Cefuroxime, a New Cephalosporin Antibiotic: Activity In Vivo

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The pharmacokinetic properties of cefuroxime have been evaluated in laboratory animals. On injection into mice, rats, and rabbits by the subcutaneous or intramuscular routes, high serum level peaks were recorded. There was no significant absorption after oral administration. After injection, the antibiotic was excreted in large amounts in the urine. It was well distributed in the body and penetrated into the tissues at a satisfactory rate. This, coupled with a low degree of serum protein binding, was correlated with a very good protective effect in animals (mice, rats, and rabbits) experimentally infected with a wide range of bacteria, including β -lactamase-producing strains. It is concluded that cefuroxime should have a good potential for treating a wide range of bacterial infections in humans.

It has been shown (2) that cefuroxime, a new antibiotic of the cephalosporin series, has good activity in vitro against a wide range of bacterial species, including many strains which, being producers of β -lactamase, are resistant to many of the existing cephalosporins and penicillins.

Following the in vitro work, an evaluation of the pharmacokinetic properties of the compound and of its ability to protect against a range of bacterial infections in various animal species was undertaken. The results are reported here.

MATERIALS AND METHODS

Cefuroxime, cephalexin, cephalothin (sodium salts), and cephaloridine were prepared in our own laboratories as pure crystalline materials. Cefazolin (Totacef intramuscular, Bristol Laboratories Inc.) was obtained commercially. The materials were all administered as solutions in physiological saline solution. In the case of the cephalosporins, which were used as sodium salts, dosages were calculated to refer to the amount of free acid present.

The animals used were albino, Charles River female mice, weighing 18 to 20 g, albino female rats, Wistar strain, weighing 100 g, and Californian rabbits, weighing 2.0 to 2.5 kg. Antibiotic injections were made in 0.2-ml volumes in mice and rats and in volumes of 0.25 ml/kg in rabbits.

Cefuroxime concentrations in tissues and body fluids. Cefuroxime, at various dose levels, was administered to mice, rats, or rabbits and, at different intervals of time thereafter, samples of serum, lung, liver, spleen, and kidney were obtained and assayed for their cefuroxime content. Urine samples were collected from animals kept in metabolism

cages arranged for the collection of urine at periods of 0 to 6 h and 6 to 24 h after giving the dose of antibiotic. Serum and urine from individual rats and rabbits were collected and assayed. Pooled samples of serum, urine, and tissues from groups of three mice were used.

Interstitial fluid was collected from silicone baskets subcutaneously implanted in the neck region (1). The baskets were made from translucent silicone rubber tubing (internal diameter, 13 mm, and wall thickness, 5 mm) (Esco Rubber Ltd., London). The open ends of the cylinders were plugged with a roll of silicone rubber, 5 mm wide, giving a closed cylinder with an external length of 50 mm and an internal length of 40 mm. Each cylinder was converted into a basket by punching 42 holes (5 mm in diameter) in the walls, leaving a central nonperforated region that was 10 mm wide. The area represented by the holes was 43% of the total internal surface area. The baskets had been implanted, four per rabbit, for 12 months prior to the experiment reported here; each contained clear, pale-colored fluid. At various times after the intramuscular injection of cefuroxime, 0.5 ml of fluid from the basket was aspirated and blood was collected from a marginal ear vein. The position of each basket was noted and they were sampled in rotation. It was found that, in any one animal, concentrations of antibiotic showed small variation from one basket to another.

Peritoneal, pleural, and pericardial fluids were removed from rabbits killed at various times after intramuscular injection with cefuroxime.

Cefuroxime assay. Concentrations of cefuroxime in serum, tissue homogenates, and body fluids were determined by microbiological assay. The medium was prepared by dissolving 5 g of Oxoid peptone, 3 g of Oxoid Lab Lemco, 10 g of sodium citrate, 5 g of sodium chloride, and 12 g of agar (Difco) in distilled water to give 1 liter at pH 7.0

and was seeded with *Bacillus subtilis* NCIB 8993. Standards (1.25, 0.625, 0.312 and 0.15 $\mu\text{g/ml}$) 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 0.05 M phosphate buffer. Sera and urines were diluted in the same buffer. Provided the serum samples were diluted at least 1:4, no interference with the assay was observed from the presence of serum protein.

Because of the small volumes of peritoneal, pericardial, and pleural fluids available, these were assayed by applying 25- μl volumes to Whatman AA disks (6 mm) by means of microcapillary pipettes. Four disks were used for each sample of fluid. Disks were prepared with standard concentrations of cefuroxime. The disks were applied to the same assay medium as used in the well-plate assay, seeded, and read as outlined above.

Experimental chemotherapy. Protection tests were done on mice and rats experimentally infected with a range of microorganisms. At the time that the tests were done, the challenge strains were tested for sensitivity to the various antibiotics by an agar plate dilution method (2). Mice were challenged by the intraperitoneal route with the test organisms suspended in 0.2 ml of 4% hog gastric mucin. Rats were challenged by the same route in a 2% suspension of killed, dried brewers' yeast. The challenge dose in both species was approximately 10 to 100 times the mean lethal dose, and the majority of untreated animals died within 24 h of challenge. Five to eight animals were used at each of the serial threefold dose concentrations of antibiotic tested, which were given subcutaneously (0.2 ml/animal) at 1 and 5 h after challenge. The number of animals surviving on day 5 was used to calculate

the median effective dose by logit transformation of the dose response curve.

Californian rabbits (2.0 to 2.5 kg) were challenged intravenously with a suspension of *Staphylococcus aureus* 630 (0.5 ml/kg of body weight). The challenge dose was such that approximately 50% of the untreated controls died between 24 and 28 h, and all died within 48 h. Five animals were used at each of the serial twofold concentrations of antibiotic, which were given intramuscularly at 1 and 5 h postchallenge. The number of survivors on day 5 was counted, and the median effective dose was calculated by logit transformation of the dose response curve.

RESULTS

Serum levels and urinary excretion. The serum antibiotic levels and urinary recoveries after subcutaneous or intramuscular injection of cefuroxime in single doses to mice, rats, rabbits, and dogs are shown in Table 1. Absorption of the antibiotic was rapid from the injection site, and peak serum levels were recorded at 15 min after injection. Elimination from the serum tended to be rapid in the smaller rodents (rats and mice), and the duration of the serum levels became more prolonged in rabbits and dogs when the size of the dose was increased. Urinary recovery values also increased in proportion to the size of the animal and in rabbits and dogs approached the values found in humans (R. D. Foord, submitted for publication). Cefuroxime gave good serum levels at feasible doses of antibiotic, the levels being sufficient to cover the mini-

TABLE 1. Serum levels and percentage of urinary recoveries of cefuroxime in mice, rats, rabbits, or dogs after subcutaneous or intramuscular administration in single doses

Species	Route	Dose (mg/kg)	No. of animals	Average serum concentrations at (h):					Overall average % urinary recovery
				0.25	0.5	1	2	4	
Mouse	Subcutaneous	25	7 ^a	19.5	13.7	3.9	<1	<1	33
	Subcutaneous	50	8 ^a	50	33	8.1	<1	<1	50
	Subcutaneous	100	5 ^a	61	53	12.1	<1	<1	31
Rat	Subcutaneous	25	5	21	13.5	4.8	1.4	<1	52
	Subcutaneous	50	13	50	41	19.2	<1	<1	51
	Subcutaneous	100	5	89	64	38	9.8	2.4	67
	Subcutaneous	500	4	345	339	236	71	3.9	50
Rabbit	Intramuscular	25	5	32	21	7.1	<1	<1	81
	Intramuscular	50	5	84	61	21	<6	<1	75
	Intramuscular	100	10	192	96	51	16.5	<2	100
Dog	Intramuscular	60	4	NT ^b	70	56	32	6	66
	Intramuscular	180	4	NT	200	154	103	22	70
	Intramuscular	540	4	NT	835	853	320	73	69

^a Each sample was a pool from six mice.

^b NT, Not tested.

mum inhibitory concentrations against many susceptible strains of bacteria. In all cases the injections were well tolerated, and the animals showed no signs of malaise after the injections.

Urine samples from representative animals were investigated by chromatography (70% propanol-30% water; Whatman no. 1 paper buffered to pH 7.0 with phosphate buffer) followed by bioautographs on nutrient agar (using the Oxford strain of *S. aureus*). No spot corresponding to the 3-hydroxymethyl analogue was seen, indicating that the esterified carbamate group at position 3 in cefuroxime is stable in the animal body.

In mice, rats, and rabbits to which cefuroxime was given orally at 100 mg/kg, only very low serum levels and urinary recoveries of 1 to 3% were recorded. It was concluded that the antibiotic is not significantly absorbed from the gastrointestinal tract.

The effect of giving probenecid along with the cefuroxime was tested on mice and rats. Serum levels and urinary recoveries were measured in both species after giving, by subcutaneous injection, cefuroxime at 50 mg/kg with and without probenecid. The probenecid, at 500 mg/kg, was given orally 30 min before giving the injection of cefuroxime. The results indicate that probenecid has only a marginal effect on the size and duration of blood levels in these species and it does not affect the overall urinary recovery of the antibiotic (Table 2). It was concluded that tubular secretion must account for only a small part of the excretion of cefuroxime in these species and that most is likely to be eliminated by glomerular filtration.

Tissue levels. Cefuroxime levels in the serum, kidney, liver, spleen, and lung after giving single injections of 100 mg/kg are shown in Table 3. The antibiotic was given subcutaneously in rats and mice and intramuscularly in rabbits. The tissues were sampled at

0.5 and 1 h after giving the cefuroxime. The solid tissues were assayed by preparing 1:4 homogenates in physiological saline in a blender. The results showed a typical distribution pattern for a cephalosporin antibiotic—higher levels in the kidney, reflecting the excretion of the antibiotic by that organ. Although there were signs of concentration of the antibiotic in the liver in rats, this was not seen in rabbits. This, together with the observed high urinary recovery in the rabbit (Table 1), suggests that excretion of cefuroxime by the liver is minimal in that species. Lung and spleen levels were lower than the serum levels at the corresponding time, a not unexpected finding after a single dose of the antibiotic.

Average cefuroxime levels in the serum, peritoneal fluid, pleural fluid, and pericardial fluid of rabbits after a single dose of 50 mg/kg intramuscularly to rabbits are shown in Fig. 1. The serum levels were higher than those in the serous fluids, although therapeutic levels were reached in all of them, especially in the peritoneal fluid. There was a suggestion that the serous fluid levels might be more persistent.

Figure 2 shows the average cefuroxime levels in the serum of rabbits given a single dose of 50 mg/kg intramuscularly. At the same times, interstitial fluid from subcutaneously implanted plastic baskets was assayed, and the results are also illustrated in Fig. 2. The persistence of the tissue fluid levels as compared with the serum levels is clearly shown in this experiment, and it led to the belief that a "staircase effect" might be obtained on repeated dosing. An interval of 3 h between the repeated injections was chosen since cefuroxime is essentially excreted from the serum in rabbits at this time. The results of giving 10 repeated doses of 50 mg/kg at 3-h intervals is shown in Fig. 3. At each sampling, the serum and interstitial fluid were withdrawn immediately before giving the next 3-h dose.

TABLE 2. Effect of probenecid (500 mg/kg, orally) on serum levels and urinary excretion of cefuroxime given subcutaneously at 50 mg/kg to rats and mice

Species	With/without probenecid ^a	Serum levels (μg/ml) at (h):						Overall % urinary recovery
		0.25	0.5	1	2	3	4	
Mouse ^b	With	59	40	20	3.8	1.3	NT ^c	35
	Without	31	30	9	1.1	1.0	NT	32
Rat ^d	With	70	59	22	1	NT	<1	43
	Without	40	36	18.9	5.7	NT	<1	44

^a Probenecid given 30 min before the cefuroxime.

^b Each serum sample represents a pool from three mice.

^c NT, Not tested.

^d Each value is the average from four rats; the sera were assayed separately.

TABLE 3. Cefuroxime concentrations in serum and tissues of laboratory animals after single injections of 100 mg/kg

Species	Tissue	Serum or tissue level ($\mu\text{g}/\text{ml}$ or $\mu\text{g}/\text{g}$) at (h):	
		0.5 ^a	1 ^a
Mouse	Serum	53	12
	Kidney	155	23
	Liver	55	16.8
	Spleen	<6	<5
	Lung	17.6	<5
Rat	Serum	76	49
	Kidney	158	60
	Liver	134	49
	Spleen	13.6	<5
	Lung	43	13.7
Rabbit	Serum	81	51
	Kidney	833	295
	Liver	25	<10
	Spleen	10	29
	Lung	38	14.9

^a Average from five samples at each time. Samples were from individual rats and rabbits and pools were from six mice.

FIG. 1. Cefuroxime levels in serum and in peritoneal, pleural, and pericardial fluids after a single dose of 50 mg/kg intramuscularly in rabbits.

The serum levels climbed very slowly, this being consistent with a very small "overlap" in the serum levels (cf. Fig. 2). The more persistent interstitial fluid levels, however,

FIG. 2. Cefuroxime levels in serum and interstitial fluid (from subcutaneous basket implants) after a single dose of 50 mg/kg intramuscularly in rabbits.

FIG. 3. Cefuroxime levels in serum and interstitial fluid after multiple 3-h doses of 50 mg/kg intramuscularly in rabbits. The samples were withdrawn at 3 h after each injection, just before the next dose was given.

climbed to a plateau of some 17 $\mu\text{g/ml}$ after giving six doses.

Protection tests. Minimum inhibitory concentrations in vitro of cefuroxime, cephalixin, cefazolin, cephaloridine, and cephalothin against the test organisms used in the protection tests are shown in Table 4. The β -lactamase-producing strains of *Proteus* spp., *Enterobacter cloacae*, and *Klebsiella aerogenes* can readily be identified by the lack of activity on the part of the β -lactamase-susceptible cephalosporins. The greater activity of the β -lactamase-stable cefuroxime is evident.

Table 5 summarizes the results of protection tests on mice and rats challenged with a range of test organisms and treated with cefu-

roxime, cephalixin, cefazolin, cephaloridine, and cephalothin. Cefuroxime was effective in protecting against all of the test organisms used, in most cases at low dose levels. The activities of the other cephalosporins were usually related to the sensitivities of the strains in vitro (Table 4), although some exceptions were recorded. These exceptions can probably be accounted for by the fact that some test organisms probably produce smaller amounts of β -lactamase. Moreover, in this kind of test, some of the cephalosporins with a very rapid antibacterial activity, e.g., cephaloridine, may protect the animals by killing the challenge organisms before their β -lactamase has had time to work. Good protection

TABLE 4. Activity of cefuroxime and other cephalosporins against bacterial species used in protection tests

Organism ^a	Minimum inhibitory concentration ($\mu\text{g/ml}$)				
	Cephalixin	Cefazolin	Cephaloridine	Cephalothin	Cefuroxime
<i>Staphylococcus aureus</i> 853	1	0.25	0.5	0.25	1
<i>S. aureus</i> 630	1	1	8	0.5	0.5
<i>Escherichia coli</i> 851	8	1	4	8	4
<i>E. coli</i> 573	16	1	4	8	4
<i>Proteus mirabilis</i> 431	16	31	31	31	4
<i>P. morganii</i> 1375	>250	>250	>250	>250	62.5
<i>P. morganii</i> 235	>250	125	>250	250	4
<i>P. morganii</i> Rooney	>250	125	>250	250	4
<i>P. vulgaris</i> 52740	>250	250	>250	>250	16
<i>Enterobacter cloacae</i> 817501	>250	250	>250	>250	16
<i>Klebsiella aerogenes</i> 46189	31	62.5	250	125	31

^a Initial inoculum level was 10^7 .

TABLE 5. Protection tests on experimentally infected mice and rats

Species	Challenge organism and strain	Median effective dose in mg/kg per dose of:				
		Cephalixin	Cefazolin	Cephaloridine	Cephalothin	Cefuroxime
Mouse	<i>Staphylococcus aureus</i> 853	4	3	<0.8	8	5
	<i>S. aureus</i> 630	44	8	2	33	2
	<i>Escherichia coli</i> 573	13	6	8	70	4
	<i>E. coli</i> 851	2	1	4	>50	5
	<i>Proteus mirabilis</i> 431	25	19	5	6	2
	<i>P. morganii</i> (Rooney)	9	19	25	19	2
	<i>P. morganii</i> 1375	NT ^a	133	133	20	8
	<i>P. vulgaris</i> 52740	>200	182	>200	>200	35
	<i>Enterobacter cloacae</i> 817501	57	43	77	106	6
	<i>Klebsiella aerogenes</i> 46189	25	6	15	>200	6
Rat	<i>Staphylococcus aureus</i> 853	9	3	<2	6	5
	<i>S. aureus</i> 630	5	1	1	4	1
	<i>Escherichia coli</i> 851	23	10	10	59	32
	<i>Proteus mirabilis</i> 431	16	40	40	84	26
	<i>P. morganii</i> 235	138	131	131	>150	5
	<i>P. morganii</i> 1375	>150	>150	>150	>150	29
	<i>P. vulgaris</i> 52740	Ca. 150	>150	Ca. 150	>150	55
	<i>Enterobacter cloacae</i> 817501	25	>150	>150	>150	7
	<i>Klebsiella aerogenes</i> 46189	NT	30	45	>150	8

^a NT, Not tested.

with cefuroxime was seen in both mice and rats and should be considered in relation to the relatively short duration of serum levels produced in these species (Table 1) by the administration of the modest dose levels required for protection.

The results of a protection test on rabbits challenged intravenously with *S. aureus* strain 630 (a penicillinase-producing strain) are shown in Table 6. Cefuroxime, cephalixin, cefazolin, and cephaloridine were compared in this test. All of the compounds tested gave satisfactory protection, but cephaloridine was the most effective, a reflection of the very rapid lytic action of that substance.

DISCUSSION

Cefuroxime, a new cephalosporin antibiotic with resistance to destruction by most β -lactamases, shows a favorable pharmacokinetic pattern in experimental animals. High serum levels result from single subcutaneous or intramuscular injections, although the substance is not absorbed when given by mouth. The antibiotic is eliminated quite rapidly in small rodents, but in larger animals it is more persistent. Excretion appears to be via the kidney and to take place mainly by glomerular filtration. The substance is stable in the animal body in that the carbamate group at position 3 is not removed by esterases. This is in contrast to 3-acetoxymethyl cephalosporins (e.g., cephalothin) in which de-esterification occurs rapidly in rodents.

TABLE 6. Protection test on rabbits challenged intravenously with *S. aureus* strain 630

Antibiotic	Median effective dose ^a (mg/kg per dose)
Cefuroxime	3
Cephalixin	13
Cefazolin	1
Cephaloridine	<1

^a Calculated from the numbers of survivors on postchallenge day 5.

From the blood stream, cefuroxime penetrates readily into the tissues and serous fluids and, in rabbits, good persistence of the antibiotic was seen such that a buildup of antibiotic levels took place when repeated doses were given just before the serum levels fell to zero. In rabbits given 50 mg/kg, this was at 3-h intervals. Work on human volunteers (R.D. Foord, submitted for publication) has shown that the corresponding period in humans given smaller doses (7 to 15 mg/kg) is about 8 h, and it seems likely that tissue fluid levels would show a stepwise increase after repeated doses in humans also.

Protection tests in mice, rats, and rabbits against a wide range of pathogenic strains of bacteria showed that the antibiotic, which is not de-esterified in the body, is able to protect efficiently against such lethal infections. In particular, since many of the test strains used were β -lactamase-producing gram-negative bacilli, it was evident that cefuroxime was an effective antibiotic in vivo where other β -lactamase-susceptible cephalosporins were ineffective or less effective. It is concluded that the antibiotic is likely to be successful in treating a wide range of infections in humans.

Toxicity tests, which will be reported elsewhere, showed that the substance is of low toxicity and, in particular, does not have any toxic action on the kidney or liver. The way is, therefore, opened for the evaluation of cefuroxime in humans.

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