Effect of Ampicillin and Chloramphenicol Against Streptococcus pneumoniae and Neisseria meningitidis

WILLIAM E. FELDMAN* AND TERESA ZWEIGHAFT

Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30303

Received for publication 30 October 1978

Antagonism, determined by isobolograms constructed from data from combinations of ampicillin and chloramphenicol at or below the minimal inhibitory or bactericidal concentrations, was observed against 13 clinical isolates of meningococcus and against one isolate of pneumococcus. Synergy occurred against six strains of pneumococcus and three of meningococcus. Additive effects were noted against 14 isolates of pneumococcus and 5 of meningococcus. There was no relationship between the minimal inhibitory or bactericidal concentrations for the isolates and the occurrence of antagonistic, additive, or synergistic effects. These data indicate that ampicillin and chloramphenicol may be antagonistic in vitro against some strains of pneumococcus or meningococcus.

The combination of ampicillin or penicillin with chloramphenicol has been recommended as initial antibiotic therapy for patients with suspected bacterial meningitis because of the emergence of ampicillin-resistant Haemophilus influenzae type b strains (2). This recommendation was supported by data from a recent in vitro study (6) which showed that ampicillin and chloramphenicol were not antagonistic against H. influenzae type b. However, limited information (1, 7–9) is available about the interaction of these antibiotics against Streptococcus pneumoniae (pneumococcus) or Neisseria meningitidis (meningococcus), the other two common causes of childhood meningitis.

The present study was done to examine the in vitro effect of ampicillin and chloramphenicol against isolates of S. pneumoniae and N. meningitidis.

MATERIALS AND METHODS

Bacterial isolates. A total of 21 strains of S. pneumoniae and 21 strains of N. meningitidis were isolated from patients or were obtained from Clyde Thornberry (Center for Disease Control). The pneumococci were isolated from either blood (12 strains) or cerebrospinal fluid (9 strains). Capsular typing was not done on the pneumococcal strains. The meningococci were isolated from cerebrospinal fluid (13 strains), blood (2 strains), throat culture (2 strains), brain, spleen, or eye culture (1 strain each), and an unknown source (1 strain). Of the meningococci 13 strains were group B and 8 were group C.

Media. Studies were done by using Mueller-Hinton broth to grow the meningococci and Todd-Hewitt broth supplemented with 5% human serum to grow the pneumococci. Bacterial colony counts of the meningococci or pneumococci were determined by plating 0.1-ml samples from serial 10-fold dilutions of broth cultures onto the surface of chocolate agar plates containing 1% IsoVitaleX (Baltimore Biological Laboratory) or plates containing 5% sheep blood, respectively (5).

Studies of antimicrobial interaction. The 42 strains were tested in duplicate for their susceptibility to ampicillin and chloramphenicol, separately and in combination, by using a microtiter apparatus (Cooke Engineering Co., Alexandria, Va.).

Tests were done in microtiter plates by using a modification of a previously described procedure (6). Final concentrations of ampicillin increased in 0.01-µg/ml increments from 0.01 to 0.15 µg/ml. Additional concentrations of 0.005, 0.0025, and 0.00125 µg/ml were used for each strain. Final concentrations of chloramphenicol increased in 0.1-µg/ml increments from 0.1 to 1.6 µg/ml for the meningococci and by 0.2-µg/ml increments from 0.2 to 6 µg/ml for the pneumococci.

The inocula of meningococci were prepared by diluting an 18-h broth culture 1,000-fold and adding approximately 5 × 10⁶ colony-forming units to each well of the microtiter plate. Overnight cultures of the pneumococcal strains sometimes underwent autoysis. To avoid this problem, a fresh broth was inoculated from the overnight culture and incubated for 6 h at 37°C. This broth was diluted to provide an inoculum of approximately 10⁴ colony-forming units per well.

Plates were incubated at 37°C for 18 h. The minimal inhibitory concentration (MIC) was the smallest concentration of antibiotic(s) which prevented a visible button of growth at the bottom of the well (6). Minimal bactericidal concentrations were determined by subculture of wells with no visible growth on chocolate or blood agar.

Results were interpreted by using two methods. First, the fractional inhibitory concentration (FIC) described by Elion et al. (4) was calculated. The FIC is the ratio of the MIC of a drug in combination compared with that of the drug alone. The FIC index is the sum of the FIC values of the two antibiotics
when used in the best concentration. Second, isobolograms were constructed from the MICs and minimal bactericidal concentrations as described by Eickhoff (3). Synergy was considered to be present if there was a concave isobologram compared with the line connecting the drugs separately and the FIC index was <0.8. If the line connecting combinations was parallel to that connecting points of drugs plotted separately and the FIC index was ≥0.8 but <1.2, the combined effect was additive; if the line connecting combined drugs was convex and the FIC index was ≥1.2, the combined drugs were interpreted as antagonistic.

RESULTS

All isolates were susceptible to both ampicillin and chloramphenicol (Tables 1 and 2).

By using isobolograms and FIC indexes derived from the MICs, synergy between ampicillin and chloramphenicol was shown to occur against three isolates of meningococcus (Table 1). Additive effects were observed against 5 strains, and antagonism occurred against 13 isolates. Of the strains showing antagonism, six were group B and seven were group C.

For the pneumococci, synergy was observed against 6 isolates, additive effects were observed against 14, and antagonism occurred against only 1 strain (Table 2).

Similar results were observed when data were interpreted by using isobolograms and FIC indexes derived from the minimal bactericidal concentrations.

DISCUSSION

Previous in vitro and animal studies indicated that antagonism between penicillin and chloramphenicol may occur against pneumococci (1, 7, 8). These studies examined only a few strains, and thus the frequency of antagonism was not clear. Results from this study indicate that antagonism of penicillin by chloramphenicol is infrequent and that most often the antibiotics have an additive effect.

To our knowledge, only one report has examined the in vitro effect of penicillin and chloramphenicol against a strain of meningococcus (9). Small concentrations of chloramphenicol appeared to slow the rate of killing by penicillin, but larger concentrations of chloramphenicol did not affect it. Our data indicate that antagonism between ampicillin and chloramphenicol occurs frequently against isolates of N. meningitidis. The mechanism(s) of the antagonism is(are) unknown at this time.

Further work is necessary to determine whether the in vitro antagonism observed in this study may happen in vivo in patients with meningococcal or pneumococcal meningitis. The recommendation to use ampicillin and chloramphenicol as initial therapy for suspected bacterial meningitis may need to be reevaluated if in vivo antagonism is demonstrated.

ACKNOWLEDGMENTS

This study was supported by a grant from Parke, Davis & Co.
LITERATURE CITED


