ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1976, p. 397-405

Vol. 9, No. 3
Printed in U.S.A.

In Vitro and Clinical Studies of Cefatrizine, a New Semisynthetic Cephalosporin

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Received for publication 18 September 1975

Cefatrizine, a new oral semisynthetic cephalosporin, was evaluated in vitro and in the treatment of 18 patients with acute urinary tract infection, pneumonia, and soft tissue infection. In vitro, it was more active than cephalexin for gram-positive and gram-negative bacteria. It was also more active than cephalothin, cefazolin, and cephapirin against most of the gram-negative bacteria but less active against the gram-positive bacteria. Of the patients treated with cefatrizine, only one failed to respond. This patient had pneumococcal conjunctivitis and hypogammaglobulinemia and neutropenia. The mean peak serum level after multiple 6-hourly doses of 500 mg was 6.2 μg/ml. The serum levels of cefatrizine necessary for inhibition of most susceptible organisms were well within the achievable range. The drug was well tolerated, and no renal, hepatic, or hematological toxicity was detected.

Among the cephalosporins available orally, cephalexin and cephradine have been shown to produce adequate serum levels and are used in the treatment of a variety of infections (3, 6, 8, 11, 14, 17). Cephaloglycin, on the other hand, is poorly absorbed from the gastrointestinal tract (12, 13). Recently, cefatrizine {7-[D-α-amino-α-(4-hydroxyphenyl)acetamido]-3-(1H-1,2,3,-triazol-5-ylthio methyl)-3-cephem-4-carboxylic acid}, at present an investigational drug (BL-S640), has been shown to be well absorbed in animals after oral and parenteral administration (10).

The present study was undertaken to evaluate the in vitro activity of cefatrizine and its efficacy and safety in the treatment of infections due to susceptible organisms.

MATERIALS AND METHODS

Laboratory studies. The in vitro susceptibility of 256 recent clinical isolates to cefatrizine, cephalexin, cephalothin, cefazolin, and cephapirin was determined by the agar dilution method (2), using Mueller-Hinton agar (BBL), except in the case of *Haemophilus influenzae*, where GC medium base (BBL) supplemented with 1% hemoglobin (BBL) and 1% IsoVilateX (BBL) was used. Inoculation of the plates was performed by use of the Steers replicator (15). Approximately 10^5 organisms were delivered to each plate for each representative organism. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that inhibited growth after 18 h of incubation (24 h for *H. influenzae*) at 37 C. The susceptibility of the following bacteria was determined: 25 strains each of *Escherichia coli* and penicillinase-producing *Staphylococcus aureus*, 24 strains each of *Proteus mirabilis*, *Streptococcus pneumoniae*, and *Streptococcus faecalis*, 23 strains of group A streptococcus, 22 strains of *H. influenzae*, 20 strains each of *Klebsiella* sp. and penicillinase-negative *S. aureus*, 17 strains of *Enterobacter* sp., and 16 strains each of alpha-streptococcus and indole-positive *Proteus*.

Disk susceptibility testing was done according to the standardized disk technique described by the Food and Drug Administration (4, 5), with 30-μg disks of cefatrizine obtained from Bristol Laboratories, Syracuse, N. Y. The zone diameter was then plotted against the MIC values and a regression curve was calculated by the method of least squares.

Serum levels from 19 patients were determined by disk plate assay (7), using *Sarcina lutea* ATCC 9341 as the test organism; when possible, assays were done at 1, 2, 3, and 6 h after the oral ingestion of multiple 6-hourly doses of 500 mg of cefatrizine, without regard to meals. The patients were receiving the drug for 2 to 4 days when the serum levels were obtained.

Clinical studies. The drug was administered orally to 33 patients with urinary tract infection, bacterial pneumonia, and skin and soft tissue infection due to susceptible organisms. Twenty-three patients were given 2 g/day and ten were given 1 g/day in four equal doses. Duration of treatment ranged from 5 to 20 days.

Appropriate bacterial cultures as well as hepatic (serum glutamic oxalacetic transaminase, bilirubin, alkaline phosphatase), renal (urinalysis, serum creatinine, blood urea nitrogen), and hematological (complete blood count) studies were done before, during, and after treatment. All patients were followed for 6 weeks after treatment was completed.

RESULTS

Antimicrobial activity. Comparison of the in vitro activity of cefatrizine with that of cephalexin, cephalothin, cefazolin, and cephapirin is shown in Fig. 1 through 10. Against most of the gram-positive organisms tested, cefatrizine was considerably more active than cephalexin, but less active than cephalothin, cefazolin, and cephapirin. Ninety-five percent of the strains of group A beta-hemolytic streptococcus were inhibited by less than 0.025 μg of cephapirin per ml and by 0.2 μg of cefatrizine, cefazolin, and cephalothin per ml. Cephalexin inhibited 90% of the strains of group A streptococcus at a concentration of 1.6 μg/ml (Fig. 1). All strains of *S. pneumoniae* were inhibited by 0.4 μg of cefazolin, cephapirin, and cephalothin per ml and by 0.8 μg of cefatrizine per ml; in comparison, 6.4 μg of cephalexin per ml was needed to inhibit all strains of *S. pneumoniae* (Fig. 2). One hundred percent of the strains of alpha-streptococcus were inhibited by 0.8 μg of cefazolin and cephapirin per ml, by 1.6 μg of cephalothin and cefatrizine per ml, and by 25.6 μg of cephalexin per ml; however, 95% of the strains of alpha-streptococcus were inhibited by only 6.4 μg of cephalexin per ml (Fig. 3). All strains of *S. aureus* (penicillinase positive and negative) were inhibited by 1.6 μg of cefatrizine and 6.4 μg of cephalexin per ml (Fig. 4 and 5). The parenteral cephalosporins were more active, especially cephalothin and cephapirin, which inhibited all strains of penicillinase-producing *S. aureus* at a concentration of 0.4 μg/ml (Fig. 4). All strains of *S. faecalis* were resistant to the five cephalosporins tested.

Cefatrizine was the most active of the cephalosporins against most of the gram-negative organisms tested. Eighty percent of the *E. coli* strains were inhibited by 1.6 μg of cefatrizine and cefazolin per ml. At the same concentration, cephalexin, cephalothin, and cephapirin inhibited less than 10% of the *E. coli* strains (Fig. 6). All strains of *Klebsiella* sp. were inhibited by 3.2 μg of cefatrizine and 6.4 μg of cefazolin per ml. Cephalothin, cephapirin, and cephalexin required a concentration of 12.8 μg/ml to inhibit all strains of *Klebsiella* sp. (Fig. 7). Only 35% of the strains of *Enterobacter* sp. were inhibited by 6.4 μg of cefatrizine per ml; less than 10% of the strains of *Enterobacter* sp. were inhibited by 25.6 μg or less of all the other cephalosporins per ml (Fig. 8); 3.2 μg of cefatrizine per ml inhibited all strains of *P. mirabilis;* the same concentration of the other cephalosporins inhibited less than 35% of the strains of this organism (Fig. 9). All strains of indole-positive *Proteus* proved resistant to all the cephalosporins tested. All strains of *H. influenzae* were inhibited by 6.4 μg of cefatrizine, cephapirin, and cephalothin per ml; the same concentration of cefazolin and cephalexin inhibited 81 and 55% of the strains, respectively (Fig. 10).

The correlation of activity of cefatrizine as determined by the agar dilution method and the standardized disk technique with a 30-μg/ml cefatrizine disk is shown in Fig. 11 and 12. All the strains inhibited by 12.5 μg/ml or more

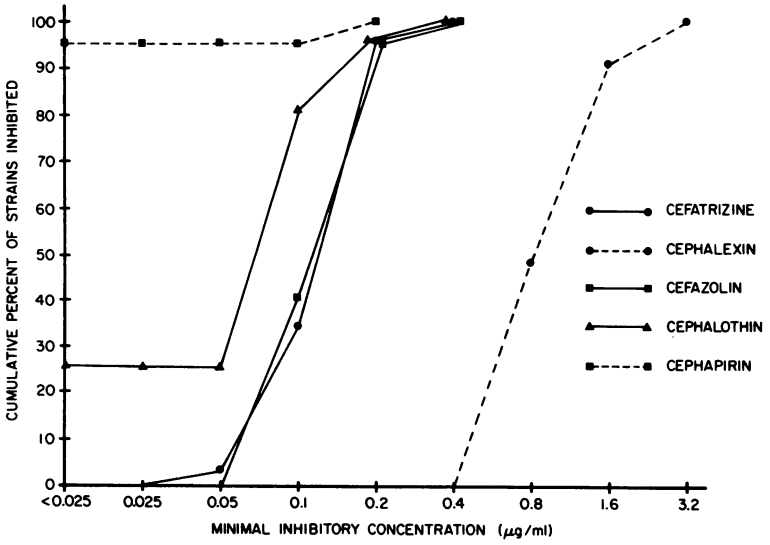

FIG. 1. *Comparison of susceptibility of 23 strains of group A beta-hemolytic streptococcus to cefatrizine and other cephalosporins.*

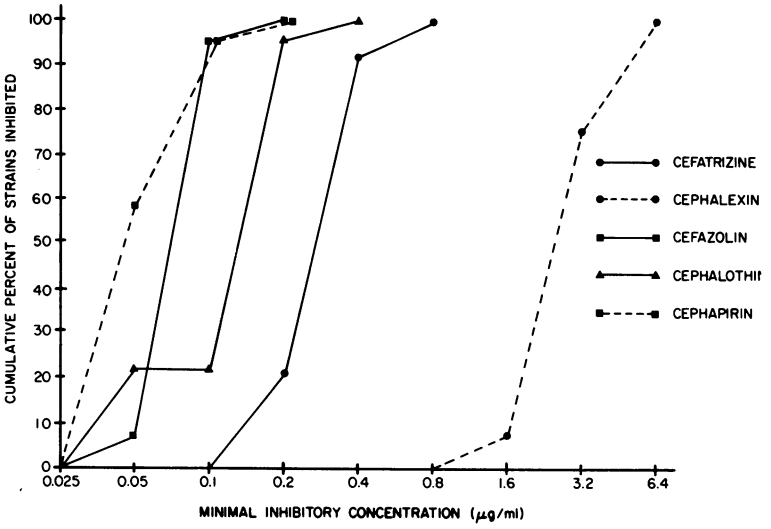

FIG. 2. *Comparison of susceptibility of 24 strains of Streptococcus pneumoniae to cefatrizine and other cephalosporins.*

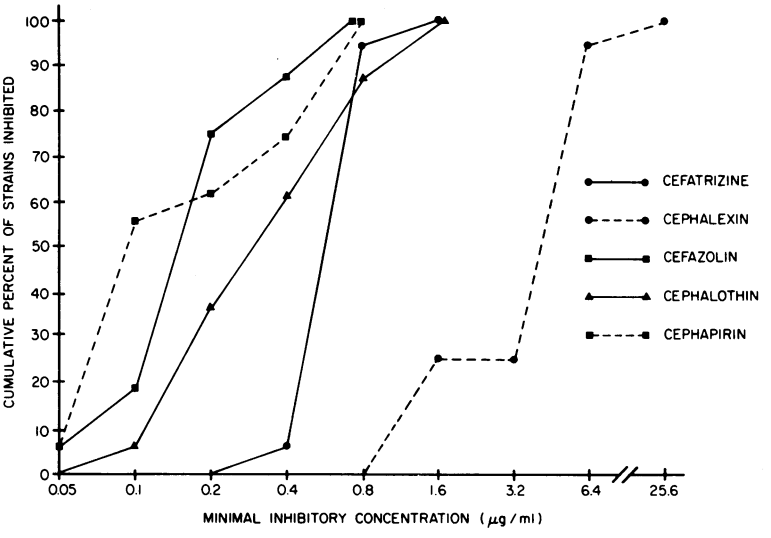

FIG. 3. *Comparison of susceptibility of 16 strains of alpha-streptococcus to cefatrizine and other cephalosporins.*

had zones of inhibition of 17 mm or less. On the other hand, 97% of strains inhibited by 6.25 μg/ml or less had zones of inhibition of 18 mm or more.

Serum concentrations. A mean peak serum level of 6.2 μg/ml was obtained 2 h after the previous dose following multiple 6-hourly doses of 500 mg of cefatrizine. After 6 hours the mean serum level was 1.6 μg/ml. Compared with our previous studies with cephalexin (1), it can be seen that the levels of cefatrizine were lower than those of cephalexin at 1 and 2 h but higher after 6 h (Table 1); these levels are, however, not entirely comparable since in the case of cephalexin they were obtained after a single dose, whereas those for cefatrizine were obtained after multiple doses.

Clinical results. A total of 33 patients were treated with cefatrizine; in 15 of these patients no pathogenic organism was isolated, but therapy was continued in 11 of them because clinical and laboratory evidence suggested a bacterial infection. These patients, however, were not included in the evaluation of the efficacy of

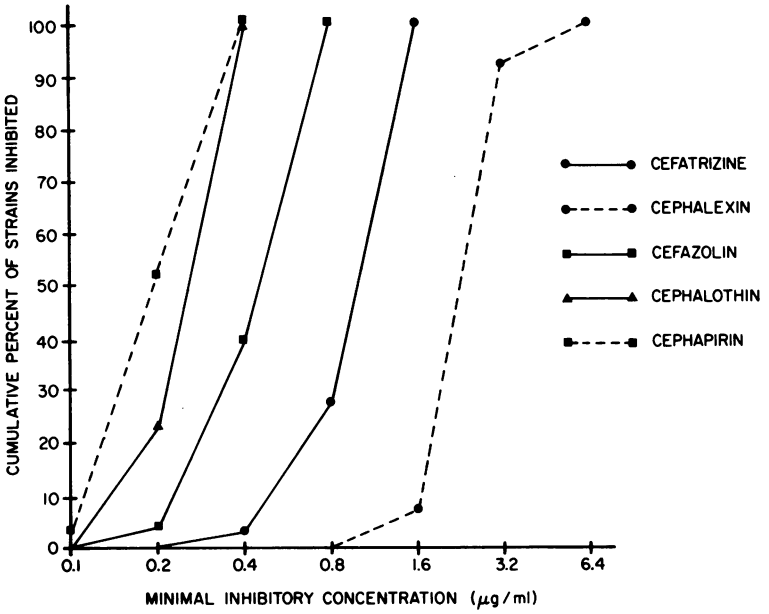

FIG. 4. *Comparison of susceptibility of 25 strains of penicillinase-producing Staphylococcus aureus to cefatrizine and other cephalosporins.*

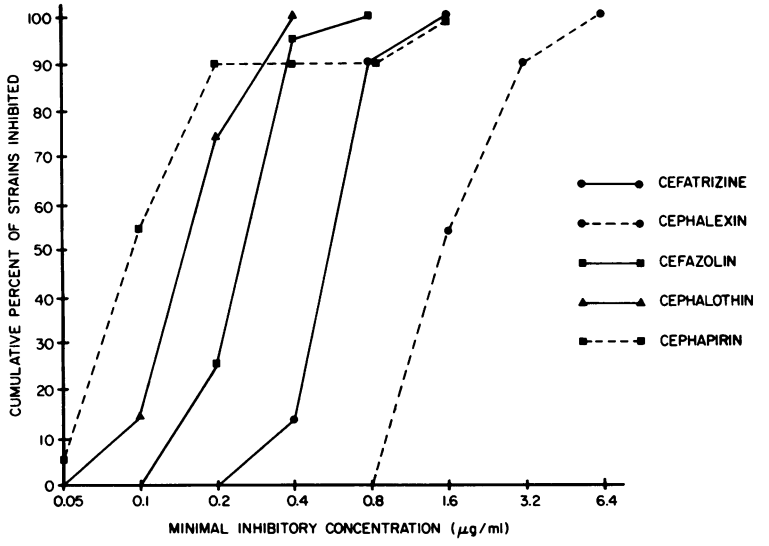

FIG. 5. *Comparison of susceptibility of 20 strains of non-penicillinase-producing Staphylococcus aureus to cefatrizine and other cephalosporins.*

the drug. Of the 18 patients in whom a bacterial pathogen was isolated, six had an acute urinary tract infection, seven had soft tissue infection, four had pneumonia, and one had bacterial conjunctivitis (Table 2).

All six patients with acute urinary tract infection became asymptomatic, and their urine cultures were negative within 48 h of treatment and remained sterile while receiving the antibiotic. Four patients were treated with 1 g daily, and two were treated with 2 g daily. Reinfection with a different organism occurred within 1 week after discontinuation of therapy in three patients. In two of them this was not unexpected, since one patient had a neurogenic bladder and history of recurrent urinary tract

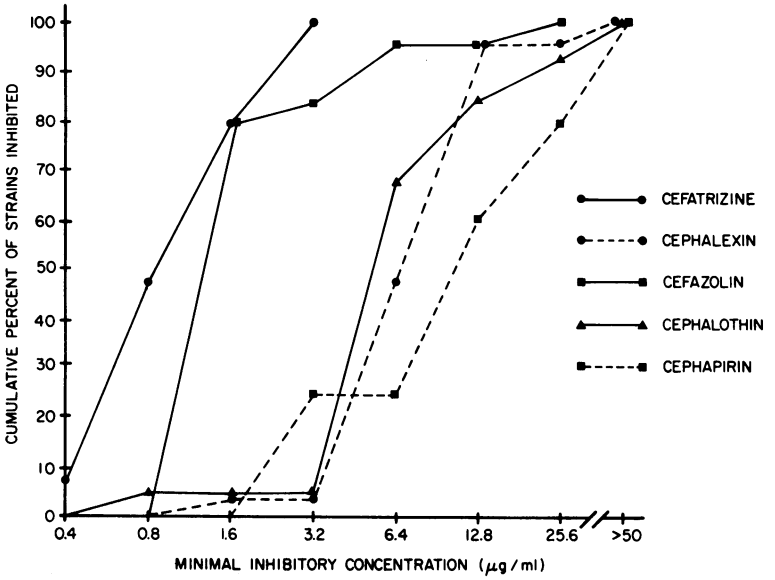

FIG. 6. *Comparison of susceptibility of 25 strains of Escherichia coli to cefatrizine and other cephalosporins.*

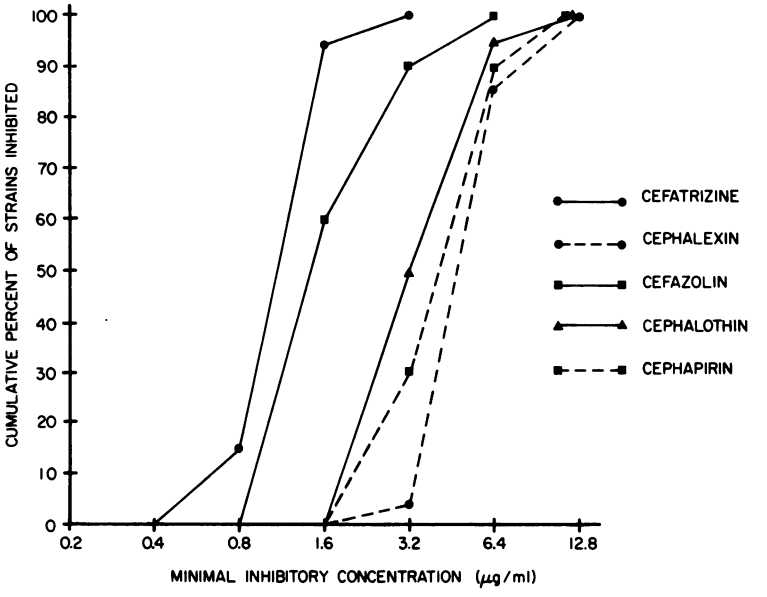

FIG. 7. *Comparison of susceptibility of 20 strains of Klebsiella sp. to cefatrizine and other cephalosporins.*

infections and the other was reinfected after a cystoscopic examination. These three patients were considered cured of their original infection. Two patients remained asymptomatic and with negative urine cultures for the 6 weeks post-treatment. Relapse with the original organism 3 days after discontinuation of therapy was encountered in one patient who had a right kidney stone and chronic pyelonephritis.

Four patients with pneumococcal pneumonia were treated with 2 g of cefatrizine daily for 7 days. All of them responded promptly and were afebrile within 24 to 48 h. Follow-up chest X rays showed complete clearing of the infiltrates in all of the patients.

Of the seven patients with soft tissue infections, four had subcutaneous abscesses and three had cellulitis. All of them were treated

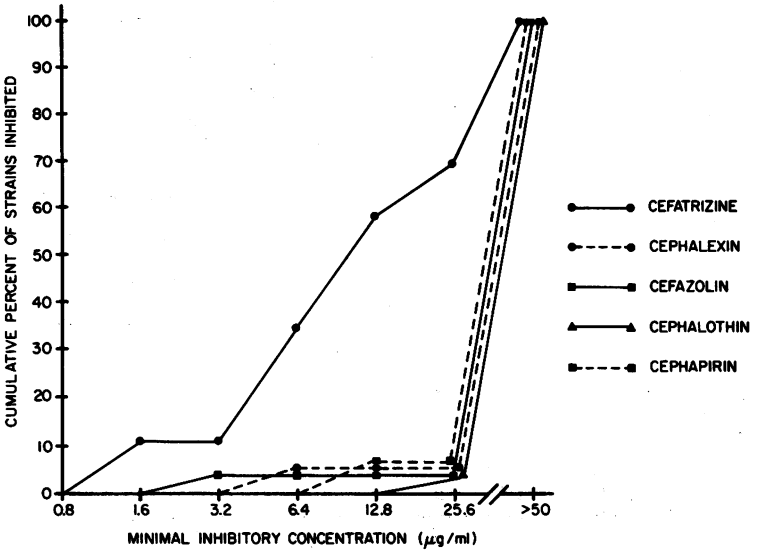

FIG. 8. *Comparison of susceptibility of 17 strains of Enterobacter sp. to cefatrizine and other cephalosporins.*

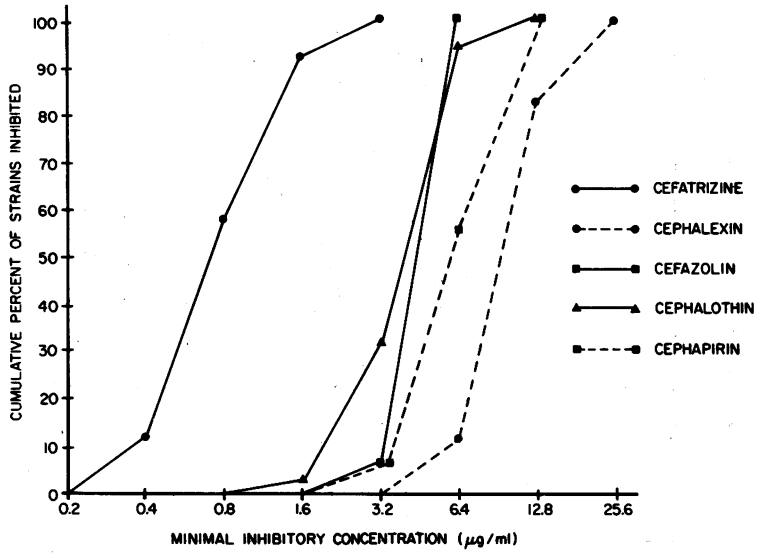

FIG. 9. *Comparison of susceptibility of 24 strains of Proteus mirabilis to cefatrizine and other cephalosporins.*

with 2 g of cefatrizine daily except one patient who was treated with only 1 g daily. Duration of treatment ranged between 5 and 20 days; the longest course of therapy was given to a patient with a subcutaneous abscess who had hypogammaglobulinemia and cyclic neutropenia. Six patients were cured and one was lost to follow-up. Two patients were cured who had incision and drainage of their abscesses in addition to antibiotic therapy. In the latter cases rare *S. aureus* was isolated throughout treatment, but their wounds healed completely.

Finally, we treated a patient with bilateral pneumococcal conjunctivitis. This was the same patient with hypogammaglobulinemia and cyclic neutropenia. She failed to respond clinically and bacteriologically to a 10-day course with 2 g of cefatrizine daily. Previously she had also failed to respond to 18 days of treatment with cephalexin.

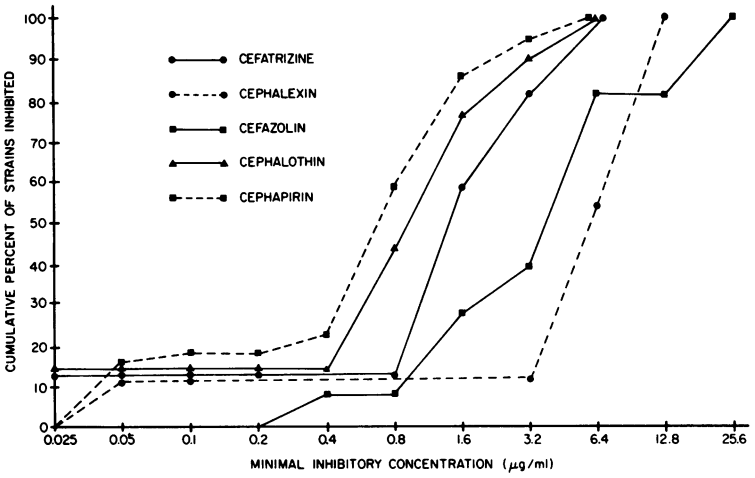

FIG. 10. *Comparison of susceptibility of 22 strains of Haemophilus influenzae to cefatrizine and other cephalosporins.*

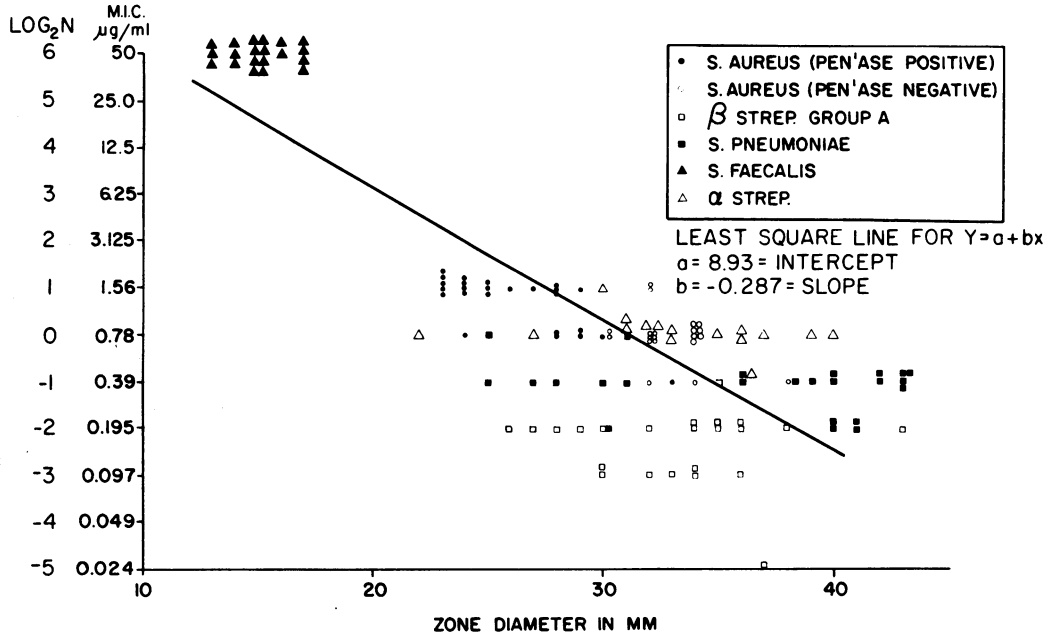

FIG. 11. *Relationship of zone diameters by the standardized disk procedure (4, 5) to agar dilution MIC values for gram-positive bacteria.*

Among the 28 patients that completed their course of treatment, no major adverse reactions were observed. Five patients had very mild diarrhea, and no treatment or termination of therapy was necessary. One patient had mild nausea without vomiting, and in another one the dose was reduced from 2 to 1 g daily because of nausea and vomiting.

Complete blood count, serum glutamic oxalacetic transaminase, bilirubin, alkaline phosphatase, serum creatinine, blood urea nitrogen, and urinalysis were determined before, during, and after therapy, and no abnormalities were detected in any case.

DISCUSSION

Cefatrizine is a new semisynthetic cephalosporin with a wide spectrum of antibacterial

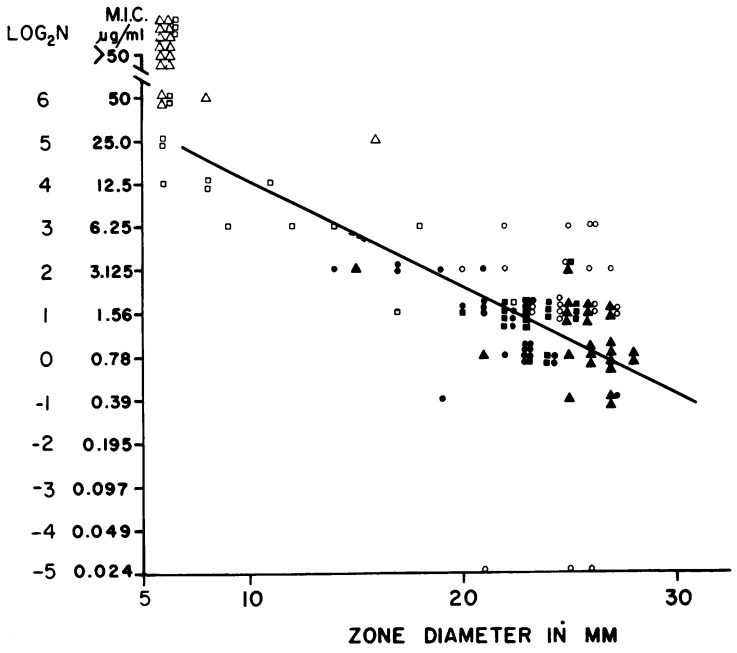

FIG. 12.. *Relationship of zone diameters by the standardized disk procedure (4, 5) to agar dilution MIC values for gram-negative bacteria. Symbols: △, Proteus (indole positive); ▲ P. mirabilis; □, Enterobacter; ■, Klebsiella; ●, E. coli; ○, H. influenzae. Least square line for y = a + bx. a = 6.59 = intercept; b = −0.249 = slope.*

TABLE 1. *Serum concentrations after 500 mg of cefatrizine (multiple doses) and cephalexin (single dose)*

Time after treatment (h)	Cefatrizine (µg/ml)	Cephalexin (µg/ml)
0.5	Not done	10.8
1	6.1	21.2
2	6.2	10.1
3	5.5	Not done
4	Not done	2.6
6	1.6	0.7

activity. It has been shown to be effective in the treatment of experimental infections of mice both orally and parenterally (10). Earlier reports revealed that cefatrizine was more active in vitro than cephalexin, cephalothin, cephaloridine, and cefazolin against most gram-negative and some gram-positive organisms (9). Studies done on mice showed that the therapeutic efficacy of cefatrizine compared with other cephalosporins was greater than predicted considering its comparative in vitro activity, and this was thought to be related to the longer half-life of cefatrizine in blood (10).

Our in vitro studies revealed that cefatrizine was more active than cephalexin for all gram-positive and -negative organisms tested. It was also more active than cephalothin, cefazolin, and cephapirin against most of the gram-negative bacteria, but less active against the gram-positive bacteria. The in vitro activity of cephradine was not tested since it has been shown to be almost identical to that of cephalexin (14). The MICs of cefatrizine obtained in this study are comparable to those formerly reported (9). Previous studies (9, 16) have revealed that 36.8% of indole-positive *Proteus* were inhibited by 8 µg or less and 50% of *S. faecalis* were inhibited by 16 µg or less of cefatrizine per ml. In our study, however, both organisms were uniformly resistant. Only 35% of strains of *Enterobacter* sp. were inhibited by 6.25 µg or less of cefatrizine per ml. However, this organism was more susceptible to cefatrizine than to the rest of the cephalosporins.

The peak serum levels obtained after oral ingestion of cefatrizine were lower than those of cephalexin, but they also declined at a slower rate. This phenomenon has also been observed in mice (10). The serum levels necessary for inhibition of most susceptible organisms were well within the achievable range for cefatrizine.

In the present study, 15 of 18 patients responded to treatment. The recurrence of bacter-

TABLE 2. *Clinical results of patients treated with cefatrizine*

Disease and no. of cases	Organism	No. cured	No. improved	No. failed or unknown
Acute urinary tract infections, 6	*Escherichia coli*	2[a]	1[b]	
	Klebsiella	2[a]		
	Proteus mirabilis	1[c]		
Pneumonia, 4	*Streptococcus pneumoniae*	4		
Soft tissue infection, 7	*Staphylococcus aureus*	4		
	β-hemolytic streptococci	1		1 unknown
	Pasteurella multocida	1		
Conjunctivitis, 1	*Streptococcus pneumoniae*			1 failed

[a] One was reinfected with a different organism.
[b] Relapsed 3 days after treatment was completed.
[c] Reinfected with a different organism after cystoscopy.

iuria in patients with urinary tract infections was almost always related to pretreatment urinary tract structural abnormalities. The only failure was a patient with pneumococcal conjunctivitis who had hypogammaglobulinemia and cyclic neutropenia, and the failure to respond in this case may have been related to the patient's underlying disease rather than to the efficacy of the drug. She finally responded to topical antibiotic therapy. In one patient the result of treatment could not be evaluated because he was lost for follow-up.

Cefatrizine was well tolerated. The side effects were limited to mild diarrhea, which was seen in five in 28 patients, and some nausea, seen in only two patients. In no instance did these side effects result in termination of treatment.

In conclusion, cefatrizine is a wide-spectrum cephalosporin, shown to be well tolerated and effective against infections caused by susceptible organisms when administered orally.

ACKNOWLEDGMENTS

This investigation was supported in part by a research grant from Bristol Laboratories, Syracuse, N.Y.

We thank Margaret Somerville for skillful technical assistance and Sandra Barry for clinical assistance.

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