Effect of Growth Phase on the Bactericidal Action of Chloramphenicol Against Haemophilus influenzae Type b and Escherichia coli K-1

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The bactericidal action of chloramphenicol against six Haemophilus influenzae type b isolates and six Escherichia coli K-1 isolates was compared. Cells were grown in antibiotic-free medium into the late-stationary and mid-exponential phases of growth, and inocula of 10^7 to 10^8 cells per ml were added to fresh media containing 1 or 10 μg of chloramphenicol per ml for H. influenzae isolates, 80 μg of chloramphenicol per ml for E. coli isolates, or no chloramphenicol (antibiotic free). Quantitative kinetic studies indicated that each chloramphenicol concentration killed H. influenzae cells in the stationary phase of growth significantly more rapidly than it did those in the exponential phase of growth (P < 0.001; analysis of variance). E. coli in either the stationary or the exponential phase were killed at the same rate by 80 μg of chloramphenicol per ml (P > 0.05). These results suggest that chloramphenicol may kill these organisms by different mechanisms.

Chloramphenicol has assumed an increasingly important role in the treatment of bacterial meningitis. It is currently the drug of choice for the treatment of meningitis caused by ampicillin-resistant Haemophilus influenzae type b isolates and for meningitis due to Streptococcus pneumoniae or Neisseria meningitidis in penicillin-allergic patients (10). It has been suggested as therapy for neonates with Escherichia coli meningitis (1, 2, 4), although clinical experience is limited.

Despite the wide clinical usage, little is known about the activity of chloramphenicol against these pathogens in vitro. Rahal and Simberkoff (10) and Turk (12) reported that chloramphenicol is bactericidal for H. influenzae isolates when concentrations above the minimal bactericidal concentration (MBC) are used. Cole et al. (3) reported significant reductions in the number of organisms of 14 H. influenzae type b isolates when the minimal inhibitory concentration (MIC) of chloramphenicol is used. Klastersky and Husson (8) observed that 30 μg of chloramphenicol per ml did not kill eight clinical isolates of E. coli. Rahal and Simberkoff (10) did not perform quantitative kinetic studies with E. coli but did report that the MBCs of chloramphenicol for E. coli isolates were markedly higher than the MIC, suggesting that chloramphenicol is bacteriostatic rather than bactericidal.

The previous studies of the interaction of chloramphenicol with H. influenzae type b and E. coli did not take into account the growth phase, nor did they include quantitative kinetic comparisons of various H. influenzae and E. coli isolates. In the present study, we compared the effect of growth phase on the bactericidal activity of chloramphenicol against meningeal isolates of H. influenzae type b and E. coli K-1.


MATERIALS AND METHODS

Isolates. Six H. influenzae type b strains and six E. coli K-1 strains were isolated from cerebrospinal fluid of infants and neonates, respectively. Isolates were identified by standard techniques (9). Serogrouping was done by a latex agglutination method (11) for H. influenzae and counterimmunoelectrophoresis (6) for E. coli or centrifuged culture supernatants after overnight growth in Mueller-Hinton broth (MHB) with or without 3% supplement C (Difco Laboratories) at 37°C and pH 7.2.

Chloramphenicol. Ten milligrams of chloramphenicol standard powder (1,000 μg/ml) was dissolved in 0.2 ml of sterile ethanol, and 9.8 ml of MHB was added to make a stock solution of 1,000 μg/ml. Serial dilutions of the stock solution were made in MHB with or without supplement C (3%; Difco) to achieve the final concentrations of chloramphenicol for the MBCs, MBCs, and kill curves.

Quantitation of bacteria. Quantitation of bacteria was done by evenly spreading 0.1-ml samples from serial 10-fold dilutions made in MHB over the surface of Mueller-Hinton and chocolate agar plates for E. coli and H. influenzae, respectively. All colony counts
were done in duplicate. Plates containing 20 to 300 isolated colonies were counted and multiplied by the dilution factor to determine the original concentration of bacteria.

**MICs and MBCs.** Inocula of approximately $10^5$ organisms were added to tubes containing 1 ml of MHB (with 3% supplement C for *H. influenzae* isolates). Final concentrations of chloramphenicol were serial twofold dilutions from 80 to 0.02 μg/ml. The MIC and MBC were defined as the smallest concentrations resulting in inhibition of visible growth and in lack of growth, respectively, after plating 0.01 ml (>99.9% kill) after incubation at 37°C for 18 h.

**Kill curves.** The stationary-phase inoculum was prepared by inoculating three isolated colonies into 20 ml of MHB (pH 7.2) with or without 3% supplement C and incubating for 16 h at 37°C. After 16 h, there were $\geq 10^6$ organisms per ml. A 100-fold dilution of the 16-h growth was made in fresh, warm MHB, and 2 ml of the dilution was added to tubes containing 18 ml of fresh MHB with final concentrations of 1 or 10 μg of chloramphenicol per ml for *H. influenzae* isolates, 80 μg of chloramphenicol per ml for *E. coli* isolates, or no chloramphenicol (antibiotic free). Cells in the exponential phase of growth were prepared from a 4-h culture in antibiotic-free MHB diluted 10-fold. Two milliliters of the dilution was added to tubes containing 18 ml of MHB with or without chloramphenicol. Each organism was tested on at least two different occasions with similar results.

**Statistical analysis.** Log conversions of bacterial concentrations were used to compare the data. Correlations were interpreted by standard methods (7).

Results are expressed as the means of all experiments, although data were analyzed by the technique of analysis of variance, in which one variable is compared while others are held constant.

**RESULTS**

For *H. influenzae* isolates, the median MIC and MBC were 0.625 μg/ml (range, 0.312 to 1.25 μg/ml) and 1.25 μg/ml (range, 0.312 to 2.5 μg/ml), respectively. For *E. coli* isolates, the median MIC and MBC were 2.5 μg/ml (range, 1.25 to 5.0 μg/ml) and 40 μg/ml (range, 10 to 80 μg/ml), respectively.

The initial inoculum of *H. influenzae* cells in the stationary phase of growth was approximately $10^5$ CFU/ml (Fig. 1). After a lag phase of approximately 1 h during which there was no evidence of multiplication in antibiotic-free medium, there was an increase in CFU, with counts of $>10^7$ CFU/ml reached by 5 h. With the same inoculum size of exponential-phase cells, there were $>10^7$ CFU/ml in chloramphenicol-free MHB by 3 h (Fig. 1B). Both 1 and 10 μg of chloramphenicol per ml killed stationary-phase cells significantly more rapidly than exponential-phase cells ($P < 0.0001$; analysis of variance).

With an inoculum of approximately $10^6$ CFU/ml, stationary-phase *E. coli* cells reached a colony count of $>10^8$ CFU/ml of antibiotic-free medium at 5 h (Fig. 2). Chloramphenicol signifi-

![Figure 1](http://aac.asm.org/)  
**FIG. 1.** Effect of chloramphenicol on *H. influenzae* cells in the stationary (A) or the exponential (B) phase of growth. Results are the means of six isolates and indicate mean concentrations ± 1 standard deviation. N.G., No growth. ○, No chloramphenicol; □, 1 μg of chloramphenicol per ml; Δ, 10 μg of chloramphenicol per ml.
cantly decreased populations in both the exponential and stationary growth phases, but the rates of decrease in cell densities were the same ($P > 0.05$; analysis of variance).

**DISCUSSION**

Our results indicate that chloramphenicol kills stationary-phase *H. influenzae* cells significantly faster than exponential-phase cells. It is well known that patients with *H. influenzae* meningitis usually have bacterial concentrations of $>10^7$ CFU/ml (5, 6). Such large populations suggest that organisms have grown for some time and probably are in the in vivo equivalent of the stationary phase of growth observed in vitro. Thus, the data from this study may provide a basis for the well-documented efficacy of chloramphenicol for *H. influenzae* meningitis.

Data from this study also confirm the previous impression (10) that smaller concentrations of chloramphenicol are more bactericidal for *H. influenzae* than for *E. coli*. Thus, these results do not support the use of chloramphenicol in neonates with *E. coli* meningitis, in whom rapid killing of the pathogen is thought to be essential for cure.

Chloramphenicol is thought to inhibit translation of protein on *E. coli* ribosomes by its interaction with the 50S ribosomal subunits (13). Results from this study suggest that chloramphenicol kills pathogenic *H. influenzae* isolates differently than it does those of *E. coli*. Further studies are necessary to correlate these in vivo differences with in vitro cell-free systems to determine the mechanism(s) of cell death in these organisms.

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**LITERATURE CITED**

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