Chloramphenicol Kills *Haemophilus influenzae* More Rapidly Than Does Ampicillin or Cefamandole

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The bactericidal effects of chloramphenicol and three β-lactams (ampicillin, cefamandole, and penicillin G) were measured for 27 strains of *Haemophilus influenzae* type b isolated from the blood or cerebrospinal fluid of infected infants. Of the ampicillin-susceptible strains, 75% were killed by <2.0 μg of each antibiotic per ml; however, the concentration of the β-lactam agents required for bactericidal activity was higher than that required for inhibitory activity. Chloramphenicol was the only agent which had no marked discrepancy between inhibitory and bactericidal concentrations regardless of β-lactamase production. Importantly, chloramphenicol was more rapidly bactericidal than either ampicillin or cefamandole. The bactericidal requirement of ampicillin was increased by the presence of chloramphenicol for about one-third of the isolates examined. Neither the inhibitory nor the bactericidal activity of chloramphenicol was influenced by ampicillin. Synergy occurred for only two β-lactamase-positive isolates. The more rapid bactericidal action of chloramphenicol persisted even in the presence of ampicillin. The rapid bactericidal action of chloramphenicol with or without ampicillin supports the use of chloramphenicol alone or with ampicillin for *H. influenzae* infections.

Determination of the optimal therapy for *Haemophilus influenzae* meningitis remains a problem. There is confusion in the clinical literature as to the relative efficacy of various regimens (1, 6, 8–10, 13, 14, 19–21). At present, the combination of chloramphenicol and ampicillin is recommended as the initial therapy for suspected *H. influenzae* meningitis (5). Consequently, understanding the combined effects of chloramphenicol plus ampicillin against *H. influenzae* is of importance.

Although many in vitro studies of these two agents have been reported, these findings are also inconsistent. Antagonism between ampicillin and chloramphenicol against *H. influenzae* strains occurred in studies of bactericidal activity reported by McBaye et al. (15) and MacKenzie (11). In contrast, Feldman (7) failed to demonstrate antagonism of inhibitory activity and showed synergism for some strains. Cole et al. (4) observed no mutual antagonism after a short incubation of ampicillin with chloramphenicol; however, in their study, the concentration of both drugs was at or below the minimal inhibitory concentration (MIC). Also working at or below inhibitory concentrations of chloramphenicol, Rocco and Overturf reported that chloramphenicol inhibits the bactericidal effect of ampicillin (18).

The apparently contradictory nature of these studies may have resulted from using different endpoints, i.e., bactericidal (11, 15), inhibitory (7), or reduction in viability at antibiotic concentrations which were less than bactericidal (4, 18). Moreover, the bactericidal rate of these agents alone and in combination has not been systematically evaluated.

To resolve some of these contradictory findings, we examined the gravimetric requirements and the rate of bactericidal action of penicillins (penicillin G and ampicillin), a cephalosporin (cefamandole), chloramphenicol, and chloramphenicol plus ampicillin. We studied 27 *H. influenzae* type b strains from the blood or cerebrospinal fluid of infected infants. Chloramphenicol had a rapid bactericidal activity which was superior to that of either ampicillin or cefamandole. The action of chloramphenicol was independent of β-lactamase production by *H. influenzae*. Although chloramphenicol may interfere with the bactericidal activity of ampicillin, such interference may be inconsequential. In our experiments, the bactericidal action of chloramphenicol and its rapidity were predominant.
MATERIALS AND METHODS

Organisms. H. influenzae type b strains were obtained from the clinical laboratories of the University of Illinois Hospital, Chicago, from the Centers for Disease Control, Atlanta, Ga., and from Carl Norden of the University of Pittsburgh School of Medicine. H. influenzae isolated at the University of Illinois Hospital were typed in the clinical laboratories, using a slide agglutination test with type b-specific antisera (Difco Laboratories, Detroit, Mich.). A reference type b strain, previously typed in the same laboratory, was included for each determination. The other isolates were typed at the Centers for Disease Control or at the University of Pittsburgh. All organisms were isolated from cerebrospinal fluid or blood. All ampicillin-resistant organisms (MIC, ≥12.5 μg/ml) were tested for β-lactamase production by the rapid chromogenic cephalosporin method (22). Each assay contained a known positive and negative control previously determined in our laboratory. The isolated organisms were suspended in skim milk and stored at -70°C for future use.

Inoculum. Mueller-Hinton broth with 1% supplement C (Difco) was used. The lots of supplement C did not produce spurious β-lactam resistance, as may occur (23). The ampicillin-resistant strains all produced β-lactamase (see above). The remaining strains were all ampicillin susceptible (see Table 1). Frozen samples of each organism were thawed, inoculated into the broth, and incubated overnight at 37°C in 5% CO2. The overnight culture dilution was to give a final working concentration of approximately 10^5 to 10^6 CFU/ml for all experiments. The inoculum size was determined by plating serial dilutions onto chocolate agar plates incubated as described above, and colonies were counted.

Antibiotics. Antibiotic laboratory standards of ampicillin (Bristol Laboratories, Syracuse, N.Y.), penicillin G (Bristol Laboratories), and cefamandole (Eli Lilly & Co., Indianapolis, Ind.) were reconstituted in sterile, distilled water to 2 mg/ml, dispensed in aliquots, and stored at -20°C. A nonesterified chloramphenicol laboratory standard (Parke-Davis, Grand Rapids, Mich.) was dissolved in ethanol (0.1 total volume of stock solution), and brought to 2 mg/ml with sterile distilled water, dispensed in aliquots, and stored at -20°C.

Susceptibility Testing. A total of 27 H. influenzae isolates were tested by the tube dilution method with Mueller-Hinton broth containing 1% supplement C. The final volume in each tube was 1 ml, containing approximately 10^5 CFU. The lowest concentration of antibiotic preventing the development of turbidity after 24 h of incubation was defined as the MIC. The minimal bactericidal concentration (MBC) was defined as the concentration of antibiotic resulting in ≤10 CFU/ml after plating in a 0.1-ml portion onto chocolate agar plates.

Antibiotic susceptibility titrations were performed for H. influenzae strains with twofold dilutions of ampicillin and chloramphenicol separately and in combination. Chloramphenicol concentrations were 0.01, 0.02, 0.04, 0.09, 0.18, 0.37, 0.75, 1.5, 3.0, 6.0, and 12.0 μg/ml. Ampicillin-susceptible H. influenzae strains were tested at ampicillin concentrations of 0.01, 0.02, 0.04, 0.09, 0.18, 0.37, 0.75, 1.5, 3.0, 6.0, 12.0, 25, and 50 μg/ml. For β-lactamase-positive strains, ampicillin concentrations were 3.1, 6.2, 12.5, 25, 50, 100, 200, and 400 μg/ml.

A synergistic effect was defined as inhibition or killing when the concentration of each drug in the combination was fourfold less than that of either antibiotic alone (16). The criterion for antagonism of inhibition or killing was that the concentrations of both antibiotics required for the endpoints be increased. Changes in antibiotic requirements occurred in combinations which did not meet the criterion for antagonism because only one of the two agents had an increased requirement for inhibition or killing. To describe these changes, the term "interference" is used. A fourfold increase in the MIC or MBC of ampicillin in the presence of subinhibitory amounts of chloramphenicol was used to identify chloramphenicol interference with ampicillin activity. Conversely, a fourfold increase in the MIC or MBC of chloramphenicol in the presence of ampicillin identified ampicillin interference with the activity of chloramphenicol.

Kinetic studies. Timed killing assays were performed for 14 of the ampicillin-susceptible isolates. Bacterial titers were determined by using a final volume of 10 ml of Mueller-Hinton broth with 1% supplement C containing 10^6 CFU of H. influenzae (10^5 CFU/ml) in the presence of 20 μg of chloramphenicol, 20 μg of ampicillin, 20 μg of chloramphenicol plus 20 μg of ampicillin, or 20 μg of cefamandole per ml. After incubation, 0.1-ml portions were withdrawn at time intervals.

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TABLE 1. Distribution of MICs and MBCs of ampicillin, penicillin, cefamandole, and chloramphenicol for H. influenzae strains

<table>
<thead>
<tr>
<th>Organism (no. of strains)</th>
<th>Agent</th>
<th>MIC (μg/ml)</th>
<th>MBC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50%</td>
<td>75%</td>
</tr>
<tr>
<td>Ampicillin-susceptible</td>
<td>Ampicillin</td>
<td>0.18</td>
<td>0.37</td>
</tr>
<tr>
<td>H. influenzae (21)</td>
<td>Penicillin</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Cefamandole</td>
<td>0.18</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>0.37</td>
<td>0.75</td>
</tr>
<tr>
<td>β-lactamase*</td>
<td>Ampicillin</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>H. influenzae (6)</td>
<td>Penicillin</td>
<td>50</td>
<td>&gt;50</td>
</tr>
<tr>
<td></td>
<td>Cefamandole</td>
<td>0.37</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>0.75</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*50%, 75%, and 90%, MIC required to inhibit 50, 75, and 90% of strains, respectively.

b50%, 75%, and 90%, MBC required to kill 50, 75, and 90% of strains, respectively.
RESULTS

Ampicillin, penicillin, cefamandole, and chloramphenicol MIC and MBC distributions for 27 H. influenzae strains are shown in Table 1. All four antibiotics had similar activities for the 21 H. influenzae strains that were ampicillin susceptible. Although 75% of these strains were killed by <2.0 μg of each antibiotic per ml, a substantial increase (four- to eightfold) in β-lactam antibiotic concentration was required for bactericidal activity as compared with inhibitory activity (e.g., penicillin 90% MIC, 0.75 μg/ml; 90% MBC, 6.2 μg/ml). Chloramphenicol was the only agent tested which had no marked discrepancy between inhibitory and bactericidal concentrations.

Six β-lactamase-positive H. influenzae strains were resistant to penicillin and ampicillin and were inhibited only by very high concentrations not attainable in patients. Both cefamandole and chloramphenicol had inhibitory and bactericidal activity against these β-lactamase-positive isolates.

Chloramphenicol had equal activity against both ampicillin-susceptible and ampicillin-resistant strains. In contrast to the other agents, chloramphenicol had a similar concentration requirement for inhibitory and bactericidal activity; this requirement was present regardless of whether the strain produced β-lactamase.

Synergy between chloramphenicol and ampicillin (for either inhibitory or bactericidal activity) was not observed for ampicillin-susceptible strains. Only two isolates, both of which produced β-lactamase, exhibited synergy when an inhibitory endpoint was used. Even in the presence of chloramphenicol, the ampicillin requirement of these two β-lactamase-producing strains was 12.5 and 25 μg/ml. Ampicillin-chloramphenicol synergy occurred only when an inhibitory endpoint was used; bactericidal activity was not improved in any instance.

No mutual antagonism was observed with any of the strains; we therefore examined the independent activity of each antibiotic in the combination to determine whether one agent produced interference with the other (i.e., unidirectional interference). The bactericidal requirement of ampicillin was increased by the presence of chloramphenicol for about one-third of the isolates examined and occurred in both β-lactamase-negative and -positive strains (Table 2). The inhibitory activity of ampicillin was not altered by chloramphenicol. The ampicillin-chloramphenicol interference was not mutual. The activity of chloramphenicol was not influenced by ampicillin regardless of whether inhibitory or bactericidal endpoints were examined.

Chloramphenicol was rapidly bactericidal for H. influenzae, with a reduction in viable bacteria of more than 4 log10 after 8 h of incubation (Table 3). In contrast, ampicillin was slower in its bactericidal action, with a reduction of less than 3 log10 after 8 h. Cefamandole kinetics of bactericidal activity resembled those of ampicillin. After 8 h of incubation, more than 2 log10 CFU/ml remained viable with either ampicillin or cefamandole. In contrast, 8-h cultures containing chloramphenicol alone or in combination with ampicillin had less than 1 log10 CFU/ml remaining viable. Although all antibiotics tested resulted in marked reduction of viability by 24 h, the rate was most rapid after incubation with chloramphenicol alone or in combination. For H. influenzae, the bactericidal rate of the chloramphenicol-ampicillin combination was as rapid as that of chloramphenicol alone.

We examined individually the kinetics of bactericidal activity for each of the strains averaged in Table 3. For every strain, chloramphenicol was more rapidly bactericidal than ampicillin. Chloramphenicol was also more rapidly bactericidal than cefamandole for six of the eight strains tested. The chloramphenicol-ampicillin combination was more rapidly bactericidal than ampicillin alone for each strain examined. Ampicillin did not retard the rapid bactericidal action of chloramphenicol, with the exception of one strain. This isolate required an increase in killing time when ampicillin was added to chloramphenicol. Only the presence of chloramphenicol resulted in total sterilization of all strains in 24 h.

DISCUSSION

By examination of several aspects of the activity of chloramphenicol and β-lactams, their
behavior in combination can be better understood, and apparent contradictions present in the literature can be explained (4, 7, 11, 15). Chloramphenicol has bactericidal activity against *H. influenzae* (2, 17). Against ampicillin-susceptible strains of *H. influenzae*, the bactericidal requirements of all four agents were similar (Table 1 and reference 2). β-Lactamase-positive and -negative isolates were equally susceptible to chloramphenicol.

The relative rates of bactericidal activity for ampicillin and chloramphenicol against *H. influenzae* have not been closely examined previously. Chloramphenicol was more rapidly bactericidal than ampicillin against *H. influenzae* (Table 3). Chloramphenicol alone or in combination with ampicillin produced a significantly greater reduction of bacterial viability after 6 and 8 h as compared with ampicillin or cefamandole alone (Table 3). Additionally, only the addition of chloramphenicol reliably sterilized all cultures.

The presence of ampicillin did not impede the rapid bactericidal effects of chloramphenicol (Table 3). The ampicillin-chloramphenicol combination was as effective as the more effective single agent, chloramphenicol. Ampicillin-chloramphenicol synergy was infrequent and occurred only with β-lactamase-positive strains. However, even in these few instances, the ampicillin requirement (12.5 to 25 µg/ml) remained too great for it to be useful clinically for treatment of infections due to β-lactamase-positive isolates.

The interference between chloramphenicol and ampicillin was unidirectional and influenced bactericidal activity of ampicillin only (Table 2). Chloramphenicol increased the ampicillin MBC requirement by at least fourfold for 8 of 26 strains tested. Since ampicillin did not interfere with chloramphenicol activity, no mutual antagonism between chloramphenicol and ampicillin was observed. These findings are consistent with those recently reported by Rocco and Overturf (18).

Some earlier observers of this phenomenon of unilateral interference described it as antagonism (11, 15). Other workers who refuted the existence of antagonism required that mutual interference between the two agents be exhibited (4, 7). Thus, the apparent conflict in the earlier literature about ampicillin-chloramphenicol antagonism depends on whether mutual interference was required. In short, chloramphenicol interfered with ampicillin, but ampicillin did not interfere with chloramphenicol.

When chloramphenicol and ampicillin were used together, chloramphenicol increased the ampicillin MBC requirement for certain *H. influenzae* strains. However, the more rapid bactericidal action of chloramphenicol remained dominant even with these strains. The more rapid action of chloramphenicol should override a possible adverse effect on the ampicillin MBC requirement.

The previous failure to demonstrate an adverse interaction of chloramphenicol with ampicillin occurred under experimental conditions in which bactericidal activity was not measured (7). An additional difficulty in the Feldman study (7) may have been the use of microtiter plates for incubations; in our study, microbroth dilution tests of *H. influenzae* did not give results consistent with those from macrobroth dilution tests (data not shown).

In other experiments (4), attempts were made to measure bactericidal activity with antibiotic concentrations at or below the MIC; evaluations were performed very early in the incubation period, when a reduction of less than 2 log₁₀ in live bacteria had taken place. In our experiments, using antibiotic concentrations that were bactericidal, differences in ampicillin and chloramphenicol activity were evident after 6 h (Table 3). The failure of some interactions to meet strict criteria for mutual antagonism may also explain why interference was not reported until recently. The lack of ampicillin effect on chloramphenicol may relate to the more rapid action of chloramphenicol.

### Table 3. Timed Killing of *H. influenzae* strains by ampicillin, chloramphenicol, cefamandole, or ampicillin plus chloramphenicol

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of strains</th>
<th>Mean ± SD log₁₀ CFU/ml at incubation time (h) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth</td>
<td>14</td>
<td>5.2 ± 0.8 5.7 ± 1.0 6.6 ± 0.8 8.1 ± 1.0 8.7 ± 1.2 8.2 ± 2.7</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>8</td>
<td>5.2 ± 0.8 4.6 ± 1.2 4.0 ± 1.0 3.0 ± 1.4 3.9 ± 1.3 2.3 ± 1.1 0.4 ± 0.7</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>14</td>
<td>5.2 ± 0.8 4.7 ± 1.0 3.8 ± 0.7 3.2 ± 1.0 3.9 ± 1.0 2.4 ± 1.2 0.6 ± 1.3</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>14</td>
<td>5.2 ± 0.8 4.0 ± 1.0 3.2 ± 1.3 1.7 ± 1.6 0.4 ± 0.9 No growth</td>
</tr>
<tr>
<td>Ampicillin + chloramphenicol</td>
<td>14</td>
<td>5.2 ± 0.8 4.2 ± 1.4 2.5 ± 1.5 1.2 ± 1.5 0.5 ± 1.1 No growth</td>
</tr>
</tbody>
</table>

*All antibiotics were used at a concentration of 20 µg/ml.

Using Schefé's multiple comparisons (3), *p* < 0.05 for ampicillin versus ampicillin plus chloramphenicol at 6 and 8 h, ampicillin versus chloramphenicol at 8 h, and cefamandole versus chloramphenicol at 8 h.*
The concentration of 20 μg/ml that we used exceeds the MBC for ampicillin-susceptible strains. This concentration was used to ensure that the rate comparisons were made only under bactericidal conditions. These concentrations are readily achievable in serum. *H. influenzae* meningitis, as well as other *H. influenzae* infections, are often bacteremic. The more rapid bactericidal effect of chloramphenicol may be an important determinant of its efficacy against *H. influenzae* infection when it is used alone or in various combinations.

Future studies of chloramphenicol–β-lactam combinations would be helpful in evaluating the biological relevance of our findings. These might include studies in vitro with higher inocula of organisms because of the inoculum-dependent behavior of some β-lactams against *H. influenzae* (24), and studies in animals infected with *H. influenzae*.

Although we demonstrated acceptable bactericidal activity with cefamandole, this agent cannot be used for patients with proved or suspected *H. influenzae* bacteremia. Several bacteremic patients have developed *H. influenzae* meningitis while receiving cefamandole (12).

No definite conclusions are possible from the clinical studies of various treatment regimens (1, 6, 8–10, 13, 14, 19–21) because of the small numbers of patients studied prospectively with each regimen. Since most of the antibiotic regimens tested are effective for most *H. influenzae* strains, an advantage of one treatment could be detected only when large numbers of patients are studied. Larger, more definitive clinical trials are needed. Our demonstration of the rapid bactericidal action of chloramphenicol in the presence or absence of ampicillin supports the use of chloramphenicol alone or in combination with ampicillin for *H. influenzae* infections.

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**LITERATURE CITED**


