinal absorption of non-lipophilic ionized drugs and pointed out the contribution of mucosal components to the absorption process of non-lipophilic dyes from rat intestine.[2-4] We also showed that absorption characteristics of cephalosporins, cephalexin and cefazolin, were not consistent with the pH-partition hypothesis and that the transfer rate of cephalexin across the lipid bilayers, liposomes, was markedly faster than predicted from its partition coefficient to the organic solvents.[5]

The aminopenicillins, ampicillin, amoxicillin, and cyclacillin, are always ionized, the zwitterion being the dominant ionic species at the physiological pH of the small intestine, and yet are significantly absorbed from the gastrointestinal tract to produce therapeutic effects.[6,7] Recently Dixon and Mizen reported the absorption of aminopenicillins by using an *in vitro* everted gut sac method and suggested the active transport of cyclacillin.[8]

The present study deals with the absorption of the three aminopenicillins from rat small intestine *in situ* in order to elucidate the mechanism involved in their transport.

MATERIALS AND METHODS

Materials——Ampicillin anhydrous*, cyclacillin anhydrous*, and amoxicillin anhydrous** were used as supplied. L-Phenylalanylglycine and glycylglycine were supplied from

* Takeda Chemical Industries, Osaka, Japan.

** Fujisawa Pharmaceutical Co., Osaka, Japan.

Professor H.Yajima and his coworkers, Faculty of Pharmaceutical Sciences, Kyoto University. Phosphatidylcholine was prepared from egg yolks according to the method of Rhodes and Lea.[9] Rat intestinal mucosa was scraped off with a cover glass and the total lipids were extracted by the method of Folch, *et al.*[10] All other reagents used in these experiments were the finest grade available.

Preparation of Drug Solution——Drugs were dissolved in isotonic buffer solution of NaH_2PO_4–Na_2HPO_4, pH 6.5.

Apparent Partition Coefficient——Apparent partition coefficients were determined by the method described in the previous report from this laboratory.[11]

Preparation of Liposomes——Two kinds of liposomes were prepared, one from phosphatidylcholine (80 μmol), cholesterol (20 μmol), and dicetylphosphate (5 μmol), and the other from total lipid extracts of the intestine (32 mg) according to the method of Kinsky, *et al.*[12]

Transfer Rate Measurement——The overall transfer rate of penicillins from aqueous dispersion of liposomes were determined by the method of dynamic dialysis of Meyer and Guttman,[13] except that the internal solution of the Visking Cellulose Tube (20/32) was 6 ml, and the external solution was 60 ml. Temperature was maintained at 37°.

Procedure of Absorption Experiments——Male Wistar rats weighing 160—200 g were used in all experiments. The procedure of absorption experiments from rat small intestine *in situ* was the same as those reported in the papers from this laboratory.[14] Rectal temperature was maintained at 37±1°. 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 perfusion solution and the combined effluent. The results were expressed as the mean±standard deviation of at least three experiments.

Analytical Methods——Ampicillin[15] and amoxicillin[16] were determined spectrofluorometrically according to the method of Miyazaki, *et al.* in some experiments. In most experiments, a high pressure liquid chromatography was used to determine the three aminopenicillins. A high pressure liquid chromatograph TRI ROTAR (Japan Spectroscopic Co., Tokyo, Japan) equipped with an ultraviolet detector (UVIDEC-100, Japan Spectroscopic Co.) was used in a reversed phase with a μBondapak C_{18} column (30.5 cm×4.0 mm I.D., Waters Assoc., Milford, Mass., U.S.A.). Mobile phases of methanol-water containing 0.01 M ammonium acetate for the assay of ampicillin, amoxicillin, and cyclacillin in perfusion solution were 7:3, 88:12, and 73:27 (by volume), respectively, and the flow rate were maintained at 1 ml/min. The wave lengths of the detector were 213, 225, and 210 nm for ampicillin, amoxicillin, and cyclacillin, respectively. An aliquot of the perfusion solution was filtered through 0.45 μm pore size triacetylcellulose membrane (Fuji Photo Film, Tokyo, Japan) and an appropriate volume of the filtrate was injected into the liquid chromatograph using a high precision microsyringe (Precision Sampling, Baton Rouge, La., U.S.A.). The drug concentration was calculated from the peak height using the calibration curve.

RESULTS

The apparent partition coefficients and the permeability rate constants for the artificial lipid membranes for aminopenicillins used in this study are summarized in Table I. The permeability rate constants of the penicillins were the regular order of their apparent partition coefficients, lipophilicity.

Absorption of aminopenicillins from rat small intestine was examined as the function of the initial concentrations, using *in situ* perfusion method; the results are given in Table II. While the intestinal absorption of ampicillin in percentage was independent of the initial concentration, the absorption processes of amoxicillin and cyclacillin were saturable. In addition the absorption of

TABLE I *The Apparent Partition Coefficients and the Permeability Rate Constants for the Artificial Lipid Membranes for Aminopenicillins*

Aminopenicillin	Ampicillin	Amoxicillin	Cyclacillin
Apparent partition coefficient[a]	0.01	0.00	0.02
Permeability rate constant:			
Absorption simulator[b]	8.36×10^{-5}	5.47×10^{-5}	2.45×10^{-4}
Liposome (PC-Ch-DP)[c]	8.66×10^{-3}	9.45×10^{-4}	1.91×10^{-2}
Liposome (TL)[d]	2.12×10^{-2}	5.92×10^{-3}	——[e]

a) pH 6.5 isotonic sodium phosphate buffer—chloroform, at 37°
b) Sartorius Absorption Simulator (Sartorius Membranefilter GmbH, Göttingen, Germany). In cm·min^{-1}.
c) Composed of phosphatidylcholine–cholesterol–dicetylphosphate (80:20:5). In min^{-1}
d) Composed of total lipid extracts from rat small intestinal mucosa
e) Undetectable.

TABLE II. *Intestinal Absorption of Aminopenicillins as the Function of the Initial Concentrations*

Aminopenicillin	% absorbed in one hour at the concentration of		
	10^{-5} M	10^{-4} M	10^{-3} M
Ampicillin	10.1±3.0 (4)	9.7±1.1 (4)	11.0±1.9 (4)
Amoxicillin	16.2±1.4 (4)	15.4±2.2 (4)	12.6±2.2 (3)
Cyclacillin	81.6±9.9 (4)	67.3±5.4 (4)	62.3±8.4 (4)

Numbers in parentheses represent the number of experiments.

amoxicillin was greater than that of ampicillin and this does not coincide with their permeability to the artificial lipid membranes. This indicates the possibility of the carrier-mediated transport systems for amoxicillin and cyclacillin.

In order to clarify the contribution of the carrier systems to the absorption processes of the penicillins, the effect of chemical modification of the mucosal surface on their absorption was investigated. Bihler and Cybulsky[17] showed that a short pretreatment with a low concentration of $HgCl_2$ strongly inhibited sugar transport at the luminal aspect without affecting the internal levels of Na^+ and K^+. In the present study, the small intestine was pretreated with 3 mM $HgCl_2$ for 5 min before the absorption experiment to modify the apical plasma membrane of the epithelial cells. The results are shown in Table III. The absorption of amoxicillin and cyclacillin from the small intestine, which were shown to be saturable, were significantly inhibited ($p<0.001$) by the pretreatment with $HgCl_2$, while the absorption of ampicillin was not affected at all. This suggests that protein and sulfhydryl groups within the mucosal membrane are involved in the absorption processes of amoxicillin and cyclacillin.

As a clue to elucidate the carrier system, mutual inhibition of the intestinal absorption between pairs of aminopenicillins was examined. As is evident from Table IV, cyclacillin and amoxicillin reduced their absorption mutually when the substrate: inhibitor ratio was 1:10. This suggests that these two penicillins could be sharing a common carrier system.

In order to make clearer the carrier system, the relationship between the transport systems for the penicillins and those for nutrients, amino acids and dipeptides, which are known to be transported by a carrier-mediated system,[18,19] was investigated. The results are listed in Table V. While amino acids, L-phenylalanine and glycine, did not affect the absorption of penicillins, dipeptides, L-

TABLE III. *Effect of Pretreatment with $HgCl_2$ on the Intestinal Absorption of Aminopenicillins*

Aminopenicillin	% absorbed in one hour		% of control
	Control	$HgCl_2$-treated[a]	
Ampicillin	11.2±1.8 (4)	10.0±0.8 (3)	89
Amoxicillin	13.4±3.8 (4)	6.8±2.3 (3)	51
Cyclacillin	63.3±2.7 (3)	17.7±2.1 (4)	28

Initial concentration of drugs = 0.1 mM.
Numbers in parentheses represent the number of experiments.
a) Pretreatment: 3 mM $HgCl_2$ for 5 min.

TABLE IV. *Mutual Inhibition of the Intestinal Absorption between Pairs of Aminopenicillins*

Substrate	Inhibitor	Concentration (mM)	Substrate absorbed (% in one hour)	Statistical Significance (p)[a]
Amoxicillin	None	—	15.4±2.2 (4)	—
	Ampicillin	0.1	14.7±4.8 (4)	N.S.
		1.0	13.0±1.1 (3)	N.S.
	Cyclacillin	0.1	11.6±3.2 (3)	N.S.
		1.0	9.6±2.4 (4)	<0.02
Cyclacillin	None	—	67.3±5.4 (4)	—
	Amoxicillin	0.1	65.0±6.1 (3)	N.S.
		1.0	42.0±9.2 (3)	<0.01
Ampicillin	None	—	9.7±1.1 (4)	—
	Amoxicillin	0.1	10.7±2.7 (4)	N.S.
		1.0	10.5±2.1 (4)	N.S.

Concentration of substrates = 0.1 mM.
Numbers in parentheses represent the number of experiments.
a) Student's t-test; N.S. = Not significant.

phenylalanylglycine and glycylglycine, significantly reduced the absorption of cyclacillin but not of others.

DISCUSSION

Carrier-mediated transport systems in rat small intestine were demonstrated for amoxicillin and cyclacillin. Saturation phenomenon of the transport of cyclacillin across the mucosal membrane *in vitro* was shown by Dixon and Mizen.[8] Tsuji, *et al.* demonstrated the saturation of the absorption of amoxicillin from the upper small intestine by using the *in situ* perfusion technique,[20] while Miyazaki, *et al.*[21] and Dixon and Mizen[8] reported that its permeation through the everted rat intestinal sacs and its mucosal uptake were proportional to the mucosal concentrations. In the present investigation, saturation of the intestinal absorption of cyclacillin and amoxicillin was observed (Table II).

When the mucosal surface was modified by the pretreatment with $HgCl_2$, the absorption of amoxicillin and cyclacillin was markedly reduced (Table III). The contribution of protein and sulfhydryl groups within the mucosal membrane to their absorption processes is suggested. The remaining portion of the absorption seems to be by the carrier-independent simple diffusion through lipoid regions of the intestinal boundary, and is closely related to the permeability to the artificial lipid membranes (Table I).

Mutual inhibition of the intestinal absorption between amoxicillin and cyclacillin may indicate the common carrier system for the two penicillins.

TABLE V. *Effect of Amino Acids and Dipeptides on the Absorption of Aminopenicillins from Rat Small Intestine*

Penicillin	Inhibitor	% Absorbed in one hour	Statistical Significance (p)[a)]
Cyclacillin	None	67.3±5.4 (4)	——
	Glycine	64.7±4.2 (3)	N.S.
	L-Phenylalanine	61.2±1.4 (3)	N.S.
	Glycylglycine	48.2±5.0 (3)	<0.01
	L-Phenylalanylglycine	38.8±8.6 (3)	<0.01
Amoxicillin	None	15.4±2.2 (4)	——
	Glycine	15.2±1.3 (3)	N.S.
	Glycylglycine	14.9±2.2 (4)	N.S.
	L-Phenylalanylglycine	15.0±0.6 (3)	N.S.
Ampicillin	None	9.7±1.1 (4)	——
	L-Phenylalanylglycine	10.5±0.2 (3)	N.S.

Concentrations of penicillins and inhibitors were 0.1 and 5.0 mM, respectively.
Numbers in parentheses represent the number of experiments.
a) Student's t-test; N.S. =Not significant.

Although no relationship between the absorption of cyclacillin and that of amino acids could be demonstrated, dipeptides, L-phenylalanylglycine and glycylglycine, significantly reduced its absorption. It is known that dipeptides are actively absorbed by using a specific transport pathway distinct from that of amino acids.[19] Interestingly, the absorption of amoxicillin was affected by none of these dipeptides and amino acids. These results may suggest that the common carrier system for cyclacillin and dipeptides is not operative for amoxicillin. Thus it seems quite feasible to consider that cyclacillin is absorbed by at least two carrier systems, one which can be blocked by amoxicillin and the other by dipeptides, in addition to the simple diffusion mechanism. Furthermore, cyclacillin can be transported against a concentration gradient; final serosal-to-mucosal concentration ratios were 1.89±0.34 in the jejunum and 2.51±0.18 in the ileum at the concentration of 0.1 mM after 30 min incubation of the everted sacs. This agrees very well with the results of Dixon and Mizen[8] and it is suggested that cyclacillin is actively transported across the mucosal membrane of rat small intestine. In contrast, neither amoxicillin nor ampicillin concentrated against a gradient. This is in agreement with the results of other investigations.[8,21] Thus the active transport may not be involved in their absorption processes. Since the intestinal absorption of amoxicillin, which was better than predicted from the permeability to the artificial lipid membranes, was shown to be saturable and to share the common carrier system with cyclacillin, it is considered that the facilitated diffusion mechanism contributes to the amoxicillin transport. No evidence of carrier-mediated transport of ampicillin could be found.

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