Pharmacodynamics of Cefazolin in the Presence of Normal and Impaired Renal Function

MICHAEL F. REIN, FREDERIC B. WESTERVELT, AND MERLE A. SANDE

Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22901

Received for publication 17 July 1973

The excretion of cefazolin, a new cephalosporin antibiotic, was studied in subjects with normal and impaired renal function. Twelve subjects with creatinine clearances ranging from 0 to 144 ml per min per 1.73 m² were given a single 500-mg intramuscular dose of cefazolin. Serum and urine levels were determined at intervals by agar diffusion. Peak serum levels in normals ranged from 44 to 70 µg/ml and occurred 30 to 60 min after injection. The mean serum half-life in normals was 1.6 h, and this was prolonged from 20 to 40% by simultaneous administration of probenecid. The total elimination constant varied linearly with the creatinine clearance in patients with renal impairment. The serum half-life in anephric patients was about 42 h. The fractional clearance of the drug varied directly with the serum level. Peak urine levels ranged from 60 to over 2,000 µg/ml, and more than 90% of the dose was recovered in the urine of normals during the first 24 h. The data suggest that cefazolin is cleared primarily by the glomerulus, with tubular and biliary secretion playing a secondary role.

Cefazolin is a new, semisynthetic cephalosporin antibiotic with several favorable properties. (i) It can be administered by relatively painless intramuscular injection. (ii) Like other cephalosporins, it is generally bactericidal at readily attainable blood and tissue levels. (iii) It is effective in vitro against a wide range of gram-positive and gram-negative organisms. It appears somewhat more effective against *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* than cephalothin or cephaloridine but slightly less active against *Staphylococcus aureus* (2, 12, 16). As with other cephalosporins, it is generally ineffective against *Proteus vulgaris*, *Enterobacter* sp., *Pseudomonas aeruginosa*, and *Streptococcus faecalis*. Its resistance to bacterial penicillinase is good (2, 12). (iv) The nephrotoxic potential of cefazolin is less than that of cephaloridine. Although renal tubular necrosis could be produced in rabbits with either drug, the required dose was more than twice as high with cefazolin as with cephaloridine (12). Since the experimental renal toxicity is dose related, unnecessarily high serum levels should be avoided.

Accumulation of the drug can be expected in patients with renal impairment. The chemical structure of cefazolin suggests that renal function will be a more important determinant of blood levels than with cephalothin. The latter

drug possesses an acetyl ester at the 3 position (6), rendering it susceptible to degradation by esterases located primarily in the liver. Extrarenal inactivation and excretion may account for between 30 and 80% of a dose of cephalothin (11). Because cefazolin, like cephaloridine and cephalexin (4, 7), lacks the 3-ester linkage, it is deprived of this major extrarenal eliminative pathway.

This study was undertaken to determine the rate of excretion of cefazolin in patients with varying degrees of renal failure, to evaluate the effect of probenecid, and to develop a simple guide for administration of the drug that would assure serum levels within acceptable therapeutic limits.

MATERIALS AND METHODS

Cefazolin. Cefazolin sodium for injection was supplied in ampoules containing 1 g of equivalent cefazolin activity. Immediately before injection, the drug was reconstituted, with bacteriostatic water containing 0.9% benzyl alcohol, to a final concentration equivalent to 250 mg of cefazolin per ml of solution. Cefazolin sodium laboratory standard was supplied in vials equivalent to 20 mg of activity. This was reconstituted with distilled water and diluted in the appropriate carrier within 24 h of use.

Human subjects. Five informed volunteers with no evidence of renal, hepatic, or infectious disease and who had received no drugs during the preceding 2

weeks were given 500 mg of cefazolin intramuscularly. Activity and oral intake were not regulated. Serum and urine specimens were collected at intervals. Simultaneous creatinine clearances were done on all subjects. Nine patients with varying degrees of renal failure of diverse etiologies gave informed consent and received 500 mg of cefazolin intramuscularly. These patients had no evidence of concurrent infection of antibiotic therapy, and all had normal values for serum bilirubin, alkaline phosphatase, amylase, lactic dehydrogenase (LDH), serum glutamic oxalacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT). Hematocrit, white blood cells, creatinine clearance (Ccr), LDH, SGOT, alkaline phosphatase, serum albumin, and blood urea nitrogen (BUN) were determined by standard methods before, during, and after administration of the cefazolin. All clearances in this study are corrected for body surface area of 1.73 m². Three normal volunteers from the initial group were given four oral doses of probenecid (500 mg) at 6-h intervals. One hour after the initial dose of probenecid, they were given 500 mg of cefazolin intramuscularly. Serum samples were collected at intervals over the next 24 h.

Collection of specimens. Venous blood was collected with Vacutainers and allowed to clot at room temperature. Serum was removed and kept frozen until just prior to assay. Fractions of fresh-voided urine were handled similarly.

Cefazolin assay in serum and urine. Drug concentrations were determined by a modification of the method of Bennett et al. (1). Two vials of *Bacillus subtilis* spore suspension (Difco) were added to 100 ml of normal saline with 40 meq of potassium phosphate. Samples of 2 ml were refrigerated. Thirty-one grams of nutrient agar (Difco; 1.5%) was mixed with 1,080 ml of distilled water, boiled to clarity, separated into 250-ml flasks, autoclaved, and maintained at 50 C. A 0.8-ml amount of the *B. subtilis* spore suspension was added, and the agar was poured into plastic petri dishes (150 by 15 mm). When the agar layer solidified, wells 4.5 mm in diameter were cut into the layer with an agar punch (1). Standards of cefazolin were prepared by serial dilution in human serum for use in assaying the serum samples and in water for assaying urine specimens. Wells were filled by using Pasteur pipettes. A separate standard curve was set up for each day's analysis, and each plate contained one or more standards. The plates were incubated at 37 C overnight, and zone sizes were read on a Fisher-Lilly antibiotic zone reader. Samples diluted to fall within the range defined by the standard curve were run in triplicate, and zone sizes were corrected whenever necessary as determined by the standards included in each plate. The standards generated a straight line when the log of the antibiotic concentration was plotted against zone size. Serum levels of less than 5 µg/ml could not be accurately determined and were excluded. The accuracy of the serum levels was checked in unselected specimens by parallel tube dilutions in broth using *S. aureus* as the test organism. Several sera were assayed after a second freezing without measurable change in antibiotic level.

Decay curves. Serum levels were plotted semilogarithmically against time, and the rate of decay of the serum levels was determined as a least-squares approximation of the descending portion of the curve. Sampling times were too short to permit determination of half-life in one renal failure patient. The renal clearance of cefazolin in normals was determined from a 2-h urine specimen collected between 2 and 4 h after administration of the drug. The geometric mean of the serum levels at the extremes of the period (S_1 and S_2) was taken as the mean serum level. The clearance was calculated as urine volume $\times$ urine level/[120 $\times$ ($S_1 \times S_2$)^{1/2}].

RESULTS

Patient tolerance. Normal volunteers and patients with impaired renal function noted relatively little discomfort at the injection site. There was no evidence of local reaction to the drug. Where tested, no impairment of renal, hematological, or hepatic function could be attributed to the single dose of cefazolin.

Serum levels of cefazolin. The results of the assay for cefazolin in the serum are presented in Table 1. The subjects had creatinine clearances ranging from 0 to 144 ml/min, and the 500-mg injection yielded cefazolin doses ranging from 5.80 to 9.78 mg/kg. Peak serum levels in the normal subjects (Ccr > 100 ml/min) occurred between 30 and 60 min after the injection and ranged from 44.0 to 70.0 µg/ml. In these same subjects, the serum half-life of cefazolin (Table 1) ranged from 1.37 to 1.96 h with a mean half-life of 1.60 h. In the patients with renal impairment (Ccr 0-47 ml/min), peak serum levels ranged from 42.0 to 81.5 µg/ml depending upon initial weight-adjusted dose and level of renal function. The serum half-life of the drug was determined in eight of the patients and ranged from 4.83 h associated with a creatinine clearance of 47.2 ml/min to 69.2 h in an anuric patient.

Effect of probenecid on excretion of cefazolin. Simultaneous administration of probenecid (500 mg) orally every 6 h slowed the rate of excretion of the drug (Table 1). Probenecid prolonged the serum half-life from 20 to 40% over its values in the same subjects who received the drug without probenecid. Peak serum levels ranged from 47.0 to 82.5 µg/ml, representing an increase of from 7 to 33%. It is interesting to note that the creatinine clearances measured during the administration of probenecid were lower than base line values in the same subjects.

Elimination constant of cefazolin. The least-squares approximations of the decay curves for 10 of the subjects are presented in Fig. 1. The slope of the descending portion of the curve is numerically equal to the total elimination constant for the drug (15). The

TABLE 1. Serum cefazolin levels after 500-mg intramuscular injection

Patient	Dose (mg/kg)	Creati- nine clearance (ml/min/ 1.73 m ²)	Serum half-life (h)	Serum levels (μg/ml)											
				½ h	1 h	2 h	4 h	8 h	12 h	24 h	36 h	48 h	53 h	72 h	
M.R.	6.48	144	1.67	39.5	47.0	26.1	13.0	<5.0	<5.0	<5.0	4.8	12.6			
M.J.	5.80	129	1.44	43.5	50.0	25.8	11.4	<5.0	<5.0	<5.0					
K.C.	8.68	122	1.37	55.0	65.0	30.9	13.6	<5.0	<5.0	<5.0					
M.S.	5.96	121	1.96	44.0	38.0	24.6	13.0	<5.0	<5.0	<5.0					
P.B.	9.18	101	1.53	70.0	65.0	34.8	16.2	5.0	<5.0	<5.0	48	31	7.8	24	
J.S.	5.70	47	4.83	38.0	42.0	30.0	24.0	16.2	7.5	<5.0					
P.N.	7.87	19	8.08	66.5	64.0	74.0	75.0	53.5	26.0	10.7					
S.L.	7.20	9	12.10	75.0	80.5	81.5	74.0	72.5	32.0	20.0					
C.L.	7.30	9	25.57		80.0	60.0	54.0	52.0	44.0		30.4				
I.W.	7.72	5	27.1	60.0	60.5	73.5	76.0	75.0	60.0	47.0					
Z.B.	9.78	1		37.0	39.0	48.0	50.0	50.0							
H.L.	8.54	1	48.5				50.0								
G.G.	6.81	0	14.82				64.0			30.4	48	31	7.8	24	
C.L.	7.30	0	69.2				49.0			35.0					
K.C. ^a	8.68	114	1.96	82.5	86.5	73.0	24.0	8.0	<5.0	<5.0					
M.R. ^a	5.79	103	2.35	50.0	58.5	40.0	22.4	7.2	<5.0	<5.0					
M.S. ^a	5.96	90.2	2.38	41.5	47.0	44.0	20.0	6.7	<5.0	<5.0					

^a During course of probenecid—500 mg by mouth every 6 h.

elimination constant is plotted against the creatinine clearance for all patients in Fig. 2. A straight-line relationship is suggested (correlation coefficient 0.94), and least-squares analysis yields a line with the formula: $k_2 + 0.0034 \text{ Ccr} + 0.017$. The symmetrical scattering of points in the region of higher creatinine clearances probably reflects experimental error in determining the creatinine clearances in patients with very low levels of serum creatinine. There is no evidence of a systematic departure from linearity.

The 2-h cefazolin clearances are presented in Table 2. The fractional clearance of cefazolin ($C_{\text{cefazolin}}/C_{\text{creatinine}}$) ranged from 0.33 to 0.80. In Fig. 3, the fractional clearance of cefazolin can be seen to increase with increased serum concentration of the drug. A linear relationship is suggested, and the least-squares line is shown.

Urine levels in normals and patients with renal impairment. Peak levels in normals ranged from about 1,000 to over 2,000 μg/ml (Table 3). Low levels of drug were detectable in the urine 24 h after administration, and total 24-h urinary excretion accounted for more than 90% of the administered dose of cefazolin in all normals.

Even with severe renal impairment ($\text{Ccr} \geq 5.0$ ml/min) urine levels greater than 60 μg/ml were consistently obtained.

DISCUSSION

Our observed peak serum levels in normal subjects after a 500-mg dose of cefazolin (44 to 70 μg/ml) are somewhat higher than those previously published (5, 13, 14, 16). The discrepancy is reduced when dosages are adjusted to account for the weight of the recipient. The difference cannot be accounted for by a systematic error in our cup-plate techniques since the method proved to be accurate within 7 μg/ml when identical samples were tested by tube-dilution assays in broth. Samples of the drug from the two American distributors of cefazolin were found to have identical concentrations when tested in parallel against a variety of microorganisms by tube-dilution and cup-plate diffusion. Thus differences in drug origin cannot account for the discrepancy in peak serum levels. Of the four papers cited, only that by Shibata and Fuji (16) specifically documents that standards were diluted in human serum. These authors demonstrate that the diffusion-dependent assays employing water-based standards yield artifactually low levels resulting from significant binding of cefazolin to plasma proteins. This binding is species dependent (25% in dog serum, 74% in human serum, 80–90% in rat serum, and 90% in rabbit serum [8, 17]), and hence only human serum should be used in the preparation of standards.

FIG. 1. Least-squares approximations of the descending portion of selected serum level curves expressed as percentage-of-peak levels. The half-lives may be approximated as the time required to drop from 100% of peak to 50% of peak. Numbers in parentheses are 24-h creatinine clearances.

FIG. 2. Total elimination constant for cefazolin (left-hand ordinate) plotted as a function of creatinine clearance with 90% confidence intervals. The serum half-life of cefazolin (right-hand ordinate) may also be determined from the creatinine clearance.

When peak serum levels are plotted against the dose (milligrams) of cefazolin given to normal subjects (kilograms), a straight-line relationship is strongly suggested. Regression analysis yields a multiple correlation coefficient of 0.99 and generates a least-squares line with the formula: peak level ($\mu\text{g/ml}$) = $7.5 \times \text{dose (mg/kg)} + 0.7$. The peak serum level (micrograms per milliliter) in patients with normal renal and hepatic function may, therefore, be roughly predicted by multiplying the dose of cefazolin (milligrams per kilogram) by 7.5.

Cefazolin, like cephaloridine and cephalixin, lacks the 3-acetyl ester linkage, and this study demonstrates that elimination of the drug is

TABLE 2. Two-hour cefazolin clearance in normal volunteers

Subject	Creatinine clearance (ml/min/1.73 m ²)	Cefazolin clearance (ml/min/1.73 m ²)	Fractional clearance of cefazolin ($C_{\text{cefazolin}}/C_{\text{creatinine}}$)	Mean serum level of cefazolin ($\mu\text{g/ml}$)
P.B.	101	81	0.80	23.7
K.C.	122	76	0.62	20.5
M.S.	121	69	0.57	17.9
M.R.	144	59	0.40	18.4
M.J.	129	43	0.33	17.1

FIG. 3. Fractional clearance of cefazolin ($C_{\text{cel}}/C_{\text{creat}}$) plotted as a function of mean serum level over the 2-h collection period. Least-squares approximation is shown.

TABLE 3. *Urinary cefazolin levels after 500-mg intramuscular injection*

Subject	Creatinine clearance (ml/min/1.73 m ²)	Urinary levels (μg/ml)					
		2 h	4 h	6 h	8 h	12 h	24 h
M.R.	144	1,120	1,110	530	91	44	1
M.J.	129	1,660	900	370	229	110	11
K.C.	122	940	580	480	122	5	1
M.S.	121	1,440	1,410	570	380	82	2
P.B.	101	2,250	1,720	400	510	7	3
J.S.	47	1,530	1,350	420	202	126	10
P.N.	19	200			670		
S.L.	9	200	400	500	470	385	
C.L.	9	69	70	80	108	62	
I.W.	5	100	174	210	128		

primarily renal. The half-life of the drug was as long as 69.2 h in an anuric patient, whereas in normals it averaged about 1.6 h. Cefazolin has been shown to be excreted by the biliary system in numerous animal studies (8, 9, 13). Patients with biliary tract disease when given cefazolin and cannulated at surgery had biliary levels about twice as high as simultaneous serum levels (18). The high urinary recovery of cefazolin in patients with normal renal function suggests that most of the drug secreted into the bile must be reabsorbed from intestinal contents.

When the log of the serum cefazolin level is plotted against time (Fig. 1) the slope of the descending portion of the curve is numerically identical to k_2 , the total elimination constant of the drug (15). This elimination constant bears the following relationship to the serum half-life ($T_{1/2}$): $T_{1/2} = \ln 2/k_2$. When the elimination constant is plotted against creatinine clearance (Fig. 2), a straight-line relationship is demonstrated. The overall rate of elimination of the drug correlates with renal function as measured by creatinine clearance with a correlation coefficient of 0.94. This supports the conclusion that the kidney is the major route of excretion of the drug in man, and the elimination constant can be predicted with all degrees of renal impairment by linear interpolation. The data, then, will allow one to approximate the serum half-life of cefazolin in a patient whose creatinine clearance is known.

The effect of probenecid on the excretion of cefazolin is qualitatively very similar to that seen with cephaloridine (17). Creatinine clearances in our subjects on probenecid were lower than when measured in these same subjects prior to receiving the drug. A diminution in creatinine clearance with probenecid has been

described in several species (10; B. Rennie, *Fed. Proc.* 25:392, 1966) and probably relates to inhibition of tubular secretion of creatinine. The renal effect of probenecid is limited to the tubules, however, and while it might account for a change in the clearance of materials delivered to the urine by tubular secretion, it does not influence glomerular function. Thus, the apparent change in creatinine clearance exhibited by our subjects in response to probenecid probably represents a tubular, rather than a true glomerular, effect, and the change in elimination constant of cefazolin likewise represents a decrease in tubular secretion rather than in glomerular filtration. However, as noted by Gibaldi and Schwartz, the effect of probenecid on the serum level (penicillins and cephaloridine) may not be a simple function of altered tubular secretion, but may additionally reflect a decrease in the volume of distribution of the drug (3).

Although the data is limited, Table 2 and Fig. 3 suggest that increasing serum levels of cefazolin are associated with an increasing fractional clearance. If secretion were solely a function of glomerular filtration rate, the plot of Fig. 3 would approximate a horizontal line. Two different mechanisms might explain this observation. (i) If cefazolin that is bound to serum protein is at least partially restricted from glomerular filtrate and the serum binding sites are nearly saturated at therapeutic levels of cefazolin, then an increase in serum levels would yield a higher fraction of unbound drug. This would result in greater filtration at the glomerulus. Kozatani et al., however, failed to demonstrate a significant difference in the fraction of cefazolin bound to rat serum proteins throughout a wide range of serum levels (8). (ii) In addition to simple glomerular filtration, delivery of cefazolin from serum to urine may be accomplished by a tubular transport system which is unsaturated at therapeutic serum levels. Higher blood levels would present more of the drug to this transport system and would result in greater tubular transfer of cefazolin into the urine at any given glomerular filtration rate. Such a tubular mechanism is suggested by the diminished excretion of cefazolin caused by probenecid. It would seem, therefore, that, with larger doses of cefazolin and consequently higher serum levels, probenecid would have a relatively greater effect on excretion of the drug.

Thus, the kidney represents the major pathway for excretion of cefazolin in the human. The drug is handled primarily by glomerular filtra-

tion with a smaller component of renal tubular secretion. Minor amounts of the drug are excreted through the biliary system in normals, but this may become a major route in renal failure. For practical purposes the serum half-life of the drug correlates well with creatinine clearance.

ACKNOWLEDGMENTS

We are indebted to the Eli Lilly Company for their support of this project and for supplying cefazolin injectable and laboratory standards. We wish to thank William M. O'Brien for his assistance with statistical analysis, Kip B. Courtney for her technical assistance, and Patricia H. Birch for her help in the preparation of the manuscript.

LITERATURE CITED

1. Bennett, J., J. Brodie, E. Benner, and W. Kirby. 1966. Simplified, accurate method for antibiotic assay of clinical specimens. *Appl. Microbiol.* **14**:170-177.
2. Eli Lilly and Company. 1972. Cefazolin sodium: clinical investigation manual. Eli Lilly and Co., Indianapolis.
3. Gibaldi, M., and M. A. Schwartz. 1968. Apparent effect of probenecid on the distribution of penicillins in man. *Clin. Pharm. Ther.* **9**:345-349.
4. Griffith, R., and H. Black. 1971. Blood, urine and tissue concentrations of the cephalosporin antibiotics in normal subjects. *Postgrad. Med. J. Suppl.* **47**:32-40.
5. Ishiyama, S., I. Nakayama, H. Iwamoto, S. Iwai, M. Okui, and T. Matsubara. 1971. Absorption, tissue concentration and organ distribution of cefazolin. *Antimicrob. Ag. Chemother.* **1970**, p. 476-480.
6. Kariyone, K., H. Harada, M. Kurita, and T. Takano. 1970. Cefazolin, a new semisynthetic cephalosporin antibiotic. I. Synthesis and chemical properties of cefazolin. *J. Antibiot. (Tokyo)* **23**:131-136.
7. Kirby, W., J. de Maine, and W. Serrill. 1971. Pharmacokinetics of the cephalosporins in healthy volunteers and uremic patients. *Postgrad. Med. J. Suppl.* **47**:41-46.
8. Kozatani, J., M. Okui, T. Matsubara, and M. Nishida. 1972. Cefazolin, a new semisynthetic cephalosporin antibiotic. VI. Excretion and metabolism of cefazolin-C¹⁴ in rats after intramuscular administration. *J. Antibiot. (Tokyo)* **25**:86-93.
9. Kozatani, J., M. Okui, K. Noda, T. Ogino, and H. Nogouchi. 1972. Cefazolin, a new semisynthetic cephalosporin antibiotic. V. Distribution of cefazolin-C¹⁴ in mice and rats after parenteral administration. *Chem. Pharm. Bull.* **20**:1105-1113.
10. Lambert, P. P. 1945. Étude comparée de l'élimination de l'inuline, de la créatinine et de l'uroselectan B par le rein des oiseaux. *Arch. Intern. Pharmacodyn.* **71**:313.
11. Lee, C. C., E. Herr, and R. Anderson. 1963. Pharmacological and toxicological studies on cephalothin. *Clin. Med.* **70**:1123-1138.
12. Nishida, M., T. Matsubara, T. Murakawa, Y. Mine, and Y. Yokota. 1970. Cefazolin, a new semisynthetic cephalosporin antibiotic. II. *In vitro* and *in vivo* antimicrobial activity. *J. Antibiot. (Tokyo)* **23**:137-148.
13. Nishida, M., T. Matsubara, T. Murakawa, Y. Mine, and Y. Yokota. 1970. Cefazolin, a new semisynthetic cephalosporin antibiotic. III. Absorption, excretion, and tissue distribution in parenteral administration. *J. Antibiot. (Tokyo)* **23**:184-194.
14. Nishida, M., T. Matsubara, T. Murakawa, Y. Mine, Y. Yokota, S. Kuwahara, and S. Goto. 1970. *In vitro* and *in vivo* evaluation of cefazolin, a new cephalosporin C derivative. *Antimicrob. Ag. Chemother.* **1969**, p. 236-243.
15. Regamey, C., and L. Humair. 1971. Pharmacokinetics of cephalixin in renal insufficiency. *Postgrad. Med. J. Suppl.* **47**:69-78.
16. Shibata, K., and M. Fuji. 1971. Clinical studies of cefazolin in the surgical field. *Antimicrob. Ag. Chemother.* **1970**, p. 467-472.
17. Tuano, S., J. Brodie, and W. Kirby. 1967. Cephaloridine versus cephalothin: relation of the kidney to blood level differences after parenteral administration. *Antimicrob. Ag. Chemother.* **1966**, p. 101-106.