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Synthesis and relation between physicochemical properties and oral absorption of 7-O-acylmandelamido-3-methyl-3-cephem-4-carboxylic acids *

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Summary

O-Acylates (IV) of 7-D-mandelamido-3-methyl-3-cephem-4-carboxylic acid (I) were prepared and their physicochemical properties and oral absorption in mice were measured to search for an orally active cephalosporin having a 7-acyl group other than phenylglycine analogs. The pK_a and log P values of IB were 2.8 and 1.02–3.54, respectively, and IV were hydrolyzed to the parent drug (I) in a homogenate of mouse small intestine. The esters, IVb (propionate), IVc (*n*-butyrate), IVd (*i*-butyrate), and IVe (*n*-valerate) produced higher plasma levels of I and improved relative bioavailability (BA) than those observed after oral dosing with I. Among the esters, IVe showed the highest BA, 40.3%. Good correlations between log P and log BA and between log P and peak plasma level (log C_{max}) were observed. The esters resulting in good BA have log P values similar to those of orally active penicillins having an acyl group other than phenylglycine analogs, e.g. propicillin

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(PP-PC) and phenethicillin (PE-PC). The present study indicates that an ester prodrug of the parenteral cephalosporin having a 7-acyl group other than phenylglycine analog can be absorbed effectively through the gastrointestinal tract when log P and the pK_a values are in a selected range.

Introduction

Among many potent broad-spectrum cephalosporins, the orally active cephalosporins used in clinical practice are limited to those having phenylglycine analogs in the 7-acyl group, e.g. cephalexin (CEX) and cephradine (CED) (O'Callaghan, 1979). These cephalosporins are completely absorbed from the gastrointestinal tract (GI tract) despite the lack of lipophilicity by the active transport and/or the carrier-mediated transport (Tsuji and Yamana, 1981). However, the cephalosporins having 7-acyl groups other than phenylglycine analogs are poorly absorbed from the GI tract (Yoshimura et al., 1985) owing to the role played by high hydrophilicity in the pH partition hypothesis (Tsuji and Yamana, 1981).

On the other hand, some penicillins having 6-acyl groups other than phenylglycine analogs, e.g. propicillin (PP-PC), phenethicillin (PE-PC), and carfecillin (CF-PC), are well absorbed from the GI tract. Previously, we found that these penicillins are absorbed following the pH partition hypothesis and an acid-stable penicillin having a log P value between 1.65 and 3.17 (P: partition coefficient between 1-octanol and water) had good bioavailability after it was administered orally to mice (Yoshimura and Kakeya, 1983).

As the β-lactam ring of a cephalosporin is generally stable compared with that of penicillin under acidic conditions (Tsuji and Yamana, 1981), improvement of the oral bioavailability of a cephalosporin is expected by increasing the lipophilicity. We have improved the oral bioavailability of cefotiam (CTM) by pivaloyloxymethylation of the carboxylic acid group at the position 4 (Yoshimura et al., 1985). However, there are no such successful reports by esterification of the hydroxyl group at the 7-acyl group of cephalosporins.

Thus, we chose 7-D-mandelamido-3-methyl-3-cephem-4-carboxylic acid (I), a parenteral broad spectrum cephalosporin (Dominguez-Gil et al., 1979), as a model compound, and synthesized several O-acylates (IV) of it to improve the gastrointestinal absorption.

Materials and Methods

Apparatus

Infrared (IR) and ultraviolet (UV) spectra were measured on a Hitachi 215 spectrophotometer and Hitachi ESP 3 spectrophotometer, respectively. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian EM 390 spectrometer with tetramethylsilane as an internal reference. For thin-layer chromatography,

Kiesel gel 60 plates, F_{254} (Merck Art No. 5715), and a Hitachi 156 spectrophotometer (scanning wavelength λ_{sample} 260 nm and $\lambda_{reference}$ 360 nm) were used. High-performance liquid chromatography (HPLC) was done using a Shimadzu LC-3A instrument equipped with the column (250 × 4 mm i.d.) of Nucleosil C_{18} (10 μm particle, Gasukuro Kogyo) and a variable wavelength UV detector.

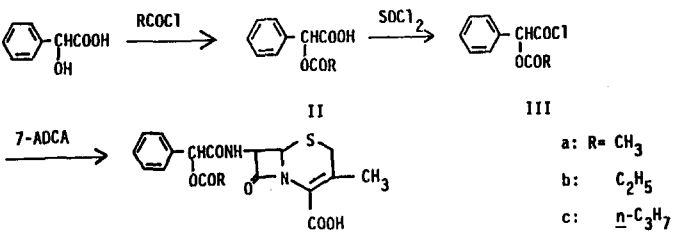
Synthesis

7-D-Mandelamido-3-methyl-3-cephem-4-carboxylic acid (I)

I was prepared by Hoover's method (1974). IR ν_{max}^{KBr} (cm^{-1}): 1780, 1765, 1707, and 1640. NMR(d_6-DMSO) δ: 2.02(s, 3H, CH_3), 3.30 and 3.57(ABq, J = 18.6Hz, 2H, C_2–CH_2), 4.99(d, J = 4.5Hz, 1H, C_6–H), 5.03(s, 1H, CHCO), 5.55(dd, J = 4.5Hz, J = 9.0Hz, 1H, C_7–H), 7.10–7.33(m, 5H, phenyl ring), 8.35(d, J = 9Hz, 1H, OCNH). Anal. Calcd. for $C_{16}H_{16}N_2O_5S$: C, 55.15; H, 4.64; N, 8.04. Found: C, 54.49; H, 4.68; N, 8.05. The purity of I was more than 98% by HPLC analysis.

Preparation of 7-(D-O-acyl mandelamido-3-methyl-3-cephem-4-carboxylic acid (IV): general procedure

Scheme 1 shows the reaction processes.



Scheme 1

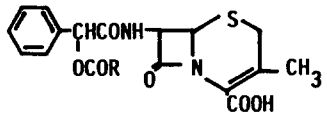
(1) D-Mandelic acid (30 mmol) was refluxed with the corresponding acyl chloride (40 mmol) in anhydrous dichloromethane (30 ml) for 3 h and the mixture was concentrated in vacuo. The O-acyl mandelic acid (II), was purified by recrystallization from isopropylether–*n*-hexane or by column chromatography on silica gel with isopropylether–*n*-hexane as an eluent. The structure of II was confirmed by IR, NMR spectra, and elemental analysis.

(2) A solution of II (10 mmol) in dichloromethane (20 ml) was stirred overnight with thionyl chloride (2 ml) and dimethylformamide (2 drops) at room temperature. Removal of the solvent in vacuo gave the acyl chloride (III) as an oily residue, which was used in the coupling reaction.

(3) A solution of 7-amino-3-methyl-3-cephem-4-carboxylic acid (10 mmol) and dibutylamine (3.4 ml) in dichloromethane (40 ml) cooled in dry ice–ethanol was

TABLE 1

ELEMENTAL ANALYSES OF 7-O-ACYLMANDELAMIDO-3-METHYL-3-CEPHEM-4-CARBOXYLIC ACID (IV)



No.	R	Formula	Yield (%)	Analyses (%)					
				Calcd.			Found		
				C	H	N	C	H	N
IV_a	CH_3	$C_{18}H_{18}N_2O_6S \cdot 1/2H_2O$	32.0	54.12	4.79	7.01	54.10	4.78	6.71
IV_b	CH_2CH_3	$C_{19}H_{20}N_2O_6S \cdot 1/2H_2O$	14.5	55.27	5.12	6.78	55.00	5.09	6.82
IV_c	$(CH_2)_2CH_3$	$C_{20}H_{22}N_2O_6S$	21.5	57.40	5.30	6.70	56.99	5.42	6.70
IV_d	$CH(CH_3)_2$	$C_{20}H_{22}N_2O_6S$	49.7	57.40	5.30	6.70	57.22	5.47	6.55
IV_e	$(CH_2)_3CH_3$	$C_{21}H_{24}N_2O_6S \cdot 1/2H_2O$	15.6	57.12	5.71	6.35	56.83	5.81	6.18
IV_f	$(CH_2)_5CH_3$	$C_{23}H_{28}N_2O_6S \cdot 1/2H_2O$	32.7	58.82	6.24	5.97	58.70	6.59	5.79
IV_g	$(CH_2)_6CH_3$	$C_{24}H_{30}N_2O_6S \cdot 1/2H_2O$	40.2	59.60	6.27	5.79	59.86	6.30	6.18

treated dropwise with a solution of III in dichloromethane (10 ml) with stirring. After 1 h, the reaction mixture was poured into a mixture of ice-cooled 10% H_3PO_4 (150 ml) and ethyl acetate (200 ml) and the aqueous layer was extracted with ethyl acetate (200 ml × 2). The combined organic layer was washed with 10% H_3PO_4 (100 ml × 2), cold water (150 ml × 2) and saturated brine, dried over anhydrous Na_2SO_4, and was concentrated in vacuo. The oily residue was triturated with isopropylether and the ester (IV) was recrystallized from acetone–isopropylether or purified by column chromatography on silica gel with isopropylether–ethyl acetate as an eluent. The analytical results of IV are shown in Tables 1 and 2. The purity of IV was more than 98% by the HPLC analysis.

Physicochemical properties

(1) Acid stability

The parent cephalosporin (I) or the ester (IV) (10 mg) and the equimolar of $NaHCO_3$ were dissolved in water (10 ml). This solution (1.0 ml) was added to a mixture of 0.2 N HCl (25 ml) and ethanol (25 ml) at 35°C, and the concentration of the cephalosporin was measured by HPLC. HPLC analysis: mobile phase (0.05 M $(NH_4)_2SO_4 : CH_3CN : CH_3CO_2H$ 400 : 200 : 1 (I), 200 : 200 : 1 (IV)) were used with a flow rate of 1.5 ml/min. The retention time: I, 3.88 min; IVa, 2.33 min; IVb, 2.72 min; IVc, 3.25 min; IVd, 3.23 min; IVe, 4.03 min; IVf, 7.12 min; IVg, 10.04 min.

(2) pK_a value

I or IV (10 mg) and the equimolar of $NaHCO_3$ were dissolved in water (10 ml). The solution (1.0 ml) was diluted to 25 ml with isotonic buffers of various pH's. The pK_a value of I was calculated from the absorbance (262 nm) at various pH's.

(3) Water solubility

I or IV (20 mg) was added to an isotonic buffer of pH 4.5 (5.0 ml) and shaken for

TABLE 2

IR AND NMR DATA OF THE ESTERS (IV)

No.	IR ν_{max}^{KBr} (cm^{-1})	NMR (d_6-DMSO) δ
IV_a	1780, 1770, 1750, 1725, 1660	1.97(s, 3H, CH_3), 2.11(s, 3H, CH_3CO), 3.18 and 3.45(ABq, J = 17.1Hz, 2H, C_2-CH_2), 4.90(d, J = 4.5Hz, 1H, C_6-H), 5.57(dd, J = 4.5Hz, J = 8.7Hz, 1H, C_7-H), 5.97(s, 1H, CHCO), 7.23–7.64(m, 5H, phenyl-H), 9.22(d, J = 8.7Hz, 1H, CONH)
IV_b	1770, 1750, 1725, 1660	1.06(t, J = 7Hz, 3H, CH_3), 1.97(s, 3H, CH_3), 2.43(q, J = 7Hz, 2H, CH_2), 3.18 and 3.43(ABq, J = 18Hz, 2H, C_2-CH_2), 4.91(d, J = 4.5Hz, 1H, C_6-H), 5.57(dd, J = 4.5Hz, J = 9Hz, 1H, C_7-H), 6.00(s, 1H, CHCO), 7.07–7.73(m, 5H, phenyl-H), 9.23(d, J = 9Hz, 1H, CONH)
IV_c	1775, 1765, 1755, 1750, 1665	0.90(t, J = 7.5Hz, 3H, CH_3), 1.32–1.88(m, 2H, CH_2), 1.98(s, 3H, CH_3), 2.39(t, J = 7.5Hz, 2H, CH_2CO), 3.16 and 3.44(ABq, J = 18.3Hz, 2H, C_2-CH_2), 4.90(d, J = 4.8Hz, 1H, C_6-H), 5.57(dd, J = 4.8Hz, J = 8.7Hz, 1H, C_7-H), 5.99(s, 1H, CHCO), 7.12–7.63(m, 5H, phenyl-H), 9.22(d, J = 8.7Hz, 1H, CONH)
IV_d	1775, 1755, 1735, 1725, 1690	1.10(d, J = 7Hz, 3H, $CHCH_3$), 1.17(d, J = 7Hz, 3H, $CHCH_3$), 1.95(s, 3H, CH_3), 2.35–2.95(m, 1H, CH), 3.17 and 3.45(ABq, J = 18Hz, 2H, C_2-CH_2), 4.90(d, J = 4.5Hz, 1H, C_6-H), 5.88(dd, J = 4.5Hz, J = 8.7Hz, 1H, C_7-H), 5.99(s, 1H, CHCO), 7.30–7.70(m, 5H, phenyl-H), 9.22(d, J = 8.7Hz, 1H, CONH)
IV_e	1775, 1760, 1740, 1710, 1665	0.86(t, J = 7Hz, 3H, CH_3), 1.03–1.78(m, 4H, $(CH_2)_2$), 1.98(s, 3H, CH_3), 2.41(t, J = 7Hz, 2H, CH_2CO), 3.17 and 3.45(ABq, J = 17.4Hz, 2H, C_2-CH_2), 4.91(d, J = 4.5Hz, 1H, C_6-H), 5.57(dd, J = 4.5Hz, J = 9Hz, 1H, C_7-H), 6.00(s, 1H, CHCO), 7.10–7.67(m, 5H, phenyl-H), 9.22(d, J = 8.7Hz, 1H, CONH)
IV_f	1780, 1765, 1740, 1725, 1695	1.00(s, 9H, $(CH_3)_3$), 1.97(s, 3H, CH_3), 3.17 and 3.48(ABq, J = 18Hz, 2H, C_2-CH_2), 4.92(d, J = 4.5Hz, 1H, C_6-H), 5.57(dd, J = 4.5Hz, J = 8.7Hz, 1H, C_7-H), 5.92(s, 1H, CHCO), 7.2–7.6(m, 5H, phenyl-H), 9.22(d, J = 9Hz, 1H, CONH)
IV_g	1770, 1740, 1705, 1670	0.70–1.77(m, 11H, $(CH_2)_4CH_3$), 2.01(s, 3H, CH_3), 2.37(t, J = 7.5Hz, 2H, CH_2CO), 3.27 and 3.54(ABq, J = 18.3Hz, 2H, C_2-CH_2), 4.96(d, J = 4.8Hz, 1H, C_6-H), 5.47(dd, J = 4.5Hz, J = 7.5Hz, 1H, C_7-H), 5.93(s, 1H, CHCO), 7.13–7.63(m, 5H, phenyl-H), 9.17(d, J = 8.7Hz, 1H, CONH)

TABLE 2 (continued)
IR AND NMR DATA OF THE ESTERS (IV)

No.	IR ν_{max}^{KBr} (cm^{-1})	NMR (d_6-DMSO) δ
IV_h	1780, 1740, 1725, 1690	0.57–1.78(m, 13H, $(CH_2)_5CH_3$), 2.01(s, 3H, CH_3), 2.32(t. J = 6.6Hz, 2H, CH_2CO), 3.29 and 3.57(ABq, J = 17.1Hz, 2H, C_2–CH_2), 4.99(d, J = 4.5Hz, 1H, C_6–H), 5.46(dd, J = 4.5Hz, J = 9Hz, 1H, C_6–H), 5.96(s, 1H, CHCO), 7.10–7.67(m, 5H, phenyl-H), 9.19(d, J = 9Hz, 1H, CONH)

2 h at room temperature. After filtration, the concentration of I or IV was measured by HPLC.

(4) Partition coefficient

I or finely ground IV (10 mg) was added to the isotonic buffers of pH 3.58 and 5.06 (20 ml). The suspension was shaken vigorously with 1-octanol (5.0 ml) for 30 min at 25°C, then centrifuged, and the concentration of the drug in the aqueous layer was measured spectrophotometrically. Log P value was calculated from the observed partition coefficient P′ at various pH's following the equation:

$$\log P = \log P' + \log(1 + 10^{pH - pK_a})$$

Hydrolysis to parent drug

Male SLC-ICR mice (4 weeks old), starved but with free access to water overnight, were killed. The small intestine was removed immediately and washed several times with an ice-cooled saline. After homogenation in an ice-cooled saline (one part small intestine to 100 parts saline) and centrifugation at 3000 rpm for 10 min, the supernatant was used as a 1% homogenate of small intestine.

The ester IV (10 mg) and $NaHCO_3$ (2.0 mg) were dissolved in a small volume of aqueous acetone, and diluted with 1/15 M phosphate buffer of pH 7.4 to 10.0 ml. This solution of IV (2.0 ml) was added rapidly to the homogenate (8.0 ml) so that the final concentration of IV was 0.2 mg/ml. The mixture was incubated at 37°C and a sample (1.0 ml) taken immediately, and at 0.5, 1, 2, 3, 4, and 6 h after incubation was added to a mixture of 1 N HCl (0.5 ml), saline (0.5 ml), and dichloromethane (6.0 ml), shaken for 5 min, centrifuged, and the organic layer (4.0 ml) was concentrated in vacuo. After development of the residue on TLC with benzene–dioxane–acetic acid (20 : 20 : 1), the amounts of I and IV were measured densitometrically with authentic standards as the references.

Hydrolysis of IVe to I in 10% tissue homogenate

The small intestine or liver removed from mice was homogenized in an ice-cooled saline (1 part tissue to 10 parts saline), centrifuged at 3000 rpm for 10 min, and the

supernatant was used as a 10% homogenate of small intestine or liver. The plasma was diluted with 9 times volume of saline.

The aqueous solution of IVe (1.0 ml; 1 mg/ml) was added to 10% homogenates or 10% plasma (9.0 ml) at 37°C so that the final concentration of IVe was 0.1 mg/ml. The mixture was incubated at the same temperature and a sample (1.0 ml) taken immediately, and at 2, 5, 10, 15 and 30 min after incubation was added to acetonitrile (1.0 ml) and centrifuged. The supernatant was injected directly to HPLC.

Oral absorption

Male SLC-ICR mice, weighing ca. 15 g (4 weeks old) were starved overnight. IV or I was administered orally to a group of 4 mice by intubation as an aqueous solution with the equimolar $NaHCO_3$ at a dose of 100 mg/kg equivalent to I. I was also administered subcutaneously in mice at the same dose. Blood was taken from inferior vena cava at 0.25, 0.5, 1 and 2 h after dosing. The relative bioavailability was calculated by the ratio of the area under the plasma level–time curve (AUC_{oral}) after oral administration and that (AUC_{sc}) after subcutaneous administration.

Assay

The plasma concentration of I was measured by the cylinder plate method using *B. subtilis* PCI 219P as the test organism. The detection limit was 1.5 μg/ml.

Identification of the ester (IV) in the plasma after oral administration of IV

The plasma sampled at 0.25 and 0.5 h after oral administration of IV in mice were added to a mixture of saline (0.5 ml), 1 N HCl (0.5 ml) and dichloromethane (6.0 ml). Having been shaken vigorously for 5 min, the mixture was centrifuged and the organic layer (4.0 ml) was dried under a N_2 gas stream. The residue was dissolved in dichloromethane (0.1 ml), spotted on the TLC plates and developed with benzene–dioxane–acetic acid (20 : 20 : 1).

HPLC method. At 0.25 h after the oral administration of IVe at a dose of 100 mg/kg equivalent to I in mice, the plasma was sampled, shaken with the same volume of acetonitrile and centrifuged. The supernatant was injected into HPLC.

Results and Discussion

Physicochemical properties of (I) and (IV)

The stability of a cephalosporin or its ester in an acid medium such as gastric juice is a prerequisite for the successful GI absorption. The acid stability of I and IV in 50% aqueous ethanol solution at pH 1.3 and 35°C were measured. The residual amount of I measured by HPLC at 4 h after incubation was 87% and those of IV were more than 90% as shown in Table 3. The β-lactam rings of I and IV were found to be quite stable and the hydrolyzations of the esters were negligible.

The pK_a of the carboxylic acid influences the absorption of an acidic drug from the GI tract (Schanker et al., 1958). The pK_a values of I and IV were the same 2.80;

TABLE 3

ACID STABILITY, WATER SOLUBILITY (S), Log P VALUE, AND THE HYDROLYSIS RATE CONSTANT OF THE ESTER (IV) TO THE PARENT CEPHALOSPORIN (I)

No.	Residual amount of IV [a] (%)	S (mg/ml) [b]	log P [c]	k [d] (h^{-1})	$t_{1/2}$ (h)
IV_a	99.4	1.86	1.02	0.212	3.27
IV_b	98.4	1.75	1.46	0.174	3.28
IV_c	90.6	1.79	1.89	0.408	1.70
IV_d	96.9	1.17	2.05	0.0636	10.89
IV_e	97.2	0.49	2.23	1.17	0.59
IV_f	99.7	0.082	3.23	0.212	2.77
IV_g	97.6	0.034	3.54	0.0665	10.42

[a] At 4 h after incubation in 50% aqueous ethanol at pH 1.3 and 35°C.

[b] At pH 4.5 buffer.

[c] Between 1-octanol and water.

[d] First-order rate constant in 1% homogenate of small intestine of mice at 37°C; initial concentration, 0.20 mg/ml.

this value is similar to those of the orally active penicillins (pK_a 2.6–2.8) and higher than those of other parenteral cephalosporins (pK_a 2.0–2.4) (Bergan, 1978; Tsuji and Yamana, 1981).

The water solubility of an ester prodrug of a parenteral cephalosporin near the so-called virtual pH affects oral bioavailability (Yoshimura et al., 1985). At pH 4.5, although the water solubility of IVf, and g was relatively low (0.034–0.082 mg/ml), that of IVa–e was relatively high (0.49–1.86 mg/ml) owing probably to the presence of intact carboxylic acid group at the position 4 of IV (Table 3). I showed the water solubility 1.35 mg/ml.

The partition coefficient P of a drug also affects GI absorption (Lien, 1975) and this principle is expected applicable to those cephalosporins that are apparently absorbed by a passive transport mechanism.

A penicillin other than having a phenylglycine analog moiety in the 6-acyl group and showing a log P value between 1.65 and 3.17, e.g. PP-PC and PE-PC, resulted in a bioavailability of more than 30% after oral administration to mice (Yoshimura and Kakeya, 1983).

Thus, we estimated the log P values of I and IV. Although the log P value of I was 0.43, that of an ester (IV) was between 1.02 and 3.54, which indicates that the lipophilicity of I was improved by esterification (Table 3).

Among the esters, $IV_{c,d,e,f,}$ and IV_g had log P values similar to those of orally active penicillins, e.g. PP-PC (log P 2.58) and PE-PC (log P 2.20).

Hydrolysis to parent cephalosporin

A cephalosporin ester must be hydrolyzed to the parent cephalosporin during or shortly after the absorption into a body. From the safety standpoint, it is desirable that the hydrolysis proceeds in the gastrointesinal wall during absorption. We measured the rates in a 1% homogenate of mouse small intestine at 37°C. In a

TABLE 4

THE HYDROLYSIS RATE CONSTANTS OF IV_e TO THE PARENT CEPHALOSPORIN (I) IN THE TISSUE HOMOGENATES AND THE PLASMA OF MICE

Medium	k [a] (min^{-1})	$t_{1/2}$ (min)
10% small intestine homogenate	0.146	4.74
10% liver homogenate	0.292	2.37
10% plasma	–	– [b]

[a] At 37°C; initial concentration was 0.1 mg/ml.

[b] The residual amount of IV_e at 30 min after incubation was 95.0%.

preliminary examination, the intact esters (IV) could not be detected in the plasma after they were administered orally to mice owing probably to the very fast hydrolysis in the gastrointestinal wall in vivo. However, to know differences among

TABLE 5

MEAN PLASMA LEVELS OF THE PARENT CEPHALOSPORIN (I), AREA UNDER PLASMA LEVEL OF I–TIME CURVE AND RELATIVE BIOAVAILABILITY AFTER ORAL ADMINISTRATION OF THE ESTERS (IV) AND THE PARENT DRUG (I) IN MICE

No.	Dosing route	Plasma levels (μg/ml)				AUC_0^{2h} (h·μg/ml)	Bioavailability (%)
		0.25 h	0.5 h	1 h	2 h		
I	s.c.	96.0 (4.0) *	94.5 (5.5)	17.3 (2.3)	3.1 (0.1)	73.8	100.0
I	p.o.	14.6 (1.7)	9.3 (0.4)	6.1 (0.2)	2.8 (0.0)	13.1	17.8
IV_a	p.o.	11.4 (1.0)	8.7 (0.4)	7.5 (0.7)	2.8 (0.0)	13.2	17.8
IV_b	p.o.	17.2 (1.0)	15.4 (2.2)	15.8 (3.5)	6.0 (1.0)	24.9	33.7
IV_c	p.o.	16.7 (1.1)	20.3 (2.1)	14.4 (1.6)	3.4 (0.3)	24.9	33.7
IV_d	p.o.	17.6 (1.3)	15.0 (1.2)	12.8 (0.9)	3.1 (0.0)	21.2	28.7
IV_e	p.o.	31.2 (1.0)	25.4 (3.8)	14.5 (0.8)	3.1 (0.1)	29.8	40.3
IV_f	p.o.	3.5 (0.2)	3.3 (0.2)	3.1 (0.1)	2.9 (0.1)	5.9	8.0
IV_g	p.o.	2.4 (0.0)	2.4 (0.1)	2.3 (0.0)	2.3 (0.0)	4.4	6.0

Relative bioavailability (%) = $100 \times AUC_{p.o.}/AUC_{s.c.}$

n = 4; dose = 100 mg/kg as I.

* S.E. in parentheses.

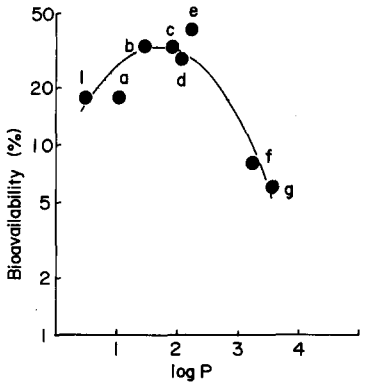


Fig. 1. Relation between log P value and relative bioavailability (BA) after oral administration of I and IV in mice at a dose of 100 mg/kg equivalent to I.

the hydrolysis rates of the esters (IV) more distinctly, we selected an in vitro experimental condition, i.e. 0.2 mg/ml of IV in a 1% homogenate of mouse small intestine. The hydrolysis took place following pseudo-first-order kinetics and the rate constants obtained as well as the half-lives ($t_{1/2}$) are shown in Table 3.

The hydrolysis rate of *n*-valerate (IVe) to I was the fastest with a half-life ($t_{1/2}$) 0.59 h, followed by *n*-butyrate (IVc), 1.70 h and *n*-heptanoate (IVf), 2.77 h. *n*-Octanoate (IVg), and *i*-butyrate (IVd) had a half-life of 10.42 and 10.89 h, respectively.

We also measured the hydrolysis rate of IVe to I in 10% homgenate of mouse small intestine, liver and 10% mouse plasma. Although IVe was hydrolyzed rapidly to I in the homogenate of small intestine and liver with $t_{1/2}$ of 4.74 min and 2.37 min, respectively, IVe was not hydrolyzed to I in the 10% mouse plasma during 30 min (Table 4).

Absorption studies

The plasma levels of the parent cephalosporin (I) after oral administration of the esters (IV) and I and after subcutaneous administration of I were measured in mice; the dose was 100 mg/kg equivalent of I.

The ester was not detected in the plasma after oral dosing of IV. The peak plasma levels of I were observed at 0.25–0.5 h after oral dosing, then decreased below the detection limit within 3 h. IVe showed the highest peak plasma level (C_{max}), 31.2 μg/ml, and IVb, c and d showed higher plasma levels than that observed after oral administration of the parent cephalosporin (I); IVa, f, and g did not show improved plasma levels (Table 5).

The relative bioavailability (BA) after oral dosage of I was 17.8%, and that of acetate (IVa), *n*-heptanoate (IV_f), and *n*-octanoate (IV_g) was in the range of 6.0–17.8%; propionate (IVb), *n*-butyrate (IVc), and *n*-valerate (IVe) resulted in a relatively higher BA, i.e. 33.7, 33.7, and 40.3%, respectively (Table 5). The ester, IVc and IVe having pK_a and log P values similar to those of the orally active penicillins showed improved BA.

Correlation between log P or $t_{1/2}$ and BA

The log P values of I and IV, were plotted against C_{max} and BA. As shown in Fig. 1, good parabolic correlations were observed following Eqns. 1 and 2 derived through the least-squares analysis.

$$\log C_{max}(\mu g/ml) = 0.687 + 0.864(\log P) - 0.270(\log P)^2 \quad (1)$$

$n = 8$, $r = 0.920$, $s = 0.183$,

$F_{2,5} = 13.8(F_{2,5;\,\alpha=0.01} = 13.3)$, $(\log P)_0 = 1.60$

$$\log BA(\%) = 0.879 + 0.763(\log P) - 0.226(\log P)^2 \quad (2)$$

$n = 8$, $r = 0.946$, $s = 0.117$,

$F_{2,5} = 21.3(F_{2,5;\,\alpha=0.005} = 18.3)$, $(\log P)_0 = 1.69$

where n = number of studies, r = correlation coefficient, s = standard deviation, F = F-statistic.

The log P value of an ester resulting in more than 30% of BA is between 1.23 and 2.14 and the optimal log P value, $(\log P)_0$, derived from the Eqns. 1 and 2 was 1.60 and 1.69, respectively. In the penicillins, log P value resulting in good BA was between 1.65 and 3.17 in mice with $(\log P)_0$ 2.41 (Yoshimura and Kakeya, 1983); the optimal log P values of the cephalosporin esters and penicillins were different.

As the hydrolysis rate ($t_{1/2}$) was supposed to be related to C_{max} or BA, the least-squares analysis was also tried, but no significant correlation was observed. This must be the result of the very fast in vivo hydrolysis of IV in the GI wall which obliterate the difference observed in vitro.

Since the t_{max}'s of plasma I levels were similar, the C_{max}'s should be closely related to BA, a plot of log BA versus log C_{max} resulted in a good linear fit (Eqn. 3).

$$\log BA(\%) = 0.347 + 0.957 \log C_{max}(\mu g/ml) \quad (3)$$

$n = 7$, $s = 0.108$, $r = 0.975$

$F_{1,6} = 96.3(F_{1,6;\,\alpha=0.005} = 22.8)$

The solubility and stability in the GI tract or wall must be additional factors controlling the bioavailability of IV. Although the low BA of IVf and g might be ascribed to their relatively low aqueous solubility, IV seems to be more soluble and stable against Δ^2 isomerization at the virtual pH of the absorption site in the GI tract than the esters at position 4 as IV has a carboxylic acid group at position 4 of the cephalosporin.

From these results, for the oral absorption of 7-O-acylmandelamido-3-methyl-3-cephem-4-carboxylic acid (IV), lipophilicity was found a critical factor so long as the hydrolysis rate, solubility, and stability of the esters were relatively high as estimated in the present study.

Conclusion

Previously, we proposed that optimization of the lipophilicity, hydrolysis rate, water solubility, and stability is necessary to design an orally active ester prodrug of a parenteral cephalosporin. As the solubility and stability of O-acylmandelamido-3-methyl-3-cephem-4-carboxylic acid (IV) in the GI tract must be improved over those with esters at position 4 of CTM by the presence of an intact carboxylic acid group at the same position, the lipophilicity was well correlated with the oral bioavailability in mice. Although the in vitro hydrolysis rates were not significantly correlated with the bioavailability, an ester IV, having pK_a 2.80 and log P 1.23–2.14, showed improved bioavailability over that of the parent cephalosporin after oral administration. These findings suggest that 7-O-acylation of a parenteral cephalosporin is also an effective way to enhance oral bioavailability.

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