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Antagonism of Ampicillin and Chloramphenicol for Meningeal Isolates of Group B Streptococci

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The increasing prevalence of ampicillin-resistant *Haemophilus influenzae* type b has led to the recommendation that ampicillin and chloramphenicol be given as the initial therapy for suspected bacterial meningitis in infants and children. However, during the first 2 months of life, *H. influenzae* type b is a rare cause of meningitis, whereas group B streptococcus is the most frequently isolated agent. Since ampicillin and chloramphenicol have been shown to be antagonistic for other streptococci, an in vitro study of their effect on group B streptococci was performed. The effect of ampicillin and chloramphenicol, alone and in combination, on 18 meningeal isolates was determined for 2 different inocula of group B streptococci, using microtiter broth dilution and growth kinetic assays. Isoboles, fractional lethal concentration indices, or both indicated antagonism for all strains. Growth kinetic assays for two representative strains demonstrated inhibition of the early bactericidal activity of ampicillin by chloramphenicol. These findings of in vitro antagonism suggest that this combination may be contraindicated for the treatment of infants with group B streptococcal meningitis.

Because of the emergence of ampicillin-resistant *Haemophilus influenzae* type b strains, a combination of ampicillin with chloramphenicol has been recommended since 1974 as initial antibiotic therapy for bacterial meningitis beyond the neonatal period (2). This recommendation may lead to the occasional treatment of infants less than 2 months of age with this combination, although *H. influenzae* type b is infrequently isolated from infants in this age group with meningitis (1, 15, 19, 20).

In vitro and some in vivo studies have indicated that chloramphenicol in combination with a penicillin is antagonistic for some bacterial species. For example, interference with the early bactericidal effect of penicillin was reported by Wallace et al. (17) in an animal model of pneumococcal meningitis. Similarly, Mathies et al. (13) observed an increased case-fatality ratio among children and adults with bacterial meningitis who were treated with the combination of ampicillin, chloramphenicol, and streptomycin compared with those who were treated with ampicillin alone.

Because group B streptococci (GBS) are the single most frequent agents isolated in our medical center from infants less than 2 months of age with bacterial meningitis (1), we believed it important to evaluate the in vitro effect of ampicillin and chloramphenicol against several meningeal isolates.

MATERIALS AND METHODS

Bacterial isolates. Eighteen cerebrospinal fluid (CSF) isolates of GBS from infants <2 months of age were serotyped by the capillary precipitin method of McCarty and Lancefield (14). The serotype distribution of these strains was representative of that reported among neonatal meningeal isolates (18) and included 1 type Ia, 2 type Ib, 1 type Ic, and 14 type III strains.

Media. Microtiter plate and growth kinetic assays were performed with Mueller-Hinton broth (MHB) (Difco Laboratories, Detroit, Mich.). Bacterial colony counts for both assays were determined by serial 10-fold dilutions of broth cultures in Todd-Hewitt agar (BBL Microbiology Systems, Cockeysville, Md.) pour plates.

Studies of antimicrobial interaction. The 18 strains were tested for their susceptibility to ampicillin and chloramphenicol, separately and in combination, by means of a microtiter plate apparatus (Dynatech Laboratories, Inc., Alexandria, Va.). Microtiter plates were prepared by adding 50 μl of MHB to each of the 96 wells, using a multichannel pipette (made for Flow Laboratories, Inc., by Labsystems, Oy, Finland). Serial twofold dilutions of ampicillin in a horizontal direction from left to right were prepared with a 50-μl microdiluter (Dynatech). A single concentration of chloramphenicol was added to each well (50 μl/well) along

each horizontal row of ampicillin dilutions. Final concentrations of ampicillin ranged from 0.025 to 25 μg/ml and final concentrations of chloramphenicol ranged from 0.8 to 250 μg/ml for low- or high-inoculum experiments.

Since the concentration of GBS in the CSF of neonates with meningitis at admission may contain 10^7 to 10^8 colony-forming units (CFU) per ml (7), both high (10^{6-7})- and standard (10^{4-5})-inoculum experiments were performed. The former was used in testing four representative strains. The inocula were prepared by diluting an 18-h MHB culture to an optical density of 0.1 at 540 nm (Coleman Junior spectrophotometer, model 6C; Coleman Instruments, Maywood, Ill.) (approximately 5×10^7 CFU/ml) and then further diluting to 1:10 or 1:1,000 for high and standard inocula of approximately 5×10^6 and 5×10^4 CFU/ml, respectively. Microtiter plates were incubated at 37°C for 18 h. The minimal inhibitory concentration (MIC) was the lowest concentration of antibiotic at which no visible growth in the well was observed. The minimal bactericidal concentration (MBC) was defined as no growth of well subcultures on blood agar plates containing 50-μl portions and incubated at 37°C overnight. The results were expressed both as the fractional lethal concentration (FLC) indices and by isobolograms. The fractional inhibitory concentration (FIC) index described by Elion et al. (6) was calculated by the formula

$$\text{FIC index} = \frac{\text{MIC of drug A + drug B}}{\text{MIC of drug A}} + \frac{\text{MIC of drug B + drug A}}{\text{MIC of drug B}}$$

Similarly, the FLC index was calculated with the MBC ratios. When possible, isobolograms were constructed from MBC results as described by Eickhoff (5). Antagonism of the drug combination was indicated by a convex isobologram when compared with the line connecting the drugs separately or when the FLC index was $\geqq 1.2$. The effect of the drug combination was considered to be additive when the line connecting the combinations was parallel to that connecting points of drugs used alone or if the FLC index fell between 0.8 and 1.2.

Growth kinetic studies were performed by incorporating antibiotics singly or in combination into MHB at a final volume of 15 ml. Serial twofold dilutions with $\sim 10^5$ CFU/ml as the inoculum were performed to determine the MIC, which was defined as the lowest concentration of antibiotic resulting in no visible turbidity after incubation for 18 h at 37°C. A standard inoculum (5×10^4 CFU/ml) of two strains, using a final concentration of drug at twice the MIC (ampicillin at 0.2 μg/ml and chloramphenicol at 6.3 μg/ml), was tested in duplicate. Results were expressed as mean and range of $\log_{10}$ CFU per milliliter. In addition, one of these two strains was tested at an inoculum of 5×10^6 CFU/ml, using theoretically achievable concentrations of ampicillin (10) (12.5 μg/ml) and chloramphenicol (16) (10 μg/ml) in the CSF of infants with meningitis. Ampicillin at 200 μg/ml and chloramphenicol at 10 μg/ml were also tested by this method alone and in combination. Equal portions were removed immediately after inoculation of the flasks, at hourly intervals times six during incubation in a shaking water bath at 37°C, and at 24 h. Equal portions of broth were serially diluted, and pour plates were used to determine the viable CFU for construction of growth curves.

RESULTS

Microtiter plate dilution studies were performed at the standard inoculum (10^{4-5} CFU/ml) for 18 strains of GBS and at the high inoculum (10^{6-7} CFU/ml) for four representative strains (Tables 1 and 2). Antagonism was demonstrated by one or both methods of analysis (isoboles or FLC index) for all strains. At the standard inoculum, the FLC index was $\geqq 1.2$ for 11 of the 18 strains. Construction of isobolograms revealed convex isoboles, indicating antagonism for 16 of the 18 strains. Figure 1 illustrates isobolograms for two representative strains, 424 and 432, at the standard inoculum. It is noteworthy that the isoboles for these two strains were convex, even though the FLC indices were not >1.2 (see Table 1). The finding of convex isoboles was also true for all strains with FLC indices of <1.2.

Because quantitative cultures of CSF from neonates with meningitis due to GBS not infrequently indicate viable bacterial counts of $>10^5$ CFU/ml, the above studies were repeated for four representative strains, using an inoculum of 10^{6-7} CFU/ml. The FLC index was $\geqq 1.2$ for three of the four strains, and all four were observed to have convex isoboles.

Growth kinetic assays were also performed at the standard and high inocula. Figure 2 shows the mean results for two experiments with a representative strain of GBS (M732), using the standard inoculum. In the absence of antibiotic, the colony count was $>10^8$ CFU/ml at 24 h of incubation, whereas ampicillin alone produced complete killing at 4 h. Chloramphenicol alone inhibited growth, but did not reduce the colony count at the end of the incubation period. The addition of chloramphenicol inhibited the early bactericidal effect of ampicillin, producing a greater than 4-log difference in CFU per milliliter when the combination was compared with ampicillin alone at 4 h of incubation. A second strain (421) was tested in duplicate under the same conditions, and similar results were observed. There was complete loss of viability with ampicillin alone at 3 h, but a greater than 4-log difference was observed at 3 h when this was compared with the combination. When a higher concentration of chloramphenicol (six times the MIC) and the same concentration of ampicillin

TABLE 1. *Effect of ampicillin and chloramphenicol on GBS (inoculum, ~5 × 10⁴ CFU/ml)*

Strain	Serotype	MBC (μg/ml)[a]				FLC index
		Ampicillin	Chloramphenicol	Combination		
				Ampicillin	Chloramphenicol	
M732	III	0.0625	6.25	0.1125	1.56	1.75
III-Bell	III	0.025	25	0.05	6.25	2.25
400	III	0.025	12.5	0.05	3.125	2.25
401	III	0.025	6.25	0.025	1.56	1.25
406	III	0.025	12.5	0.025	3.125	1.25
408	III	0.025	12.5	0.025	3.125	1.25
420	III	0.1	25	0.1	6.25	1.25
421	III	0.025	3.125	0.05	1.56	2.5
422	III	0.025	25	0.05	6.25	2.25
423	Ic	0.025	25	0.1	6.25	2.25
424	Ib	0.1	50	0.1	1.56	1.03
425	Ia	0.1	12.5	0.1	1.56	1.12
426	Ib	0.05	50	0.2	3.125	4.06
428	III	0.025	25	0.025	1.56	1.06
429	III	0.025	50	0.025	0.8	1.02
430	III	0.025	25	0.025	3.125	1.25
431	III	0.025	25	0.025	1.56	1.06
432	III	0.05	50	0.05	1.56	1.03

[a] Mean of duplicate experiments.

TABLE 2. *Effect of ampicillin and chloramphenicol on GBS (serotype III; inoculum, ~5 × 10⁶ CFU/ml)*

Strain	MBC (μg/ml)[a]				FLC index
	Ampicillin	Chloramphenicol	Combination		
			Ampicillin	Chloramphenicol	
M732	3.905	156.25	6.25	31.25	1.80
III-Bell	6.25	250	6.25	7.8	1.03
400	0.8	250	3.125	62.5	4.15
401	0.4	250	3.125	62.5	8.06

[a] Mean of duplicate experiments.

(two times the MIC) were tested, comparable data were accumulated.

Concentrations of ampicillin (12.5 μg/ml) and chloramphenicol (10 μg/ml) which are theoretically achievable in the CSF of infants were tested, using strain M732 and the high inoculum. Loss of bacterial viability occurred at 24 h with ampicillin alone, whereas approximately 10^4 CFU of GBS per ml remained after 24 h of incubation both with the combination and with chloramphenicol alone. Ampicillin reduced the viable bacterial count at 6 h by approximately 2 logs more than the combination. Although a variety of test conditions regarding bacterial inoculum and antibiotic concentrations were used

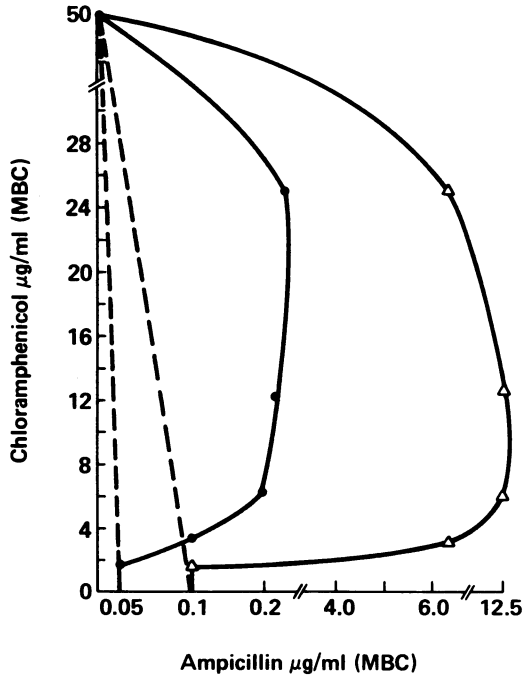

FIG. 1. *Isobolograms for the standard inoculum (5 × 10⁴ CFU/ml) of representative strains of GBS, 424 and 432, demonstrating antagonistic effects of ampicillin and chloramphenicol. Dotted lines represent theoretical additive isoboles.*

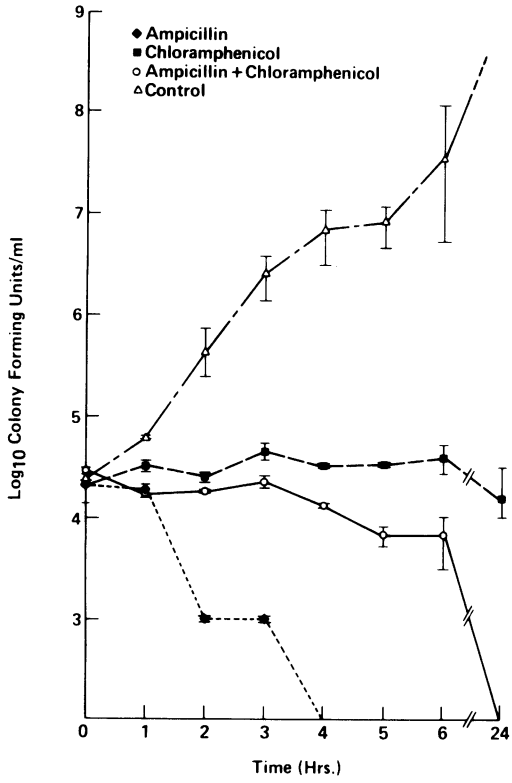

FIG. 2. *Mean and range of two growth kinetic studies of GBS strain M732 at the standard inoculum (~5 × 10^4 CFU/ml) with 0.2 μg of ampicillin per ml (twice the MIC) and 6.3 μg of chloramphenicol per ml (twice the MIC), alone and in combination.*

for these growth kinetic assays, in vitro antagonism between ampicillin and chloramphenicol was always observed.

Since the combination of moxalactam (a 1-oxa beta-lactam antibiotic) and ampicillin is presently being evaluated for its efficacy in the treatment of neonatal meningitis by the Third Neonatal Meningitis Cooperative Study Group, a growth kinetic study with GBS strain M732 at the standard inoculum was performed with these drugs alone and in combination. There was complete loss of bacterial viability at 3 h for each antibiotic alone and for the combination, suggesting an additive and not antagonistic effect.

DISCUSSION

The combination of ampicillin and chloramphenicol is the recommended initial antibiotic therapy for bacterial meningitis in children. The possible use of this combination in infants less than 2 months old with meningitis, who commonly have GBS isolated from the CSF, is of concern in light of this investigation, which clearly demonstrates in vitro antagonism of ampicillin and chloramphenicol for meningeal isolates of GBS. The bacterial inoculum and antibiotic concentrations were varied for each of the two in vitro methods used, microtiter plate and growth kinetic assays, and antagonism was observed regardless of the method or test conditions. These findings were not unexpected, however, since several previous reports described antagonism for streptococcal species other than groups B and D when chloramphenicol was added to a penicillin.

Jawetz and Gunnison (8) observed that bacteriostatic antibiotics such as chloramphenicol and tetracycline may act antagonistically with bactericidal antibiotics such as penicillin, streptomycin, bacitracin, and neomycin. This was later confirmed by Manten and Wisse (12). Jawetz et al. (9) clearly demonstrated that certain bacteria were killed more slowly by the combination of penicillin and a bacteriostatic antibiotic such as chloramphenicol than by penicillin alone. Interference by chloramphenicol with the early bactericidal effect of penicillin was also reported in experimental group A streptococcal infection in mice (9) and pneumococcal meningitis in dogs (17).

The relationship of these in vitro and experimental results indicating antagonism is not precisely known. However, several clinical studies in which the use of a combination of antibiotics, usually bactericidal and bacteriostatic, resulted in a poorer outcome than with the bactericidal antibiotic alone have been recorded. Lepper and Dowling (11) observed a higher mortality in patients with pneumococcal meningitis receiving a combination of penicillin and chlortetracycline than in those receiving penicillin alone (79 versus 30%). In a later study by Mathies et al. (13), 264 patients >2 months of age with acute bacterial meningitis were assigned to treatment with either a single drug (ampicillin) or a combination of drugs (ampicillin, chloramphenicol, and streptomycin). The groups were comparable with respect to age, severity of disease, and etiology. There were 6 deaths among 140 patients treated with ampicillin, or a case fatality ratio of 4.3%. In contrast, 124 patients were treated with the multiple-drug regimen and 13 died, giving a case fatality ratio of 10.5%. When neurological sequelae, such as hemiparesis, cranial nerve palsies, and deafness, were combined with fatalities, the difference was 12.1 versus 22.6% for the single- and multiple-drug treatment groups, respectively. These studies support the thesis that antibiotic antagonism may influence clinical outcome (11, 13).

The present study demonstrates chloram-

phenicol inhibition of the early bactericidal effect of ampicillin against GBS, an effect which was not found when the combination of ampicillin and moxalactam was tested. This latter observation is important, since moxalactam plus ampicillin may have wide clinical use if clinical trials demonstrate efficacy for this combination in the treatment of meningitis due to gram-negative enteric bacilli in neonates or *H. influenzae* type b in older infants. In view of these in vitro data and the possibility of antagonism between ampicillin and chloramphenicol for GBS in vivo, this combination is contraindicated for the treatment of GBS meningitis. Since GBS meningitis occurs infrequently beyond 8 weeks of age, however, no change is suggested in the current recommendation that ampicillin and chloramphenicol be used for the initial treatment of meningitis in infants and children. Similarly, since in vitro (3) and in vivo (4) studies have demonstrated synergy for ampicillin and an aminoglycoside against GBS, this combination seems to be a reasonable choice for the initial therapy of neonatal meningitis until the efficacy of potentially useful new agents can be established.

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