INTESTINAL ABSORPTION OF SEVERAL β-LACTAM ANTIBIOTICS. III. 1) COMPETITIVE INHIBITION BEHAVIOR AMONG ZWITTERIONIC β-LACTAM ANTIBIOTICS IN THE RAT INTESTINAL ABSORPTION*

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Competitive inhibitory behavior among zwitterionic β-lactam antibiotics in the rat intestinal absorption process was examined. Intestinal absorption of cephadrine and cephalexin was significantly inhibited by cyclacillin. The mutual inhibition between these amino-cephalosporins was also observed. On the other hand, in the pretreatment experiments with cyclacillin, it was found that absorption of cephadrine was significantly inhibited, but the inhibitory effect of cyclacillin was not observed for cephalexin. These results showed that cephadrine had a common carrier-mediated transport mechanism with cyclacillin which is not present in the absorption process of cephalexin. It was postulated that the mutual inhibition between cephadrine and cephalexin is based on the competitive inhibition in the accumulation or uptake by the intestinal mucosa.

Keywords — ampicillin; amoxicillin; cyclacillin; cephadrine; cephalexin; intestinal absorption; accumulation; competitive inhibition

INTRODUCTION

It is well known that amino-penicillins and amino-cephalosporins are absorbed from the small intestine even though they are completely ionized over entire pH range and have very low lipid solubility. The oral absorption of these zwitterionic β-lactam antibiotics would not be predicted from the pH partition hypothesis2) which concluded that most drugs were absorbed from the gastrointestinal tract by passive diffusion of the unionized drug species. We have investigated that the mechanisms of the intestinal absorption of these amino-penicillins (ampicillin, amoxicillin) and amino-cephalosporins (cephalexin, cephadrine) in the rat and pointed out1) that the amino-cephalosporins were absorbed by special transport mechanism. We also showed1) that the accumulations of cephalexin and cephadrine by the mucosal tissue were significantly greater than those of ampicillin and amoxicillin. Dixon and Mizen,3) and Kimura et al. 4) reported that cyclacillin was absorbed by active transport mechanism in the rat. And the saturable absorption processes were observed for amoxicillin, cephalaxin, and cephadrine by Kimura et al. 5) and Tsuji et al. 6) However, the differences in the absorption mechanisms of these zwitterionic β-lactam antibiotics were not shown in detail. The present study was undertaken to clarify the absorption characteristics of cephalaxin and cephadrine from the standpoint of the competitive inhibition behavior between them and by other zwitterionic β-lactam antibiotics.

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MATERIALS AND METHODS

Materials and Reagents — The chemical structure of β-lactam antibiotics covered in this report is presented in Chart 1. Ampicillin anhydrous, cephalaxin monohydrate, cephradine dihydrate, and amoxicillin trihydrate were used. All the reagents were of the special grade, and were prepared with redistilled water.

Procedure for Absorption Experiments — Wistar male rats (180–250 g) were anesthetized with ether and sodium pentobarbital (2 mg/100 g). Whole small intestine, loops, and everted sacs were used in the absorption experiments.

1) Loop Experiments: The drugs were dissolved in the modified Ringer solution, pH 7.4 (150 μM). Two loops (10 cm) at 1 cm interval below 15 cm from pylorus were prepared according to the method of Levine and Pelikan. After washing the loop gently with 10 ml of the drug-free solution mentioned above, 1 ml of drug solution was injected into the loop using a syringe. At selected time (15 min) before the animal was sacrificed, the loop was removed and the contents were emptied into a 5-ml volumetric flask. The mucosal side of the loop was rinsed with the modified Ringer solution to give a volume of 5 ml. In the determination of tissue concentration, the homogenate was prepared in a Teflon homogenizer with 3.0 ml of the internal standard solution (cephalexin or cephradine solution).

2) In Situ Recirculating Perfusion Experiments: The technique of Kakami et al. was employed. The drugs were dissolved in modified Ringer solution with pH 7.4 (150 μM). The bile duct was ligated in all experiments. Before experiments, the intestine was rinsed with 30 ml of warmed modified Ringer solution by syringe to exclude the intestinal contents, and perfused with 50 ml of drug-free solution, maintained at

CHART 1. Structure of β-Lactam Antibiotics Tested

Takeda Chemical Industries, Osaka, Japan.

II. Shionogi & Co., Osaka, Japan.

III. Sankyo Co., Tokyo, Japan.

IV. Kyowa Hakko Kogyo Co., Tokyo, Japan.
37°C, to wash and bufferize the intestine. The perfused solution (50 ml) was then circulated at a rate of 5 ml/min. One half milliliter of the sample solution was pipetted at the selected time after the start of recirculation.

Pretreatment by cyclacillin was performed as follows: After the intestines were rinsed with warmed modified Ringer solution, cyclacillin (3 mM) was circulated for 10 min. And then the perfused drug solution was circulated as mentioned above.

3) Everted Sac Experiments: A large section of small intestines, starting below the pyloric sphincter was removed from the rat, and rinsed with iced saline. The first 15 cm segment was discarded. The next 25 cm segment was everted, and 10 cm segments of two sacs were prepared. Drug solution (150 μM) was prepared with modified Ringer solution with pH 7.4. The serosal volume was 1.0 ml. These two sacs were

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FIG. 1. *Effects of Amino-penicillins on the Disappearance and Tissue Accumulation of Cephradine in Rat Intestinal Loops at pH 7.4 in 15 min*

Results are expressed as mean of three or four loops with S.E.M.
Significance; a) p < 0.01, b) p < 0.001.

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FIG. 2. *Effects of Amino-penicillins on the Disappearance and Tissue Accumulation of Cephalexin in Rat Intestinal Loops*

Results are expressed as mean of four to ten loops with S.E.M.
Significance; a) p < 0.01, b) p < 0.001.
placed in a 100 ml Erlenmeyer flask containing 100 ml of the drug solution (mucosal side), previously equilibrated at 37°C, and the medium was continually gassed with 5% carbon dioxide in oxygen. After 15 min, each sac was emptied into a test tube and the sample solution was filtered through 0.45 μm pore size membrane (TM-2, Toyo Roshi Co., Tokyo, Japan).

Pretreatment by cyclacillin was proceeded as follows: Each sac containing 1 ml of drug-free solution was placed in mucosal medium containing 3 mM cyclacillin, and the medium was continually gassed for 10 min. After the sac was rinsed with iced solution, it was introduced into another mucosal medium containing the test drug, and then followed as mentioned above.

**Analytical Method**—A high performance liquid chromatography was used for determination of the drugs. A high performance liquid chromatograph, Hitachi 635 A, equipped with a multi-wavelength UV detector (635-0090) was used in a reversed phase with a LiChrosorb RP 18 column (25 cm x 4 mm I.D., 5 μm, Merck). Mobile phase was a mixture of potassium dihydrogen phosphate (KH₂PO₄) solution-methanol. A mobile phase of 0.1 M KH₂PO₄ solution-methanol (4:1, by volume) was used for the determination of amino-cephalosporins in the presence of ampicillin or amoxicillin. For the determination of amino-cephalosporins in the presence of cyclacillin, a mixture of 0.05 M KH₂PO₄ (adjusted pH to 5.5 with 4 N sodium hydroxide solution)-methanol (4:1, by volume) was used. The flow rate was maintained at 0.6 ml/min, and the column was warmed at 55°C using a water bath circulator. The wavelength of the detector was set at 260 nm. For the assay of the drug within the intestinal tissue, 6 ml of methanol was added to the homogenate and then centrifuged at 3000 rpm for 10 min. The super-

![Graph 3](image1.png)  
**FIG. 3.** Effects of Amino-penicillins on the Disappearance of Cephradine from Rat Intestinal Lumens at pH 7.4 by Simultaneous Perfusion  
○: control (150 μM), ●: with cyclacillin (3 mM), △: with amoxicillin (3 mM), ▲: with ampicillin (3 mM).  
Results are expressed as mean of two experiments with range.

![Graph 4](image2.png)  
**FIG. 4.** Effects of Amino-penicillins on the Disappearance of Cephalexin from Rat Intestinal Lumens at pH 7.4 by Simultaneous Perfusion  
○: control (150 μM), ●: with cyclacillin (3 mM), △: with amoxicillin (3 mM), ▲: with ampicillin (3 mM).  
Results are expressed as mean of two experiments with range.
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nantant was filtered through 0.45 μm pore size membrane and an appropriate volume of the filtrate was injected into the liquid chromatograph. All the sample solutions for the determination were filtered through the membrane mentioned above. The peak height ratio was calculated for the determination of the drug concentration.

RESULTS
Inhibitory Effects of Amino-penicillins on the Intestinal Absorption of Amino-cephalosporins

The Amino-penicillins chosen for the inhibition studies were cyclacillin, amoxicillin, and ampicillin, whose absorption mechanisms were investigated in several laboratories. Fig. 1 shows the results for cephradine absorption. Cyclacillin reduced both the rate of disappearance from intestinal loop and tissue accumulation of cephradine markedly. And the inhibitory effects of ampicillin and amoxicillin were less than that of cyclacillin.

Similar inhibitory effect was also observed in the absorption of cephalixin to a smaller extent (Fig. 2).

In order to clarify the time-course of the inhibitory effects of the amino-penicillins on the absorption of cephradine and cephalixin, the in situ perfusion technique was adopted in the absorption study. The results for cephradine and cephalixin are shown in Fig. 3 and Fig. 4, respectively, and each value was not corrected with respect to the water absorption. While the degree of the inhibitory effects of these amino-penicillins on the absorption of cephradine and cephalixin was shown to be different with the results obtained by the loop method (Fig. 1 and 2), the absorption of both cephradine and cephalixin were reduced by the simultaneous perfu-

FIG. 5. Effects of Various Concentrations of Cyclacillin on the Disappearance of Cephradine from Rat Intestinal Lumens at pH 7.4 by Simultaneous Perfusion
○ : control (150 μM), concentration of cyclacillin;
● : 3 mM, △ : 1.5 mM, ■ : 750 μM,
△ : 450 μM, □ : 150 μM.
Results are expressed as mean of two experiments with range.

FIG. 6. Effects of Various Concentrations of Cyclacillin on the Disappearance of Cephalixin from Rat Intestinal Lumens at pH 7.4 by Simultaneous Perfusion
○ : control (150 μM), concentration of cyclacillin;
● : 3 mM, △ : 1.5 mM, Δ : 450 μM.
Results are expressed as mean of two experiments with range.
FIG. 7. Effects of Cephalexin on the Disappearance of Cephradine from Rat Intestinal Lumens at pH 7.4 by Simultaneous Perfusion
○: control (150 μM), ●: with cephalixin (3 mM). Results are expressed as mean of two experiments with range.

FIG. 8. Effects of Cephradine on the Disappearance of Cephalexin from Rat Intestinal Lumens at pH 7.4 by Simultaneous Perfusion
○: control (150 μM), ●: with cephradine (3 mM). Results are expressed as mean of two experiments with range.

FIG. 9. Effects of Pretreatment with Cyclacillin on the Disappearance of Cephradine from Rat Intestinal Lumens at pH 7.4
○: control (150 μM), concentration of cyclacillin; △: 3 mM.
Results are expressed as mean of at least three experiments with S.E.M.
Significance; a) p<0.05, b) p<0.01, c) p<0.001.

Absorption of all amino-penicillins. And the inhibitory effects of cyclacillin were greater than those of ampicillin and amoxicillin.

During the course of this study, the concentrations of cephradine and cephalixin in the perfused medium were gradually increased in the presence of higher concentration of cyclacillin (3 mM). Although the mechanism is in question, it is likely to be related to the competitive inhibition which resulted in the back diffusion of amino-cephalosporins from the intestinal mucosa. Because the drug disappeared from the lumen is always supplied from the reservoir by using the in situ perfusion technique, this method seems to be a better method to examine the inhibition of absorption.

The effects of different amount of cyclacillin on the absorption of cephradine and cephalixin were also examined by the in situ perfusion technique. The result for cephradine is shown in Fig.
5. The inhibition of cephadrine absorption by cyclacillin tended to increase as the concentration of cyclacillin in the perfusion medium increased. Similar result was obtained for cephalexin (Fig. 6).

**Mutual Inhibition of the Intestinal Absorption between Cephadrine and Cephalexin**

In order to make clear the absorption mechanism common to cephadrine and cephalexin, mutual inhibition was examined by the in situ perfusion technique. As shown in Fig. 7 and 8, there was a significant mutual inhibition between cephadrine and cephalexin. It was also found that a considerable inhibitory effect appeared during the time-course of 20—60 min in the presence of 20 times concentration of inhibitory drug. It appeared that the inhibitory effect was based on the significantly accumulated drug which is simultaneously present, and the lag time was observed because it took about 20 min for drug to accumulate in the tissue.

**Effect of Pretreatment with Cyclacillin on the Intestinal Absorption of Amino-cephalosporins**

In order to examine the difference of absorption mechanism between cephadrine and cephalexin, a pretreatment by the drug which has a competitive inhibitory effect was performed before the absorption studies. Cyclacillin was selected for its strong inhibitory effect, and the in situ perfusion technique and the in vitro permeation method using an everted intestinal sac were employed for these objects.

The disappearance profiles from the lumen of cephadrine and cephalexin after pretreatment with 3 mM cyclacillin for 10 min are shown in Fig. 9 and 10, respectively. There was a different inhibitory phenomenon between cephadrine and cephalexin. And as shown in Fig. 11, the permeation from the mucosal side to serosal side of cephadrine was inhibited significantly by the pretreatment with cyclacillin. This inhibitory phenomenon was not observed for the permea-

**FIG. 10. Effects of Pretreatment with Cyclacillin on the Disappearance of Cephalexin from Rat Intestinal Lumens at pH 7.4**

○ : control (150 μM), concentration of cyclacillin; △ : 3 mM.

Results are expressed as mean of at least three experiments with S.E.M.

**FIG. 11. Effects of Pretreatment of Cyclacillin on the Transport of Cephadrine and Cephalexin across Everted Rat Intestinal Sacs at pH 7.4**

a) concentration of amino-cephalosporins: 150 μM, b) concentration of cyclacillin: 3 mM.

Results are expressed as mean with S.E.M. Number of experiments is given in parentheses. Significance; **) p<0.01.
tion of cephalixin. These results may indicate that cephradine has a closer absorption mechanism with that of cyclacillin.

DISCUSSION

In order to elucidate particular transport mechanisms shared among zwitterionic β-lactam antibiotics, the interrelationship between the transport system of amino cephalosporins and that of amino penicillin was investigated. In the present study, the absorption of cephradine and cephalixin was inhibited by amino penicillins (Fig. 1, 2, 3, and 4) and mutual inhibition between these amino cephalosporins was observed (Fig. 7 and 8). By comparison of the results between the effects of amino penicillins on the amino cephalosporins (Fig. 3 and 4), and mutual inhibitory effects of amino cephalosporins (Fig. 7 and 8), it was found that the inhibitory effects of cyclacillin were significantly greater than those of amino cephalosporins, ampicillin, and amoxicillin. Furthermore, amino cephalosporins had a greater inhibitory effect than that of ampicillin and amoxicillin.

In the previous paper, it was shown that the accumulation of amino cephalosporins by the mucosal tissue was marked in comparison with ampicillin and amoxicillin. In another experiment, the results that were obtained showed that one of the macromolecular fractions isolated from the intestinal mucosa had a remarkable binding ability to ampicillin, amoxicillin, cephradine, and cephalixin. It was also found that there was a good correlation between the degree of affinity to this fraction and absorption characteristics such as tissue wall uptake or disappearance from the lumen. From the results, it seemed reasonable to assume that the binding to this macromolecular fraction played an important role in the accumulation of these drugs in the intestinal mucosa. It also appeared that these zwitterionic β-lactam antibiotics shared a common absorption system such as specific accumulation by the intestinal mucosa or the binding to the macromolecular fraction mentioned above.

As shown in Fig. 9, 10, and 11, it seemed that cephradine and cyclacillin had a common transport mechanism which was not present in the absorption process of cephalixin. It was reported that cyclacillin had an active transport mechanism. Based on the results shown in the previous paper, cephalixin was not absorbed by an active transport mechanism. Thus, it is possible that the mutual inhibition between cephradine and cephalixin observed in this study is due to the competitive inhibition of the accumulation or uptake by the intestinal mucosa.

The detail characteristics of the inhibitory effects among zwitterionic β-lactam antibiotics are under investigation by using the macromolecular fraction mentioned above.

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