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Bactericidal and Bacteriostatic Action of Chloramphenicol Against Meningeal Pathogens

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The bacteriostatic and bactericidal effects of chloramphenicol, ampicillin, tetracycline, and sulfisoxazole were compared against several potential meningeal pathogens. Chloramphenicol is bactericidal at clinically achievable concentrations against *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis*. It is bacteriostatic against gram-negative bacilli of the family *Enterobacteriaceae* and against *Staphylococcus aureus*. Chloramphenicol has proven highly efficacious in the treatment of bacterial meningitis caused by those organisms against which it is bactericidal at low concentrations. Because leukocytic phagocytosis in the subarachnoid space is inefficient, we propose that bactericidal activity in cerebrospinal fluid is important for optimal therapy of bacterial meningitis. Chloramphenicol does not provide such activity in meningitis caused by enteric gram-negative bacilli.

Chloramphenicol has assumed an increasingly important role in the therapy of pyogenic meningitis. It is considered the drug of choice for meningitis due to ampicillin-resistant *Haemophilus influenzae* and for pneumococcal and meningococcal meningitis in patients allergic to penicillin. Recently, enteric gram-negative bacillary meningitis has been noted more frequently among adults undergoing neurosurgical procedures and in those with debilitating disease (7). Antibiotic susceptibility of these pathogens is often limited to chloramphenicol and aminoglycosides. When chloramphenicol susceptibility is known, this drug has been favored as the treatment of choice because of its greater penetrability from serum to cerebrospinal fluid (11). To evaluate the potential efficacy of chloramphenicol against meningitis due to gram-negative bacilli, we have compared the bacteriostatic and bactericidal action of chloramphenicol, ampicillin, tetracycline, and sulfisoxazole against the more common meningeal pathogens (*Streptococcus pneumoniae*, *Neisseria meningitidis*, and *H. influenzae*) and against enteric gram-negative bacilli and *Staphylococcus* aureus.

MATERIALS AND METHODS

Bacterial pathogens, with the exception of meningococci, were obtained from clinical specimens and identified by standard microbiological techniques. Emil Gotschlich of Rockefeller University provided 10 strains of *N. meningitidis*. Tube dilution sensitivity studies were carried out with Mueller-Hinton broth and an inoculum of 10^5 organisms per ml. One-half milliliter of inoculum was added to 0.5 ml of each antibiotic dilution, and the suspensions were incubated for 18 h at 37°C. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic preventing visible turbidity. All clear tubes were subcultured with a calibrated loop (0.01 ml) onto antibiotic-free agar for 18 h at 37°C. The minimal bactericidal concentration (MBC) was defined as that yielding growth of fewer than five colonies (>99% killing). Supplement C (2.5%, Difco) was added for determination of *H. influenzae* susceptibility. Quantitative studies of bacterial killing rates were performed with 2-ml suspensions containing 10^5 organisms per ml. Aliquots of 0.1 ml were removed at timed intervals, and colony-forming units were counted by the standard plate dilution technique.

RESULTS

Eleven strains of *H. influenzae*, 10 strains of *S. pneumoniae*, and 10 strains of *N. meningitidis* were tested for their bacteriostatic and bactericidal susceptibility to chloramphenicol, ampicillin, and tetracycline. Susceptibility of the *N. meningitidis* strains to sulfisoxazole was also tested.

Against *H. influenzae*, the MIC of chloramphenicol was at least twofold lower than that of ampicillin for 10 of 11 strains. The MBC of chloramphenicol was at least twofold lower than that of ampicillin for six strains and equal to that of ampicillin for four strains. In general, bactericidal activity of chloramphenicol occurred at concentrations comparable to those at

which ampicillin was bacteriostatic. Tetracycline, at clinically achievable concentrations, demonstrated only bacteriostatic activity against most strains (Fig. 1).

In contrast to *H. influenzae, S. pneumoniae* were severalfold more susceptible to the bactericidal action of ampicillin than to that of chloramphenicol. Nevertheless, the MICs and MBCs of chloramphenicol were not widely separate for most strains, and nine of ten were killed by the clinically achievable concentration of 6.25 μg/ml. The MICs and MBCs for tetracycline differed by four- to eightfold among susceptible strains, but several strains were highly resistant to both the bacteriostatic and the bactericidal activities of this antibiotic (Fig. 2).

Meningococci, like pneumococci, were more susceptible to ampicillin than to chloramphenicol, but the latter drug again exhibited bactericidal activity against most isolates at clinically achievable concentrations (Fig. 3). Six of ten isolates were killed by 6.25 μg or less per ml, and the MBC for three others was 12.5 μg/ml. All strains were inhibited by less than 1.56 μg of tetracycline per ml, but only three were killed by 6.25 μg/ml. Thus, tetracycline was essentially bacteriostatic for the majority of meningococci. Sulfisoxazole demonstrated bactericidal activity at 62.5 μg/ml against six of ten meningococcal strains, indicating susceptibility at clinically achievable concentrations. The remaining four were sulfonamide resistant, requiring 31.2 to 62.5 μg/ml for inhibition and 250 to 500 μg/ml for bactericidal activity.

In contrast to the bactericidal action of chloramphenicol at low concentrations against *H. influenzae, S. pneumoniae,* and *N. meningitidis,* studies with a variety of *Enterobacteriaceae* and *Staphylococcus aureus* demonstrated only bacteriostatic activity against almost all strains (Table 1). Members of the family *Neisseriaceae* other than *N. meningitidis,* namely, *Acinetobacter, Moraxella,* and *N. catarrhalis,* were variably susceptible to the bactericidal effect of low concentrations of chloramphenicol. *Acinetobacter* were not killed by such concentrations, whereas *N. catarrhalis* and a single *Moraxella* isolate were susceptible. A strain of *Cardiobacterium hominis* isolated from the blood of a patient with endocarditis was killed by 1.56 μg of chloramphenicol per ml (Table 2).

Quantitative kinetic studies with a single strain of *H. influenzae* demonstrated almost identical rates of killing by 1.56 μg of chloramphenicol or ampicillin per ml against an inoculum of 10^5 organisms per ml (Fig. 4). With inocula of 10^2 to 10^6 organisms per ml, the MIC and MBC of chloramphenicol against *H. influenzae* showed no significant change. With 10^7 organisms per ml, the MBC also remained within clinically achievable concentrations.

DISCUSSION

Alexander et al., in 1949, described the rapidly lethal effect of chloramphenicol against *H. influenzae* at a concentration of 10 μg/ml (1). Nevertheless, chloramphenicol, because of its inability to sterilize cultures of staphylococci and enteric bacilli at concentrations below 500 μg/ml, is generally described as a bacteriostatic antibi-

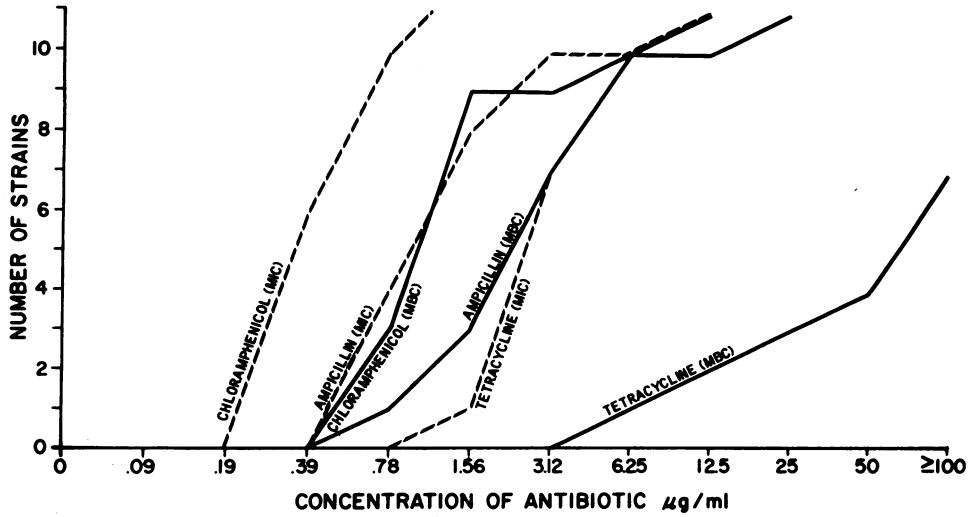

FIG. 1. *Bactericidal activity of chloramphenicol against H. influenzae compared with those of ampicillin and tetracycline. Interrupted lines represent cumulative MICs and solid lines represent cumulative MBCs against 11 strains.*

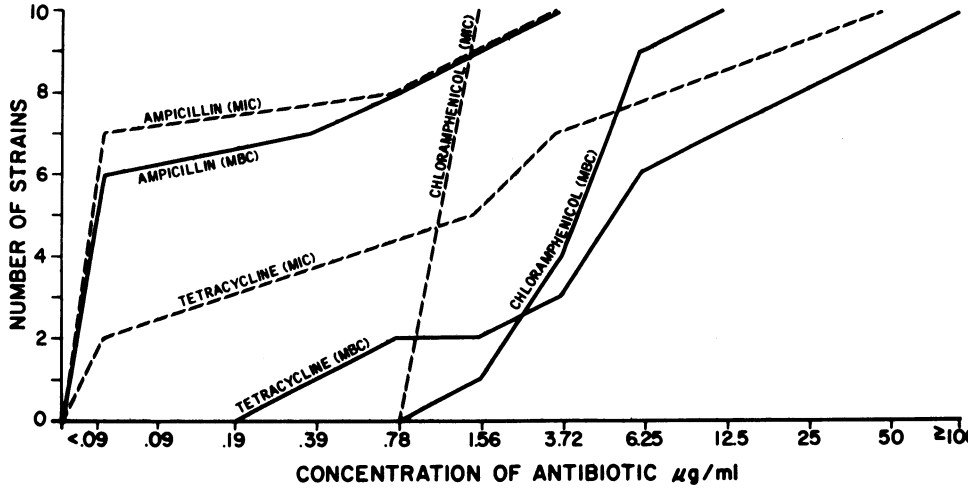

FIG. 2. *Bactericidal activity of chloramphenicol against S. pneumoniae compared with those of ampicillin and tetracycline. Interrupted lines represent cumulative MICs and solid lines represent cumulative MBCs against 10 strains.*

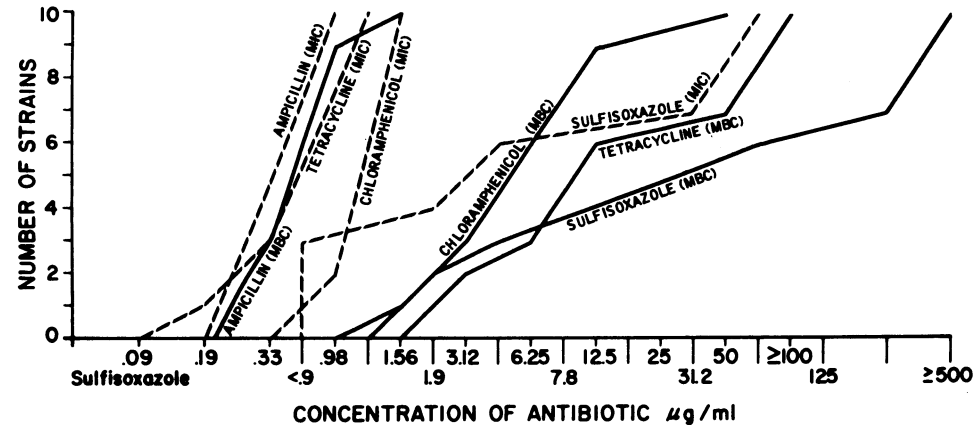

FIG. 3. *Bactericidal activity of chloramphenicol against N. meningitidis compared with those of ampicillin, tetracycline, and sulfisoxazole. Interrupted lines represent cumulative MICs and solid lines represent cumulative MBCs against 10 strains.*

otic. These divergent effects of the same antibiotic against different bacterial species reaffirm the concept that antibiotics cannot be broadly classified as bacteriostatic or bactericidal on the basis of their activity against selected microbial strains. For clinical purposes, the relationship between the antibiotic concentration required to kill >99% of a bacterial population in 24 h and that concentration which can be achieved in body fluids represents a more useful definition of "bactericidal" activity for a specific drug against a specific organism. In essence, each antibacterial agent possesses finite "bacteriostatic" and bactericidal concentration ranges which differ against various bacteria. Agents such as the penicillins and cephalosporins are considered bactericidal because inhibitory and lethal concentrations are almost identical against most susceptible organisms. Enterococci are exceptional because they are not uniformly killed by low concentrations of penicillin. Recently, certain strains of *Staphylococcal aureus* have proven resistant to the lethal action of semisynthetic penicillins and susceptible only to their inhibitory effect (12). The data we have presented indicate that the antibacterial action of chloramphenicol (as well as of tetracycline and sulfisoxazole in some instances) cannot be broadly characterized as bacteriostatic against all organisms. Thus, if one defines the bactericidal concentrations of chloramphenicol as a minimal lethal concentration which can be

TABLE 1. *Bacteriostatic susceptibility of Enterobacteriaceae and Staphylococcus aureus to chloramphenicol*

Organism	MIC (μg/ml)	MBC (μg/ml)
E. coli	1.9	250
Salmonella (2 strains)	0.9, 1.9	62.5, >500
Klebsiella (3 strains)	0.1, 1.9, 1.9	15.6, 250, >500
Enterobacter (2 strains)	3.9, 7.8	>500, >500
Serratia (3 strains)	3.9, 15.6, 31.2	>500, >500, >500
Proteus	3.9	>500
Providencia (2 strains)	15.6, 15.6	62.5, >500
Staphylococcus aureus (6 strains)	3.12–12.5	25–>50

TABLE 2. *Variable bacteriostatic and bactericidal susceptibility of Neisseriaceae and a single strain of Cardiobacterium hominis to chloramphenicol*

Organism	MIC (μg/ml)	MBC (μg/ml)
Acinetobacter (4 strains)	3.1, 6.2, 50, >50	25, >25, >50, >50
Neisseria catarrhalis (2 strains)	0.78, 3.1	0.78, 6.2
Moraxella	0.78	0.78
Cardiobacterium hominis	0.78	1.56

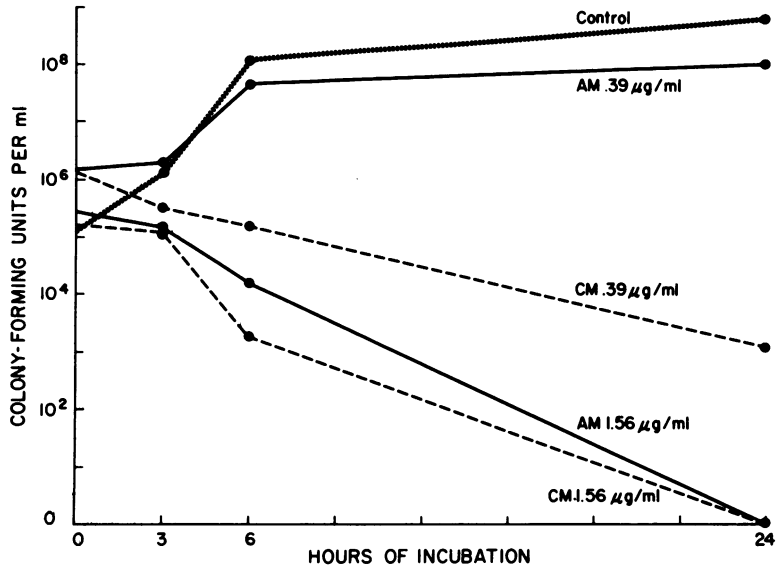

FIG. 4. *Comparative rate of bactericidal activities of chloramphenicol (CM) and ampicillin (AM) against a susceptible strain of H. influenzae (MBC, 1.56 μg/ml).*

achieved in most body fluids (15 to 60 μg/ml), then this drug is bactericidal against most strains of *H. influenzae*, pneumococcus, and meningococcus but bacteriostatic against *Staphylococcus aureus* and enteric gram-negative bacilli. Whether such variable antibacterial effects against different species are due to separate biochemical actions remains to be determined.

These considerations hold important therapeutic implications for infections which require treatment with bactericidal antimicrobial agents. Bacterial endocarditis is a widely accepted example because of the paucity of phagocytic leukocytes in infected cardiac vegetations. Deficient phagocytosis of bacteria at the site of infection thus mandates lethal antibiotic activity for cure. In bacterial meningitis, an abundance of phagocytic leukocytes usually appears in the cerebrospinal fluid. However, the number of viable bacteria rapidly rises to approximately 10^7 organisms per ml, suggesting relatively inefficient leukocytic phagocytosis (4). In 1946, Wood and his associates postulated a state of inefficient phagocytosis of pneumococci in meningitis due to the lack of type-specific antibody in the early stages of infection (18). Petersdorf et al.

later confirmed the poor phagocytic function of meningeal leukocytes against encapsulated pneumococci in dogs (10), whereas O'Toole and colleagues documented the absence of specific antipneumococcal opsonic antibody in most cases of pneumococcal meningitis studied (18). It appears now that early phagocytosis of pneumococci and other encapsulated organisms is dependent upon nonspecific, heat-labile, complement-mediated opsonization (13, 14). Complement activity in normal and infected spinal fluid is either undetectable or present in concentrations which represent small fractions of those in serum (3, 6, 17). Thus, absent or minimal concentrations of specific antibody and complement in spinal fluid may explain the inefficient phagocytosis and strikingly high populations of bacteria found in the subarachnoid space of patients with purulent meningitis.

These factors suggest that bacterial meningitis, like endocarditis, requires bactericidal antibiotic activity for optimal therapy. That chloramphenicol and sulfonamides have been highly succsssful in the therapy of purulent meningitis would argue against this hypothesis if these drugs were universally bacteriostatic. Our data indicate that chloramphenicol is bactericidal for the three major meningeal pathogens against which it has been consistently successful and that sulfonamides are bactericidal against susceptible strains of meningococci. It is of interest that recent studies by Brotherton et al. (2), Turk (16), Paredes et al. (9), and Feldman (5) have demonstrated low MBCs of chloramphenicol against *H. influenzae* and *S. pneumoniae*.

The possible requirement for bactericidal antibiotic activity in purulent meningitis raises a question regarding the role of chloramphenicol in the treatment of meningitis due to gram-negative enteric bacilli against which this antibiotic is only bacteriostatic at clinically achievable concentrations. The use of chloramphenicol for gram-negative bacillary meningitis in adults has not been highly successful despite its excellent diffusion into cerebrospinal fluid. Resistance has developed during therapy in 30% of the strains in one series (Z. A. McGee, A. B. Kaiser, C. Rubens, and W. E. Farrar, Jr., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., Abstr. no. 4, 1977). This lesser efficacy of chloramphenicol in gram-negative enteric bacillary meningitis as compared with *H. influenzae*, pneumococcal, and meningococcal meningitis may be due to host factors as well as antibiotic activity. Nevertheless, Sande and co-workers have provided instructive data in a well-defined rabbit model which compares the action of chloramphenicol in two types of meningitis. In a study of *H. influenzae* meningitis, chloramphenicol and the combination of ampicillin plus chloramphenicol cleared the spinal fluid of viable organisms as rapidly as ampicillin alone (J. Bodine, T. Murray, and M. A. Sande, Clin. Res. **25**: 27A, 1977). These findings are consistent with Feldman's in vitro studies which demonstrate that chloramphenicol does not antagonize the bactericidal activity of ampicillin against *H. influenzae* (5). In contrast, bacterial counts in spinal fluid of experimental *Proteus* meningitis remained constant after chloramphenicol therapy, indicating a bacteriostatic effect. Furthermore, bacteriostatic activity resulted from the combination of chloramphenicol and gentamicin, indicating antagonism of gentamicin by chloramphenicol (15).

In conclusion, chloramphenicol is bactericidal in clinically achievable concentrations against certain meningeal pathogens against which it has proven highly efficacious. This supports the concept that bactericidal activity in cerebrospinal fluid is important for optimal therapy because of inefficient phagocytosis in the subarachnoid space. Chloramphenicol does not provide such activity when used for the therapy of meningitis due to gram-negative enteric bacilli.

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