Antagonism by Chloramphenicol of Broad-Spectrum β-Lactam Antibiotics Against Klebsiella pneumoniae

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Received 11 October 1983/Accepted 12 January 1984

Chloramphenicol combined with cefotaxime, moxalactam, cefoperazone, aztreonam, or imipenem was tested in vitro against clinical isolates of Klebsiella pneumoniae. By time-kill cultures (killing curves), chloramphenicol interfered with activity of all five β-lactams. When chloramphenicol was added before the β-lactams, the action of cefotaxime, moxalactam, or cefoperazone against all isolates was antagonized at all times tested. The action of aztreonam was antagonized against four of six isolates. With imipenem, antagonism occurred against half of the isolates at some time during 24 h when chloramphenicol was added simultaneously, provided that a sufficient inoculum of K. pneumoniae was employed. Generally, less antagonism resulted when chloramphenicol was added after the cephalosporins. Interference of bactericidal activity of three new cephalosporins by chloramphenicol has potential clinical relevance to the therapy of gram-negative bacillary meningitis. The lesser antagonism of aztreonam and imipenem by chloramphenicol is of uncertain clinical relevance but indicates that this in vitro phenomenon may apply to a wide range of β-lactam antibiotics.

Chloramphenicol therapy of gram-negative bacillary meningitis (GNBM) has been associated with high mortality (4, 11). This is likely due in part to the bacteriostatic activity of chloramphenicol against most gram-negative bacilli in concentrations obtainable in cerebrospinal fluid (CSF) (12). In bacillary meningitis, a cure appears to be related to achieving or exceeding by 10 to 20 times in CSF the MBC for the infecting organism (1, 2, 12–14). New cephalosporins, including cephaplexin antibiotics, achieve these necessary high bactericidal ratios against gram-negative bacilli in CSF and are now considered by some authorities to be drugs of choice for this disorder in adults (3, 5, 9). In GNBM caused by susceptible organisms, monotherapy with one of these new extended-spectrum cephalosporins is usually recommended (5). However, empirical therapy or certain other clinical situations might appear to necessitate use of chloramphenicol concomitantly with an extended-spectrum cephalosporin for central nervous system infections.

Clinical experience with concomitantly administered chloramphenicol and an extended-spectrum cephalosporin in GNBM has not been previously reported, and in vitro studies of synergy or antagonism with chloramphenicol and cephalosporins, in particular the extended-spectrum cephalosporins, are also lacking.

We investigated in vitro the activities of three commercially available extended-spectrum cephalosporins (cefotaxime, moxalactam, cefoperazone) and two investigational β-lactams, a carbapenem (imipenem) and a monobactam (aztreonam), specifically evaluating their potential for antagonism by chloramphenicol against clinical isolates of Klebsiella pneumoniae.

MATERIALS AND METHODS

Bacteria. Six clinical isolates of K. pneumoniae were obtained from blood or spinal fluid of patients at the Nashville Veterans Administration Medical Center. Identification was by standard microbiological techniques (10). Organisms were lyophilized and stored at −15°C or frozen on agar and stored at −70°C until used. At the start of testing, organisms were reconstituted in Mueller-Hinton broth and kept on brain heart infusion agar slants at 4°C for daily usage. Tube dilution susceptibility tests to determine the MIC for each isolate were carried out in Mueller-Hinton broth with an inoculum of 10^5 organisms from an 18-h growth (19).

Antimicrobial agents. Chloramphenicol base (Parke-Davis Co., Morris Plains, N.J.), cefotaxime sodium (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), cefoperazone sodium (Pfizer Laboratories Division, New York, N.Y.), moxalactam disodium (Eli Lilly & Co., Indianapolis, Ind.), and aztreonam base (E. R. Squibb & Sons, Princeton, N.J.) were reconstituted in concentrations of 1,000 μg/ml; portions were stored at −70°C until used. Imipenem (Merck Sharp & Dohme, West Point, Pa.) was reconstituted in 0.5 M morpholinopropanesulfonic acid buffer (pH 6.8)-ethylene glycol–distilled water in a concentration of 100 μg/ml, and portions were snap-frozen and stored at −70°C until used.

Killing curves. Mueller-Hinton broth cultures (1-ml volumes in test tubes) were inoculated with 3 × 10^5 organisms from an overnight culture for all antibiotics and additionally with 1.5 × 10^6 organisms in specified tests with imipenem and incubated aerobically for 24 h at 35°C without shaking. Cultures were initiated without antibiotics, with chloramphenicol (10 μg/ml), with one of the β-lactam antibiotics (10 μg/ml), or with a combination of chloramphenicol and a β-lactam (both 10 μg/ml). Chloramphenicol was added 1 h before, simultaneously with, or 1 h after the β-lactam.

At 3, 6, and 24 h, samples of 0.01 or 0.1 ml were removed from each culture and serially diluted in sterile saline. Portions (0.01 or 0.1 ml) of three dilutions were plated on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). Plates were incubated aerobically for 18 to 24 h at 35°C, and CFU were enumerated in triplicate. Tissue-killed cultures (killing curves) were repeated with four to six susceptible isolates of K. pneumoniae for each chloramphenicol–β-lactam combination at three different times of chloramphenicol addition to cultures. Antagonism was defined as a decrease in CFU of −2 log_{10} for the combination compared with the β-lactam alone.

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RESULTS

Susceptibilities. MICs required to inhibit 90% of *K. pneumoniae* isolates (MIC₉₀) were as follows: cefotaxime, 0.049 μg/ml; moxalactam, 0.195 μg/ml; cefoperazone, 0.39 μg/ml; aztreonam, 0.098 μg/ml; and imipenem, 0.195 μg/ml. One isolate was resistant to cefoperazone (MIC, >50 μg/ml) but susceptible to the other β-lactams. All isolates were inhibited by chloramphenicol (MIC₉₀, 6.25 μg/ml).

Timed-kill studies. The five β-lactams were all antagonized by chloramphenicol.

Cephalosporins. Antagonism was most marked when chloramphenicol was added to cultures 1 h before the cephalosporins (Fig. 1). In the figure, results with cefotaxime, moxalactam, and cefoperazone are graphically represented as killing curves from a composite of geometric means for the several isolates tested against each cephalosporin alone, chloramphenicol alone, or with the cephalosporin preceded by chloramphenicol. Interference with the activity of cefotaxime occurred at 3 h with three of six isolates, at 6 h with five of six isolates, and at 24 h with four of six isolates. Its activity against all six isolates was antagonized at some time during the 24-h test period. Interference with moxalactam occurred at 3 h in three of six isolates, at 6 h in five of six isolates, and at 24 h in all six isolates. Antagonism of cefoperazone occurred at 3 h in four of five isolates and at 6 and 24 h in all five isolates tested.

When chloramphenicol was added simultaneously with cephalosporins, the frequency of antagonism was similar. Cefotaxime antagonism resulted at 3, 6, and 24 h with all six isolates. Antagonism against moxalactam occurred in three of six isolates at 3 h, in four of six isolates at 6 h, and in five of six isolates at 24 h. The activity of cefoperazone was interfered with in three of five isolates at 3 and 6 h and in all five isolates at 24 h.

Chloramphenicol added 1 h after cephalosporins caused less interference than when added earlier. Antagonism of cefotaxime was present in one of four isolates at 3 and 6 h and in two of four isolates only at 24 h. Moxalactam was antagonized only at 24 h in one of four isolates. Also, cefoperazone was antagonized only at 24 h in all four isolates when the addition of chloramphenicol was deferred.

Other β-lactams. The investigational non-cephalosporin β-lactams aztreonam (a monobactam) and imipenem (a carbapenem) were also antagonized by chloramphenicol in vitro, but the frequency and degree of antagonism were less than with the cephalosporins. When chloramphenicol was added first, interference with the action of aztreonam occurred with four of six isolates tested (Fig. 2) at both 3 and 6 h, whereas at 24 h, antagonism occurred in two of six isolates. Aztreonam was less markedly interfered with during simultaneous addition of chloramphenicol. Three of six isolates at 3 h and only one of six isolates at 6 and 24 h demonstrated antagonism.

When chloramphenicol was added first, imipenem was not appreciably antagonized at any time during the 24-h test period with a low inoculum of *K. pneumoniae* (Fig. 2). A higher inoculum of organisms resulted in demonstrable antagonism of imipenem by chloramphenicol. With an inoculum of 1.5 × 10⁶ CFU and simultaneous addition of chloramphenicol, interference occurred in three of six isolates at 3 h, in two of six isolates at 6 h, and in one of six isolates at 24 h.

DISCUSSION

In general, bactericidal antimicrobial activity is necessary in the cerebrospinal space because it is an area of relatively impaired host resistance (15, 16). Besides its bacteriostatic activity, another important mitigating factor in the use of chloramphenicol for GNB is its potential for antagonism of concomitantly used bactericidal antibiotics with resultant bacteriostasis. Antibiotic antagonism was first demonstrated
in vitro for penicillin by chloramphenicol against streptococci and \textit{K. pneumoniae} (8). Chloramphenicol also antagonizes the action of penicillin in vivo. In dogs with pneumococcal meningitis, chloramphenicol administered first antagonizes penicillin (18).

First- and second-generation cephalosporins are rarely used in combination with chloramphenicol, as reflected by the paucity of studies regarding their synergy or antagonism (17). Since the introduction of new cephalosporins that can be used reasonably in bacillary meningitis, the coadministration of a cephalosporin with chloramphenicol might be considered. In vitro antagonism of these new cephalosporins by chloramphenicol in concentrations clinically achievable in CSF occurred in our study of susceptible isolates of \textit{K. pneumoniae}. There were no significant differences among the three cephalosporins in degree of antagonism.

The intermediate-sized inocula used were sufficient to demonstrate interference, and studies showing an in vitro inoculum effect for cephalosporins suggest that an even more pronounced effect might have occurred with larger inocula (6, 20). Indeed, an inoculum of 1.5 $\times$ 10$^5$ CFU was required for clear-cut demonstration of antagonism of imipenem. In vivo antagonism of cephalosporins by chloramphenicol in CSF could be realistically anticipated, since relatively greater bacterial concentrations are present in bacillary meningitis (7).

Our results support the notion that concomitant therapy with chloramphenicol plus an extended-spectrum cephalosporin for GNBM should be used with caution. If both drugs are deemed necessary, administration of chloramphenicol should be delayed. The potential role of the other two new $\beta$-lactams in GNBM is not clear, but if used, these in vitro results suggest that perhaps similar caution should be exercised in concomitant administration of chloramphenicol with aztreonam or imipenem. Conclusions cannot be made regarding the use of chloramphenicol plus any of these new $\beta$-lactam antibiotics in other clinical settings in which higher levels of the $\beta$-lactams exist and host immunity is greater.

ACKNOWLEDGMENTS

This study was supported by the Veterans Administration.

We thank Beverly Hosse for advice and technical assistance and Muriel Burnette for assistance in preparing the manuscript.

LITERATURE CITED