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Studies on Absorption Promoters for Rectal Delivery Preparations. I. Promoting Efficacy of Enamine Derivatives of Amino Acids for the Rectal Absorption of β-Lactam Antibiotics in Rabbits[1)]

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Enamine derivatives of amino acids were synthesized and their promoting efficacies for the rectal absorption of β-lactam antibiotics were studied in rabbits. Ethyl acetoacetate (EtAA) enamine derivatives were preferentially studied because of the low toxicity of EtAA generated by the hydrolysis of the enamine moiety in the rectum and blood stream. The EtAA enamine of sodium D-phenylglycine (PG EtAA Na) was found to have remarkable promoting efficacy for the rectal absorption of sodium ampicillin, cephalothin, cephapirin, cefazolin and cephaloridine. Other enamine derivatives of amino acids also showed promoting efficacy for the rectal absorption of penicillins and cephalosporins which could not permeate alone due to their poor lipid affinity. The promoting efficacy of PG EtAA Na for the rectal absorption of antibiotics increased with decrease in their partition coefficients. The present results suggest that the enamine derivatives of amino acids are clinically applicable for use in rectal delivery preparations of penicillins and cephalosporins which cannot permeate unaided through the rectal membrane.

Keywords——ampicillin, cephaloridine, cephalexin, cephalothin, cephapirin and cefazorin; enamine derivatives of amino acids; enamine prodrugs of amino acid-like β-lactam antibiotics; improvement of bioavailability; rectal absorption promoter

In the previous report,[2)] we showed that the enamine derivatives of amino acid-like β-lactam antibiotics failed to show improved bioavailability compared with their parent antibiotics on oral administration to rabbits owing to rapid hydrolysis of the enamine moiety in the acidic gastric juice. However, the enamine derivatives were found to permeate through the rectal membrane in rabbits while the parent antibiotics did not. Further, the bioavailability of an enamine derivative of ampicillin, sodium N-(1-methyl-2-ethoxycarbonylvinyl)-ampicillin (ABPC EtAA Na), following rectal administration was much larger than that of sodium ampicillin after oral administration. This improvement in the bioavailability was explained on the basis of a moderate stability to hydrolysis of the enamine moiety at the neutral pH of the rectal fluid[3)] and an enhanced lipid affinity of the enamine derivative; such affinity is indispensable for the rectal absorption of drugs as well as for gastrointestinal absorption.

Thus, enamine derivatives of β-lactam antibiotics were considered to be potentially useful prodrugs for clinical use in rectal delivery preparations. In addition to these findings, ABPC EtAA Na was found to promote the rectal absorption of sodium ampicillin (ABPC Na) following concomitant administration of ABPC Na and ABPC EtAA Na in the rectum of rabbits.

Thus, some enamine derivatives having no remarkable intrinsic biological activity may possess promoting activity similar to that of ABPC EtAA Na for the rectal absorption of drugs which do not permeate through the rectal membrane unaided.

To promote the rectal absorption of poorly absorbable or nonabsorbable drugs, surface-active agents[4)] and chelating agents[5)] have been tested as adjuvants of rectal suppositories. However, little work has been done on the development of absorption promoters to improve the rectal absorption of drugs.

In the present report, enamine derivatives of amino acids were synthesized and their efficacies for enhancing the rectal absorption of β-lactam antibiotics were studied.

Experimental

Materials——Samples of sodium ampicillin (ABPC Na), sodium cephalothin (CET Na), sodium cephalexin (CEX Na), sodium cephapirin (CEP Na), sodium cephaloridine (CER Na), and sodium cefazolin (CEZ Na) were used as supplied from commercial sources. All other reagents and solvents were commercial products of reagent grade and were used without further purification.

Synthesis of Enamine Derivatives——Reaction of Sodium D-Phenylglycine with Ethyl Acetoacetate: The enamine derivative of D-phenylglycine with ethyl acetoacetate (EtAA) was synthesized following the method of Dane *et al.*[6] with a small modification. EtAA (1.1 mol) was added to a methanol suspension of sodium D-phenylglycine (1 mol) and the mixture was stirred for 30 min at 50°C. The resulting clear solution with small amounts of precipitate was filtered, poured into in excess of ethyl ether and allowed to stand overnight at room temperature. The precipitate was collected and crystallized from ethanol solution to give sodium N-(1-methyl-2-ethoxycarbonylvinyl)-D-phenylglycine (PG EtAA Na) in 80% yield.

Other enamine derivatives were similarly prepared with some modifications, if necessary, from sodium salts or methyl esters of amino acids and β-dicarbonyl compounds such as EtAA and acetyl acetone (AA).

Reaction of Sodium D-Phenylglycine with Diethyl Ethoxymethylenemalonate: Sodium D-phenylglycine (1 mol) was boiled with diethyl ethoxymethylenemalonate (1.5 mol) in benzene, and the by-product ethanol was azeotropically removed. After reaction for 3 h, a pale yellow solution with small amounts of precipitate was obtained. The solvent was evaporated off under reduced pressure. The residue was dissolved in methanol and filtered to remove the insolubles, then the methanol was distilled off and the residue was washed with petrolatum ether. The yield of N-[2,2-bis(ethoxycarbonyl)vinyl]-D-phenylglycine sodium (PG DEMM Na) was 50%.

The enamine derivatives synthesized are listed in Table I with their abbreviations and some physicochemical properties.

Table I. Properties of Enamine Derivatives

Amino acid	Enamine	NMR data[a] –NH–	NMR data[a] =CH–	mp, °C	λ_{max}[b]	$t_{1/2}$[c]
λ-Aminobutyric acid	GABA EtAA Na	8.95(d, 8)	4.35(s)	110—115	282.5	18.8
Glycine	Gln EtAA Na	8.73(d, 8)	4.17(s)	168—171	281.0	3.7
L-Leucine	Leu EtAA Na	8.70(d, 8)	4.15(s)	147—149	287.0	7.2
L-Lysine	Lys EtAA Na	α 9.70(d, 8)	4.63(s)	77—79	285.5	22.9
		ω 8.75(d, 8)	4.63(s)			
L-Phenylalanine	Phe EtAA Na	9.25(d, 9)	4.24(s)	96—98	283.5	15.7
D-Phenylglycine	PG EtAA Na	9.50(d, 7)	4.48(s)	199—201	289.0	26.1
D-Phenylglycine	PG AA Na	8.85(d, 7)	5.00(s)	124—126	311.0	Stable
D-Phenylglycine	PG DEMM Na	8.00(d, 14)	—	128—129	283.0	Stable
D-Phenylglycine methyl ester	PGM EtAA	9.50(d, 6)	4.22(s)	98—101	283.0	—
L-Tryptophan	Trp EtAA Na	8.95(d, 9)	4.25(s)	118—120	288.0	7.2
Taurine	Tau EtAA Na	9.18(d, 10)	4.62(s)	113—115	285.5	24.0

a) Each sample was measured in dimethyl sulfoxide-d_6. Letters s and d in parenthesis designate singlet and doublet peaks, respectively. Numbers in parenthesis designate the coupling constant (Hz). Each proton disappeared upon D_2O addition.
b) λ_{max} was measured in ethyl alcohol.
c) $t_{1/2}$ (min) was measured in pH 7.4 phosphate buffer (μ=0.15) at 25°C.
EtAA: ethyl acetoacetate. AA: acetylacetone. DEMM: diethyl ethoxymethylenemalonate.

Physicochemical Properties——NMR Measurements: The spectra were obtained at 31°C using a Hitachi R-22 spectrometer (90 MHz). Samples were dissolved in dimethyl sulfoxide-d_6 at a concentration of 5 w/w%. Tetramethylsilane was used as an internal reference. The addition of D_2O and the proton decoupling technique were used to assign some protons.

Hydrolysis of the Enamine Moiety: A spectrophotometric method was used to determine the stability of enamine derivatives to hydrolysis at 25°C in phosphate buffer at pH 7.4 (ionic strength, 0.15) employing a Shimadzu recording spectrophotometer, model UV-200.

The hydrolysis of the enamine moiety followed apparent first-order kinetics and the values of half-life for the hydrolysis were obtained.

Preparation of Rectal Delivery Preparations——Three bases were used: a mixture of equal amounts of liquid paraffin and white petrolatum (Nikko Chemicals Co., Ltd.), Witepsol H-15 (Dynamit Novel Chemicals, Troisdorf-Oberlar, West Germany), and Mglyol 812 (Dynamit Novel Chemicals).

Suitable amounts of an enamine derivative and antibiotic were taken into a mortar, ground and mixed with a pestle. A sufficient amount of a base kept at 40°C was taken into the mortar and mixed well with the pestle. Suppositories of Witepsol H-15 were administered after solidification at 10°C in disposable plastic molds (Nippon Elanco Co., Ltd.). The oil suspensions were prepared immediately prior to the experiments and were administered from 1 ml disposable syringes.[3]

Animal Study——Five to ten male albino rabbits, 2.5—3.0 kg, were used for each experiment. The dose of antibiotics was fixed at 15 mg/kg for penicillins and 50 mg/kg for cephalosporins on the basis of their clinical doses and the sensitivities of the microbiological assay. The animals were fasted for 12 h before the experiments but water was given freely. After the animal had been secured in a supine position, aliquots of a preparation were administered to the rectum following the method described in the previous paper.[3]

At 0, 10, 20, 40, 60, 80, 100, 120 and 150 min after the administration, 0.2 ml blood samples were collected from a marginal ear vein. The blood samples were kept at 4°C until assay.

Microbiological Assay——Blood samples were hemolyzed by the addition of an appropriate amount of water. Microbiological assay was performed following the method described in the previous paper[3] with *Sarcina lutea* ATCC 9341 for penicillins and *Bacilus subtilis* ATCC 6633 for cephalosporins.

Results and Discussion

NMR Spectra

Dane *et al.*[6] synthesized derivatives of potassium D-phenylglycine with β-dicarbonyl compounds and assigned enamine structures on the basis of their IR and NMR spectra.

To confirm the enamine structure of β-dicarbonyl compounds of amino acids, proton resonance studies were carried out. Assignments of protons in the derivatives were performed by employing deuterium exchange and decoupling experiments (Table I). The doublet signal of the N–H proton in the enamine moiety and the singlet signal of the C–H proton in the vinyl moiety disappeared on the addition of D_2O. The C–H proton in the α-carbon of α-amino acids also changed from a doublet to a singlet on the addition of D_2O.

From these results, the reaction products were confirmed to be present in the enamine form in dimethyl sulfoxide solution, and the presence of Schiff base was not observed.

Physicochemical Properties

The enamine derivatives were found to have a strong absorption band at around 280 nm with a molar absorptivity of about 2×10^4. Their half-lives for the hydrolysis of the enamine moiety at pH 7.4 and 25°C were obtained following the spectral changes (Table I). The enamine derivatives obtained with EtAA were found to hydrolyze rapidly but the derivatives of acetylacetone and diethyl ethoxymethylenemalonate were very stable and little change in the absorption spectra was observed during the experimental period of 4 h.

Sodium salts of enamine derivatives were readily soluble in water and they were also soluble in ethanol, suggesting an amphoteric nature that should favor their permeation through the biological membrane. This consideration is supported by the large apparent partition coefficients of EtAA derivatives of D-phenylglycine, L-phenylalanine (Phe EtAA), and L-tryptophan (Trp EtAA) between *n*-butanol and Menzel's buffer at pH 10 (7.4, 6.5 and 4.9, respectively).

Thus, the enamine derivatives of amino acids are considered to possess an appropriate lipid affinity for permeation through biological membranes, including the rectal membrane. The EtAA enamine derivatives are easily hydrolyzed in the rectum during the absorption process and in the blood stream to produce amino acids and EtAA.

It requires a comprehensive study to check the safety of these enamine derivatives for use as adjuvants for rectal delivery preparations. However, the acute toxicity of the enamine derivatives of EtAA was found to be very low with LD_{50} of 1.0—1.5 g/kg in rats (intravenous administration), and EtAA is officially approved for use as a flavoring agent in some kinds of food in Japan. Thus, they are considered to be suitable as absorption promoter adjuvants for rectal delivery preparations designed for long-term administration.

Rectal Absorption

The promoting efficacies of EtAA and the sodium salt of PG EtAA (PG EtAA Na) were compared for the rectal absorption of antibiotics in rabbits by employing rectal preparations of ABPC Na or CET Na suspended in paraffin base (Figs. 1 and 2). In Fig. 1, for the sake of comparison, the results of the oral administration of ABPC Na in rabbits are also presented.

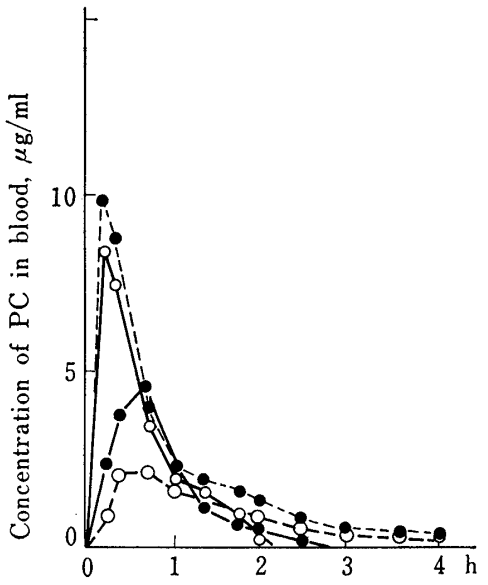

Fig. 1. Effect of PG EtAA Na or EtAA on the Rectal Absorption of ABPC Na in Rabbits

Rectal administration: ···●···; ABPC EtAA Na (10 w/w%), —●—; ABPC Na (10 w/w%)+PG EtAA Na (10 w/w%), —○—; ABPC Na (10 w/w%)+EtAA (5 w/w%), in liquid paraffin-white petrolatum base (50: 50, w/w).
Oral administration: ···○···; ABPC Na in hard gelatin capsule.
Dose: equivalent to 15 mg/kg of ABPC Na. 0.15 g/kg of suppository for ABPC Na. 0.196 g/kg of suppository for ABPC EtAA Na.
Each point represents the mean value of five rabbits.
The coefficient of variation of each point is less than 24%.

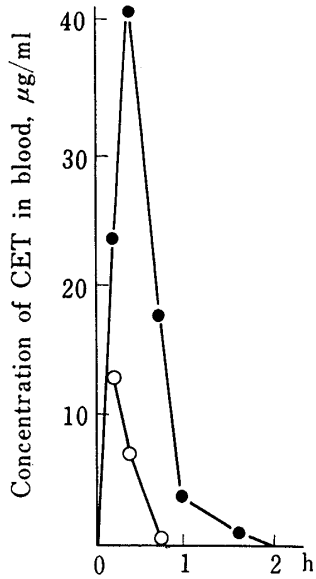

Fig. 2. Effect of PG EtAA Na or EtAA on the Rectal Absorption of CET Na in Rabbits

—●—; CET Na (20 w/w%)+PG EtAA Na (10 w/w%), —○—; CET Na (20 w/w%)+EtAA (5 w/w%), in liquid paraffin-white petrolatum base (50: 50, w/w).
Dose: 50 mg/kg of CET Na. 0.25 g/kg of suppository for CET Na.
Each point represents the mean value of five rabbits. The coefficient of variation of each point is less than 30%.

Because D-phenylglycine was used as an acyl moiety in ampicillin, the EtAA enamine derivative of D-phenylglycine was tentatively used. However, no difference was observed in the promoting efficacy of the rectal absorption of antibiotics between D- and DL-phenylglycine. Thus, the results for D-phenylglycine (PG EtAA Na) are presented in this paper.

Aliquots of a suspension of ABPC Na and PG EtAA Na at the same concentration of 10 w/w% were administered in the rectum of rabbits at a dose of 15 mg of ABPC Na/kg. The values of AUC and peak blood level in terms of ABPC Na were about twice those obtained following the oral administration of ABPC Na in hard gelatin capsules at the same dose in rabbits.[3)]

PG EtAA Na was found to have a remarkable promoting efficacy in rabbits for the rectal absorption of ABPC Na, which did not permeate through the rectal membrane unaided.

In contrast to the results for PG EtAA Na, the incorporation of EtAA into a suspension of ABPC Na in the paraffin base at a concentration of 5 w/w%, which was approximately equivalent to 10 w/w% of PG EtAA Na, gave a bioavailability to 52.9±8.5%. This value is similar to that obtained by rectal administration of ABPC EtAA Na.[3)]

To clarify the nature of the strong promoting efficacy of EtAA for the rectal absorption of ABPC Na, possible enamine formation in the paraffin base during preparation of the suspension was studied.

ABPC Na and EtAA were suspended in the paraffin base and kept at 40 °C under stirring; periodically, aliquots of the suspension were dissolved in acetone and the amount of enamine formed in the base was measured. From the results of spectroscopic and TLC measurements, ABPC Na contained in the suspension was found to change to its enamine derivative within 10 min. Thus, the remarkable rectal absorption of ABPC Na in the presence of EtAA can be explained on the basis of the present observation of enamine formation.

To compare the true promoting efficacy of EtAA with that of PG EtAA Na, the rectal absorption of CET Na was studied because of the absence of an active amino group in the molecule and its inability to permeate through the rectal membrane due to its poor lipid affinity.

A preparation of 20 w/w% of CET Na and 10 w/w% of PG EtAA Na or 5 w/w% of EtAA suspended in the paraffin base was administered into the rectum of rabbits at the dose of 50 mg of CET Na/kg (Fig. 2). PG EtAA Na was found to have a stronger promoting efficacy than EtAA for the rectal absorption of CET Na. The enhancements of bioavailability and peak blood level of CET Na by PG EtAA Na were five times and three times larger than those by EtAA, respectively.

Here, it must be noted that EtAA is soluble in lipids but its water solubility is only 2% at physiological temperature. Thus, it may be considered that most of the EtAA incorporated into the suspension is confined to the base and a small portion present at the interface between the base and rectal fluid is effective for the promotion of the rectal absorption of CET Na released from the base into the rectal fluid at the interface.

The promoting efficacies of enamine derivatives of amino acids for the rectal absorption of CET Na suspended in the paraffin base are presented in Table II.

Table II. Effect of Promoters on the Rectal Absorption of Cephalothin Na

Promoter	Peak level, μg/ml	Bioavailability, %
Control	0	0
EtAA	13.3± 4.0	9.1±2.6
γ-Aminobutyric acid EtAA Na	13.2± 2.2	27.1±8.2
Glycylglycine EtAA Na	7.2± 4.8	8.4±1.7
L-Leucine EtAA Na	39.9± 4.2	51.3±5.8
L-Lysine EtAA Na	18.0± 7.3	33.6±4.7
L-Phenylalanine EtAA Na	49.7±11.1	49.7±6.9
D-Phenylglycine EtAA Na	40.3± 1.0	47.7±1.1
D-Phenylglycine AA Na	25.2± 3.3	48.1±0.9
D-Phenylglycine DEMM Na	12.7± 7.4	25.9±6.1
D-Phenylglycine methyl ester EtAA	8.0± 2.3	6.0±1.5
L-Tryptophan EtAA Na	38.6± 7.1	45.9±4.0
D-Glucosamine EtAA Na	14.7± 1.0	13.6±4.9
p-Aminobenzoic acid EtAA Na	3.7± 1.8	6.2±2.4
Taurine EtAA Na	37.2±19.4	31.7±8.6

Bioavailability was calculated as AUC after rectal/AUC after *i.v.*
Rp: CET Na 20%, promoter 10%, liquid paraffin 35%, white petrolatum 35%.
Dose: 50 mg of CET Na/kg

The enamine derivatives of PG with acetylacetone (PG AA Na) and PG DEMM Na are very stable in water and even in blood at 37 °C. They also exhibited a remarkable promoting efficacy for the rectal absorption of CET Na. It is considered that both EtAA enamines of amino acids and EtAA regenerated by hydrolysis play an important role in the promotion

of rectal absorption of antibiotics. The methyl ester of PG EtAA also showed only a slight promoting effect on the rectal absorption of antibiotics in rabbits. The methyl ester of PG EtAA is not soluble in water, so, it will not be sufficiently released from the suppository bases. From the above results, it may be concluded that water-soluble enamine derivatives will promote the rectal absorption of CET Na.

The effect of concentration of enamines on the bioavailability of CET Na by rectal absorption was studied (Fig. 3).

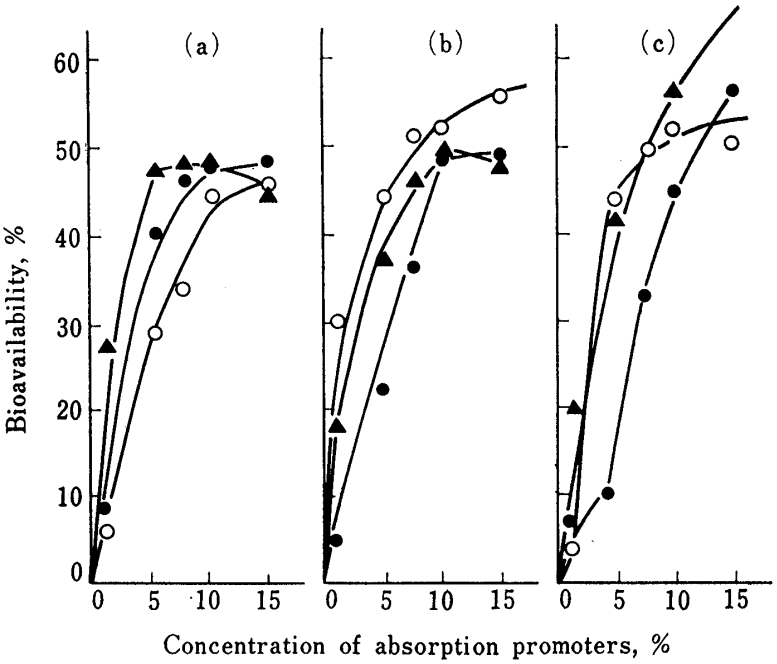

Fig. 3. Effect of the Concentration of Absorption Promoters in the Suppository on the Bioavailability of CET Na in Rabbits

(a): PG EtAA Na, (b): Phe EtAA Na, (c): Trp EtAA Na.
A mixture of CET Na and enamine promotor was suspended in liquid paraffin-white petloratum base (50: 50, w/w); (—○—), Witepsol H-15 base; (—●—), or Miglyol 812 base; (—▲—), respectively.
Dose: 50 mg/kg of CET Na. 0.25 g/kg of suppository for CET Na.
Each point represents the mean value of five rabbits. The coefficient of variation of each point is less than 22%.

Among many promoters studied, EtAA enamines of PG Na, Phe Na and Trp Na were chosen because of their strong promoting efficacy. In Fig. 3, the results with two glyceride bases and the paraffin base are presented.

The bioavailability of CET Na reached a maximum in the presence of 10—15% of each promoter, and no remarkable differences was observed among the bases.

To study the applicability of these enamine promoters for rectal delivery preparations of water-soluble antibiotics which do not permeate through the rectal membrane unaided and which have been clinically used by the parenteral route alone, three cephalosporins, CEZ Na, CEP Na and CER Na were administered in rabbits as suspensions in the paraffin base containing PG EtAA Na (10%) (Fig. 4).

These cephalosporins were well absorbed in the presence of 10% PG EtAA Na with bioavailabilities of 90%, 100% and 60%, respectively.

To compare the promoting efficacy of PG EtAA Na for the rectally poorly absorbable β-lactam antibiotics, the bioavailabilities of six antibiotics suspended in the paraffin base with 10% PG EtAA Na were plotted against the logarithm of the partition coefficients, log P, between n-octanol and water (Fig. 5).

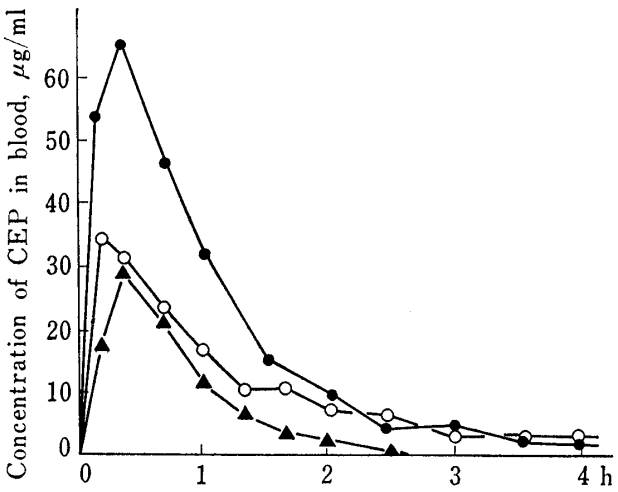

Fig. 4. Effect of PG EtAA Na on the Rectal Absorption of Cephalosporins in Rabbits

—○—; cephaloridine Na, —▲—; cephapirin Na, —●—; cefazorin Na.

Dose: 50 mg/kg of antibiotics. 0.25 g/kg of suppository for each antibiotics.

Each antibiotic and PG EtAA Na were suspended in liquid paraffin-white petrolatum base (50: 50, w/w) at concentrations of 20 w/w% and 10 w/w%, respectively.

Each point represents the mean value of five rabbits. The coefficient of variation of each point is less than 18%.

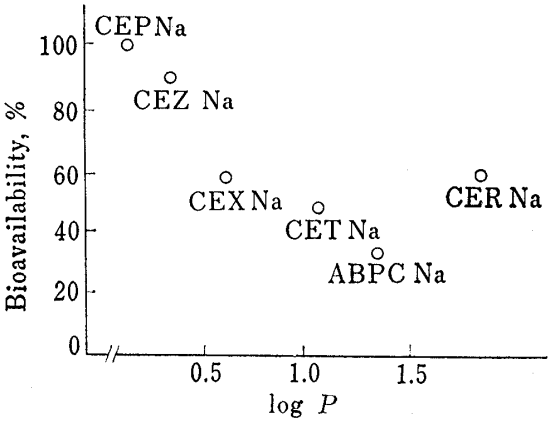

Fig. 5. Relations between Bioavailability following Rectal Administration of Antibiotics with PG EtAA Na in Rabbits and Their Octanol-Water Partitioning Properties (log *P*)

Dose: 0.15 g/kg of suppository for penicillin. 0.25 g/kg of suppository for cephalosporin.

Each antibiotic was suspended in liquid paraffin-white petrolatum base (50: 50, w/w) at a concentration of 10 w/w% for penicillins or 20 w/w% for cephalosporins in the presence of 10 w/w% of PG EtAA Na.

Each point represents the mean value of five rabbits. The coefficient of variation of each point is less than 22%.

Potassium penicillin G (PC–G K) permeated through the rectal membrane in rabbits without PG EtAA Na with a bioavailability of about 15%. However, enhancement of the absorption of PC–G K by PG EtAA Na was not observed.

The six antibiotics (Fig. 5) did not permeate through the rectal membrane in rabbits without PG EtAA Na within the limit of sensitivity of the assay. A linear correlation was obtained between the bioavailability and log *P* except in the case of CER Na.

In the previous reports,[2] we showed that many penicillins having log *P* larger than 1.5 permeated through the rectal membrane in rabbits, and the logarithm of AUC was linearly correlated with log *P* with a correlation coefficient of 0.9936. The rectal absorption of these penicillins which were easily absorbed without enamine promoter were only slightly influenced by the incorporation of PG EtAA Na in the paraffin suspension. Thus, it may be considered that the rectal absorption of antibiotics is also strictly controlled by their lipid affinity, but that enamine derivatives of amino acids effectively promote the rectal absorption of water-soluble antibiotics having small values of log *P*.

The promoting mechanism of the rectal absorption of antibiotics by enamine derivatives is not known at present. However, the results presented in this paper suggest that the enamine derivatives of amino acids are clinically applicable for use in rectal delivery preparations of penicillins and cephalosporins which cannot permeate through the rectal membrane unaided.

References and Notes

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