In Vitro Evaluation of the New Oral Cephalosporin Cefatrizine: Comparison with Other Cephalosporins

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Cefatrizine (BL-S640), a semisynthetic, orally administered cephalosporin, was found to have an in vitro spectrum of activity comparable to those of four other cephalosporins tested. It is as effective as cephalixin, the other orally administered cephalosporin evaluated, against most species, and it appears to be more effective than cephalixin against many Enterobacter, Haemophilus, and Proteus strains isolated in our hospital. It is not inactivated by the plasmid-determined β-lactamases of 14 strains of ampicillin-resistant Salmonella typhimurium or the ampicillin resistance determinant of an H. influenzae strain from the Center for Disease Control. No synergy was observed between cefatrizine and gentamicin, kanamycin, carbenicillin, or polymyxin when tested against selected strains.

The need for an effective orally administered cephalosporin has been apparent for sometime. Cefatrizine (CF), BL-S640, is a new semisynthetic cephalosporin that is absorbed well from the gastrointestinal tract. The other orally administered cephalosporin in present use, cephalixin (CX), has less antibacterial activity than other members of this group of compounds: cephalothin (KF), cephaloridine (CL), cefazolin (CZ), and cephaloglycin (3, 7). In conjunction with clinical trials of this new cephalosporin, sensitivities of 272 local bacterial isolates were determined and compared for CF, KF, CL, CX, and CZ. In addition, Enterobacter, Pseudomonas, and Serratia strains were tested for synergistic effects of CF with other antibiotics.

MATERIALS AND METHODS

Antibiotic agents. CF propylene glycolate (Bristol Laboratories, Syracuse, N.Y.) was supplied as the crystalline dipolar ion for use in antibiotic susceptibility testing (2). KF, CL, CX, and CZ, all from Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, Ind., were used for comparative studies. Gentamicin sulfate (Garamycin, Schering Corp., Bloomfield, Mass.), disodium carbenicillin (Pyopen, Beecham-Massengill, Bristol, Tenn.), polymyxin sulfate (Aerosporin, Burroughs-Wellcome), and kanamycin sulfate (Kantrex, Bristol Laboratories) were used in synergy studies. All stock solutions of antibiotics were prepared daily.

Bacterial isolates. Standard strains of Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used in each trial as controls. Representative strains of gram-negative (190 isolates) and gram-positive (82 isolates) organisms were obtained from the clinical laboratory of the Hospital of the Medical College of Ohio at Toledo. All were stored in sterilized skimmed milk at −60°C. The strains to be tested were thawed and subcultured on solid medium to insure adequate growth. In addition, seven strains of Neisseria meningitidis and an ampicillin-resistant Haemophilus influenzae strain sent to us from the Center for Disease Control, Atlanta, Ga., were included.

Susceptibility studies. Minimal inhibitory concentrations (MICs) were determined by standard methods (6). Todd-Hewitt broth (BBL) was used to test Streptococcus species. Tryptic soy broth (Difco) was tried, but more reproducible results were obtained with the Todd-Hewitt medium. Clear GC medium used for Neisseria gonorrhoeae was composed of: soluble starch, 1 g; KH₂PO₄, 1 g; KCl, 5 g; K₂HPO₄, 4 g; proteose peptone, 15 g; water, 1 liter; glucose (20%, wt/vol), 25 ml; NaHCO₃ (4.2%, wt/vol), 10 ml; and supplement B (Difco), 10 ml. Mueller-Hinton medium (Difco) with 1% supplement B was used for H. influenzae and N. meningitidis. Mueller-Hinton medium without any supplement was used for all other strains tested. Twofold serial dilutions of the antibiotics were prepared (128 to 0.125 μg/ml). Tubes were inoculated with 10⁶organisms per ml and were incubated at 37°C for 18 to 24 h. Neisseria, Streptococcus, and Haemophilus strains were incubated in 10% CO₂. The MIC was recorded as the lowest concentration of antibiotic at which there was no visible growth.

Broth from tubes in which there was no visible growth was inoculated to an agar plate by using a calibrated platinum loop (3-mm diameter), and the lowest concentration at which there was 99.9% killing of the inoculum was recorded as the minimal bactericidal concentration (MBC). Duplicate plates
were inoculated with 0.1 ml of an appropriate dilution to determine the number of colony-forming units in the inoculum. Disk susceptibilities to each of the cephalosporins were determined by the Kirby-Bauer method (1). Zone sizes, indicating susceptibility or resistance, were recorded for KF, CL, CX and CZ. The size of the zone surrounding the CF disk was recorded in millimeters.

Synergy studies. Synergy studies were performed on Serratia, Pseudomonas, and Enterobacter species by the agar dilution method. Plates containing antibiotic (0.125 to 128 µg/ml) or antibiotic plus one of three concentrations of CF (0.25, 2, or 16 µg/ml) were inoculated with a Steers replicator (9). Approximately 100 to 300 colony-forming units per spot were inoculated by each prong of the replicator. Plates were prepared each day from fresh stock solutions of antibiotics and dried for 2 h in an incubator before inoculation. The inoculated plates were incubated for 18 to 24 h at 37°C. Growth of fewer than five colonies per inoculum indicated susceptibility.

RESULTS

Activity of cephalosporins against gram-negative isolates. Table 1 records the MICs for some of the more common gram-negative organisms. Each strain was tested at least twice under the conditions described above. Most strains of E. coli and Klebsiella were inhibited by low concentrations (≤8 µg/ml) of BL-S640: 81% of E. coli and 70% of K. pneumoniae tested. As predicted, based on previous studies by other workers (5, 8, 10, 11), Serratia and Pseudomonas species were uniformly resistant to >128 µg of each of the cephalosporins per ml. Each of 15 strains of S. typhimurium, 14 of which contained plasmids mediating resistance to ampicillin, was inhibited by 8 µg of BL-S640 per ml. Forty-two percent of the Proteus species tested were inhibited at this concentration of CF; similar values were found for KF (42%) and CZ (47%). CL and CX appeared less effective in their abilities to inhibit these strains. CF was not as effective against Enterobacter at 8 µg/ml (31% of strains inhibited) as CZ (50%) but definitely more so than the other three cephalosporins. Both N. meningitidis and gonorrhoeae were inhibited by low concentrations of CF. All isolates of H. influenzae were inhibited by 8 µg of CF and KF per ml. CL and CZ inhibited 89% of the strains, but only 22% of the strains had MICs of 8 µg/ml or less with CX. An ampicillin-resistant strain (25 to 50 µg of ampicillin per ml) from the Center for Disease Control was sensitive at 8 µg of BL-S640 per ml, 2 µg of KF per ml, 8 µg of CL per ml, 16 µg of CX per ml, and 8 µg of CZ per ml. MICs were usually within one or two dilutions of the MIC. With disks containing 30 µg of CF per ml, the zone of inhibition ranged from 0 to 30 mm. The strains that were susceptible to 8 µg/ml or less usually had zones ranging from 18 to 30 mm. The majority of strains that were resistant to 8 µg/ml or greater had zones ranging from 0 to 16 mm.

Activity of cephalosporins against gram-positive isolates. Emphasis, reflected in the numbers of each species tested, was on the organisms that are frequently resistant to antibiotics in common use. Each strain of Streptococcus pyogenes and Streptococcus pneumoniae was sensitive to concentrations of CF readily obtainable in serum (Table 2). For both S. pyogenes and S. pneumoniae, the diameter of the zone of inhibition surrounding the disk containing 30 µg of CF per ml ranged from 20 to 30 mm. The MIC was usually the same as the MIC or within one- or two-tube dilutions of the MIC.

One strain of methicillin-resistant S. aureus, DU 4916, was resistant to >128 µg of each of the cephalosporins tested per ml, except for CL (MIC = 16 µg/ml). Zone diameters for those staphylococcal strains susceptible to ≤8 µg of CF per ml ranged from 18 to 40 mm. Those that required >8 µg of BL-S640 per ml had a diameter of 16 mm or less. MIC values varied; not all MBCs were within the one- or two-tube dilution range of the MIC. The only consistent observation for S. aureus was that size of the original inoculum influenced the MIC value; larger inocula usually resulted in higher values for both the MIC and MBC.

The enterococci were resistant to these compounds, 16 µg/ml being the lowest MIC. The MBC was not reached at the concentrations used. Zone diameters determined by the Kirby-Bauer method ranged from 10 to 16 mm, the majority being 14 or 15 mm.

Synergy studies. Enterobacter, Pseudomonas, and Serratia strains were tested for synergistic effects of CF with other antibiotics. Pseudomonas and Serratia were examined for susceptibilities to gentamicin and carbenicillin and for the effect of the addition of CF at three concentrations (0.25, 2, and 16 µg/ml). As in the tube dilution experiments, each of the strains was resistant to CF. All but two strains, both Pseudomonas, were inhibited by 1 µg of gentamicin per ml; these two strains required 2 µg/ml. No synergistic effect was noted for the majority of the strains; in fact, in several instances, a slightly antagonistic effect was seen in the presence of CF. There was a slight amount of growth, a thin film or a few tiny colonies, on plates containing both gentamicin and CF, but no growth on gentamicin alone. The results obtained with carbenicillin were
Table 1. MICs of cephalosporins of gram-negative organisms

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Enterobacteriaceae. It well with VOL. S. pyogenes (10) and some of H. losporins or trium gram-positive organisms, a variety of S. pneumoniae and Enterococci groups (5, pital antimicrobial agent, 8-lactamases are isolated from, 8-lactamase, pneumoniae a aureus, and Enterococci tested. MICs obtained from the agar dilution method. Our findings from the same, Mueller-Hinton broth containing 1% supplement B, differences may reflect variations in the local strains. The MICs in these studies were somewhat higher for the streptococci, but this may reflect the differences in the media. Our data agree with the findings of Kayser (4), who reported that CL was the most effective cephalosporin tested against S. aureus. CZ is certainly as effective as CX, the orally administered cephalosporin, in most species evaluated, and it appears to be more effective than CX against many strains of Enterobacter, Haemophilus, and Proteus isolated in our hospital.

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LITERATURE CITED