ORALLY ACTIVE ESTERS OF CEPHALOSPORIN ANTIBIOTICS. II

SYNTHESIS AND BIOLOGICAL PROPERTIES OF THE ACETOXYMETHYL ESTER OF CEFAMANDOLE

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The synthesis of the acetoxymethyl (AOM) ester of cefamandole (CM) is described. The sparingly soluble ester is shown to be well absorbed orally by mice, but only when administered in solution in a partially non-aqueous vehicle, 50% propylene glycol. Neither the ester in aqueous suspension nor the sodium salt of CM in solution is well absorbed orally. The rate of oral absorption of the ester from solution is very rapid as shown by the early peak time and shape of the plasma level curve. Oral bioavailability from solution is at least 60% and is apparently limited only by hydrolysis or precipitation of a variable portion of the ester dose in the intestinal lumen prior to absorption.

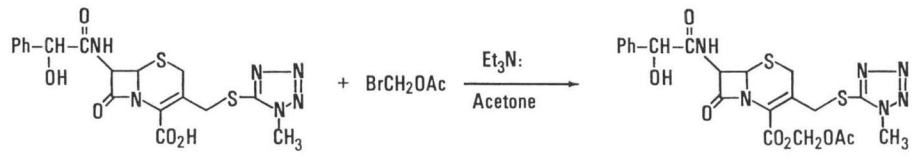
Esterification of the carboxyl group of penicillins and cephalosporins can result in significant improvement in the oral absorption of the parent drug. Pivampicillin[1], bacampicillin[2] and talampicillin[3] are examples of such successful penicillin derivatives, and the acetoxymethyl ester of cephaloglycin[4] and other cephalosporin derivatives[5] illustrate the applicability of this pro-drug principle to cephalosporins. Although the intact esters are biologically inactive, they can be hydrolyzed to the active parent drug either during passage through the intestinal wall or after absorption has occurred[6].

Cefamandole nafate is a cephalosporin recently introduced into clinical use as a parenteral broad-spectrum antibiotic. Since free cefamandole is not absorbed well enough to be clinically useful as an oral preparation, we have prepared a 4-carboxyl acetoxymethyl ester pro-drug in an attempt to improve oral efficacy. This report describes the efficient oral absorption of such a cefamandole ester but only when presented to the G.I. tract in solution in a partially nonaqueous vehicle.

Chemistry

Cefamandole (**1**) was converted to the acetoxymethyl ester **2** by reaction of its triethylammonium salt with bromomethyl acetate in acetone. In order to circumvent formation of the Δ^2-ester as a by-product, cefamandole and an excess of bromomethyl acetate were mixed together in acetone, and a dilute acetone solution of triethylamine was added very slowly over a 2-hour period. In this manner the triethylammonium salt of cefamandole was consumed as it was formed. BENTLEY *et al.* recently attributed the isomerization of Δ^3-cephalosporin esters during their formation to the carboxylate anion acting as a base promoting loss of a proton at C-2 thereby facilitating isomerization[9]. The ester **2** was isolated and crystallized from either methanol or chloroform.

Scheme 1.



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Materials and Methods

Dose Solutions

Dose solutions of sodium cefamandole were prepared in saline at 2 mg/ml for subcutaneous or oral administration. In addition, dose solutions of sodium cefamandole for oral use were prepared in 50% propylene glycol - water. Since the acetoxymethyl ester of cefamandole is only sparingly soluble in water, dose solutions were prepared by first dissolving the ester in propylene glycol at about 50°C, and then diluting with an equal volume of warm saline to produce a final dose concentration of 2 mg/ml. Suspensions of the acetoxymethyl ester at 2 mg/ml were prepared for oral dosing in 10% acacia. In all cases the compounds were administered at 15 mg/kg on an equal weight basis.

Biological Assay

Biological activity was determined by a conventional paper disk-agar plate procedure using *Micrococcus luteus* ATCC 9341 as the test organism. In experiments with the sodium salt, standard curves were prepared from aliquots of the dose solution either in saline, for the assay of urine and intestinal washes, or in fresh mouse plasma diluted with an equal volume of water for assay of plasma. Since the ester is biologically inactive until hydrolyzed, solutions used for construction of the standard curves of the ester were subjected to a hydrolysis step to release the parent compound cefamandole. An aliquot of the ester dose diluted to 10 μg/ml was treated for 15 minutes at room temperature with 1 mg/ml of a preparation of freeze-dried hamster intestinal villi[8]. Serial dilutions for the standard curve were then prepared from this incubation solution using the appropriate diluent containing 1 mg/ml of the villi. These conditions were found to result in complete hydrolysis of the ester to free cefamandole and permitted the use of the ester as its own standard.

Chromatography of the Ester

In addition to the physical methods used to establish the purity of the acetoxymethyl ester of cefamandole (see Experimental Section) the ester was dissolved in acetone and chromatographed on Whatman No. 1 paper buffered with 0.1 molar sodium acetate, pH 4.6, using a solvent system of 2-butanone - water at 92: 8. This system separated the ester from free cefamandole, the former running near the front and the latter remaining near the origin. Standard solutions of sodium cefamandole and ester were applied at a series of concentrations, chromatographed, and plated on *Bacillus subtilis* ATCC 6633. Visual comparison of the zone sizes of the cefamandole standards and the zones of free cefamandole remaining near the origin in the ester lanes established that the level of free cefamandole in the sample of ester used in these experiments was not greater than 2.0%.

Distribution Ratios

Ratios for the distribution of free cefamandole and the AOM ester between chloroform and 0.1 M pH 7.0 sodium phosphate buffer were determined by repeated equilibration between equal volumes of solvents at 0.125 mg/ml of combined phase volume. Chloroform samples were evaporated and redissolved in buffer for microbiological assay; buffer samples were assayed without evaporation. Ester samples were treated with villi as described in the section on Biological Assay.

Animal Preparation, Housing and Processing

Cox Male Standard Mice Lai:COX(Standard)BR were housed overnight in a wire bottom cage with free access to water and a liquid diet[5]. The purpose of the liquid diet was to provide nutritional

intake and to avoid coprophagy. The result was a G.I. tract free of solids, yet without the nutritional shock and coprophagy that often accompanies overnight starvation of rodents. After dosing, the mice were housed individually in ventilated wide mouth glass jars containing a layer of saline beneath an elevated wire screen floor. At intervals after dosing, mice were removed from the jars and heparinized blood, stomach, small intestine and urinary bladder were removed for assay of antibiotic activity. Plasma was separated from the blood samples and diluted with an equal volume of water for assay against a standard curve of identical composition. This procedure is described in more detail in a previous publication[5].

Results and Discussion

Although a reduction in aqueous solubility was expected due to esterification, the degree to which such a reduction would affect rate of solution and oral absorption could not be determined without *in vivo* testing. Thus, the purpose of varying the dose routes and vehicles was to test the effect of dosing the relatively water insoluble ester either in aqueous suspension or in solution in a partially nonaqueous solvent. The short half-life of cefamandole in mice made the running of experiments beyond one hour unnecessary.

Table 1. Plasma levels of cefamandole after dosage of sodium cefamandole or the AOM ester to mice.

Compound	Dose route	Vehicle	μg/ml at intervals after dose of 15 mg/kg			
			5 min.	20 min.	40 min.	60 min.
Na CM	i.v.	Saline	34.4 (1.4)*	12.7 (1.5)	1.7 (0.4)	0.8
Na CM	s.c.	Saline	20.3 (3.5)	18.5 (1.9)	3.9 (0.7)	1.1 (0.2)
Na CM	Oral	Saline	<0.9	<0.9	<0.9	<0.9
Na CM	Oral	50% Prop. glycol	<0.9	<0.9	<0.9	<0.9
AOM Ester	Oral	10% Acacia (suspension)	<0.9	<0.9	<0.9	<0.9
AOM Ester	Oral	50% Prop. glycol	15.9 (4.3)	8.2 (1.3)	3.2 (0.8)	0.84

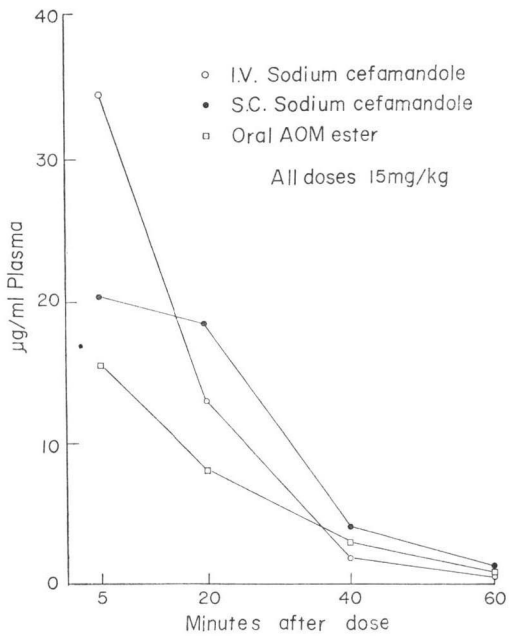
* ( )=±S.E.M.; N=4

Plasma Levels

Sodium cefamandole when dosed intravenously or subcutaneously in normal saline gave early and high plasma levels but dropped to near the minimum detection level within one hour (Table 1 and Fig. 1). When dosed orally in the same vehicle, the oral absorption of cefamandole was not efficient enough to result in detectable plasma levels at any time point up to one hour.

When the sparingly soluble cefamandole ester was dosed orally in 10% acacia suspension, no detectable levels were reached in the plasma in one hour. However, when the ester was dissolved in 50% propylene glycol and dosed orally in solution, very early and high plasma levels resulted and were followed by a rapid decline similar to that seen with parenteral cefa-

Fig. 1. Plasma levels of cefamandole after administration of sodium cefamandole or the AOM ester to mice.



mandole (Table 1). The shape of the plasma curve after the oral ester, *i.e.* the early peak followed by a rapid decline, is more typical of an intravenous bolus dose than an oral dose (Fig. 1). We interpret this behavior to represent very rapid oral absorption of the intact ester for a relatively short time followed by relatively little oral absorption for the remainder of the experimental period. Examination of the contents of the intestinal tract at intervals after dosage supports this interpretation. (See the section on G.I. tract recovery). Competition between the events of absorption prior to hydrolysis and hydrolysis prior to absorption may be occurring. The results could also be explained by a competition between absorption prior to precipitation of the dose from the vehicle and precipitation prior to absorption.

In order to determine if the propylene glycol vehicle was improving oral absorption of the ester by a direct action on the intestinal wall, 50% propylene glycol was also used as an oral vehicle for sodium cefamandole. No plasma levels could be detected either with this vehicle or with saline (Table 1). Thus, we conclude that the vehicle increases oral absorption through solubilization of the sparingly water soluble ester and not by a direct effect on the intestinal wall.

Urinary Excretion

The excretion of cefamandole in the urine expressed as percent of dose, is shown in Table 2. After intravenous or subcutaneous dosage of the sodium salt, excretion into the urine is rapid and essentially complete in one hour. More than half of the administered biological activity can be recovered in the urine. When either the sodium salt in solution or the ester in suspension is administered orally, less than 5% of the dose appears in the urine in one hour. On the other hand, oral administration of the ester in solution results in the excretion of about one-third of the dose in the urine in one hour. Compared with parenteral dosage, this represents a bioavailability of about 60% *via* the oral route. The urinary excretion data reported in Table 2 are consistent with the plasma levels reported in Table 1 of the preceding section.

Table 2. Urinary excretion of cefamandole after dosage of sodium cefamandole or the AOM ester to mice.

Compound	Dose route	Vehicle	% of Dose in the urine at intervals after dose of 15 mg/kg			
			5 min.	20 min.	40 min.	60 min.
Na CM	i.v.	Saline	11.8 (1.3)*	47.8 (3.3)	58.5 (6.1)	53.9 (5.0)
Na CM	s.c.	Saline	2.8 (0.3)	24.8 (1.5)	49.3 (5.6)	56.4 (5.1)
Na CM	Oral	50% Prop. glycol	<0.9	<0.9	<0.9	2.0 (0.4)
AOM Ester	Oral	10% Acacia (suspension)	1.5	1.5	2.2 (0.3)	4.2 (0.3)
AOM Ester	Oral	50% Prop. glycol	1.2	15.0 (1.3)	22.2 (2.6)	34.3 (10.1)

* ( )=±S.E.M.; N=4.

In a supplementary experiment intended to assure that the urinary excretion at one hour adequately reflects the comparative performance of the oral ester and the subcutaneous sodium salt, the urinary excretion by separate groups of four mice were monitored out to two hours. The percent of dose excreted in two hours was 55.2% for the subcutaneous sodium salt and 39.0% for the oral ester. These values are consistent with the one hour values reported in Table 2 for these two preparations, and represent an oral bioavailability of the ester of 70% when compared with subcutaneous dosage of the sodium salt at two hours.

G.I. Tract

The fraction of the dose found in the combined stomach and small intestine after oral or parenteral dosage is shown in Table 3. Seven to 10% of a subcutaneous dose of sodium cefamandole appears in the intestinal lumen *via* biliary excretion. When cefamandole is dosed orally either as a sodium cefamandole solution or as an ester suspension, most of the dose remains in the lumen of the G.I. tract. However, when the ester is dosed in solution in 50% propylene glycol, a substantial fraction of the dose leaves the G.I. tract, mostly in the first few minutes after dosage. This behavior is consistent with the plasma level data reported in Table 1 and the urinary excretion data reported in Table 2.

Table 3. Recovery of cefamandole from the G.I. tract after dosage of sodium cefamandole or the AOM ester to mice.

Compound	Dose route	Vehicle	% of Dose in the G.I. tract at intervals after dose of 15 mg/kg			
			5 min.	20 min.	40 min.	60 min.
Na CM	s.c.	Saline	1.25 (0.03)*	5.9 (0.05)	9.8 (2.4)	6.7 (1.3)
Na CM	Oral	50% Prop. glycol	77.8 (5.5)	76.0 (3.3)	78.5 (4.3)	71.3 (4.0)
AOM Ester	Oral	10% Acacia (suspension)	69.2 (4.9)	71.2 (4.5)	83.6 (2.0)	75.8
AOM Ester	Oral	50% Prop.	54.2 (5.8)	36.5 (3.5)	30.1 (4.5)	22.4 (2.8)

* ( )=±S.E.M.; N=4.

In a separate set of *in vitro* control experiments, doses of sodium cefamandole were introduced into the lumen of the excised intestine of mice, incubated for either 5 or 60 minutes at 37.5°C in saline, and assayed. Under these conditions, 14% of the dose at 5 minutes and 19% of the dose at 60 minutes could not be recovered, probably due to interference by tissue and contents and decomposition. Thus, we feel that the fraction of dose that could not be recovered from the G.I. tract after oral sodium cefamandole (Table 3) probably represents losses due more to method rather than absorption.

Distribution Ratios

As a result of masking the strongly acidic 4-carboxyl group by esterification, the distribution ratio (chloroform/pH 7.0 buffer) is changed from less than 0.0008 for free cefamandole to greater than 113 for the AOM ester. This favorable change in lipid solubility occurs at the expense of aqueous solubility (less than 1 μg/ml for the AOM ester in pH 7.0 buffer). The aqueous solubility of the ester is so low that it offsets the advantages of increased lipid solubility and lack of ionization unless the ester is presented to the intestine in solution in a partially non-aqueous solvent. All of the data from Tables 1, 2 and 3 comparing the suspension and solution of the ester and the solution of the sodium salt are consistent with these changes in physical properties.

Conclusions

The sparingly soluble acetoxymethyl ester of cefamandol is rapidly and efficiently absorbed orally by mice but only when administered in solution in a partially non-aqueous vehicle. Neither the ester in aqueous suspension nor the sodium salt of cefamandole in solution is well absorbed. The efficient oral absorption of the ester can be demonstrated not only by plasma level comparisons with parenteral dosage, but also by disappearance from the gastrointestinal tract and appearance in the urine. Comparisons of urinary excretion after oral and parenteral dosage indicate an oral bioavailability of at least 60% for the ester dosed in solution. Limitations on bioavailability could be due to hydrolysis of the ester or to precipitation of a portion of the ester dose, with either or both of these events occurring in the gastrointestinal tract prior to absorption. Although the present experiments illustrate that the

oral absorption of the AOM ester of cefamandole is very efficient when the ester is dosed in solution, a practical oral dosage form would require a higher concentration of the ester in solution than that which can be achieved with this vehicle.

Experimental Section

All melting points are uncorrected. The NMR spectrum was obtained on a Varian Associates T-60 spectrometer. The elemental analysis was performed by the microanalytical group of the Lilly Research Laboratories. The bromomethyl acetate was prepared by the method of ULICH and ADAMS[9] and the cefamandole by the method of RYAN[10].

7-(D-Mandelamido)-3-[[1-methyl-1H-tetrazol-5-yl)-thio]methyl]-3-cephem-4-carboxylic acid, Acetoxymethyl Ester (**2**)

A mixture of **1** (18 g, 39 mmol) and bromomethyl acetate (10 ml, 100 mmol) was dissolved in acetone (100 ml), stirred, and treated dropwise with triethylamine (3.94 g, 5.4 ml, 39 mmol) in 10 ml of acetone. The triethylamine was added slowly over a 2-hour period. After the addition was complete stirring was continued for an additional 1 hour. The triethylamine hydrobromide (4.4 g, 62%) was collected by filtration. The filtrate was evaporated *in vacuo* and the residue was dissolved in EtOAc and washed twice each with water, 1 N HCl, 10% aqueous $NaHCO_3$, and finally saturated aqueous NaCl. The EtOAc solution was dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. The residue was crystallized from $CHCl_3$ to yield 7.0 g (34%) of **2** as a white crystalline solid: m.p. 126~130°C (decomp.), NMR ($CDCl_3$/DMSO/d_6), δ 2.15 (s, 3H), 2.97 (s, 1H, removed by D_2O), 3.71 (s, 2H), 3.97 (s, 3H), 4.08 (d, 1H, J=7Hz), 4.52 (d, 1H, J=7Hz), 5.01 (d, 1H, J=3Hz), 5.18 (s, 1H), 5.8 (m, 3H), 7.4 (m, 5H), and 8.25 ppm (d, 1H, J=5 Hz).

Anal. Calcd. for $C_{21}H_{22}N_6O_7S_2$: C 47.18, H 4.15, N 15.72, S 12.0
C 47.03, H 4.11, N 15.45, S 12.05.

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