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# Chloramphenicol Inhibition of the Bactericidal Effect of Ampicillin Against *Haemophilus influenzae*

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The effects of combinations of ampicillin and chloramphenicol against seven strains of *Haemophilus influenzae* type b (five beta-lactamase-negative and two beta-lactamase-positive strains) were evaluated by killing-kinetic methods. Growth of strains was assessed in modified Levinthal broth against an inoculum of $10^5$ organisms per ml; colony counts were performed immediately and at 6 and 20 h postinoculation. Ampicillin and chloramphenicol were completely bactericidal at 20 h, reducing bacterial densities to 0 colony-forming units per ml at concentrations equivalent to the ampicillin inhibitory concentration and twofold the chloramphenicol inhibitory concentration. Chloramphenicol at its inhibitory concentration or at one-half of its inhibitory concentration prevented the normally bactericidal activity of ampicillin at 20 h incubation, but not at 6 h.

Results of studies published since 1965 have yielded conflicting results regarding the in vitro and clinical activity of combinations of chloramphenicol and ampicillin against *Haemophilus influenzae* type b. Conclusions of investigators completing in vitro studies have varied, suggesting an antagonistic effect (6), an indifferent or additive effect (2), or synergy (3). Mathies and co-workers (5) concluded that the use of combinations of chloramphenicol and ampicillin (in addition to 48 h of streptomycin treatment) resulted in a higher mortality rate in children treated for meningitis caused by *H. influenzae* than that in children treated with ampicillin alone. Currently, the American Academy of Pediatrics recommends the use of ampicillin and chloramphenicol as the initial regimen for *H. influenzae* meningitis until the results of beta-lactamase testing of the responsible organism can be completed (1). Because it remains conceivable that antibiotic antagonism may occur in the clinical setting and since recommendations for the combined use of antibiotics exist, we again attempted to examine the bactericidal activity of combinations of ampicillin and chloramphenicol against *H. influenzae* in an in vitro system. A modification of the killing-kinetics technique as originally proposed by Jawetz and Gunnison in 1952 (4) was used in this study.

Five beta-lactamase-negative and two beta-lactamase-positive strains of *H. influenzae* type b, obtained from patients with acute bacterial meningitis, were used in these studies. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration of ampicillin and chloramphenicol against each strain were determined in serial dilution of antibiotics (0.1 to 200 μg/ml); ending dilutions were in a total volume of 1.0 ml of modified Levinthal broth (6, 7). Inhibitory concentrations were determined against an inoculum of $10^5$ organisms per ml in 10% $CO_2$ at 37°C. Beta-lactamase activity was determined by the capillary tube method as described by Thornsberry and Kirvin (9). The minimal bactericidal concentration was defined as the lowest concentration yielding ≤10 colonies on chocolate agar after overnight growth from a subculture of 0.01 ml from clear MIC dilutions.

The effect of combinations of ampicillin and chloramphenicol against *H. influenzae* was determined during growth in modified Levinthal broth. In a total volume of 5 or 10 ml, the growth of each strain was evaluated in combinations of ampicillin and chloramphenicol after the inoculation of $10^5$ organisms per ml prepared from cultures grown overnight. Serial colony counts were performed at 0, 6, and 20 h postinoculation. The following regimens of ampicillin or chloramphenicol or both were evaluated against selected strains: ampicillin alone at each respective strain's MIC of ampicillin, at two times the ampicillin MIC, and at 25 μg of ampicillin per ml; chloramphenicol alone at each respective strain's MIC of chloramphenicol, at two times the chloramphenicol MIC, at one-half of the chloramphenicol MIC, at 5.0 μg of chloramphenicol and 10 μg of chloramphenicol per ml. Ampicillin and chloramphenicol in combination at all concentrations listed were tested with at least five strains each.

The five beta-lactamase-negative strains had

median MICs of 0.4 μg of ampicillin and 0.8 μg of chloramphenicol per ml and median minimal bactericidal concentrations of 0.8 μg of ampicillin and 1.6 μg of chloramphenicol per ml. Two beta-lactamase-positive strains had median MICs of 25 μg of ampicillin and 0.8 μg of chloramphenicol per ml and median minimal bactericidal concentrations of 50 μg of ampicillin and 1.6 μg of chloramphenicol per ml.

With the exception of a single strain, ampicillin at the determined MIC reduced bacterial density to ≤10 colony-forming units (CFU) per ml at 20 h in killing-kinetic assays against all beta-lactamase-negative strains of *H. influenzae* type b (Table 1). Chloramphenicol did not reduce bacterial density to ≤100 CFU/ml at 20 h in a concentration at its determined MIC but was uniformly bactericidal (i.e., 0 CFU/ml at 20 h) at twofold its MIC and at concentrations of 5 and 10 μg of chloramphenicol per ml. Ampicillin and chloramphenicol alone were equivalent in activity (at each respective MIC) at 6 h, reducing colony counts ($\log_{10}$) to 3.28 and 3.54, respectively. Combinations of chloramphenicol and ampicillin in concentrations at the respective MIC of each antibiotic produced a killing effect at 20 h equivalent to that of chloramphenicol alone, thus neutralizing the otherwise bactericidal activity of ampicillin alone. Chloramphenicol in a concentration of one-half of its determined MIC also inhibited the activity of ampicillin at 6 and 20 h when combined with ampicillin at its MIC; this combination failed to yield less than 1,000 CFU/ml at 20 h in four of the five strains tested. Beta-lactamase-positive strains were not inhibited by incubation in any concentration of ampicillin, whereas the activity of chloramphenicol at its MIC, as assessed by colony counts at 6 and 20 h, was equivalent to that observed among beta-lactamase-negative strains. No inhibition or addition of activity to chloramphenicol was noted in the presence of ampicillin.

Prior in vitro studies (2, 3, 6) of the interaction of chloramphenicol and ampicillin against populations of *H. influenzae* have stressed the effects of combinations of these antibiotics on the inhibitory activity of each respective antibiotic. Killing-kinetic assays as described in our studies stress the effect of these combinations on bactericidal activity. In these studies, chloramphenicol antagonized the bactericidal activity of ampicillin such that normally bactericidal concentrations of ampicillin (0.4 to 0.8 μg/ml) failed to reduce bacterial density to 0 CFU/ml in the presence of chloramphenicol in concentrations equal to or at half of its MIC. This inhibition of ampicillin activity by chloramphenicol was expressed only at 20 h and was not observed at 6 h. McBryde et al. (6) described antagonistic effects

TABLE 1. Mean bacterial densities of five ampicillin-susceptible *H. influenzae* strains in the presence of ampicillin and chloramphenicol at 0, 6, and 20 h of incubation

| Chloramphenicol concn | Mean bacterial density ($\log_{10}$ CFU/ml) at following time (h) with ampicillin at indicated concn: | | | | | |
|---|---|---|---|---|---|---|
| | 0 | | 6 | | 20 | |
| | MIC | 0 | MIC | 0 | MIC | 0 |
| MIC | 4.81 | 4.85 | 3.26 | 3.54 | 3.27 | 2.85 |
| 50% MIC | 4.99 | ND[a] | 5.06 | ND | 4.23 | ND |
| 2 × MIC | 5.66 | 5.72 | 3.10 | 3.14 | 0 | 0 |
| 0 | 4.83 | 5.78 | 3.28 | 8.45 | 0 | 8.72 |

[a] ND, Not done.

of ampicillin activity by chloramphenicol in 101 of 107 tests, using plate and tube methods for demonstrating combined activity. In contrast, Feldman (3) concluded that synergy occurred in 6 of 13 strains based upon MIC isobolograms and noted no antagonism. He concluded that chloramphenicol is bactericidal against *H. influenzae*, and therefore antagonism would not be expected to occur; however, the methods used in these studies would not have recognized the interference with the bactericidal activity of ampicillin, since the effect on inhibitory activity was used as the endpoint. Cole et al. (2) used methods similar to ours and concluded that the effect of ampicillin and chloramphenicol combinations was largely indifferent. However, these investigators examined the effect of combinations of ampicillin and chloramphenicol by measuring bacterial densities at 4 and 8 h only. They did not examine colony counts at 20 h. They observed that ampicillin alone consistently reduced bacterial densities to a lower level in the absence of added chloramphenicol and that there was a linear correlation between increasing amounts of chloramphenicol from 0 to MIC concentrations and a decreasing bactericidal effect of ampicillin at 8 h.

In the killing-kinetic assay utilized here, chloramphenicol in a concentration equal to or at half of its determined MIC inhibited the normally bactericidal effect of ampicillin at 20 h of incubation when combined with ampicillin in concentrations equal to the ampicillin MIC. The clinical significance of such observations are unknown. Recent studies by Yogev et al. have documented chloramphenicol cerebrospinal fluid trough levels during the treatment of bacterial meningitis of 4.2 ± 0.3 μg/ml (range, 1.0 to 7.5 μg/ml) after intravenous administration and 6.6 ± 0.73 μg/ml (range, 1.5 to 16.5 μg/ml) after oral administration (10). Tuomanen and co-workers observed no significant change in achievable cerebrospinal fluid concentrations over the course of 10

days of therapy (E. Tuomanen, A. Smith, K. Powell, and M. Marks, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 186, 1980). Although in both of these studies cerebrospinal fluid chloramphenicol levels were above that of the median MIC for chloramphenicol against *H. influenzae* strains (8), these levels were not consistently above either the minimal bactericidal concentration (≥1.6 μg of chloramphenicol per ml) or the bactericidal concentrations (two-fold MIC) used in this study. It is probable that antagonistic concentrations of ampicillin and chloramphenicol, as defined here, would be only transiently achieved during therapy. However, since the effects of the in vitro and clinical interaction of ampicillin and chloramphenicol combinations remain unresolved, it would seem that chloramphenicol and ampicillin should be used alone whenever possible in the treatment of *H. influenzae* type b meningitis.

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