INTESTINAL ABSORPTION OF SEVERAL β-LACTAM ANTIBIOTICS. V.1) EFFECT OF AMINO β-LACTAM ANALOGUES AND DIPEPTIDES ON THE ABSORPTION OF AMINO β-LACTAM ANTIBIOTICS*

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The absorption mechanism of amino β-lactam antibiotics was investigated by using the whole small intestine of a rat. Mutual inhibition among amino β-lactam analogues and the effects of dipeptides were studied. The influences of glycyglycine on the absorption of cefradine at the four different parts of intestine were also studied. Similarly to the case of cephalixin and cephadine,2) the absorption of amoxicillin was significantly inhibited by cyclacillin, cephadine, and cephalixin, but the absorption of ampicillin was not reduced by any tested antibiotics. In the experiments using dipeptides (6.0 mM), the absorption of cyclacillin was reduced significantly by glycyglycine, not by L-carnosine. And cephadine absorption was influenced by L-carnosine (6.0, 10 mM), not by glycyglycine (6 mM). On the contrary, the absorption of cephadine was not reduced at all by these dipeptides. And from the experiment using the four different parts of intestine, it was shown that the transport interaction of glycyglycine with cephadine was observed in only one segment (the upper part of jejunum). These results suggest that the carrier-mediated transport system correlated to dipeptides participates only to a small degree in the common absorption mechanisms of these amino β-lactam antibiotics.

**Keywords** — amino β-lactam antibiotics; peptide; intestinal absorption; carrier-mediated transport; perfusion; inhibition

INTRODUCTION

It is well known that amino β-lactam antibiotics are efficiently absorbed from the small intestine even though they are ionized over entire pH range and poorly lipophilic. Many studies on the intestinal absorption of amino β-lactam antibiotics have been reported to evaluate the absorption mechanisms.

Tsuji et al.3,4) observed the saturated absorption phenomena of amoxicillin, cyclacillin, cephalixin, cephadine, and cefadroxil, and postulated that the carrier-mediated transport played an important role as a common system on the absorption of amino β-lactam antibiotics.

Recently, Kimura et al.5,6) reported that the intestinal absorption of amino β-lactam antibiotics was inhibited by HgCl₂ pretreatment, and postulated the participation of protein and/or sulphydryl groups within the brush border membrane. They also showed that cephadine, but not cefadroxil, was significantly inhibited by amino acids, and these aminoccephalosporins were not inhibited by the presence of 50 times higher concentration of dipeptides.

In previous papers, we showed that the degree of intestinal absorption of these antibiotics (ampicillin, amoxicillin, cephadine, and cephalixin) was proportional to the amount of

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drug in the intestinal tissue,\textsuperscript{1,7} and that the mutual inhibitory behaviour was observed among cephalexin, cephradine, and cyclacillin.\textsuperscript{2} And it was found that the carrier-mediated transport system participated in cyclacillin and cephradine, in part. Furthermore, we also showed that these amino \( \beta \)-lactam antibiotics, but not monobasic cephalosporins, had affinity to the F1 fraction which was one of the soluble fractions obtained from the intestinal mucosa, and there was a significant correlation between the binding to this fraction and the absorption behaviour of the antibiotics.\textsuperscript{11}

As shown in these investigations and the results of other investigator,\textsuperscript{8–11} there is no agreement for the participation of the carrier-mediated transport system such as amino acids and dipeptides on the absorption of the amino \( \beta \)-lactam antibiotics. Moreover, the common transport characteristics which offer a explanation for the difference of the absorption of the antibiotics are unclear at this point.

The present study was undertaken to examine the existence of the common transport system of these amino \( \beta \)-lactam antibiotics. For this purpose, the extent of inhibitory effects of amino acids and dipeptides on the absorption of these amino \( \beta \)-lactam antibiotics were compared with those of these drugs.

**MATERIALS AND METHOD**

*Materials* — Ampicillin anhydrous,\textsuperscript{*} cyclacillin anhydrous,\textsuperscript{*} amoxicillin trihydrate,\textsuperscript{**} cephallexin monohydrate,\textsuperscript{***} and cephradine dihydrate\textsuperscript{*4} were used as supplied. L-Phenylalanine, L-phenylalanylglycine, and glycylglycine were purchased from Sigma Chemical Co., St. Louis, Mo. U.S.A. L-Carnosine was from Fulka AG, Chemische Fabrik Switzerland. All the reagents were of special grade and used without further purification.

*Procedure for Absorption Experiment* — The absorption experiment using the whole small intestine of a rat was studied according to the technique of Kakemi et al.\textsuperscript{12} The drugs were dissolved in modified Ringer solution\textsuperscript{13} with pH 6.8 (150 \( \mu \)M). To recognize the inhibitory effect of drugs and dipeptides clearly, twenty times concentration of inhibitors were used as reported previously.\textsuperscript{2} The bile duct was ligated. Before the experiment, the intestine was rinsed with 30 ml of warmed modified Ringer solution by syringe to exclude the intestinal content, and perfused with 50 ml of drug-free solution maintained at 37\(^\circ\)C, to wash the intestine. The perfused solution (50 ml) was then circulated at a rate of 4.5 ml/min. One fifth milliliter of sample solution was pipetted at the selected time after the start of recirculation.

Intestinal absorption of cephradine at four different segments was also examined. Each 10 cm of upper (segment 1), middle (segment 2 and 3), and distal (segment 4) segment were used, and each segment was prepared respectively as follows. Segment 1 was prepared under the pylorus. Segment 2 and 3 were prepared at the beginning of 15 and 30 cm below the pylorus, respectively. And segment 4 was prepared at the beginning of 10 cm above the ileo-cecal junction. After the segment was rinsed with about 5 ml of warmed solution at 37\(^\circ\)C as mentioned above, 0.1 ml of the sample solution was pipetted at the selected time.

*Analytical Method* — Ampicillin and amoxicillin were determined by the fluorometric methods described previously.\textsuperscript{14,15} For the determination of these drugs in the presence of other drugs, amino acids, and dipeptides, a high performance liquid chromatography was used. Cyclacillin and aminophenylboronin were also determined by this chromatographic method. A high performance liquid chromatograph (Hitachi 638-
0500) equipped with a high pressure sampling valve (638-0801) and a multiwave-length ultraviolet detector (638-0900) were used. The column, 25 cm × 4 mm i.d. stainless steel, was packed with Hitachi gel 3053 (5 μm, Hitachi Ltd., Tokyo, Japan) or Hitachi gel 3056 (5 μm).

The mobile phase was a mixture of methanol-0.05M KH₂PO₄ solution, and a mixture was adjusted to pH 6.0 with 4 N sodium hydroxide solution. The flow rate was maintained at 0.7 ml/min, and the column was warmed at 55°C using a bath circulator. A mixture of methanol-0.05M KH₂PO₄ solution (15:85, by volume) was used for the assay of ampicillin, cyclocillin, cephalexin, and cephradine. And a mixture of these solutions (8:92, by volume) was used for amoxicillin. For the determination of ampicillin in the presence of cephalexin, another column packed with Hitachi gel 3056 (5 μm) was employed, and a mixture of these solutions (20:80, by volume) was used as a mobile phase.

The wavelength of the detector was set at 210 and 260 nm for penicillins and cephalosporins, respectively. The drug concentrations were calculated from the peak height ratios using calibration curve, and the internal standard was selected from the drugs tested in this experiment.

RESULTS AND DISCUSSION
Inhibition between Amino β-Lactam Antibiotics
In order to clarify the inhibitory effects of cyclocillin and amino-cephalosporins (3.0 mM) on the absorption of amoxicillin and ampicillin (150 μM), in situ perfusion technique in whole small intestine was used. The results of amoxicillin and ampicillin are shown in Fig. 1 and 2, respectively, and each value was not corrected with respect to the water absorption.

The rate of disappearance from the intestinal lumen of amoxicillin was reduced significantly by cyclocillin, cephalexin, and cephradine, and the inhibitory effect of cyclocillin was considerably larger than those of ampicillin, cephradine, and cephalexin. There were significant inhibitions in the absorption of amoxicillin during the time-course of 30-60 min in the presence of aminocephalosporins. These inhibitory effects of aminocephalosporins had been obtained in the mutual inhibition between aminocephalosporins. And it was shown that the concentration of amoxicillin in the perfused medium was gradually increased in the presence of cyclocillin. It is assumed that the water absorption was not changed in all conditions, since the osmolarities of the perfused medium in the presence of these drugs were almost the same as the control (290 mOsm). On the contrary, the absorption of amoxicillin was significantly inhibited by the presence of cyclocillin, and it can be considered that these results offer increased concentration of amoxicillin.

Fig. 2 shows the percentages remaining in one hour for ampicillin, since the absorption of ampicillin was not so large. It was found that ampicillin was not inhibited by the simultaneous perfusion of all tested antibiotics.

In our previous study, it was shown that the absorption of cephalexin and cephradine was reduced by ampicillin, amoxicillin, and cyc-

![Time (min)](attachment:image)

![Percent of drug remaining](attachment:image)

**FIG. 1. Intestinal Absorption of Amoxicillin Perfused in Combination with Amino β-Lactam Analogues**
- ○: control (150 μM), ▼: with cyclocillin (3 mM), ■: with cephalexin (3 mM), ▽: with cephradine (3 mM), ◄: with ampicillin (3 mM).
- Each point represents the mean of 3-4 experiments with S.E.M.
- Significance: a) $p < 0.025$, b) $p < 0.01$, c) $p < 0.005$. 
lacillin. And the mutual inhibition between these aminocephalosporins was also observed. In addition to these facts, the intestinal absorption of amoxicillin was inhibited by cyclacillin and aminocephalosporins in the present study. And the inhibitory tendency of ampicillin on amoxicillin absorption was also shown. These mutual inhibitions between amino β-lactam antibiotics were also reported by Kimura et al.5,6 It is reasonable to postulate that a common transport system participates in the transport system of these amino β-lactam antibiotics. And, as shown in Fig. 1 and 2, it is assumed that the contribution of this system on the absorption of ampicillin is to a small extent.

Inhibitory Effects of Dipeptides

In order to elucidate the participation of carrier-mediated transport system, the inhibitory effects of dipeptides (L-phenylalanylglucose and glycylglycine, 3.0 mM) on the intestinal absorption of cyclacillin, cephalaxin, and cephadrine were examined by the simultaneous perfusion in the whole small intestine. The results for cyclacillin, cephalaxin, and cephadrine were shown in Fig. 3, 4, and 5, respectively. There were no reduction in the absorption of these amino β-lactam antibiotics by the presence of these dipeptides (3.0 mM). In the case of cyclacillin, and cephadrine, but not cephalaxin, the time course of the absorption was rather enhanced to a small extent (Fig. 3 and 5). It is not clear for this reason.

The effects of L-phenylalanine on the absorption of cephalaxin and cephadrine were also examined, and the results were illustrated in Fig. 4 and 5, respectively. The absorption of cephadrine after one hour was inhibited by L-phenylalanine (p < 0.05). On the contrary, cephalaxin was not inhibited at all. It was also found that the inhibitory effect of cyclacillin was extremely larger than those of amino acids and dipeptides, as shown in Fig. 4 and 5.

In order to investigate the participation of the carrier-mediated transport system in more detail, the effects of higher concentration of dipeptides (glycylglycine and L-carnosine, 6.0 mM) were studied. It is well known that these dipeptides are appreciably stable in the intestinal lumen.16,17 The results for cyclacillin, cephalaxin, and cephadrine were shown in Fig. 6, 7, and 8, respectively.

In the absorption of cyclacillin, glycylglycine was reduced significantly (Fig. 6a), however, such significant influence was not obtained by the pre-

![Graph](image-url)

**FIG. 3. Intestinal Absorption of Cyclacillin Perfused in Combination with Dipeptides**

○: control (150 μM), □: with glycylglycine (3 mM), △: with L-phenylalanylglucose (3 mM).

Each point represents the mean of 3—6 experiments with S.E.M.

Significance; a) p < 0.05.
sence of L-carnosine (Fig. 6b). Contrary to the results for cyclacillin, L-carnosine (Fig. 7b), but not glycylglycine (Fig. 7a), significantly reduced the absorption of cephalaxin. It was also found that the inhibitory effect of L-carnosine was not enhanced even though the concentration was increased (Fig. 7b). On the other hand, the absorption of cephradine was not inhibited at all by both

**FIG. 4. Intestinal Absorption of Cephalexin Perfused in Combination with Dipeptides and Amino Acid**
- ○: control (150 μM), □: with glycylglycine (3 mM), Δ: with L-phenylalanylglycine (3 mM), ●: with L-phenylalanine (3 mM).
- Each point represents the mean of 3—4 experiments with S.E.M.
- The data for cyclacillin (▼) are redrawn from the previous report. 2)

**FIG. 5. Intestinal Absorption of Cephradine Perfused in Combination with Dipeptides and Amino Acid**
- ○: control (150 μM), □: with glycylglycine (3 mM), Δ: with L-phenylalanylglycine (3 mM), ●: with L-phenylalanine (3 mM).
- Each point represents the mean of 3—5 experiments with S.E.M.
- The data for cyclacillin (▼) are redrawn from the previous report. 2)
- Significance; a) p < 0.05, b) p < 0.01.

**FIG. 6. Intestinal Absorption of Cyclacillin Perfused in Combination with Glycylglycine (Panel a) and Carnosine (Panel b)**
- ○: control (150 μM), □: with glycylglycine (6 mM), ●: with L-carnosine (6 mM).
- Each point represents the mean of 3—5 experiments with S.E.M.
- Significance; a) p < 0.01.
glycylglycine and L-carnosine (Fig. 8).

The kinetic parameters of glycylglycine transport in the everted intestine of guinea-pig have been discussed in detail by Himukai et al.18–20 They reported that the value of the maximum influx and the half saturation concentration \((K_i)\) for glycylglycine was 36.2 nmol/min-cm² and 1.66 mM, respectively. In the present study, the higher concentration of glycylglycine than that of the \(K_i\) value was selected even though \(K_i\) value was obtained from the guinea-pig. Therefore, it is expected that the marked inhibitory effects of glycylglycine occur if the transport mechanism of dipeptides are mainly involved in the absorption system of these amino \(\beta\)-lactam antibiotics. In the present results, however, the absorption of cephalixin and cephradine was not inhibited at all (Fig. 7a and 8a), and the inhibitory effect of glycylglycine on the absorption of cyclacillin was small (12.7%)

**FIG. 7. Intestinal Absorption of Cephalexin Perfused in Combination with Glycylglycine (Panel a) and Carnosine (Panel b)**

○ : control (150 \(\mu\)M), □ : with glycylglycine (6 mM), ● : with L-carnosine (6 mM), ▽ : (10 mM).
Each point represents the mean of 3—8 experiments with S.E.M.
Significance; a) \(p < 0.001\).

**FIG. 8. Intestinal Absorption of Cephradine Perfused in Combination with Glycylglycine (Panel a) and Carnosine (Panel b)**

○ : control (150 \(\mu\)M), □ : with glycylglycine (6 mM), ● : with L-carnosine (6 mM).
Each point represents the mean of 5—6 experiments with S.E.M.
inhibition). These results for the interaction between amino β-lactam antibiotics and glycyglycine were in agreement with the results of Kimura et al.\textsuperscript{5,6}

Quay reported the transport interaction of L-phenylalanylglycine and glycine with cephaloxin in rat jejunum.\textsuperscript{10,11} We also observed that cephradine absorption was significantly inhibited by glycyglycine in rat upper jejunum (about 10 cm) in the experiment using the vascular single-pass perfusion method (unpublished data). In whole intestine, however, there were no changes in the rate of absorption of cephradine by the presence of glycyglycine. In order to estimate the transport interaction in the part of whole intestine, the inhibitory effects of glycyglycine and cyclacillin on the absorption of cephradine were studied by using the four different small intestinal segments.

As shown in Fig. 9, the inhibitory effect of glycyglycine was observed in only one segment (segment 2, upper part of jejunum), and the significant inhibitory effects were not observed in other three segments. It was also shown that the influence of cyclacillin was greater than that of glycyglycine in two segments (segment 1 and 2, duodenum and upper part of jejunum). These results suggest that the transport interaction of glycyglycine with cephradine occurs in a small part of intestine, and support the results obtained.

**FIG. 9.** Comparison of Intestinal Absorption Characteristics of Cephradine at Four Different Segments 1. duodenum, 2. proximal part of jejunum, 3. distal part of jejunum, 4. ileum. ○: control (150 μM), ▼: with cyclacillin (3 mM), □: with glycyglycine (6 mM). Each point represents the mean of 2 - 4 experiment with S.E.M. Significance: a) \( p < 0.025 \).
Intestinal Absorption of β-Lactam Antibiotics

in the experiments using the whole intestine (Fig. 5 and 8a). The inhibitory effects of dipeptides on the absorption of these drugs were smaller compared with the mutual inhibitory effects between amino β-lactam antibiotics. Moreover, we confirmed that there were no comparable inhibition in the absorption of these antibiotics in the presence of several amino acids (10.0 mM), and it was also found that L-phenylalanine was significantly reduced by the presence of L-methionine (10.0 mM) in the experiment using the whole intestine (Data was not shown). From these results, it is reasonable to postulate that the carrier-mediated transport system correlated to dipeptides participates in the absorption mechanisms of β-lactam antibiotics only to a small degree.

The detailed characteristics of the absorption mechanisms in these antibiotics are under investigation from the point of view of the uptake by the intestinal brush border membrane vesicles and the binding to the soluble fraction (F1) obtained from the intestinal mucosa. 1)

REFERENCES


