In Vitro Susceptibility of Selected Bacteria to Cefaclor

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Cefaclor is an orally absorbed cephalosporin antibiotic chemically and pharmacologically similar to cephalaxin. It appears to be more active than cephalaxin against susceptible strains. The in vitro sensitivity of 230 clinical bacterial isolates to cefaclor was studied. Most isolates of S. aureus, K. pneumoniae, E. coli, and indole negative Proteus species were inhibited at clinically attainable serum and urine concentrations. Like cephalaxin, cefaclor was less active against isolates of Enterobacter species, indole positive Proteus species and enterococci although many of these isolates were inhibited at concentrations achievable in urine.

INTRODUCTION

Cefaclor, 3-chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxilic acid, is a new cephalosporin antibiotic absorbed by mouth and chemically related to cephalaxin and cephaloglycin. The methyl group of cephalaxin is replaced by a chlorine group. This report describes the results of in vitro susceptibility studies utilizing this antibiotic against 230 gram-positive and gram-negative bacterial isolates.

MATERIALS AND METHODS

Antibiotic. Cefaclor monohydrate standard powder and impregnated discs (30 ug) were supplied by Eli Lilly and Co., Indianapolis, Indiana.

Bacterial Strains. Thirty isolates each of Escherichia coli, Klebsiella pneumoniae, Enterobacter species, indole positive Proteus, indole negative Proteus, enterococci, penicillin-resistant Staphylococcus aureus and twenty isolates of penicillin-sensitive S. aureus were collected from different patients at the Yale-New Haven Hospital. All isolates were re-identified in our research laboratory. Penicillin susceptibilities of S. aureus strains were determined by Bauer-Kirby technique [1].

Procedures. For minimal inhibitory concentrations (MIC), twofold serial dilutions of cefaclor in Mueller-Hinton Broth (MHB) were prepared utilizing an automated microtitration system (Autotiter III, Canalco Co., Rockville, MD) as previously reported [2]. Each well was inoculated with a dilution of an 18 hour culture in MHB such that approximately $10^3$ to $10^4$ colony forming units were delivered to each well, or $10^4$ to $10^5$ cfu/ml. The final volume in each well was 0.1 ml. The MIC of cefaclor for each isolate was defined as the lowest concentration of cefaclor that prevented growth (detected as turbidity on visual inspection of the microtitration plates) after 18 hours of incubation at 37°C. Minimal bactericidal concentrations (MBC) were determined, after an aliquot from each well was subcultured in antibiotic-free MHB, and defined as the lowest concentration of cefaclor that prevented growth after incubation at 37°C for 18 hours. The inhibitory zone around a 30 ug cefaclor impregnated disc was determined for each isolate on Mueller-Hinton agar and compared to that isolate's broth dilution susceptibility [1,3].

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RESULTS

Broth Dilution Susceptibility Studies. The MIC and MBC of cefaclor against the various bacterial isolates are represented in Figs. 1 and 2 as the cumulative percentage of organisms inhibited or killed at each concentration of cefaclor studied. The most susceptible organisms were *S. aureus*, *K. pneumoniae*, *E. coli*, and the indole negative *Proteus* species. More than 90 percent of these isolates were inhibited by 32 ug/ml of cefaclor or less. The most sensitive organisms were the penicillin-sensitive *S. aureus*, all of which were inhibited by cefaclor concentrations of 4.0 ug/ml or less. The enterococcal isolates, indole positive *Proteus* species and *Enterobacter* isolates appeared less sensitive to cefaclor. Only 40 percent of indole positive *Proteus* and 40 percent of enterococci were inhibited by 32 ug/ml or less. *Enterobacter* species were the least sensitive as only 53 percent were inhibited by concentrations of cefaclor less than or equal to 128 ug/ml.

Disc Susceptibility Studies. The diameter of the zone of inhibition of bacterial growth around a cefaclor (30 ug) impregnated disc is plotted against the MIC of cefaclor for the gram-negative organisms in Fig. 3 and for the gram-positive organisms in Fig. 4. The 6 mm diameter of the disc is included in the final reading and, therefore, a zone diameter of 6 mm indicates no inhibition of growth. A zone of inhibition of greater than 20 mm correlated well with MIC's of 16 ug/ml or less for the *E. coli*, *K. pneumoniae* and indole negative *Proteus* species tested. Three of thirty
FIG. 2. Susceptibility of three species of gram-positive bacteria to increasing concentrations of cefaclor.

FIG. 3. Correlation of results of 30 µg disc and broth-dilution sensitivities of cefaclor against gram-negative bacteria.
strains of Enterobacter had no zones of inhibition despite MIC's equal to or less than 8 \mu g/ml. Seven isolates of the indole positive Proteus species had MIC's of 8 \mu g/ml and zones of inhibition of 29 mm or greater, but for isolates with MIC's of 16 \mu g/ml or greater there was no useful correlation with zone size. Of the gram-positive organisms, only penicillin-sensitive staphylococci showed a relationship between zone size and MIC. A zone of inhibition of 31 mm or greater correlated with MIC's of 4 \mu g/ml or less for these organisms.

DISCUSSION

Cefaclor has an antibacterial spectrum similar to that of cephalaxin. In the present study this antibiotic was bactericidal for most isolates of E. coli, P. mirabilis, and K. pneumoniae tested. Although conclusions regarding the relative efficacy of currently available orally absorbed cephalosporins should only be drawn from studies which compare these agents simultaneously, comparison of results from the present study with data from previous work with cephalaxin done in our laboratory suggests that cefaclor may be slightly more active than cephalaxin against susceptible strains of E. coli, K. pneumoniae and Proteus (indole negative and positive species) [3]. Cefaclor was also effective against most isolates of S. aureus tested, particularly penicillin-sensitive strains. Although we did not test for methicillin resistance among our isolates, methicillin-resistant strains of S. aureus are known to be less susceptible to cefaclor (personal communication, Dr. R. Kammer) as with other cephalosporins. Like cephalaxin, the drug is not as active against isolates of enterococci, indole positive Proteus and Enterobacter, although several of these isolates were inhibited by concentrations of cefaclor achievable in urine.

Pharmacokinetic studies in animals have shown that cefaclor is rapidly absorbed after oral administration and eliminated virtually unchanged in the urine [4].
appears to be true in humans as well. In single-dose studies conducted by Black and co-workers (presented at the 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, 27–29 October 1976, Chicago, IL.), 250, 500 mg and 1 gram oral doses of cefaclor gave mean peak plasma concentrations of 6.9, 12.5 and 24 ug/ml respectively. Sixty to eighty percent of the dose was recoverable from the urine in two to four hours. Currently, available data suggest that cefaclor achieves slightly lower blood levels than equivalent doses of cephalexin [5,6,7]. However, its greater antibacterial activity and the high levels of active drug achieved in the urine may make it a useful agent in the treatment of urinary tract infections due to susceptible organisms. Further study will be necessary to demonstrate a clinical advantage over currently available orally absorbed cephalosporins.

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REFERENCES

1. Bauer AW, Kirby WM, Sherris JC, et al: Antibiotic susceptibility testing by a standardized single dose method. Am J Clin Path 45:493–496, 1966
2. Andriole VT: Synergy of carbenicillin and gentamicin in experimental infection with Pseudomonas. J Infect Dis 124:46–55, 1971
3. Lyons RW, Andriole VT: Cephalexin: clinical and laboratory studies. Yale J Biol Med 44:187–198, 1971
4. Sullivan HR, Due SL, Kau DLK, et al: Metabolism of [14C] cefaclor, a cephalosporin antibiotic, in three species of laboratory animals. Antimicrob Ag Chemother 10:630–638, 1976
5. Braun P, Tillotson JR, Wilcox C, et al: Cephalexin and cephaloglycin activity in vitro and absorption and urinary excretion of single oral doses in normal young adults. Appl Micro 16:1684–1694, 1968
6. Clark H, Turck M: In vitro and in vivo evaluation of cephalexin. Antimicrob Ag Chemother 296–301, 1968
7. Griffith RS, Black HR: Cephalexin. Med Clin North Amer 54:1229–1244, 1970

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