CEFUROXIME—A NEW CEPHALOSPORIN ANTIBIOTIC

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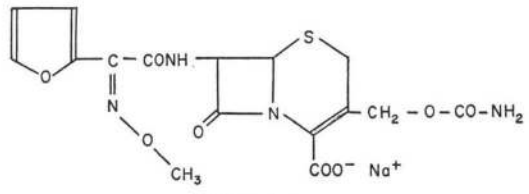
Cefuroxime is a new broad spectrum cephalosporin antibiotic for administration by injection. It is stable to most β-lactamases. It is active against gram-positive organisms, including penicillinase-producing staphylococci, and has wide activity against gram-negative bacilli including *Enterobacter* and many strains of indole-positive *Proteus* spp. The substance is also highly active against *Haemophilus influenzae* and *Neisseria gonorrhoeae*. Studies on human volunteers showed that it produced high, long-lasting blood levels with virtually complete recovery of unchanged antibiotic in the urine. No evidence of toxicity due to cefuroxime was found. Slight, short-lived pain followed intramuscular injection, and the compound was well tolerated intravenously.

Since 1964, cephalosporin antibiotics have proved to be highly effective in the treatment of a wide range of bacterial infections. All those at present in clinical use have limitations in their antibacterial spectra which, with gram-negative bacilli, can often be attributed to destruction by β-lactamases. Few are very active against *Neisseria* spp. or *Haemophilus influenzae*. Deficiencies of a pharmacological nature are also encountered among this group; these include high binding to serum proteins, low serum concentrations in man, very rapid excretion, metabolic instability and more severe pain on intramuscular injection.

Cefuroxime [(6R, 7R)-3-carbamoyloxy-methyl-7-[(2Z)-2-methoxyimino (fur-2-yl) acetamido]-ceph-3-em-4-carboxylic acid] is a new semisynthetic cephalosporin analogue which overcomes many of these disadvantages. The sodium salt was used in all of the experiments described here; its structure is given in Fig. 1.

Fig. 1. Structural formula of cefuroxime, sodium salt



Cefuroxime

Physical properties and stability: Cefuroxime sodium is a creamy white crystalline solid with a molecular weight of 446.4. It is approximately 20% soluble in water; 500 mg will dissolve in 2.5 ml water for injections to give 2.9 ml solution with a pH of 7.0. Solutions of cefuroxime are stable at room temperature for 12 hours; less than 10% decomposition occurs in 48 hours at 25°C. The substance is stable as a solid.

Toxicology: Extensive tests on mice, rats, rabbits and dogs have revealed no adverse effects. These results will be reported in detail elsewhere. In particular, no adverse effects were found on the kidney or liver in rabbits given repeated doses of cefuroxime, even at high levels. There was no evidence of sensitisation in the guinea pig. It was therefore considered safe to proceed to studies on human volunteers.

Experimental Methods

Minimum inhibitory concentration determinations

Minimum inhibitory concentrations (MICs) were determined by an agar dilution technique, in parallel tests with cephalothin. Serial two-fold dilutions of freshly prepared standard antibiotic solutions were made into nutrient agar with or without added enrichment and poured into petri dishes. Plates were inoculated with a replicate inoculating device (Denley Instruments Ltd., Bolney, Sussex) with inocula containing approximately 10 or 10^5 colony forming units. The MIC in micrograms/ml was read after 18 hours' incubation at 37°C as the lowest concentration which inhibited growth.

Bactericidal activity

Minimum bactericidal concentrations were determined from dilution tests in broth. All tubes with concentrations above the MIC were plated out on agar to detect any viable cells remaining after 18 hours' incubation.

Antibiotic assays

Concentrations of cefuroxime in body fluids were determined by microbiological assay. The medium was prepared by dissolving 5 g Oxoid peptone, 3 g Oxoid Lab Lemco, 10 g sodium citrate, 5 g sodium chloride and 12 g Bacto agar in distilled water to give 1 litre at pH 7.0 and seeded with *Bacillus subtilis* NCIB 8993. Standards (1.25, 0.625, 0.312 and 0.15 μg/ml) and samples were plated in a random design into holes punched in the agar so that each appeared four times on the plate. Standard solutions were prepared in pH 7.0 M/20 phosphate buffer. Sera and urines were diluted in the same buffer. Provided the serum samples were diluted at least 1 in 4, no interference with the assay was observed from the presence of serum protein.

Serum binding determinations

Serum samples were taken at 1 hour from volunteers who had received a 500 mg intramuscular injection. These samples were subjected to ultrafiltration, and the degree of binding of cefuroxime calculated from the amount of compound in the filtrate.

β-Lactamase preparation and assay

Crude β-lactamase preparations were obtained by subjecting overnight broth cultures of the bacterial strains used (10 $\times$ concentrated in 0.1 M phosphate buffer pH 7.0) to sonic disruption followed by centrifugation at 30,000 g for 30 minutes. The supernatant fluids constituted the crude enzyme preparations. With the exception of the *E. coli* and *K. aerogenes* strains, it was necessary to induce the production of a β-lactamase by adding 1,000μg benzyl penicillin/ml after 3 hours' incubation, and incubation was continued for a further 2 hours. Crude β-lactamase was then prepared as previously described. Susceptibility of the cephalosporins to the various β-lactamase preparations was determined by the method described by O'CALLAGHAN *et al.*[1] In this method, cephaloridine is used as the standard substrate. The enzyme concentration was adjusted so that in the standard experimental mixture of 3 ml enzyme plus cephaloridine solution (final concentration cephaloridine M/10,000 or 41.5μg/ml), the cephaloridine would be completely hydrolysed in 5~10 minutes. This concentration of enzyme was then used to test the stability of cefuroxime and cephalothin at equivalent concentration, *i. e.* M/10,000.

Human pharmacokinetics

Cefuroxime was given to 44 male human volunteers aged 19~57 years. Physical examination and laboratory tests before each study were undertaken to exclude volunteers with any abnormality. Subjects with a history of allergy were excluded. Sterile filtered aqueous solutions of sodium cefuroxime were prepared immediately before use, and the dose given calculated as the free acid. Thirty-three volunteers were given intramuscular injections deep into the lateral aspect of the thigh; nine volunteers received intravenous injections of sodium cefuroxime in 10 ml of aqueous solution over a period of 3 minutes. Two volunteers had 1 g

Table 1. Comparative *in vitro* activities of cefuroxime (CXM) and cephalothin (CET)

Organism	Number of strains	Compound	Number of strains with MIC (μg/ml)*						Geometric mean MIC
			<0.125	0.125 ~0.5	1~4	8~16	32~62	≧125	
Staph. aureus, methicillin sensitive	56	CXM	8	45	3				0.25
	56	CET	45	7	4				<0.125
Staph. aureus, methicillin resistant	25	CXM	3	2	2	12	6		5.9
	25	CET	1	4	16	4			2.0
Strep. pyogenes	7	CXM	7						<0.125
	7	CET	7						<0.125
Strep. pneumoniae	6	CXM	6						<0.125
	6	CET	6						<0.125
Strep. viridans	7	CXM	7						<0.125
	7	CET	4	3					<0.125
Strep. faecalis	6	CXM						6	>125
	6	CET				3	3		25
E. coli	129	CXM		7	108	12	2		2.9
	129	CET		4	48	63	12	2	7.0
Klebsiella spp.	73	CXM		1	12	24	13	3	8.2
	73	CET			18	22	10	23	24.3
Acinetobacter spp.	6	CXM		1	3	1	1		3.6
	6	CET					6		58
Enterobacter spp. (*E. aerogenes*, *E. cloacae*, *E. liquefaciens*)	138	CXM			31	91	8	8	10.2
	138	CET			6	5	19	108	>125
Enterobacteriaceae, others (*Hafnia*, *Citrobacter*, *Providencia*)	13	CXM		1	4	5	1	2	11.5
	13	CET				1		12	>125
Serratia spp.	8	CXM				1	1	6	116
	8	CET						8	>125
Proteus mirabilis	27	CXM			24	2	1		2.2
	27	CET			20	3	4		6.3
Pr. morganii	9	CXM			1	3	3	2	16
	9	CET					1	8	>125
Pr. vulgaris	21	CXM				6	12	3	39
	21	CET						21	>125
Pr. rettgeri	4	CXM	1			1	1	1	**
	4	CET						4	>125
Salmonella spp.	40	CXM			38	1	1		3.6
	40	CET			34	6			3.1
Shigella spp.	10	CXM			9		1		4.6
	10	CET			4	3		3	17
B. fragilis	16	CXM			1	4	11		53
	16	CET				2	14		60
H. influenzae, ampicillin-sensitive	16	CXM		16					0.5
	16	Amp†		16					0.25
H. influenzae, ampicillin-resistant	15	CXM		15					0.5
	15	Amp						15	250
N. gonorrhoeae	22	CXM	22						<0.125
	22	CET		22					0.4
N. meningitidis	2	CXM		2					0.125
	2	CET		2					0.125
Ps. aeruginosa	12	CXM						12	>125
	12	CET						12	>125

* Inoculum 10^5 colony forming units
** MIC's spread too widely, average not meaningful
† Amp = ampicillin

of the antibiotic by mouth.

Blood was taken by venepuncture and urine was collected at intervals throughout the experiments, and assayed for antibiotic activity. Toxicity studies were made two days before and one day after each dose. Blood samples were tested for haemoglobin, total RBC's, packed cell volume, mean corpuscular haemoglobin concentration, mean corpuscular volume, total and differential WBC's, blood urea, plasma creatinine, plasma bilirubin, alkaline phosphatase, serum alanine and aspartate aminotransferases, lactic acid and α-hydroxy butyric dehydrogenases. Urine was examined for pH, specific gravity, protein, blood sugar, ketones and by microscopy. Creatinine clearance was measured in each volunteer on the day of the drug dose.

Results

Antibacterial Activity *in vitro*

The activity of cefuroxime against 629 bacterial strains, most of them recent clinical isolates, is given in Table 1. The pattern and level of activity of cefuroxime against gram-positive organisms were similar to cephalothin. Cefuroxime was very active against staphylococci, regardless of whether or not they produced a penicillinase; like cephalothin, cefuroxime exhibited a high degree of stability to this enzyme. Both compounds were much less active against most methicillin-resistant strains. The activity of cefuroxime against other gram-positive organisms in general was high including *Streptococcus pyogenes*, *Strep. viridans* and *Strep. pneumoniae*; it had little or no activity against *Strep. faecalis*.

Cefuroxime was very active against members of the Enterobacteriaceae. Against *Proteus mirabilis* and most strains of *Enterobacter cloacae*, *E. aerogenes* and *Citrobacter*, it was many times more active than cephalothin; all *Serratia* strains were resistant to cephalothin and tended to be resistant to cefuroxime also. While many strains of indole-positive *Proteus* were sensitive to 32 μg/ml or less of cefuroxime, virtually all were resistant to cephalothin. Cefuroxime had high activity against *Klebsiella*, *Salmonella* and *Shigella* spp. and most strains of *Escherichia coli*. It was very active against *Neisseria gonorrhoeae* and *N. meningitidis*, and also against *Haemophilus influenzae*, including ampicillin-resistant strains[2,3]. Cefuroxime showed activity against some strains of *Bacteroides fragilis* but no activity against *Pseudomonas aeruginosa*.

The action of the compound was bactericidal and for most sensitive organisms, the minimum bactericidal concentration was the same as the MIC.

Compatibility with Other Antibiotics

In *in vitro* tests by the isobologram method[4], cefuroxime was tested in combination with several commercially available antibiotics, including tetracycline, carbenicillin and gentamicin. In every case, the effects of the mixtures were merely additive. Neither antagonism nor significant synergy was observed.

Activity against β-Lactamase-producing Organisms

Many of the gram-negative organisms tested produced a β-lactamase which could be detected in growing cultures by use of the chromogenic cephalosporin, 87/312[5]. Ten strains, producing different types of β-lactamase[6] were selected for further study and the effects of inoculum concentration on the activity of cefuroxime and cephalothin against these organisms were compared. The results, given in Table 2, show that cefuroxime was very active against the majority of strains at both low and high inoculum concentrations. In contrast, cephalothin

Table 2. Antibacterial activity and enzyme stability of cefuroxime and cephalothin against β-lactamase-producing organisms

Organism	MIC (μg/ml) at inoculum concentration (cfu/ml)†				Relative rate of hydrolysis (%)	
	Cefuroxime		Cephalothin			
	10	10^5	10	10^5	Cefuroxime	Cephalothin*
E. coli (TEM+)	4	4	4	31	<1	100
E. coli (RGN238)	4	4	8	8	10	100
K. aerogenes 447	4	125	125	>250	10	100
P. morganii 1375	4	8	>250	>250	<1	100
P. vulgaris 1352	8	31	250	>250	<1	100
E. cloacae 1085	8	8	125	250	<1	100
E. hafniae 1325	2	4	16	>250	<1	100
S. marcescens 1324	8	31	>250	>250	10	100
C. freundii 159	2	2	16	250	<1	100
Providence sp.	16	16	>250	>250	<1	100

* Value of 100 assigned arbitrarily for comparison purposes
† Colony forming units

was much less active against the majority of strains even when a single cell inoculum was used.

The results were related to the relative rates of hydrolysis of the two compounds by crude β-lactamase preparations from the same strains; at concentrations of about 40 μg/ml, cefuroxime was at least 100 times more stable to most of the enzymes than was cephalothin.

Whilst the stability of cefuroxime to some of the enzymes is not absolute, it is probably the major factor in the higher activity of the compound against most β-lactamase-producing organisms.

Human Pharmacokinetics

(1) Intramuscular injection

The mean assayed serum concentrations of cefuroxime obtained after single intramuscular injections of various doses are given in Table 3. The serum results together with timed urinary recoveries from each volunteer were computer calculated to produce a best-fit concentration-time curve. From each curve various pharmacokinetic parameters were calculated and the averages of these at each dose are shown in Table 4. In addition, the computer was used to construct the average curves for each intramuscular dose level. This was done by superimposing all the individual curves so that the times of each peak concentration coincided; this peak time was then taken as the origin of the graph (adapted from O'CALLAGHAN *et al.*[7]). The average curves obtained by this procedure are shown in Fig. 2.

The mean peak serum levels were high following intramuscular doses; they did not increase in linear relationship with the dose, but with the log dose. The mean times to peak concentrations occurred 29~45 minutes after the various intramuscular doses. The serum levels were long lasting, the 500 mg and higher doses giving measurable concentrations at 8 hours after injection. The mean ultimate serum half-lives were between 65 and 83 minutes, depending on the dose. For the lower doses, the area under the concentration-time curve nearly

Table 3. Mean serum concentrations of cefuroxime in human volunteers

Time (min.)	Serum concentration in μg/ml after						
	Intramuscular injection				Intravenous injection		
	250 mg 8 vols	500 mg 14 vols	750 mg 6 vols	1000 mg 5 vols	250 mg 3 vols	500 mg 3 vols	1000 mg 3 vols
3	*	*	*	*	39.0	66.3	99.2
10	*	*	*	*	26.6	44.0	75.4
15	*	18.4	*	*	*	*	*
20	14.2	*	32.3	*	*	*	*
30	14.5	25.3	*	37.5	15.8	21.6	43.2
40	14.0	*	32.8	*	*	*	*
60	12.8	21.6	29.6	39.1	9.7	15.0	27.2
90	10.0	15.9	*	30.4	*	*	*
120	7.0	11.9	19.1	23.0	4.8	6.6	11.7
180	3.3	7.2	*	12.2	2.3	3.6	7.4
240	1.9	4.0	6.1	7.5	1.4	2.1	3.6
360	0.7	1.5	1.5	2.8	0.5	0.9	1.1
480	*	0.5	0.7	1.7	*	*	0.3
Mean assayed peak	14.8	25.7	34.6	40.0			

* Not tested

Table 4. Computer calculated mean serum parameters after parenteral cefuroxime

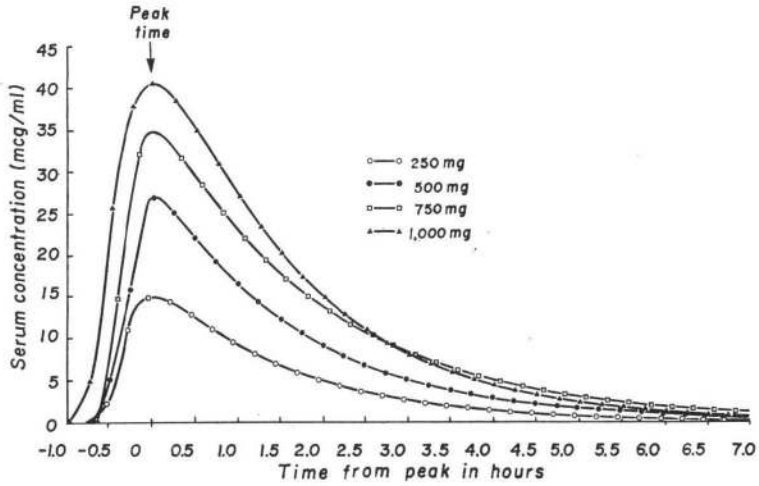
Route Dose (mg)	Peak conc. (μg/ml)	Peak time (min.)	Peak to half peak (min.)	Ultimate half-life (min.)	Area under the curve μg/ml hr	Apparent volume of distribution L/1.73 m²
Intramuscular						
250	15.0	32.1	81.3	65.0	32.9	11.07
500	26.9	29.0	84.3	70.4	59.2	13.45
750	34.9	31.3	96.0	82.3	88.6	15.81
1,000	40.4	45.0	93.2	65.6	101.3	15.05
Intravenous						
250	46.7	—	18.0	61.6	32.6	9.29
500	82.7	—	12.0	65.7	50.4	11.09
1,000	181.4	—	9.6	63.3	90.8	12.83

doubled as the dose doubled, but this relationship did not hold for the 1,000 mg dose. The apparent volume of distribution also increased with increase in dose.

(2) Intravenous injection

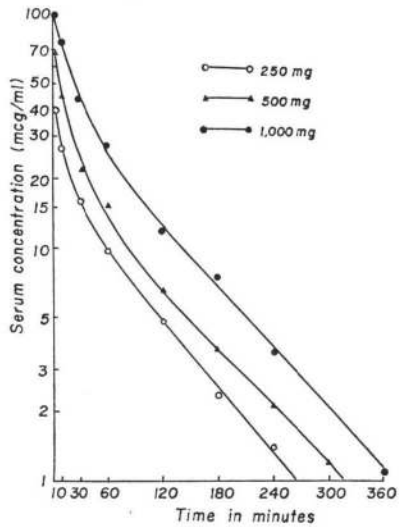
The mean assayed serum concentrations after single intravenous doses are given in Table 3. The concentrations assayed at 3 minutes following the 250, 500 and 1,000 mg intravenous doses were 39, 66 and 99 μg/ml respectively. Computer calculated average curves were constructed in a manner similar to that used for curves after intramuscular injection (Fig. 3). The mean parameters calculated from these curves are given in Table 4. The ultimate half-lives were between 62 and 66 minutes, and the areas under the serum concentration-time

Fig. 2. Serum concentrations in male human volunteers after single intramuscular doses of 250, 500, 750 and 1,000 mg of cefuroxime



curves were 33, 50 and 91 respectively.

Fig. 3. Serum concentrations in male human volunteers after single intravenous doses of 250, 500 and 1,000 mg of cefuroxime



Serum Protein Binding

Cefuroxime was found to be about 33 % bound to serum protein in samples from five volunteers. This compares with 15 % for cephaloridine 67 % for cephalothin and 85 % for cefazolin.

Urinary Recovery

Cefuroxime was rapidly excreted in high concentration through the kidney, over 90 % of the given dose being recovered in the urine within 6 hours of injection (Table 5). In all volunteers the renal clearance of cefuroxime was found to be more rapid than the simultaneously measured creatinine clearance. Mean ratios of the two clearances varied from 1.17 to 1.45 suggesting that, after allowing for 33 % protein binding, between 43 and 54 % of the drug is cleared through the kidney tubules. High pressure liquid chromatography of urine samples revealed that at least 95 % of the administered dose was excreted as unchanged cefuroxime. In the two volunteers given 1 g of cefuroxime by mouth only 1 % of the dose appeared in the urine and the serum concentrations were barely measurable.

Toxicity Studies and Tolerance in Volunteers

There was neither clinical nor laboratory evidence of toxicity in any of the volunteers in any of the trials. The intramuscular injection was slightly or moderately painful in some of the volunteers but this pain disappeared in 2~5 minutes and did not recur. Cefuroxime is

Table 5. Urinary recovery and renal clearance of cefuroxime

Route Dose (mg)	Assayed per cent recovered			Renal clearance mls/min/1.73 m²		Ratio: Creatinine / Cefuroxime
	0~2 hrs	0~6 hrs	0~24 hrs	Creatinine	Cefuroxime	
Intramuscular						
250	58.0	91.3	95.8	95.4	114.3	1.20
500	59.6	92.4	96.6	111.0	128.9	1.17
750	64.8	98.1	101.9	102.5	137.9	1.35
1,000	57.4	98.2	103.4	112.8	146.3	1.33
Intravenous						
250	83.4	110.0	114.2	103.9	138.9	1.36
500	64.9	90.4	95.1	116.3	136.0	1.21
1,000	75.1	96.4	99.2	117.6	169.6	1.45

Number of volunteers as in Table 3

marginally more painful than cephaloridine but much less so than cephalothin or cefazolin. The single intravenous injections were tolerated very well with no pain, burning, phlebitis or thrombosis either immediately or later.

Discussion

Cefuroxime, a new cephalosporin, appears to overcome some of the deficiencies of the previous members of this group of antibiotics. In addition to its stability to staphylococcal penicillinase, it is stable to most of the β-lactamases produced by gram-negative bacilli. Unlike cefoxitin[8], cefuroxime does not have absolute stability to all β-lactamases from gram-negative organisms. However, the degree of enzyme stability shown by cefuroxime has the effect of widening its antibacterial spectrum so that many organisms resistant to the cephalosporins at present in clinical use become sensitive. Tests on laboratory animals, results of which will be reported elsewhere[9], showed that the antibiotic gives effective protection whether the challenge bacteria are β-lactamase producers or not. The good protective effect is attributed to a combination of potent bactericidal action, high blood and tissue concentrations, and metabolic stability in the body. Consequently, it is hoped that infections due to strains of *Enterobacter* and indole-positive *Proteus*, as well as most β-lactamase-producing strains of *E. coli* and *Klebsiella*, may be treated successfully with cefuroxime in human patients.

The antibiotic shows unusually good activity against *Haemophilus influenzae* including the ampicillin-resistant strains. These organisms owe their resistance to a transferable β-lactamase identical with that mediated by the plasmid R_{TEM}[10]. Together with its high activity against the gram-positive cocci, this activity against *H. influenzae* may facilitate the effective treatment of more severe infections of the respiratory tract. Cefuroxime is also unusual among the cephalosporins in having very high activity against *N. gonorrhoeae* and also against the few strains of *N. meningitidis* tested. Providing the antibiotic can reach the infected site in adequate concentration, its high activity against *N. meningitidis*, *H. influenzae* and the streptococci may prove to be of benefit in the treatment of meningitis. It is noteworthy that cephamandole[11], which has increased stability to some β-lactamases, also has enhanced activity against *H. influenzae* and *Neisseria* spp.[12,13]

Cefuroxime appears to be of very low toxicity in laboratory tests. In particular, it seems to have no toxic action on the kidney, in contrast to some other cephalosporins which at high doses do have this effect. It produces neither severe nor prolonged pain on intramuscular injection and is well-tolerated intravenously. It is hoped therefore, that cefuroxime can be

given to patients with severe or deep-seated infections where higher doses may be required. The blood levels are high, and, for a cephalosporin, are prolonged, the ultimate half-life being about 70 minutes. These high levels are coupled with a low degree of serum protein binding; in contrast, cefazolin, a cephalosporin which gives high serum levels, shows a very high degree of serum protein binding which greatly reduces its effective antibacterial concentration in the serum[14]. The favourable human pharmacokinetic properties of cefuroxime, together with almost total metabolic stability and good urinary recovery, suggest that it is likely to be effective in treating human infections. It is now being made available for clinical trial and its usefulness in the infections mentioned above will be fully evaluated.

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