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Activity of Moxalactam and Cefotaxime Alone and in Combination with Ampicillin or Penicillin Against Group B Streptococci

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The activities of moxalactam and cefotaxime, alone and combined with ampicillin or penicillin, against 40 isolates of group B streptococci were assessed by using the microtiter broth dilution, checkerboard, and time-kill techniques. Penicillin and cefotaxime were bactericidal for all isolates at concentrations of 0.06 μg/ml or less. Ampicillin was slightly less active. Moxalactam was bactericidal for all strains at concentrations of 4 to 8 μg/ml. The ampicillin-moxalactam combination was partially synergistic for 60% of the isolates tested; the ampicillin-cefotaxime combination was partially synergistic for 35% of these isolates. No instances of antagonism were observed. In time-kill evaluations, ampicillin (3.0 μg/ml) and penicillin (0.75 μg/ml) effected 2.5 to 3.5 $\log_{10}$ reductions in numbers of colony-forming units. The addition of 4 μg of cefotaxime per ml or 8 to 16 μg of moxalactam per ml to penicillin or ampicillin did not alter killing kinetics. Moxalactam and cefotaxime neither enhanced nor decreased the activity of ampicillin or penicillin against group B streptococci.

Neonatal meningitis, when caused by group B streptococci or *Escherichia coli*, has a mortality rate of 15 to 50% (3, 4, 9, 10, 15, 21). Ampicillin plus a parenteral aminoglycoside, standard therapy for this disease, does not always effect clinical and microbiological cure. Although numerous factors, e.g., prematurity, play roles in determining the poor outcome of neonatal meningitis, there is definitely room for improvement in the antimicrobial therapy of this disease.

Moxalactam and cefotaxime, two relatively new beta-lactam derivatives, hold promise for therapy of neonatal meningitis caused by gram-negative bacteria. The minimal inhibitory concentrations (MICs) of these agents for most strains of *E. coli* range from 0.06 to 0.125 μg/ml (7, 17, 18). Both drugs appear in the cerebrospinal fluid in high concentrations (5 to 15 μg of cefotaxime per ml; 15 to 35 μg of moxalactam per ml) (2, 5, 14, 19). There is relatively little information on the in vitro activity of moxalactam and cefotaxime against group B streptococci. There is likewise little information on the activities of these agents in combination with penicillin or ampicillin. Such data should be useful to those concerned with therapy of neonatal meningitis or other infections caused by these streptococci.

We describe here the activities of moxalactam and cefotaxime alone and in combination with ampicillin and penicillin against group B streptococci.

MATERIALS AND METHODS

Bacteriology. Forty clinical isolates of group B streptococci were used in this study. The organisms were presumptively identified as group B streptococci by Gram stain, colony morphology, a positive test for hippurate hydrolysis, and a positive aerobic CAMP test, and they were definitively identified by the capillary precipitin method (13).

Antibiotics. The drugs used in this study and their sources were moxalactam (Eli Lilly & Co., Indianapolis, Ind.), cefotaxime (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), penicillin (Pfizer Pharmaceuticals Inc., New York), and ampicillin (Bristol Laboratories, Syracuse, N.Y.)

Susceptibility tests. Antibiotic susceptibility was determined by a microtiter broth dilution technique using twofold dilutions of the antibiotic in tryptic soy broth (6). The inoculum of each isolate was prepared from a 6-h culture grown in tryptic soy broth and diluted to contain 10^5 to 10^6 colony-forming units (CFU) per ml. Each well contained 0.05 ml of this inoculum and 0.05 ml of diluted drug. The MIC, determined after overnight incubation at 37°C, was the lowest concentration of antibiotic showing no visible growth. The minimal bactericidal concentration

(MBC) was the lowest concentration from which subculture of 0.0015 ml on Mueller-Hinton agar was sterile.

Synergy studies. Checkerboard titrations were performed by the serial twofold microdilution method (12), with each well of the microtiter plate containing 0.05 ml of a different concentration of each drug. Volumes of 0.05 ml of log-phase cultures diluted in tryptic soy broth to contain 10^5 to 10^6 CFU/ml were added to the wells. Plates were incubated overnight at 37°C and then read for visible growth.

Five strains of group B streptococci against which ampicillin-moxalactam and ampicillin-cefotaxime exhibited partial synergy in the checkerboard study were used in the time-kill evaluations. Stationary-phase (overnight) cultures of these strains were diluted in tryptic soy broth to a density of 10^5 to 10^6 CFU/ml. Tubes containing 5 ml of the appropriate drugs in tryptic soy broth were inoculated with 5 ml of these diluted cultures and incubated without CO_2 at 37°C for 24 h. Samples of 0.1 ml removed at various intervals were diluted serially in normal saline for viable counts, and 0.1-ml volumes of each dilution were streaked on sheep blood agar. Colony counts were made after overnight incubation at 37°C.

The combinations and concentrations chosen for the killing curves were (i) 0.75 μg of penicillin per ml with either 4 μg of cefotaxime or 8 μg of moxalactam per ml and (ii) 3.0 μg of ampicillin per ml with either 4.0 μg of cefotaxime per ml or 8 or 16 μg of moxalactam per ml. These concentrations approximated levels attainable in the central nervous system (5, 11, 14, 20).

Definitions. The checkerboard titration combinations were considered synergistic if inhibition occurred at one-fourth or less of the MICs of both drugs. Partial synergy was equivalent to a decrease to one-fourth or less of the MIC of one drug with a decrease to one-half of the MIC of the second antibiotic. Reduction by one-half in the MICs of both antibiotics was regarded as an additive effect. When neither drug or only one of the agents exhibited a decrease in the MIC the result was classified as indifference. If in combination the MIC of either antibiotic exceeded the MICs of the individual drugs, the result was antagonism (1). In the time-kill evaluations, 2 log decreases in CFUs when antibiotics were used in combination, as compared with when they were used alone, was considered evidence of synergy.

RESULTS

Susceptibility tests: MICs and MBCs. Since MBCs of penicillin, ampicillin, moxalactam, and cefotaxime for all 40 isolates differed from MICs by only one dilution, only the MBC data have been presented in Table 1. Penicillin and cefotaxime, with an MBC for 90% of isolates of 0.06 μg/ml, were the most active of the four agents. They were slightly more active than ampicillin, which had an MBC for 90% of isolates of 0.25 μg/ml, and significantly more active than moxalactam, which had an MBC for 90% of isolates of 8 μg/ml.

TABLE 1. *Susceptibility of group B streptococci (40 strains) to penicillin G, ampicillin, cefotaxime, and moxalactam*

Antibiotic	MBC (μg/ml)		
	Range	MBC_{50}[a]	MBC_{90}[a]
Penicillin G	0.008–0.06	0.03	0.06
Cefotaxime	0.008–0.125	0.03	0.06
Ampicillin	0.06–0.25	0.125	0.25
Moxalactam	4–8	8[b]	8

[a] MBC_{50} and MBC_{90}, MBCs for 50 and 90% of isolates, respectively.

[b] At 4 μg/ml, 40% of the strains were inhibited.

Synergy studies. In the checkerboard titration, the ampicillin-moxalactam combination was partially synergistic for 12 isolates (60%), additive for 3 (15%), and indifferent for 5 (25%). The ampicillin-cefotaxime combination was partially synergistic for 7 isolates (35%), additive for 12 isolates (60%), and indifferent for 1 (5%).

In the time-kill evaluations, ampicillin (3.0 μg/ml), penicillin (0.75 μg/ml), cefotaxime (4.0 μg/ml), and moxalactam (8 or 16 μg/ml) alone caused a 2.5 to 3.5 $\log_{10}$ reduction in CFU at 24 h. When cefotaxime (Fig. 1a) or moxalactam (Fig. 1b) was combined with penicillin or ampicillin at the concentrations listed above, killing kinetics were not altered. The rate of killing of group B streptococci by the penicillin-moxalactam combination (not shown in Fig. 1) was identical to that by the ampicillin-moxalactam combination. After 24 h of incubation, the reduction in the $\log_{10}$ CFU of group B streptococci per milliliter with any of the drug combinations (ampicillin-moxalactam, ampicillin-cefotaxime, penicillin-cefotaxime, and penicillin-moxalactam) was nearly identical (less than 0.5 $\log_{10}$ CFU/ml difference) to that produced by penicillin or ampicillin alone. Moxalactam and cefotaxime, at concentrations equal to or above the MBC for the test organisms, were as effective as penicillin or ampicillin in reducing the $\log_{10}$ CFU of group B streptococci per milliliter.

DISCUSSION

In the study on group B streptococci described above, cefotaxime was as active as penicillin and slightly more active than ampicillin. Neu's observations with cefotaxime were similar, with 90% of the strains being inhibited by 0.1 μg/ml (17). Concentrations of cefotaxime ranging from 5 to 15 μg/ml have been found in the cerebrospinal fluid of children (age, 3 to 12 years) and adults with inflamed meninges (5). These concentrations are higher than the MBCs for the group B streptococci examined in our study and

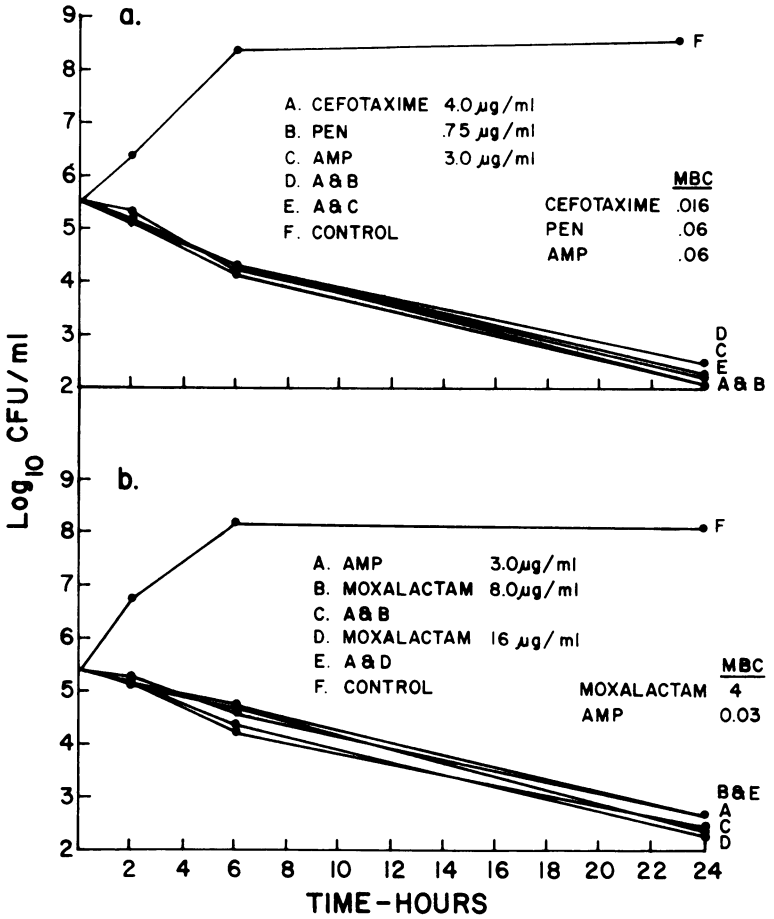

FIG. 1. *Kinetics of bacterial killing of group B streptococci by penicillin (PEN) or ampicillin (AMP) in combination with cefotaxime or moxalactam. The vertical positions of the letters correspond to the respective vertical positions of the points on the graph at 24 h.*

that of Neu et al. (17) as well as the MBCs for *E. coli* recorded by others (7).

Moxalactam, with MICs and MBCs of 4 to 8 μg/ml, was less active than cefotaxime against the group B streptococci examined in this study. These values are similar to those recorded by Fass (8) but slightly higher than those recorded by Neu et al. (18), who used an agar dilution technique (18). The concentrations of moxalactam in the cerebrospinal fluid of adults with inflamed meninges, ranging from 15 to 35 μg/ml, are clearly in excess of the MBCs recorded above (14). The killing curves indicate that when the concentration of moxalactam exceeds the MBC for group B streptococci, killing of the organism occurs at a rate comparable to that by ampicillin.

The killing curve and checkerboard synergy studies indicated no change in the rate of killing of group B streptococci when cefotaxime was used in combination with either ampicillin or penicillin; neither synergy nor antagonism was observed. As with cefotaxime, there appears to be no synergy against group B streptococci when moxalactam is combined with either penicillin or ampicillin. Although the checkerboard studies indicated partial synergism for the ampicillin-moxalactam combination against several strains of group B streptococci, no increased killing of group B streptococci could be demonstrated by the killing curves.

The slight disparity between the checkerboard studies and killing curves underscores the problems of measuring and interpreting the results of synergy studies (16). Even so, our data seem to support the conclusion that neither cefotaxime nor moxalactam exhibits synergy or antagonism against group B streptococci when combined with ampicillin or penicillin.

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