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Effect of Isoxazolyl-penicillin on the Rectal Absorption of Ampicillin in Rabbits and Humans¹⁾

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The effects of isoxazolyl-penicillins on the rectal absorption of ampicillin were investigated in rabbits and humans. The bioavailability of ampicillin following administration of a suppository in the presence or absence of an isoxazolyl-penicillin was determined from the blood level in rabbits and from the urinary excretion (0–6 h) in humans.

Rectal absorption of ampicillin from the suppository was enhanced by the presence of an isoxazolyl-penicillin in both rabbits and humans, though the effect was much greater in rabbits than in humans. The absorption-promoting effects of isoxazolyl-penicillins appeared to be related to their partition coefficients between *n*-octanol and buffer solution (pH 7.4).

Keywords—rectal absorption; isoxazolyl-penicillin; penicillin; bioavailability of ampicillin; promoting effect of isoxazolyl-penicillins for rectal absorption of ampicillin

Many workers have demonstrated the importance of lipid affinity of drugs for permeation through rectal membrane.²⁾ Pharmaceutical and chemical modifications have been performed to enhance the bioavailability following the rectal administration of poorly absorbable or non absorbable drugs.^{3–5)}

It has been reported that membrane-permeable compounds having calcium ion binding ability promote the rectal absorption of watersoluble and unabsorbable drugs of small molecular weight.⁶⁾ Isoxazolyl-penicillins have high lipid affinity and are known to have calcium ion binding ability.^{7,8)} Thus in the present work, we investigated the effectiveness of isoxazolyl-penicillins as an absorption promoter for the rectal absorption of ampicillin (ABPC). ABPC was adopted as a model compound which is poorly absorbable through the rectal membrane.

Experimental

Materials—Sodium ampicillin (ABPC Na, 912 µg/mg), ampicillin trihydrate (ABPC·3H₂O, 850 µg/mg), potassium benzylpenicillin (PCG K, 1600 units/mg), potassium propicillin (PPPC K, 993 µg/mg), sodium oxacillin (MPIPC Na, 851 µg/mg), sodium cloxacillin (MCIPC Na, 978 µg/mg), sodium flucloxacillin (MFIPC Na, 893 µg/mg), and sodium dicloxacillin (MDIPC Na, 900 µg/mg) were obtained from commercial sources and used without further purification.

Suppository Bases: Three different bases were used, a mixture of equal amounts of liquid paraffin and white petrolatum (LP-WP), SBAM (Kanegafuchi Chemicals Ind. Ltd.) and Witepsol H-15 (Dynamit novel Chemicals). SBAM and Witepsol H-15 are triglycerides of high fatty acids. Liquid paraffin and white petrolatum were commercial products of JP IX grade, and SBAM and Witepsol H-15 were gifts from Kanegafuchi Chemicals Ind. and Mitsuwa Trading Co., respectively.

Preparation of Suppository—Suitable amounts of ABPC Na and/or an isoxazolyl-penicillin were mixed in a melted suppository base (40°C) and then dispersed well by sonification with an ultrasonic cleaner (Branson, B-220) for 30 sec at 40°C. Except in the case of LP-WP, the melted base containing antibiotics was poured into disposable plastic molds (Nippon Elanco Co., Ltd.) and left to solidify at room temperature. For the human study, commercial disposable plastic molds for human use (containing 1.4 g of suppository) were used to prepare suppositories. For the animal study, the molds were used after reducing their size to one equivalent to about 0.45 g of suppository by cutting off the upper parts of the molds. An appropriate amount of melted suppository was poured into the molds according to the body weight of the animals. LP-

WP suppositories for rabbits were administered with a 1-ml disposable syringe.

Animal and Human Study—Five to six albino rabbits, 2.5–3.0 kg, and five male healthy humans, 29–57 years old, were used. Rabbits and humans were fasted for 12 h before the experiments but water was given freely. For rabbits, suppositories containing 10% (w/w) ABPC Na and/or an isoxazolyl-penicillin were prepared and administered at a dose of 15 mg/kg (equivalent to 0.15 g suppository/kg). Rectal preparations for rabbits were administered following the methods described in the previous paper.⁵⁾ Samples (0.2 ml) of blood were collected from an ear vein at 10, 20, 40, 60, 90, 120, 150, 180, 210, and 240 min after the administration. The apparent bioavailability of each penicillin in rabbits was determined by dividing the *AUC* following rectal administration by the *AUC* obtained following intravenous administration of same dose of the penicillin. For the human study, a suppository (1.4 g) containing 125 mg ABPC Na or 125 mg ABPC Na and 62.5 mg MDIPC Na was administered to each subject. A suppository (1.4 g) containing 140 mg of an isoxazolyl-penicillin and/or 70 mg ABPC Na was administered to one subject. For all human studies, Witepsol H-15 was used as a suppository base. The amount of ABPC Na or isoxazolyl-penicillins excreted in the urine in 6 h was used as an index of bioavailability.

Analytical Method—The concentration of penicillins in blood and urine samples was determined by microbiological assay with *Staphylococcus aureus* ATCC 6538 P for isoxazolyl-penicillins and *Bacillus subtilis* ATCC 6633 for ABPC Na.⁹⁾ The separation of ABPC and isoxazolyl-penicillin in blood and urine samples was performed following the method of Goto *et al.*¹⁰⁾ with a slight modification. Two-tenths ml of blood or urine was deproteinized by adding 0.8 ml of 10% trichloroacetic acid solution and centrifuged at 3000 rpm for 10 min. Five-tenths ml of the supernatant was further acidified with 0.02 N HCl to pH 2 if necessary and extracted with 6 ml of ethyl ether three times. The aqueous layer containing ABPC alone was separated and neutralized with 1% NaHCO₃ solution, then diluted with pH 7.4 phosphate buffer if necessary. The concentration of ABPC was microbiologically determined by the disc method. The ether layer containing isoxazolyl-penicillin was collected and concentrated under reduced pressure. The residue was dissolved in 0.5 ml of pH 7.4 phosphate buffer. The concentration was also determined microbiologically using the disc method. The concentration of ABPC in blood after administration of ABPC Na alone was microbiologically determined after six-fold dilution of blood with deionized water. ABPC in urine after administration of ABPC Na alone was similarly determined after appropriate dilution with deionized water.

Calcium Ion Sequestration Capacity—The calcium ion sequestration capacity of each penicillin was measured at pH 10.0 (1/200 M NH₄OH–NH₄Cl) at 20°C following the method described previously⁶⁾ with a slight modification. To a mixture of 0.1 ml of 1/400 M CaCl₂ (pH 10.0) and 0.1 ml of 0.01% eriochrome black T solution (pH 10.0), 10 μ l of penicillin sample solution (2%, pH 10.0) was added in a stepwise manner.

Results and Discussion

In preliminary experiments, the rectal absorption of various penicillins having lipid affinity was examined in rabbits and a human. In Fig. 1, the logarithms of the area under the concentration-time curve (*AUC*) and the peak blood level (*C*_{max}) after rectal administration of each penicillin in rabbits are plotted against the logarithm of the partition coefficient between *n*-octanol and water ($\log P$). The values of $\log P$ are those of Yamana *et al.*¹¹⁾

The logarithms of *AUC* and *C*_{max} increased with increase of $\log P$ of penicillins and the plots showed good linearity. These results suggest that rectal absorption of penicillins in rabbits depends upon their partition coefficients, as observed in rectal absorption of sulfonamides in rats,¹²⁾ and that penicillins having $\log P$ less than 1.5 are poorly absorbed through the rectal membranes of rabbits.

A similar experiment was performed in one human subject (30 years old). The urinary excretions (percent) of microbiologically active penicillins for 6 h after the rectal administration were plotted against $\log P$ (Fig. 2). The relationship between urinary recovery and $\log P$ was similar to that of rabbits, except in the case of MDIPC Na.

Among penicillins having $\log P$ less than 1.5 and being poorly absorbable, ABPC Na was adopted as a model compound. From previous observations,⁶⁾ MCIPC and MDIPC were selected as possible absorption promoters because they have higher $\log P$, and oral preparations combined with ABPC Na are already in clinical use. The oral combination preparations have been developed from the viewpoint that combination of ABPC Na and MCIPC Na or MDIPC Na is effective on *aeruginosae*.^{13,14)} However, their effect on ABPC absorption has not been examined.

The blood level-time curves of each penicillin after rectal coadministration of ABPC Na

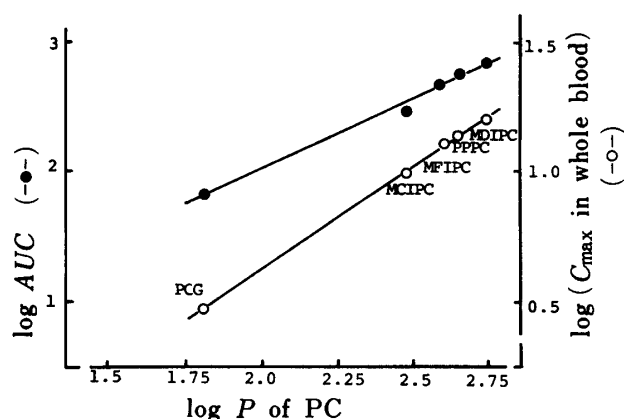


Fig. 1. Relationship between the Rectal Absorption of Penicillins (PC) in rabbits and Their Lipid-water Partitioning Properties ($\log P$)

Abbreviations in the figure indicate the sodium or potassium salts of dicloxacillin (MDIPC Na), propicillin (PPPC K), flucloxacillin (MFIPC Na), cloxacillin (MCIPC Na), and penicillin G (PCG K). Each point represents the mean value for five to six rabbits. Each penicillin was suspended in liquid paraffin-white petrolatum base (50—50 w/w) at a concentration of 10% (w/w), and given at a dose of 15 mg/kg.

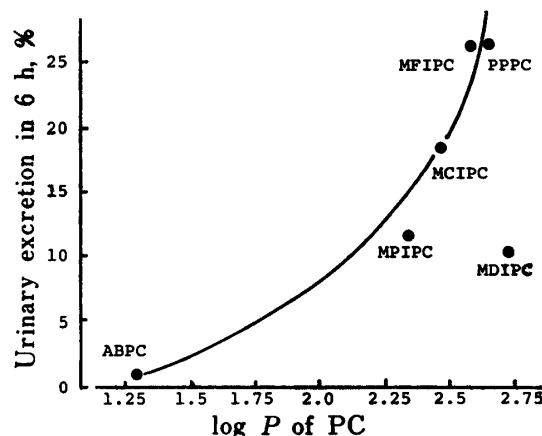


Fig. 2. Relationship between the Rectal Absorption of Penicillins (PC) in One Human Subject and Their Lipid-water Partitioning Properties ($\log P$)

Each point represents the urinary excretion (percent) in 6 h of a penicillin after rectal administration in one human subject. Each penicillin was suspended in SBAM base at a concentration of 10% (w/w). The dose of each penicillin was 140 mg/body.

and MDIPC Na in rabbits are shown in Fig. 3(a) and Fig. 3(b).

The results in Fig. 3(a) and (b) were obtained by employing LP-WP and Witepsol H-15 as suppository bases, respectively. ABPC Na was not absorbed by itself from either base.

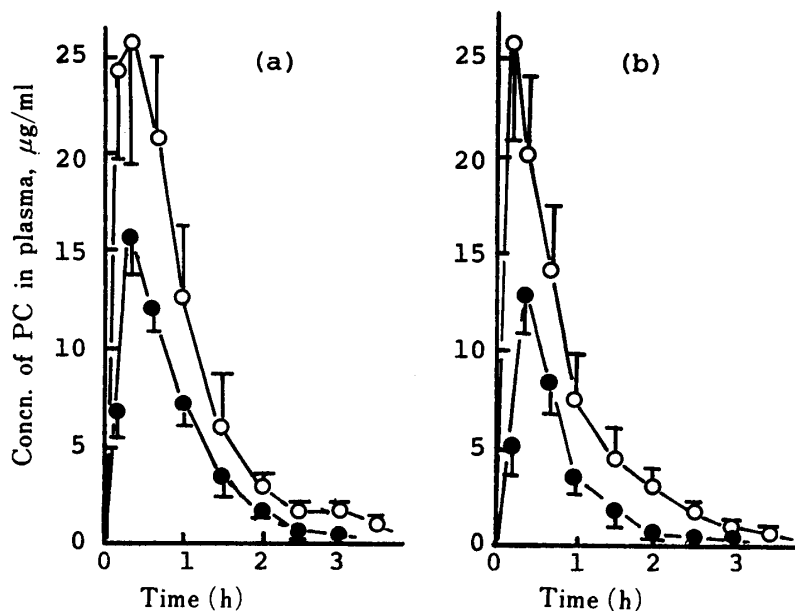


Fig. 3. Plasma Levels of ABPC (—●—) and MDIPC (—○—) in Rabbits After Rectal Administration of a Combination of ABPC Na and MDIPC Na (1:1)

(a): ABPC Na and MDIPC Na were suspended in liquid paraffin-white petrolatum base (50—50 w/w) each at a concentration of 10% (w/w). Each point represents the mean \pm S.E. for five rabbits. The doses of ABPC Na and MDIPC Na were each 15 mg/kg.

(b): ABPC Na and MDIPC Na were suspended in Witepsol H-15 base each at a concentration of 10% (w/w). Each point represents the mean \pm S.E. for five rabbits. The doses of ABPC Na and MDIPC Na were each 15 mg/kg.

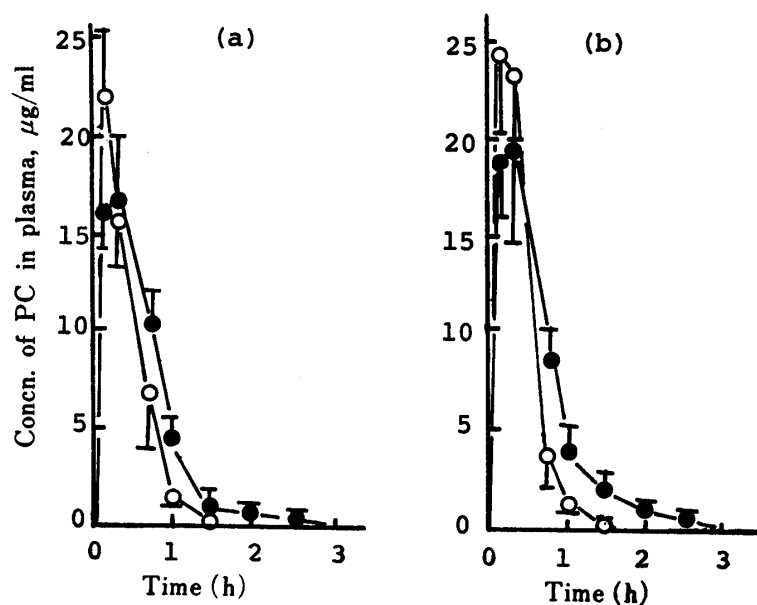


Fig. 4. Plasma Levels of ABPC (—●—) and MCIPC (○) in Rabbits after Rectal Administration of a Combination of ABPC Na (a) or ABPC·3H₂O (b) and MCIPC Na (1:1)

(a) ABPC Na and MCIPC Na were suspended in Witepsol H-15 each at a concentration of 10% (w/w). Each point represents the mean \pm S.E. for five rabbits. The doses of ABPC Na and MCIPC Na were each 15 mg/kg.

(b) ABPC·3H₂O and MCIPC Na were suspended in Witepsol H-15 each at a concentration of 10% (w/w). Each point represents the mean \pm S.E. for five rabbits. The doses of ABPC·3H₂O and MCIPC Na were each 15 mg/kg.

TABLE I. Urinary Excretions of ABPC and MDIPC in Healthy Human Volunteers after Rectal Administration of a Combination of ABPC : MDIPC (2 : 1)

Rp. : ABPC Na 125mg+MDIPC Na 62.5mg+SBAM 612.5mg						
Name	PC	0—2 h mg	2—4 h mg	4—6 h mg	Total amount mg	Recovery %
Y.N.	AB	15.91	2.29	0.77	18.92	15.2
	MDI	3.53	0.51	0.33	4.37	7.0
A.S.	AB	10.35	2.78	1.03	14.16	11.3
	MDI	2.08	1.25	0.84	4.17	6.7
H.M.	AB	12.33	2.62	0.84	15.79	12.6
	MDI	3.45	1.10	0.53	5.08	8.1
K.K.	AB	17.89	2.43	0.78	21.10	16.9
	MDI	3.92	0.73	0.31	4.96	7.9

Mean urinary excretion
 Mean \pm S.D. ABPC : 14.0 \pm 2.5%
 MDIPC : 7.4 \pm 0.7%.

The absorption of ABPC Na is remarkably enhanced by MDIPC Na. In case of the preparation 10% (w/w) of ABPC Na and 5% (w/w) of MDIPC Na in LP-WP suppository (15 mg ABPC Na/kg and 7.5 mg MDIPC Na/kg, equivalent to 0.15 g of suppository/kg), the extent of enhanced absorption of ABPC Na was smaller than that in the preparation containing 10% (w/w) of ABPC Na and MDIPC Na (Fig. 3(a)). These observations suggest that a suitable amount of MDIPC Na in the suppository (strictly speaking, a suitable amount of MDIPC Na released from the suppository in the rectum) is required to enhance the rectal absorption of ABPC Na.

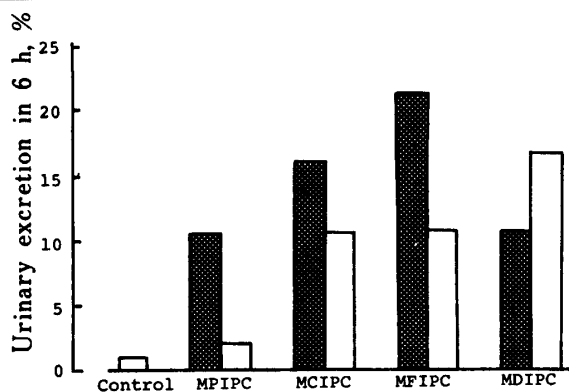


Fig. 5. Urinary Excretions (Percent) in 6 h of ABPC and Each Isoxazolyl-penicillin in One Human Subject after Rectal Administration of a Combination of ABPC Na and Each Isoxazolyl-penicillin (1: 2)

ABPC Na and each sodium isoxazolyl-penicillin were suspended in SBAM base at concentrations of 5% (w/w) and 10% (w/w), respectively. The doses of ABPC Na and each sodium isoxazolyl-penicillin were 70 mg/body and 140 mg/body, respectively. The result for ABPC Na only is also presented.

□: ABPC, ▨: Isoxazolyl-PC.

results are shown in Table I. Enhanced rectal absorption of ABPC Na was observed after administration of the combined preparations, whereas rectal absorption of ABPC Na without coexisting MDIPC Na was only 1—3%. Thus, considerable promoting efficacy of MDIPC Na for the rectal absorption of ABPC Na was observed in humans as well as in rabbits, although the extent in humans was less than that obtained in rabbits.

From the results that urinary excretions (percent) in the initial 2 h after rectal administration to humans were 80% for ABPC Na and 50.7% for MDIPC Na of the total amount excreted in 6 h, the promoting effect of MDIPC Na seems to be exhibited mainly in the early stage after administration.

The effects of other isoxazolyl-penicillins were examined in the same subject as Fig. 2. The results are shown in Fig. 5. Urinary recovery in 6 h of each isoxazolyl-penicillin following rectal administration with ABPC Na was almost the same as the value of urinary recovery following rectal administration of isoxazolyl-penicillin alone. The absorption-promoting effects were markedly scattered. Urinary excretions (percent) of unchanged ABPC Na were 2, 11, 11, and 17% for MPIPC Na, MCIPC Na, MFIPC Na, and MDIPC Na, respectively. These results suggest that the absorption-promoting effects of isoxazolyl-penicillins depend on their lipid affinities, shown as log *P*.

The calcium ion sequestration capacities (Ca^{2+} gram ion/M compound) of MDIPC Na, MFIPC Na, MCIPC Na, and MPIPC Na at pH 10.0 were 0.031, 0.017, 0.020, and 0.014, respectively. That of ABPC Na was very low (not detectable). Isoxazolyl-penicillins having higher calcium ion sequestration capacity seem to enhance the rectal absorption of ABPC Na more than those having low calcium ion sequestration capacity.

One of the clinically important problems is irritation by the suppository. In the present human study, only a tolerable (slight) irritation was felt in the early stage following the rectal administration of a suppository containing ABPC Na and isoxazolyl-penicillin or isoxazolyl-penicillin alone.

The mechanisms of the rectal absorption-promoting effect of isoxazolyl-penicillins in rabbits and humans are not yet understood in detail.

References and Notes

- 1) A part of this work was presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1980.

The absorption-promoting efficacy of MCIPC Na was also examined for the rectal absorption in rabbits of ABPC incorporated in Witepsol H-15 as the sodium salt or trihydrate ($\text{ABPC} \cdot 3\text{H}_2\text{O}$). As shown in Fig. 4(a) and (b), MCIPC Na showed a remarkable enhancement of the absorption of ABPC. No differences were observed in the absorption-promoting effect of MCIPC Na on the absorptions of ABPC Na and $\text{ABPC} \cdot 3\text{H}_2\text{O}$ in rabbits.

Next, the promoting efficacy of MDIPC Na for the rectal absorption of ABPC Na was examined in humans. A preparation having a mixing ratio of 2 (ABPC Na): 1 (MDIPC) Na in Witepsol H-15 suppository was administered to 4 normal healthy humans. This mixing ratio of 2:1 is clinically used in an oral dosage form. The

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