International Journal of Pharmaceutics, 23 (1985) 117–129
Elsevier

IJP 00780

Research Papers

Synthesis and relationship between physicochemical properties and oral absorption of pivaloyloxymethyl esters of parenteral cephalosporins

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(Received March 15th, 1984)
(Modified version received August 29th, 1984)
(Accepted September 20th, 1984)

Summary

To obtain fundamental information for designing orally active prodrugs of parenteral cephalosporins, the pivaloyloxymethyl esters of ten parenteral cephalosporins were prepared and their physicochemical properties and oral bioavailability in mice were measured. By esterification, lipophilicity was improved with a decrease in solubility, but all the esters were hydrolyzed to the parent cephalosporins rapidly in homogenates of mouse small intestine. These esters, except cefamandole, showed improved relative bioavailability (BA) when compared with the parent cephalosporins. Quantitative correlation of the partition coefficient (P), hydrolysis rate ($t_{1/2}$) to the parent cephalosporin and water solubility (S) to the BA of the esters were attempted. A good linear relation between log S and log BA, but no significant relation between log P and log BA or between log $t_{1/2}$ and log BA was observed. Among the prodrugs, the ester of Cefotiam, '1', which has log P 1.57, and the highest water solubility, 2.71 mg/ml, showed the best BA, 41.8%. The results of this study indicate the importance of water solubility in designing an orally active ester prodrug of the parenteral cephalosporin, if the lipophilicity and hydrolysis rate are sufficiently high.

Introduction

In contrast to the wide variety of parenteral cephalosporins, orally active cephalosporins in current use are limited to cephalexin, (7-(D-2-amino-2-

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phenyl)acetamido-3-methyl-3-cephem-4-carboxylic acid); and its analogs incorporating a glycyl amido moiety such as *p*-hydroxyphenylglycyl amido (cefatrizine and cefadroxyl) or D-2-amino-2-(1,4-cyclohexadien-1-yl)acetamido (cephradine) in the 7-acyl side-chain (Brogard et al., 1978; O'Callaghan, 1979; Brogard and Comte, 1982). Since many parenteral cephalosporins have broader antibacterial spectra than those of the cephalexin analogs, transforming the former into orally active prodrugs without affecting the original antibacterial spectra has been attempted in various ways. Esterification at the 4-carboxylic group of cephalosporins has been tried frequently in an attempt to duplicate the success achieved with penicillin esters (Bindrup et al., 1971; Chauvette and Flynn, 1966; Clayton et al., 1975; Foresta et al., 1977; Wheeler et al., 1977; Wheeler et al., 1979; Wright et al., 1979). However, few successful attempts to improve the oral absorption of parenteral cephalosporins by esterification have been reported.

To obtain relevant information for designing orally active prodrugs of parenteral cephalosporins, we prepared a number of pivaloyloxymethyl esters (Table 1) and investigated the relation between their physicochemical properties and oral bioavailability in mice.

Materials and Methods

Apparatus

IR and UV spectra were recorded with a Hitachi 215 spectrometer and a Hitachi ESP-3 spectrophotometer, respectively. NMR spectra were recorded on a Varian EM 390 spectrometer using tetramethylsilane as an internal reference.

Materials

Parent cephalosporins

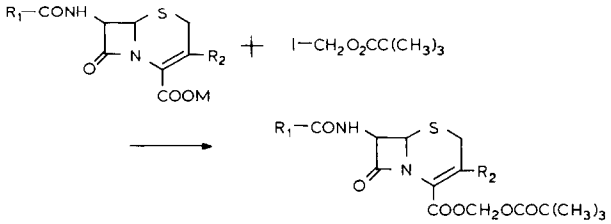
The following cephalosporins were used: cefotiam (CTM), 7β-(2-(2-aminothiazol-4-yl)acetamido)-3-(((1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl)thio)methyl)-3-cephem-4-carboxylic acid dihydrochloride; SCE-785, 7β-(2-(2-aminothiazol-4-yl)acetamido-3-((1-methyl-1H-tetrazol-5-yl)thio)methyl-3-cephem-4-carboxylic acid sodium salt; cefmenoxime (CMX), 7β-(2-(2-aminothiazol-4-yl-(Z)-2-methoxyimino-acetamido)-3-((1-methyl-1H-tetrazol-5-yl)thio)methyl-3-cephem-4-carboxylic acid hemi hydrochloride; cefotaxime (CTX), 7β-(2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid sodium salt; cephapirin (CEPR), 7β-(2-(4-pyridylthio)acetamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid sodium salt; cefazolin (CEZ), 7β-(2-(1H-tetrazol-1-yl)acetamido)-3-((5-methyl-1,3,4-thiadiazol-5-yl)thio)methyl-3-cephem-4-carboxylic acid sodium salt; ceftizoxime (CZX), 7β-(2-(2-aminothiazol-4-yl)-(Z)-methoxyimino-2-acetamido)-3-cephem-4-carboxylic acid sodium salt; ceftezole (CTZ), 7β-(2-(1H-tetrazol-1-yl)acetamido)-3-((1,3,4-thiadiazol-2-yl)thio)methyl-3-cephem-4-carboxylic acid sodium salt; cephalothin (CET), 7β-(2-(thiophen-2-yl)acetamido)-3-acetoxylmethyl-3-cephem-4-carboxylic acid sodium salt; and cefamadole (CMD), 7β-D-mande-

lamido-3-((1-methyl-tetrazol-5-yl)thio)methyl-3-cephem-4-carboxylic acid sodium salt.

Methods

Synthesis of the pivaloyloxymethyl esters of the cephalosporins; general method

The pivaloyloxymethyl esters were prepared as shown in Scheme 1. The sodium or potassium salt of a cephalosporin (5 mmol) was dissolved in dimethylformamide



Scheme 1

(20 ml), and cooled to -10 to -20°C. To this solution was added DMF solution of iodomethyl pivalate (5.5 mmol in 5 ml) during 5 min with stirring, which was continued for another 10 min. The mixture was poured into a mixture of ethyl acetate (150 ml) and ice-water (70 ml), and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (100 ml × 2). The combined organic layer was washed with chilled water (100 ml × 3) and saturated brine, then dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was triturated with isopropylether. Ester '1' was dissolved in a small volume of 0.5 N HCl, absorbed on a column of Amberlite XAD-II (100–200 mesh), and eluted successively with 0.01 N HCl and 5% acetonitrile in 0.01 N HCl. The eluate was concentrated in vacuo and to this residual solution was added sodium chloride. After cooling, the crystalline precipitate was collected and recrystallized from 0.5 N HCl. Ester '2', '8' and '9' were recrystallized from acetone-isopropylether, and ester '10' from ethyl acetate. Ester '3', '4', '5', '6' and '7' were chromatographed on a silica gel column with ethyl acetate as an eluent. After removal of the solvent in vacuo, the residue was reprecipitated with acetone–isopropylether as an amorphous form. The formation of Δ^2 ester, a side reaction reported for similar cephalosporins (Chauvette et al., 1966; Bently et al., 1976) was not serious throughout the process. The structures and analytical results of the esters are shown in Tables 1, 2 and 3.

Water solubility

An ester (50 mg) was added to 1/15 M phosphate buffer of pH 4.5 and shaken vigorously for 30 min at room temperature. After filtration, the concentration of the ester in the filtrate was measured spectrophotometrically. As the esters were unstable at neutral or alkaline pHs, pH 4.5 was selected for measuring water solubility.

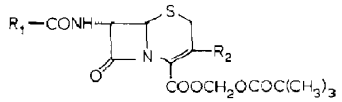
Partition coefficient

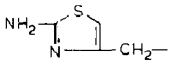
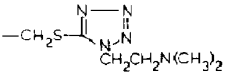
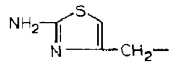
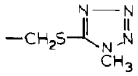
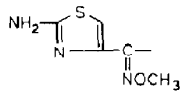
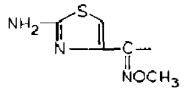
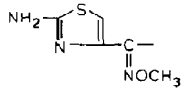
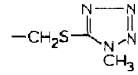
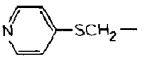
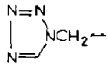
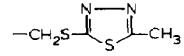
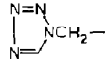
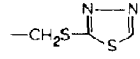
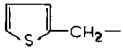
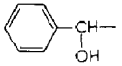
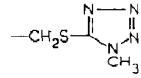
A 1/15 M phosphate buffer solution of an ester at pH 4.5 and 5.4 (ca. 50 μg/ml)

was shaken vigorously with one tenth volume of 1-octanol for 20 min. The concentration of the ester in the aqueous layer was measured by spectrophotometry. Log P values were calculated from the observed partition coefficient P′ at these pHs. The

TABLE 1

STRUCTURES OF THE PIVALOYLOXYMETHYL ESTERS OF PARENTERAL CEPHALOSPORINS



Ester No.	Parent[a] Drug	R_1	R_2
1	CTM		
2	SCE-785		
3	CTX		$-CH_2OCOCH_3$
4	CZX		$-H$
5	CMX		
6	CEPR		$-CH_2OCOCH_3$
7	CEZ		
8	CTZ		
9	CET		$-CH_2OCOCH_3$
10	CMD		

[a]See Materials and Methods

pK_a values of the aminothiazole and dimethylamino group of '1' are 4.6 and 7.0, respectively (Itakura et al., 1978) and that of the aminothiazole group of '3–5' is 3.68.

Hydrolysis to parent cephalosporins from the esters

Male SLC-ICR mice (4 weeks old), starved, but with free access to water for 16–18 h before the experiments, were killed. The small intestine was removed immediately, washed several times with ice-cooled saline to expell the luminal contents, and homogenized with saline (1 part of the small intestine to 100 parts of saline). After centrifugation at 3000 rpm for 10 min, the supernatant was used as a 1% small intestine homogenate.

An ester (10 mg equivalent of the parent cephalosporin) was dissolved in 1 N HCl (0.04 ml) and dioxane (2 ml), then diluted with saline to 50.0 ml. The solution (0.5 ml) was rapidly added to the 1% small intestine homogenate (9.5 ml) preheated at 37°C so that the final concentration of the ester was equivalent to 0.01 mg/ml of the parent cephalosporin. Sampling was carried out at 2, 5, 10, 15 and 30 min after incubation at 37°C. The sample (1.0 ml) was added to a mixture of 1/15 M phosphate buffer, pH 7.4 (2.0 ml) and dichloromethane (5.0 ml) to shake vigorously for 1 min. The concentration of the parent cephalosporin in the aqueous layer was determined by bioassay.

Absorption studies

Male SLC-ICR mice, weighing about 15 g (4 weeks old), were starved but free access to water for 16–18 h before the experiment. The esters were administered orally to a group of 4 mice by intubation either as a suspension or an aqueous solution ('1') at a dose of 100 mg/kg equivalent to the parent cephalosporin. Blood was taken from the inferior vena cava at 0.25, 0.5, 1, and 2 h after dosing. The

TABLE 2

IR SPECTRA AND ELEMENTAL ANALYSES OF THE PIVALOYLOXYMETHYL ESTERS OF PARENTERAL CEPHALOSPORINS

Ester no.	Yield (%)	IR_{max}^{KBr} (cm^{-1})	Formula	Analyses					
				Calcd.			Found		
				C	H	N	C	H	N
1	50	1775, 1730, 1690	$C_{24}H_{33}N_9O_6S_3 \cdot 2HCl \cdot 2H_2O$	38.50	5.25	16.84	38.36	5.19	16.85
2	73	1785, 1755, 1685	$C_{21}H_{26}N_8O_6S_3$	43.29	4.50	19.23	43.34	4.49	18.69
3	66	1785, 1745, 1680	$C_{22}H_{27}N_5O_9S_2 \cdot 1/2H_2O$	45.67	4.88	12.10	46.12	5.13	11.72
4	71	1790, 1750, 1685	$C_{19}H_{23}N_5O_7S_2$	45.87	4.66	14.08	45.97	4.93	13.21
5	68	1780, 1750, 1670	$C_{22}H_{27}N_9O_7S_3 \cdot 1/4H_2O$	41.93	4.40	20.00	42.29	4.43	19.42
6	54	1780, 1740, 1685	$C_{23}H_{27}N_3O_8S_3 \cdot 1/2H_2O$	50.54	5.16	7.69	50.79	5.03	7.65
7	55	1785, 1755, 1705, 1680	$C_{20}H_{24}N_8O_6S_3 \cdot 1/2H_2O$	41.59	4.36	19.40	41.86	4.12	19.13
8	68	1780, 1735, 1705, 1660	$C_{19}H_{22}N_8O_6S_3 \cdot 1/5C_6H_{14}O$	42.18	4.36	19.49	41.93	4.28	19.27
9	54	1795, 1775, 1750, 1660	$C_{22}H_{26}N_2O_8S_2$	51.74	5.13	5.49	51.61	5.26	5.33
10	50	1785, 1740, 1690	$C_{24}H_{28}N_6O_7S_2$	49.99	4.89	14.57	49.83	4.84	14.24

TABLE 3

NMR DATA OF THE PIVALOYLOXYMETHYL ESTERS OF PARENTERAL CEPHALOSPORINS

Ester no.	NMR (in d_6-DMSO) [a] δ value
1	1.26(s, 9H, $CH_3 \times 3$), 3.16(s, 6H, $N(CH_3)_2$), 3.7–4.1(m, 4H, CH_2CO and C_2-CH_2), 3.93(t, J = 6Hz, 2H, NCH_2), 4.26 and 4.44(ABq, J = 13.5Hz, 2H, C_3-CH_2), 5.00(t, J = 6Hz, 2H, NCH_2), 5.23(d, J = 4.5Hz, 1H, C_6-H), 5.74(d, J = 4.5Hz, 1H, C_7-H), 5.81 and 6.00(ABq, J = 6Hz, 2H, OCH_2O), 6.77(s, 1H, thiazole-H)
2	1.15(s, 9H, $CH_3 \times 3$), 3.36(s, 2H, CH_2CO), 3.57 and 3.84(ABq, J = 18.5Hz, 2H, C_2-CH_2), 3.90(s, 3H, NCH_3), 4.15 and 4.43(ABq, J = 13.5Hz, 2H, C_3-CH_2), 5.07(d, J = 4.5Hz, 1H, C_6-H), 5.60–5.98(m, 3H, OCH_2O and C_7-H), 6.70(s, 1H, thiazole-H), 6.80(b-s, 2H, NH_2), 8.83(d, J = 8.7Hz, 1H, CONH)
3	1.16(s, 9H, $CH_3 \times 3$), 2.02(s, 3H, CH_3CO), 3.46 and 3.68(ABq, J = 18Hz, 2H, C_2-CH_2), 3.83(s, 3H, OCH_3), 4.65 and 4.92(ABq, J = 13.5Hz, 2H, C_3-CH_2), 5.15(d, J = 4.5Hz, 1H, C_6-H), 5.68–5.98(m, 3H, OCH_2O and C_7-H), 6.71(s, 1H, thiazole-H), 6.8–7.6(b-s, 2H, NH_2), 9.55(d, J = 8.7Hz, 1H, CONH)
4	1.16(s, 9H, $CH_3 \times 3$), 3.4–3.8(m, 2H, C_2-CH_2), 3.84(s, 3H, OCH_3), 5.10(d, J = 4.5Hz, 1H, C_6-H), 5.7–5.95(m, 3H, OCH_2O and C_7-H), 6.61(t, J = 4.5Hz, C_3-H), 6.71(s, 1H, thiazole-H), 6.8–7.5(b-s, 2H, NH_2), 9.56(d, J = 9Hz, 1H, CONH)
5	1.18(s, 9H, $CH_3 \times 3$), 3.4–4.0(m, 2H, C_2-CH_2), 3.84(s, 3H, OCH_3), 3.92(s, 3H, NCH_3), 4.16 and 4.45(ABq, J = 13.5Hz, 2H, C_3-CH_2), 5.12(d, J = 4.5Hz, 1H, C_6-H), 5.62–6.0(m, 3H, OCH_2O and C_7-H), 6.69(s, 1H, thiazole-H), 7.10(b-s, 2H, NH_2), 9.51(d, J = 9Hz, 1H, CONH)
6	1.16(s, 9H, $CH_3 \times 3$), 2.02(s, 3H, CH_3CO), 3.58(b-s, 2H, C_2-CH_2), 3.85(s, 2H, SCH_2CO), 4.67 and 4.93(ABq, J = 13.5Hz, 2H, C_3-CH_2), 5.10(d, J = 5Hz, 1H, C_6-H), 5.57–5.97(m, 3H, OCH_2O and C_7-H), 7.08–7.44(m, 2H, pyridine-H), 9.23(d, J = 9Hz, 1H, CONH)
7	1.15(s, 9H, $CH_3 \times 3$), 2.68(s, 3H, CH_3), 3.60 and 3.87(ABq, J = 18.5Hz, 2H, C_2-CH_2), 4.16 and 4.93(ABq, J = 13.5Hz, 2H, C_3-CH_2), 5.13(d, J = 5Hz, 1H, C_6-H), 5.34(s, 2H, NCH_2), 5.65–6.02(m, 3H, OCH_2O and C_7-H), 9.31(s, 1H, tetrazole-H), 9.44(d, J = 9Hz, 1H, CONH)
8	1.14(s, 9H, $CH_3 \times 3$), 3.63 and 3.87(ABq, J = 18Hz, 2H, C_2-CH_2), 4.21 and 4.62 (ABq, J = 13.5Hz, 2H, C_3-CH_2), 5.13(d, J = 5Hz, 1H, C_6-H), 5.34(s, 2H, NCH_2), 5.57–6.08(m, 3H, OCH_2O and C_7-H), 9.32(s, 1H, tetrazole-H), 9.49(d, J = 9Hz, 1H, CONH), 9.53(s, 1H, thiadiazole-H)
9	1.14(s, 9H, $CH_3 \times 3$), 2.01(s, 3H, CH_3CO), 3.46 and 3.69(ABq, J = 18Hz, 2H, C_2-CH_2), 3.73(s, 2H, CH_2CO), 4.64 and 4.93(ABq, J = 13.2Hz, 2H, C_3-CH_2), 5.10(d, J = 4.5Hz, 1H, C_6-H), 5.58–5.97(m, 3H, OCH_2O and C_7-H), 6.82–7.02(m, 2H, thiophen-H), 7.19–7.40(m, 1H, thiophen-H), 9.08(d, J = 9Hz, 1H, CONH)
10	1.15(s, 3H, $CH_3 \times 3$), 3.23(s, 1H, OH), 3.57 and 3.97(ABq, J = 18Hz, 2H, C_2-CH_2), 3.90(s, 3H, NCH_3), 4.14 and 4.42(ABq, J = 13.5Hz, 2H, C_3-CH_2), 5.03(s, 1H, CH), 5.07(d, J = 5Hz, 1H, C_6-H), 5.5–6.2(m, 3H, OCH_2O and C_7-H), 7.17–7.6(m, 5H, phenyl-H), 8.66(d, J = 9Hz, 1H, CONH)

[a] Ester '1' was measured in D_2O.

parent cephalosporins were administered subcutaneously or orally as the 1% aqueous solution at a dose of 100 mg/kg. The relative bioavailability was calculated from the areas under the plasma level–time curves after oral administration (AUC_{oral}) and after subcutaneous administration (AUC_{sc}).

Assay

The plasma concentration of the parent cephalosporin was measured by the cylinder-plate method using *B. subtilis* PCI 219 or *P. mirabillis* ATCC 21100 as the assay organism.

Results and Discussion

It is well known that the physicochemical properties of a drug, such as lipophilicity and water solubility, affect its absorption from the gastrointestinal tract (GI tract).

A prodrug, per se, is required to be hydrolyzed to the parent drug after absorption. In a previous report, we found that the hydrolysis rate was also an important factor in the gastrointestinal absorption of a cephalosporin ester (Yoshimura et al., submitted).

Thus, we measured the partition coefficients, water solubilities and hydrolysis rates of the esters, and attempted correlation of these factors with their bioavailability.

Partition coefficient

Table 4 shows the partition coefficient (P) of the undissociated form of the esters

TABLE 4

WATER SOLUBILITY, log P, AND HALF-LIFE OF HYDROLYSIS OF THE PRODRUG TO THE PARENT CEPHALOSPORINS IN VITRO

Ester no.	Water solubility [a] (mg/100 ml)	log P	Half-life [b] $t_{1/2}$ (min)
1	271	1.57	22.6
2	157	1.36	3.1
3	10.7	2.62	7.9
4	22.2	2.42	4.8
5	4.9	2.40	4.3
6	43.8	1.63	4.7
7	14.2	1.34	12.4
8	30.3	1.45	6.9
9	2.0	1.57	5.3
10	2.0	0.85	7.9

[a] At pH 4.5.

[b] In 1% homogenate of mouse small intestine at 37°C at a concentration of 10 μg/ml equivalent of the parent drug.

TABLE 5

PLASMA LEVELS OF THE PARENT CEPHALOSPORIN, THE AREA UNDER PLASMA PARENT CEPHALOSPORIN LEVELS–TIME CURVE AFTER ORAL ADMINISTRATION, AND BIOAVAILABILITY IN MICE

Compound	Dosing route	Plasma levels (μg/ml)				AUC_0^{2h} (h·μg/ml)	Bioavailability [a] (%)
		0.25	0.5	1	2 h		
CTM	s.c.	69.2 (6.1) *	29.0 (1.3)	13.2 (1.5)	1.5 (0.6)	38.8	100.0
	p.o.	2.3 (0.4)	2.8 (0.1)	1.1 (0.1)	0.0	2.45	6.3
Ester 1	p.o.	21.0 (1.3)	16.2 (3.1)	6.1 (0.5)	0.6 (0.1)	16.2	41.8
SCE-785	s.c.	129.0 (7 7)	114.0 (2.0)	6.3 (1.1)	0.0	79.7	100.0
	p.o	1.5 (0.3)	1.2 (0.2)	0.5 (0.1)	0.5 (0.3)	1.45	1.8
Ester 2	p.o.	28.5 (1.4)	15.6 (2.5)	7.7 (2.2)	2.3 (1.2)	19.9	25.0
CTX	s.c.	183.1 (7.3)	117.0 (1.7)	15.4 (1.6)	0.0	101.2	100.0
	p.o.	2.1 (0.3)	2.2 (0.6)	1.5 (0.3)	0.5 (0.2)	2.73	2.7
Ester 3	p.o.	8.5 (0.3)	6.7 (0.4)	2.7 (0.5)	0.0	6.66	6.6
CZX	s.c.	117.3 (10.1)	51.0 (7.4)	11.7 (0.3)	1.5 (0.3)	57.9	100.0
	p.o.	3.2 (0.8)	3.9 (1.5)	1.5 (0.0)	1.1 (0.5)	3.94	6.8
Ester 4	p.o.	13.7 (1.9)	10.0 (1.0)	2.8 (0.6)	0.7 (0.0)	9.63	16.6
CMX	s.c.	240.0 (30.0)	113.0 (18.6)	66.0 (13.3)	6.2 (0.7)	155.0	100.0
	p.o.	2.6 (0.3)	2.1 (0.2)	2.2 (0.2)	0.3 (0.2)	3.24	2.1
Ester 5	p.o.	6.1 (1.6)	11.8 (1.6)	3.6 (1.3)	5.7 (2.0)	11.5	7.4
CEPR	s.c.	62.0 (7.6)	13.2 (5.4)	0.4 (0.2)	0.1 (0.0)	20.8	100.0
	p.o.	0.9 (0.2)	0.3 (0.1)	0.2 (0.0)	0.2 (0.0)	0.59	2.8
Ester 6	p.o.	8.7 (1.6)	2.6 (0.4)	0.2 (0.1)	0.1 (0.0)	3.35	16.1
CEZ	s.c.	185.0 (24.7)	173.0 (10.1)	39.4 (4.2)	7.9 (0.3)	144.6	100.0
	p.o.	2.7 (0.1)	2.5 (0.1)	2.5 (0.1)	1.9 (0.1)	4.44	3.1
Ester 7	p.o.	7.4 (0.4)	6.5 (0.4)	5.9 (0.3)	2.8 (0.1)	10.1	7.0

(Table continued on following page)

TABLE 5 (continued)

Compound	Dosing route	Plasma levels (μg/ml)				AUC_0^{2h} (h·μg/ml)	Bioavailability [a] (%)
		0.25	0.5	1	2 h		
CTZ	s.c.	133.8	89.5	6.8	2.2	73.2	100.0
		(8.8)	(6.2)	(0.9)	(0.0)		
	p.o.	3.0	3.2	3.0	0.0	4.20	5.7
		(0.5)	(0.9)	(0.2)			
Ester 8	p.o.	11.1	9.5	4.3	1.8	10.5	14.3
		(0.6)	(1.4)	(0.8)	(0.0)		
CET	s.c.	83.3	13.2	0.8	0.4	26.6	100.0
		(6.4)	(1.2)	(1.2)	(0.4)		
	p.o.	1.7	0.3	0.4	0.0	0.83	3.1
		(0.4)	(0.1)	(0.1)			
Ester 9	p.o.	1.8	1.6	0.3	0.0	1.28	4.8
		(0.2)	(0.3)	(0.3)			
CMD	s.c.	115.0	47.8	7.1	0.9	52.5	100.0
		(7.6)	(12.3)	(1.4)	(0.2)		
	p.o.	3.3	3.6	1.4	0.9	3.70	7.1
		(0.5)	(0.4)	(0.4)	(0.20)		
Ester 10	p.o.	0.9	0.9	0.9	0.7	1.59	3.0
		(0.0)	(0.0)	(0.0)	0.0)		

[a] Bioavailability (%) = $100 \times (AUC_{p.o.}/AUC_{s.c.})$.
* ( ) = S.E., n = 4; Dose 100 mg/kg equivalent of the parent cephalosporin.

'1–10' between 1-octanol and water. The log P values of the esters are between 0.85 and 2.62, indicating that lipophilicity is improved by esterification of the parenteral cephalosporins. Lien (1975) found that optimal log P values resulting in good absorption from the intestine of rat were about 2 for several series of drugs.

Water solubility

The water solubility of the esters of cephalosporins at pH 4.5 are shown in Table 4. '1', having two basic functional groups (i.e., N,N-dimethylaminoethyl and 2-aminothiazolyl), showed the highest water solubility, 271 mg/100 ml; it was followed by '2', which has a 2-aminothiazole group, with a solubility of 157 mg/100 ml. The solubilities of the other esters '3–10' were less than 50 mg/100 ml.

Hydrolysis to parent cephalosporins

Table 4 also shows the half-lives of the esters '1–10' in 1% small intestine homogenate of mice at 37°C. The hydrolyses from the esters to the parent cephalosporins followed pseudo-first order kinetics with half-lives of less than 30 min.

Oral absorption studies

The plasma level of the parent cephalosporin and the area under the plasma level–time curve (AUC) for 0–2 h after oral administration of the esters or the parent cephalosporins at a dose of 100 mg/kg equivalent to the parent cepha-

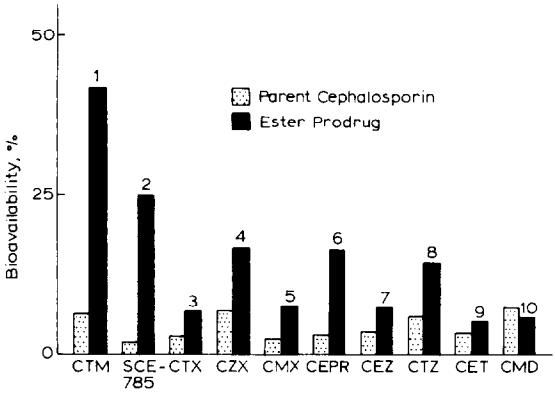


Fig. 1. Relative bioavailability after oral administration of the parenteral cephalosporins or their pivaloyloxymethyl esters in mice at a dose equivalent to 100 mg/kg of the corresponding cephalosporins.

losporin were measured in mice with the subcutaneous administration of the parent cephalosporins as references.

As shown in Table 5, the relative bioavailability of the parent cephalosporins after oral administration was between 1.8 and 7.1%, and most esters, except for '10', showed improved oral bioavailability than those of the parent cephalosporins (Fig. 1). Among them, the ester of CTM '1' showed the best oral bioavailability, 41.8%, followed by '2', 25.0%. The other esters had BAs less than 20%.

Correlation of the physicochemical properties with the bioavailability

When the log P value was plotted against log bioavailability (BA), a significant relationship was not observed between them using a least-squares analysis (Fig. 2), due probably to the sufficient lipophilicity of each ester.

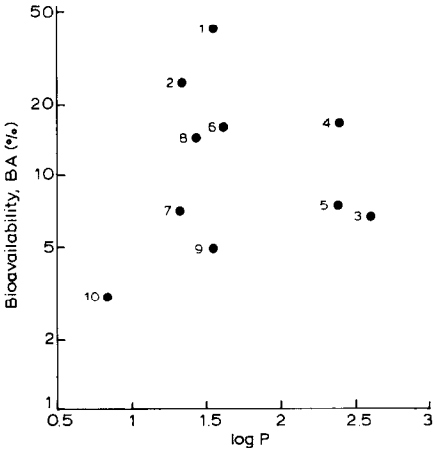


Fig. 2. Relation between log P and relative bioavailability of pivaloyloxymethyl esters of cephalosporins in mice.

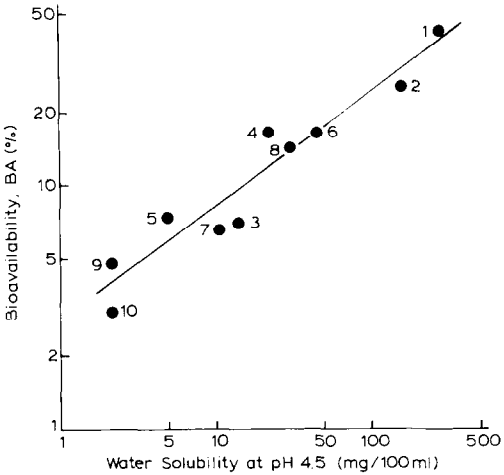


Fig. 3. Relation between water solubility (S) and relative bioavailability (BA) of pivaloyloxymethyl esters of cephalosporins in mice.

Next, the relation between in vitro hydrolysis rates of the esters and BA was examined by least-squares analysis, but no significant relation was obtained. The hydrolysis rate was not a critical factor for the oral absorption of the esters '1–10' as these esters were hydrolyzed rapidly to the parent cephalosporins.

$$\log \mathrm{BA}(\%) = 0.4641 \log(\mathrm{S}) + 0.446 \quad (1)$$

Then, the water solubility (S) of the esters was plotted against BA (Fig. 3) and a good linear relation was found and is expressed as Eqn. 1 through a least-squares analysis. The solubility of the esters contributes significantly to the BA of the parent cephalosporins.

$n = 10$, $r = 0.958$, $s = 0.106$, $F_{1,8} = 89.3$ ($F_{1,8;\alpha=0.005} = 14.7$) where n = number of studies, r = correlation coefficient, s = standard deviation, F = F-statistic.

Among the esters, the ester of CTM, '1' with the highest S value showed the best BA of 41.8%. The protonation of the dimethylamino group (pK_a 7.0) at pH 4.5, near the functional pH of small intestine (virtual pH) (Hogben et al., 1959), must contribute to the good water solubility and BA of '1'. It is interesting to note that orally active prodrugs of penicillins, such as bacampicillin and pivmecillinam, also have basic functional groups of a pK_a value between 6.9 and 8.1 (Fig. 4) (Hattori et al., 1976; Itakura et al., 1978; Tsuji and Yamana, 1981).

Good absorption of a prodrug from the GI tract appears to require the function of 3 processes: (1) dissolution of the prodrug in the GI fluid; (2) transportation across the GI membrane; and (3) hydrolysis of the prodrug to the parent drug (Yalkowsky and Morozowich, 1980). In the present study, pivaloyloxymethylation of the parenteral cephalosporins increased the lipophilicity sufficiently, keeping the hydrolysis rate high. Thus, the dissolution of the ester, the first step of the absorption, affected the BA.

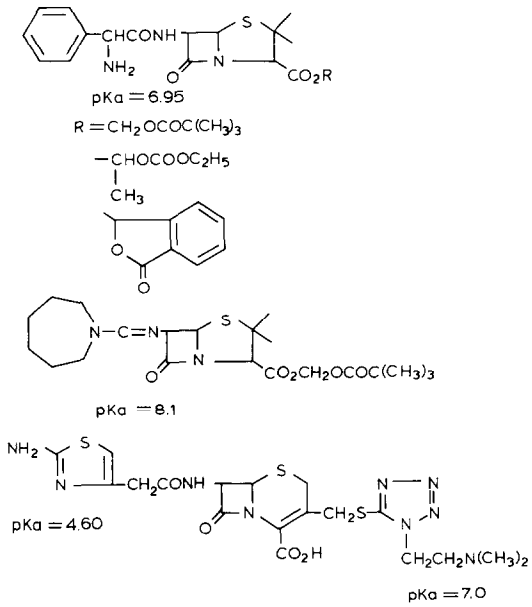


Fig. 4. Orally active prodrugs of β-lactam antibiotics.

In conclusion, it seems reasonable to propose that water solubility must be considered primarily in designing an orally active ester prodrug of a parenteral cephalosporin if the lipophilicity is improved enough by esterification and the hydrolysis rate is rapid.

Acknowledgements

We are grateful to Drs. K. Morita, H. Mima, M. Fujino, T. Shimamoto, N. Kakeya and M. Numata for their encouragement throughout this work.

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