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LABORATORY AND CLINICAL STUDIES OF A NEW ANTIBIOTIC, CEPHALOGLYCIN IN THE TREATMENT OF URINARY TRACT INFECTIONS†

Cephaloglycin, the dipolar ion of 7- (D-a-aminophenylacetamido) cephalosporanic acid, is a phenyl-glycine analogue of the antibiotic cephalothin. Preliminary studies *in vitro,* indicate that the drug has bactericidal activity against Gram-negative as well as Gram-positive bacteria.[1] The broad spectrum of antibacterial activity observed with cephaloglycin is similar to two earlier derivatives of cephalosporanic acid, cephalothin and cephaloridine,[2,3] which must be administered parenterally. In contrast, cephaloglycin is thought to be well absorbed from the gastrointestinal tract because it is excreted in high concentration in the urine after oral administration in man,[3] which suggests that the drug may be useful in the treatment of certain bacterial infections, particularly those involving the urinary tract.

The present report describes the absorption, urinary excretion, clinical effect and toxicity of oral cephaloglycin in patients with urinary tract infections. In addition, sensitivity of 151 Gram-negative organisms to cephaloglycin was studied *in vitro* by comparing the single high-concentration disc method with the tube-dilution method.

MATERIALS AND METHODS

Clinical studies

Twenty-four patients (22 males and two females from 25 to 79 years of age) with significant bacteriuria, and who consented to the study, received oral cephaloglycin treatment. Seven patients were treated for first episode urinary tract infection, whereas a history of chronic urinary tract infection was obtained from 17. However, 18 of the 24 patients had acute symptoms referable to infection of the urinary tract and six patients had asymptomatic bacteriuria. Urines were obtained by the clean catch technique and cultured as previously described.[4] Significant bacteriuria was defined as 100,000 colonies or more per milliliter of urine per species of bacteria. Urinary tract infection was arbitrarily defined in each patient as the presence of significant bacteriuria in at least two consecutive urine cultures. Intravenous pyelograms and residual volumes of bladder urine were obtained for each patient to evaluate the presence of

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urinary tract obstruction. The volume of residual bladder urine was determined without catheterization by a modification of the radioisotope method of Mulrow, *et al.* using Ortho-Iodohippuric acid.[5] No patient had an indwelling catheter or received other antibiotic treatment during the period of study.

Cephaloglycin was supplied in 250 mg. capsules* and was administered orally in doses of 500 mg. every six hours for ten days. In four patients, the dose was decreased to 250 mg. during the latter five days of treatment. Serum and urine concentrations of cephaloglycin were determined in 15 patients twenty-four hours after treatment had been initiated. Blood was drawn from fasting patients two hours after the 6 a.m. dose of cephaloglycin. Urine was collected for six hours after discarding the 6 a.m. specimen, and the volume was measured. Aliquots of urine and serum were frozen and subsequently analyzed for cephaloglycin activity by Lilly Research Laboratories.

Toxic effects of the drug were assessed by white blood cell count, differential cell count, hematocrit, platelet count, total and direct bilirubin, serum glutamic oxaloacetic acid transaminase, alkaline phosphatase, blood urea nitrogen, and urinalysis values obtained prior to, during, and after therapy. Each patient was questioned and examined daily for evidence of nausea, vomiting, diarrhea, pruritis, and skin rash.

The effect of treatment was evaluated by means of quantitative clean catch urine cultures obtained during treatment, and at 4 days, 2, 4, and 6 weeks after treatment was discontinued.

Clinical response to cephaloglycin was arbitrarily divided into three categories. *Cures* were defined as the eradication of bacteriuria during and for at least four weeks after cephaloglycin treatment. *Relapses* were defined as the recurrence of bacteriuria (eradicated during treatment) within four weeks after cephaloglycin was discontinued. Treatment *failures* were defined as the persistence of bacteriuria during cephaloglycin therapy.

Laboratory studies

One hundred fifty-one strains of Gram-negative bacteria isolated from patients with clinical infections, including the organisms isolated from the patients with bacteriuria, were studied. Organisms were collected from the Clinical Bacteriology Laboratory, subcultured, and re-identified according to the scheme of Schaub, Foley, Scott, and Bailey[6] in our Research Bacteriology Laboratory before antibiotic sensitivity tests were performed.

All organisms were tested with cephaloglycin by both tube-dilution and antibiotic disc methods. Each bacterial strain was tested against cephaloglycin discs containing 5, 15, and 30 μg. of antibiotic. As previously reported,[3] a modification of the single-disc method of Kirby, Yoshihara, Sundstedt, and Warren,[7] and Turck, Lindemeyer, and Petersdorf[8] was used in the present study. Tube dilution sensitivities were also performed as previously described[2] except that nutrient broth at pH 6.6 was used to stabilize the antibiotic thus permitting accurate interpretation of the minimum inhibitory and minimum bactericidal concentrations 24 hours after incubation at 37° C. Nutrient agar at the same pH was also used for the single-disc cephaloglycin sensitivity studies.

* Supplied by Dr. R. S. Griffith of Eli Lilly and Company, Indianapolis, Indiana.

RESULTS

Clinical studies:

The effectiveness of cephaloglycin in the treatment of significant urinary tract infection is demonstrated in Table 1. Bacteriuria was eradicated during cephaloglycin treatment in all patients with nonobstructive uropathy. Twelve patients relapsed with the same organism within two to four weeks after cessation of therapy. Only four patients were considered cured. Eight additional patients had an abnormal residual volume of bladder urine (> 20 ml.) detected after the initiation of therapy and were analyzed

TABLE 1. EFFECT OF ORAL CEPHALOGLYCIN ON BACTERIURIA

Patients	*Urine Cultures*			
Nonobstructive	*Pretreatment*	*During treatment*	*Post-treatment**	*Remarks*
W.L.	K. Aerobacter	0	0	cure
N.A.	K. Aerobacter	0	0	cure
F.S.	E. coli	0	0	cure
C.T.	P. mirabilis	0	0	cure
E.G.H.	E. coli	0	+	relapse†
J.G.	E. coli	0	+	relapse†
A.K.	E. coli	0	+	relapse
E.H.	E. coli	0	+	relapse
E.Ha	E. coli	0	+	relapse†
R.G.	E. coli	0	+	relapse†
S.K.	Paracolon	0	+	relapse
R.H.	Paracolon	0	+	relapse
A.G.	P. mirabilis	0	+	relapse
J.K.	P. mirabilis	0	+	relapse
W.M.	P. mirabilis	0	+	relapse
	K. Aerobacter	0	+	relapse
R.C.	K. Aerobacter	0	+	relapse
Obstructive				
L.B.	P. mirabilis	0	Serratia	relapse
F.L.	P. mirabilis	0	K. Aerobacter	relapse
L.H.	K. Aerobacter	0	+	relapse
J.Ma	P. mirabilis	+	+	failure
S.M.	P. rettgeri	+	+	failure
W.Ma	Serratia	+	+	failure**
G.L.	Serratia	+	+	failure**
J.M.	Serratia	+	+	failure**
	Pseudomonas	+	+	failure**

* + signifies relapse with same organism unless indicated.
** Original isolates were resistant to cephaloglycin.
† Dose of cephaloglycin reduced to 250 mg. during latter 5 days of treatment.

separately. There were no cures in this group. Furthermore, elimination of bacteriuria was achieved in only three patients during treatment and each relapsed in the follow-up period, two with a strain different from the pre-treatment isolate. In addition, bacteriuria persisted during treatment in the remaining five patients with obstructive uropathy. However, only two of these five patients were infected with organisms that were sensitive to the antibiotic. Three patients in this latter group were infected with strains of Pseudomonas and Serratia that were initially resistant to cephaloglycin. The sensitivity to cephaloglycin of the organisms isolated from the initial culture were compared with the sensitivity of the same organisms obtained following treatment (Table 2). Pre- and post-treatment sensitivities did not differ by more than one tube-dilution for any given strain, indicating that no significant increase in drug resistance occurred.

Serum levels of cephaloglycin determined in 15 patients, 2 to 2½ hours following an oral dose of 500 mg., ranged from 0.40 to 3.09 μg/ml. with a mean value of 1.46 (Fig. 1). In two additional patients, 250 mg. were given and serum levels were 0.383, and 0.483 μg/ml. Two values fell outside the above range of values (9.0 μg/ml. after 250 mg. and 15.0 μg/ml.

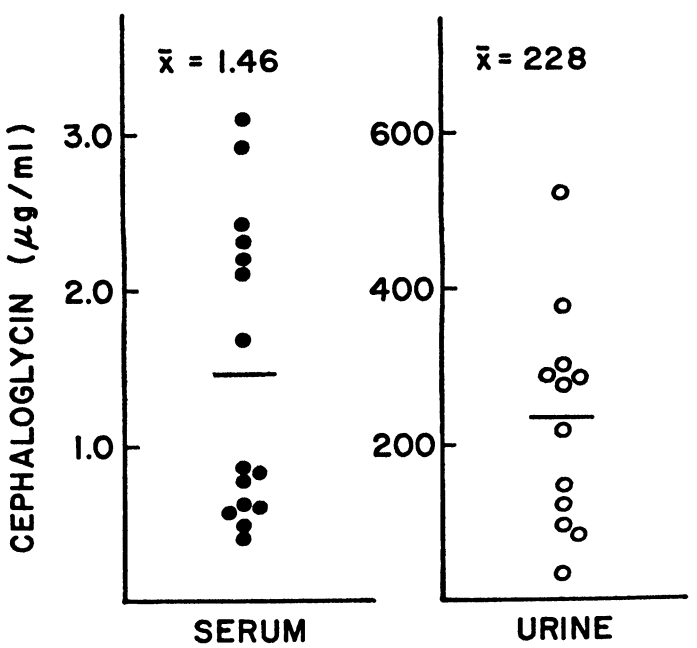

FIG. 1. Serum and urine concentrations of cephaloglycin in patients receiving a 500 mg. oral dose.

TABLE 2. CEPHALOGLYCIN TUBE DILUTION SENSITIVITY OF URINARY PATHOGENS ISOLATED BEFORE AND AFTER THERAPY

Patient	*Organism*	*Initial culture*		*Follow-up culture*	
		*MIC**	*MBC***	*MIC**	*MBC***
A.G.	P. mirabilis	12.50	50.00		>50.00
E.Ha	E. coli	1.56	3.13	3.13	6.25
E.G.H.	E. coli		50.00	>50.00	>50.00
R.H.	Paracolon	3.13	3.13	3.13	
S.K.	Paracolon	1.56	6.25	3.13	6.25
W.L.	K. Aerobacter	12.50	12.50	6.25	12.50
W.Ma	Serratia	>50.00	>50.00	>50.00	>50.00
J.Ma	P. rettgeri		50.00		25.00
W.M.	K. Aerobacter	25.00	50.00	25.00	50.00

* MIC—minimum inhibitory concentration.
** MBC—minimum bactericidal concentration.

after 500 mg. oral doses) and occurred in patients with chronic renal insufficiency and uremia.

Urine concentrations of cephaloglycin determined in 12 patients without obstructive uropathy ranged from 35.6 to 524 μg/ml. with a mean value of 228 μg/ml. following oral doses of 500 mg. (Fig. 1), and 11.5 and 28 μg/ml. in two patients after one oral dose of 250 mg. of drug. The concentration of cephaloglycin in the urine of one patient with an abnormal residual urine volume was 1232 μg/ml.

Gastrointestinal intolerance was the major side effect of the drug observed during the study. Diarrhea developed in four patients during treatment with a dose of two grams per day but was controlled in each instance by reducing the dose to one gram per day.

Eosinophilia occurred in three patients during treatment. No other hematologic, renal or liver toxicity was observed. Three patients developed mild erythematous skin rashes during the course of therapy and two patients developed balanitis one week after cessation of treatment. In general, the drug was well tolerated and in no instance was it necessary to discontinue therapy. Three patients gave a history of penicillin sensitivity but eosinophilia or allergic reactions were not observed.

Laboratory studies

Tube-dilution sensitivity of Gram-negative isolates to cephaloglycin: The bacteriostatic and bactericidal activity of cephaloglycin tested against 10^4 bacterial cells of *Escherischia coli,* and species of Klebsiella-Aerobacter, Proteus, Paracolon, Pseudomonas and Serratia are summarized in Table

3 and Figure 2. Strains of Pseudomonas and Serratia were uniformly resistant to high concentrations of cephaloglycin. Furthermore, only 26 per cent of the remaining bacterial strains tested were inhibited by cephalo-

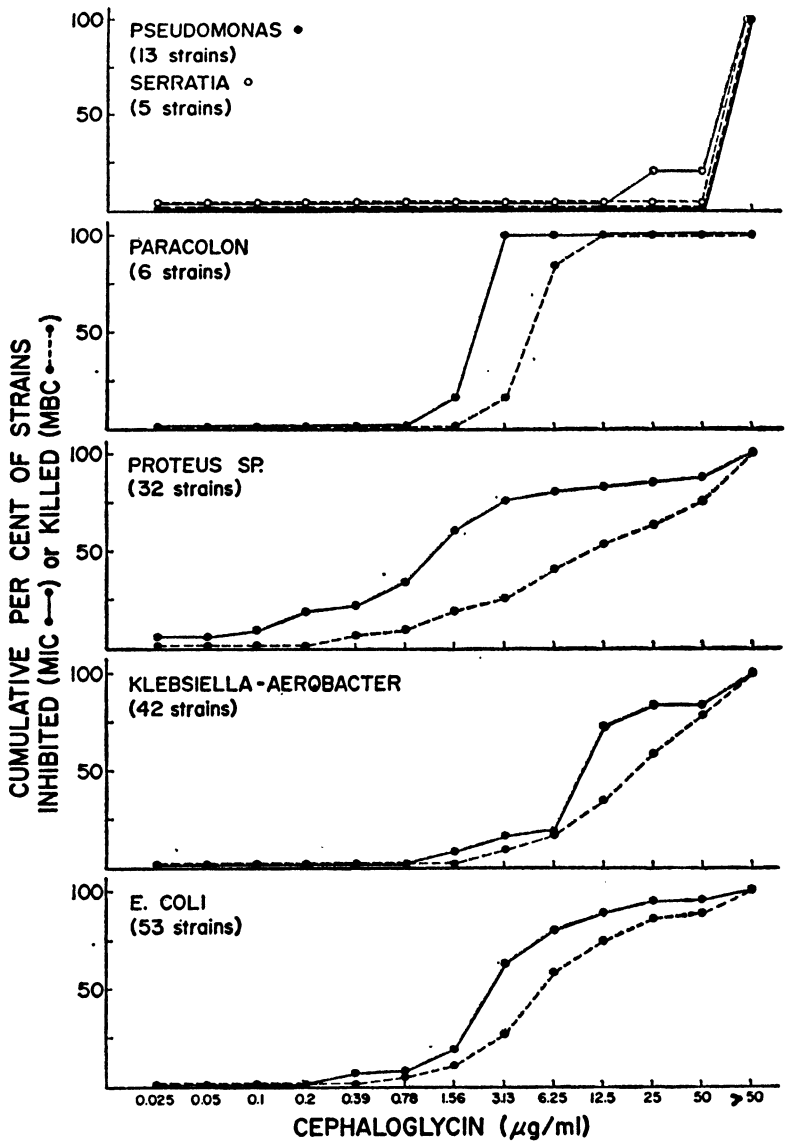

FIG. 2. Susceptibility of Gram-negative bacteria to increasing concentrations of cephaloglycin.

TABLE 3. ANTIMICROBIAL ACTIVITY OF CEPHALOGLYCIN IN VITRO

Organisms	Number tested	Number of Bacterial Strains with Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) (μg/ml.)																					
		0.025		0.05		0.01		0.2		0.39		0.78		1.56		3.13		6.25		12.5		25		50		>50	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	53									3		1	2	6	3	23	9	10	15	5	10	2	6		1	3	7
Klebsiella-Aerobacter	42											1	1	2		4	3	10	2	13	9	6	10		9	6	8
Proteus	32	2				1		3		1	2	4	1	9	3	4	2	1	5	1	4	1	3	1	4	4	8
Pseudomonas	13																									13	13
Paracolon	6													1		5	1		4		1						
Serratia	5																					1				4	5

glycin in concentrations (1.56 μg/ml.) equal to peak levels observed in serum after a 500 mg. oral dose in man.[8] However, 53 per cent of these strains were inhibited by 3.13 μg/ml. of drug, indicating that greater inhibition rates might be achievable with larger oral doses of cephaloglycin. This latter concentration of drug was most effective against the majority of strains of *E. coli* (62%), Proteus (75%), and Paracolon (100%) organisms. Nevertheless, there was a poor correlation between peak serum levels of cephaloglycin achievable in man after a 500 mg. oral dose and the minimum concentration of this antibiotic necessary for inhibiting bacterial growth *in vitro*. In contrast, 90 per cent of the bacterial strains studied,

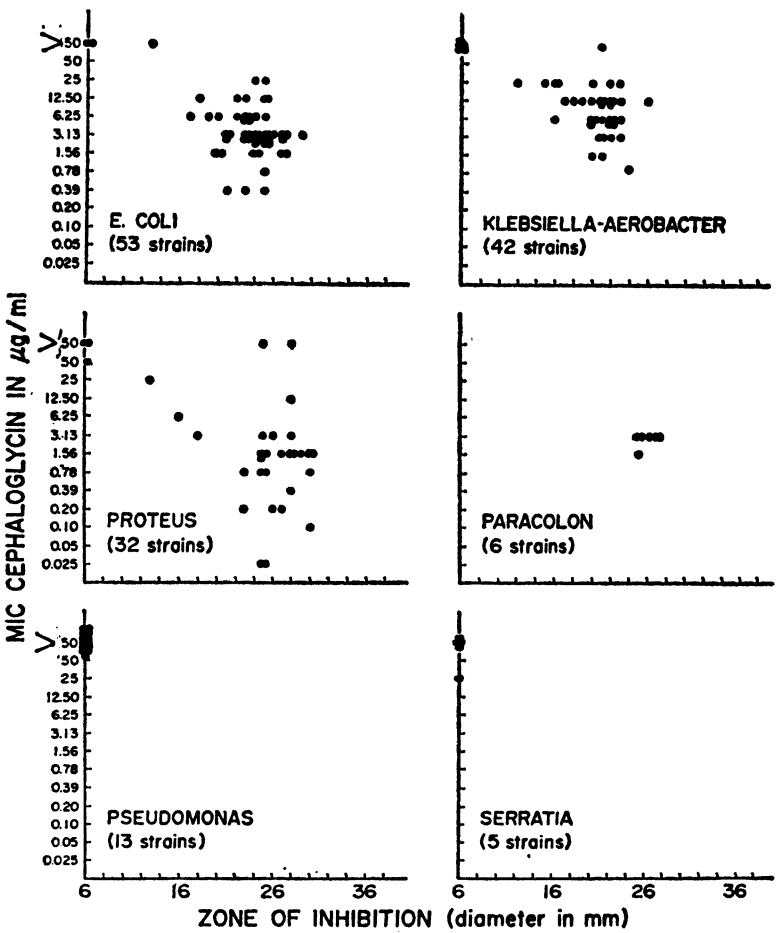

FIG. 3. Correlation of results of 30 μg. disc and tube-dilution sensitivities of cephaloglycin against Gram-negative bacteria.

except for strains of Pseudomonas and Serratia, were inhibited by 50 μg/ml. of cephaloglycin. This concentration of drug was achieved in the urine of the majority of our patients after an oral dose of 500 mg.

Comparison of disc and *tube dilution sensitivities:* The zone diameter of inhibition of Gram-negative bacteria tested with 30 μg. of cephaloglycin is illustrated in Figure 3. Since the antibiotic disc has a diameter of 6 mm., which is included in the final reading, a zone size of 6 mm. indicates no inhibition of the growth of the organism. The zone of inhibition for each strain studied is plotted against the minimum concentration (MIC) of cephaloglycin, as determined by tube-dilution sensitivity, necessary to inhibit the growth of that bacterial strain. Excellent correlation was observed between disc and tube-dilution sensitivities of cephaloglycin against strains of Serratia, Pseudomonas, and Paracolon organisms. In addition, equally good correlation was observed with 29 of 32 (90%) strains of Proteus. Twenty-four strains of Proteus had disc zone diameters of inhibition of 18 mm. or more and were inhibited by tube-dilution concentrations of 3.13 μg/ml. or less of cephaloglycin. Five Proteus strains had disc zones of inhibition less than 18 mm. and required concentrations of 6.25 μg/ml. or more. In contrast, the tube-dilution sensitivities of only 36 of 53 (68%) strains of *E. coli* correlated well with the disc sensitivities. Seventeen strains of *E. coli* had disc zone diameters of inhibition of 18 mm. or more but required concentrations of cephaloglycin of 6.25 micrograms or greater per milliliter to inhibit growth. The greatest discrepancies between disc and tube-dilution sensitivities were observed with strains of Klebsiella-Aerobacter species. Twenty-four of the 42 strains studied had disc zone diameters of inhibition of 18 mm. or more but required concentrations of cephaloglycin of 6.25 micrograms or more per milliliter before bacterial growth was inhibited.

Additional comparisons between disc and tube-dilution sensitivities were made utilizing 5 and 15 μg. discs of cephaloglycin. Smaller zones of inhibition were observed with lower disc concentrations of cephaloglycin. However, independent correlations between minimum inhibitory tube-dilution concentrations and zone diameters of inhibition produced by both the 5 and 15 μg. discs were identical to the results observed with the 30 μg. disc of cephaloglycin.

DISCUSSION

The introduction of an oral cephalosporin, cephaloglycin, which possessed a broad spectrum of antimicrobial activity was initially reviewed as a major development in antimicrobial therapy. Preliminary studies by others indicated that significant gastrointestinal absorption occurred in

man, despite low serum levels, since high concentrations of cephaloglycin were observed in the urine of patients following an oral dose of 500 mg.[8] These observations suggested that cephaloglycin might be an effective agent in the treatment of bacteriuria.

The results of the present study confirm some of these earlier observations. The oral administration of cephaloglycin does produce a high concentration of drug in the urine, although serum levels, obtained at the time of maximum absorption,[8] remain low. Furthermore, the data indicate that cephaloglycin is effective in eradicating Gram-negative organisms from the urine of patients with nonobstructive uropathy *during* treatment. However, bacteriuria recurred in the majority of these patients shortly after treatment was discontinued.

The high relapse rate observed in the present study was not due to inadequate concentrations of antibiotic in the urine nor to the emergence or acquisition of organisms resistant to the drug. Furthermore the relapses observed in patients with nonobstructive uropathy occurred with the same species and presumably the same strain of bacteria initially recovered from the urine, which suggests that cephaloglycin merely suppressed bacterial growth without sterilizing the urine.

The importance of high antibacterial serum levels in the treatment of renal parenchymal infection has not been precisely defined. Although no attempt was made in the present study to determine the localization of bacteriuria to either the upper or lower tracts, the high relapse rate might be related to the low serum levels achieved after oral administration of the drug. The tube dilution sensitivity data clearly indicate that the concentration of cephaloglycin necessary to inhibit or kill the infecting organisms exceeded the peak concentration of drug which could be achieved in the serum after an oral dose of 500 mg. Higher daily doses of cephaloglycin or longer treatment periods might result in better cure rates. Gastrointestinal intolerance, the major side effect observed, must be considered, however, and was controlled by reducing the initial dose used in the present study. Additional side effects observed during treatment included eosinophilia in three patients and skin rashes in three others. Furthermore, two patients developed balanitis one week after treatment had been discontinued. Nevertheless, none of the reactions observed was severe or life-threatening and in no instance was it necessary to discontinue treatment.

The administration of large doses of cephalothin to patients with renal insufficiency has been associated with a Coombs-positive hemolytic anemia.[9] Similarly the use of large doses of cephaloridine has resulted in renal insufficiency in some patients.[10] Normal doses of cephaloglycin were administered to two patients with chronic renal insufficiency in the present study.

Although high serum levels of drug were observed in both, neither patient developed adverse reactions to the drug. In particular, cephaloglycin was not associated with further renal insufficiency nor did either patient experience a fall in hematocrit values. Nevertheless, the drug should be used cautiously in patients with renal impairment. Furthermore, the low serum levels observed in patients with normal renal fuction suggest that cephaloglycin should not be used to treat infections outside the urinary tract.

Finally, the results of the laboratory data observed in the present study indicate that, with the exception of some strains of Klebsiella-Aerobacter and a few strains of *E. coli,* the single high-concentration disc method correlates closely with the tube-dilution method. The strains separated into two distinct populations, sensitive and resistant, with few intermediate strains, and exhibited a "bimodal distribution." The single high-concentra- disc method is reliable in determining the antimicrobial sensitivity or resistance of strains of Serratia, Pseudomonas, Paracolon, and Proteus and the majority of strains of *E. coli* to cephaloglycin.

SUMMARY

The oral administration of cephaloglycin, a new semisynthetic antibiotic, resulted in high concentrations of drug in the urine, although serum levels remained low. However, high concentrations of cephaloglycin were observed in the serum of two patients with renal insufficiency following oral administration of the drug. Although cephaloglycin was effective in eradicating Gram-negative organisms from the urine of patients with non-obstructive uropathy during treatment, bacteriuria recurred in the majority shortly after treatment was discontinued. Gastrointestinal intolerance occurred in four patients and was the most significant adverse effect observed.

In vitro sensitivity studies of 151 Gram-negative bacteria indicated that, with the exception of some strains of Klebsiella-Aerobacter, the single high-concentration disc method compared favorably with the tube-dilution method and was reliable in determining the antimicrobial sensitivity of strains of Serratia, Pseudomonas, Paracolon, Proteus, and the majority of strains of *E. coli* to cephaloglycin. Furthermore, cephaloglycin was not effective against Pseudomonas or Serratia organisms.

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REFERENCES

1. Wick, W. E. and Boniece, W. S.: *In vitro* and *in vivo* laboratory evaluation of cephaloglycin and cephaloridine. *Appl. Microbiol.,* 1965, *13,* 248-253.
2. Thornton, G. F. and Andriole, V. T.: Laboratory and clinical studies of a new antibiotic, cephaloridine, in the treatment of Gram-positive infections. *Yale J. Biol. Med.,* 1966, *39,* 9-20.

3. Griffith, R. S.: Personal communication.
4. Thornton, G. F., Lytton, B., and Andriole, V. T.: Bacteriuria during indwelling catheter drainage. *J. Amer. med. Ass.,* 1966, *195,* 179-183.
5. Mulrow, P. J., Huvos, A., and Buchanan, D. L.: Measurement of residual urine with I^{131}-labeled diodrast. *J. Lab. clin. Med.,* 1961, *57,* 109-113.
6. Schaub, I. G., Foley, M. K., Scott, E. G., and Bailey, W. R.: *Diagnostic Bacteriology,* 5th Ed. St. Louis, C. V. Mosby Company, 1958.
7. Kirby, W. M. M., Yoshihara, G., Sundstedt, K., and Warren, J.: Clinical usefulness of a single disc method for antibiotic sensitivity testing. In: *Antibiotics Annual,* 1956-1957. New York, Antibiotica, Inc., 1957, p. 892.
8. Turck, M., Lindemeyer, R. I., and Petersdorf, R. G.: Comparison of single-disc and tube dilution techniques in determining antibiotics sensitivities of gram-negative pathogens. *Ann. intern. Med.,* 1963, *58,* 56-65.
9. Gralnick, H. R., Wright, L. D., and McGinniss, M. H.: Coombs' positive reactions associated with sodium cephalothin therapy. *J. Amer. med. Ass.,* 1967, *199,* 725-726.
10. Hinman, A. R. and Wolinsky, E.: Nephrotoxicity associated with the use of cephaloridine. *J. Amer. med. Ass.,* 1967, *200,* 724-726.