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Activities of Eight New β-Lactam Antibiotics and Seven Antibiotic Combinations Against *Neisseria meningitidis*

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Each of eight new β-lactam antibiotics was highly active in vitro against *Neisseria meningitidis*, and activity was not reduced by combining some of these drugs with penicillin, ampicillin, or tobramycin. Antibacterial activity and lack of antagonism between moxalactam and ampicillin was confirmed in a model of lethal meningococcal infection in mice.

Consideration of a new antibiotic for the initial treatment of bacteremia and meningitis requires that it have adequate activity against the pathogens commonly encountered in those conditions. In addition, there should be no antagonism of the antibacterial activity when the new agent is used in combination with other antimicrobial agents. Because *Neisseria meningitidis* is a common cause of bacteremia and meningitis, we evaluated the activity of eight new β-lactam drugs and seven combinations against 30 clinical isolates. Reports on the susceptibility of *N. meningitidis* to these agents either are lacking or include small numbers of strains (1–4, 6).

Pure antibiotic powders were reconstituted in water and used immediately or stored at −70°C for up to 4 weeks. *N*-Formimidoyl thienamycin was freshly reconstituted for each experiment. Thirty clinical isolates were obtained from blood or cerebrospinal fluid of ill children. Twenty-two of these isolates were from ill children in Montreal during the period 1976–78; the remaining eight isolates were recovered in Oklahoma Children's Hospital during the period 1979–81. These organisms were from isolated cases and included groups A, B, C, X, and Y, as well as three nontypable strains.

The minimum inhibitory concentration (MIC) of each antibiotic was determined by the twofold dilution method using Mueller-Hinton agar. The inoculum consisted of approximately 10^4 colony-forming units, prepared from a log-phase culture in Trypticase soy broth, delivered to the agar surface by a Steers replicator (7). The MIC was defined as the lowest concentration of antibiotic that allowed no visible growth. Control organisms were included with each set of single drug and combination plates and were consistent within one twofold dilution of established values.

Combination studies were performed by the microtiter checkerboard technique using Mueller-Hinton broth. The final concentration of inoculum was approximately 10^5 colony-forming units per ml. All incubations were in 5 to 10% CO_2 at 34 to 36°C for 24 h. MIC endpoints were read as the lowest concentration of antibiotic that showed no visible turbidity; minimum bactericidal concentration (MBC) was defined as 99.9% killing approximately 18 h after subculture. Combination effects were defined as follows: synergy, at least a fourfold decrease in the MIC for each drug; addition, a twofold decrease in either or both drugs; indifference, no change in either MIC; and antagonism, a fourfold increase in either MIC.

Groups of eight female CF-1 mice (Sasco, Omaha, Nebr.) were infected intraperitoneally with 2×10^9 colony-forming units of *N. meningitidis* in 1 ml of an equal volume of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and 10% hog gastric mucin (Sigma Chemical Co., St. Louis, Mo.). The MICs for this cerebrospinal fluid isolate, determined as described above, were: moxalactam, 0.015 μg/ml; ampicillin, 0.05 μg/ml. A control group was injected with 1 ml of equal volumes of brain heart infusion broth and 10% mucin. In preliminary experiments, dosages of moxalactam and ampicillin were determined by administration of each drug to separate groups of mice; serum concentrations were determined by an agar diffusion assay using the Blake strain of *Escherichia coli* as an indicator organism for moxalactam and *Sarcina lutea* ATCC 9341 for ampicillin. Peak serum concentrations of 23.6 and 12 μg/ml were achieved by moxalactam at 37.5 mg/kg and ampicillin at 50 mg/kg, respectively. A single 1-ml dose of each antibiotic alone, the combination, or saline was administered subcutaneously

TABLE 1. Activity of β-lactam and tobramycin antibiotics against 30 isolates of *N. meningitidis* (agar dilution method)

Antibiotic	MIC range (μg/ml)	MIC_{50}[a] (μg/ml)	MIC_{90}[a] (μg/ml)
Ceftazidime	0.004–0.03	0.008	0.015
Ceftriaxone	≤0.0005–0.002	≤0.0005	0.001
Cefotaxime	≤0.0005–0.002	≤0.0005	0.001
Ceftizoxime	≤0.0005–0.004	0.002	0.002
Cefmenoxime	≤0.0005–0.016	0.004	0.008
N-Formimidoyl thienamycin	0.008–0.06	0.015	0.03
Cefoperazone	0.004–0.016	0.008	0.016
Moxalactam	0.004–0.008	0.004	0.008
Penicillin	0.03–0.25	0.12	0.12
Ampicillin	0.03–0.25	0.12	0.12
Tobramycin	0.06–0.5	0.12	0.25

[a] MIC_{50} and MIC_{90} indicate concentrations effective against 50 and 90% of isolates, respectively.

1 h after infection. Mice were observed for 7 days. Presence of *N. meningitidis* in dead animals was confirmed by culture of heart blood and brain.

The 30 isolates of *N. meningitidis* were highly susceptible to all the β-lactam drugs tested (Table 1). Ceftriaxone, ceftizoxime, and cefotaxime showed extremely high activity and were chosen for combination testing. Moxalactam and cefoperazone were included in combination studies because of their use in current and impending therapeutic trials in meningitis. No evidence of antagonism was found in the combination studies except for moxalactam plus ampicillin, which was antagonistic for one strain (Table 2). MBCs were within one dilution of MICs in all cases. The percentage of isolates inhibited synergistically ranged from 3% for ceftizoxime plus ampicillin and cefoperazone plus tobramycin to 20% for moxalactam plus penicillin and cefoperazone plus ampicillin. However, these were not significantly different by the Kolmogorov-Smirnov one-sample test (5).

TABLE 2. Percentage of 30 *N. meningitidis* isolates inhibited by combinations of antibiotics (checkerboard microtiter broth dilution method)

Combination	% of isolates for which the combination showed:			
	Synergy	Addition	Indifference	Antagonism
Moxalactam + penicillin	20	37	43	0
Moxalactam + ampicillin	7	31	59	3
Cefoperazone + tobramycin	3	60	37	0
Cefoperazone + ampicillin	20	50	30	0
Ceftizoxime + ampicillin	3	33	64	0
Ceftriaxone + ampicillin	10	33	57	0
Cefotaxime + ampicillin	10	40	50	0

Mice receiving ampicillin or moxalactam alone had survival rates of 75 and 71%, respectively, whereas the group receiving both antibiotics had a survival rate of 86%. These differences were not significant by the Fisher exact probability test ($P > 0.8$). None of the saline-treated infected mice survived, whereas all of the controls receiving only mucin survived. *N. meningitidis* was recovered from heart blood and meninges of all animals that died except for one saline-treated infected control mouse whose meningeal culture was negative.

We have demonstrated considerable activity of all eight β-lactams tested against *N. meningitidis*. Our MICs for ceftriaxone, cefotaxime, and moxalactam are comparable to those of Eickhoff and Ehret (2), but are considerably lower than those reported by Neu et al. (4). We noted that cefmenoxime was approximately 16 times more active against *N. meningitidis* than Stamm et al. (6) observed. Since our methodology was essentially the same, these differences might possibly be explained by strain differences due to geographic location. Mitsuhashi et al. (3) reported a cefoperazone MIC of ≤0.1 μg/ml for a single strain of *N. meningitidis*. All 30 isolates we tested were inhibited by 0.016 μg of cefoperazone per ml. To our knowledge, ours is the first report of the activity of ceftazidime, ceftizoxime, *N*-formimidoyl thienamycin, or the antibiotic combinations against *N. meningitidis*. The combinations showed no antagonism, a reassuring result considering the current practice of initiating multiple antibiotic therapy for bacteremic illness.

Although not all combinations were tested, there is little to suggest that other β-lactams or

aminoglycosides would antagonize the antibacterial activity of these new derivatives. We felt it useful to demonstrate that our in vitro results would be reflected by in vivo activity. We chose moxalactam plus ampicillin because the regimen is currently under study in the treatment of bacterial meningitis in children. Again, we expect other combinations to behave in a similar fashion in vivo. Taken together, our results reassure us that these new β-lactam drugs have considerable activity against *N. meningitidis*, that this activity would not be reduced by concomitant antibiotics, and that these effects are reflected in vivo. These data, along with pharmacokinetic and toxicity results, can be used to support clinical evaluations of these drugs in infections where *N. meningitidis* may be present.

LITERATURE CITED

1. **Brown, W. M., and R. J. Fallon.** 1979. Cefotaxime for bacterial meningitis. Lancet i:1246.
2. **Eickhoff, T. C., and J. Ehret.** 1981. Comparative in vitro studies of Ro 13-9904, a new cephalosporin derivative. Antimicrob. Agents Chemother. **19**:435–442.
3. **Mitsuhashi, S., S. Minami, N. Matsubara, A. Yotsuji, S. Kurashige, and I. Saikawa.** 1980. *In vitro* and *in vivo* antibacterial activity of cefoperazone. Clin. Therapeut. **3**(special issue):1–13.
4. **Neu, H. C., N. J. Meropol, and K. P. Fu.** 1981. Antibacterial activity of ceftriaxone (Ro 13-9904), a β-lactamase stable cephalosporin. Antimicrob. Agents Chemother. **19**:414–423.
5. **Siegel, S.** 1956. Nonparametric statistics for the behavioral sciences, p. 47–52. McGraw-Hill, New York.
6. **Stamm, J. M., R. L. Giorlami, N. L. Shipkowitz, and R. R. Bower.** 1981. Antimicrobial activity of cefmenoxime (SCE-1365). Antimicrob. Agents Chemother. **19**:454–460.
7. **Steers, E., E. L. Foltz, and B. S. Graves.** 1959. An inoculum-replicating apparatus for routine testing of bacterial susceptibility of antibiotics. Antibiot. Chemother. **9**:307–311.