

Effect of Clindamycin on the In Vitro Activity of Amikacin and Gentamicin Against Gram-Negative Bacilli

STEPHEN H. ZINNER,* RICHARD B. PROVONCHEE, KATHY S. ELIAS, AND GEORGES PETER
Division of Infectious Diseases, Department of Medicine, Roger Williams General Hospital, and Sections of Medicine and Human Growth and Development, Brown University, Providence, Rhode Island 02908*

Received for publication 24 November 1975

The in vitro effect of clindamycin on the inhibitory and bactericidal activity of amikacin (BB-K8) and gentamicin against *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* was examined by the checkerboard technique in microtiter plates. Clindamycin (15 $\mu\text{g/ml}$) produced statistically significant increases in the minimal bactericidal concentrations of amikacin and gentamicin against *E. coli* and *Klebsiellae* at 2 and 4 h of incubation. The minimal bactericidal concentration against *P. aeruginosa* was not affected. Higher concentrations of clindamycin (20 to 25 $\mu\text{g/ml}$) reduced the minimal inhibitory and bactericidal concentrations of amikacin and gentamicin for *E. coli* at 18 h of incubation. The synergistic bactericidal activity of amikacin and carbenicillin against *E. coli*, but not *P. aeruginosa*, was also inhibited slightly by clindamycin (15 $\mu\text{g/ml}$). The clinical implications of this inhibition of the early bactericidal in vitro activity of aminoglycosides by clindamycin remain to be determined. Although these in vitro results have not been studied in clinical infections, it is conceivable that slight interference in early bacterial killing could alter the outcome of infection in the immunosuppressed patient.

The increasing use of multiple antibiotics in hospitalized patients with serious sepsis has prompted consideration of possible synergy or antagonism of the antimicrobial activity of specific antibiotics. In many hospitals, clindamycin is added frequently to regimens of an aminoglycoside and a penicillin or cephalosporin in patients with suspected sepsis. This study examines the effect of clindamycin on the in vitro activity of two aminoglycoside antibiotics, gentamicin and amikacin (BB-K8), alone or in combination with carbenicillin, against strains of *Escherichia coli*, *Klebsiella*, and *Pseudomonas* isolated from hospitalized patients.

(This paper was presented in part at the 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, 12 September 1974, San Francisco, Calif.)

MATERIALS AND METHODS

Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) at 2, 4, and 18 h of incubation were determined for amikacin (generously supplied by Karl Agre, Bristol Laboratories, Syracuse, N.Y.) and gentamicin in the presence of increasing concentrations of clindamycin (1 to 25 $\mu\text{g/ml}$) (generously supplied by William Gouse, Upjohn Co., Kalamazoo, Mich.). Twenty strains of *E. coli*, 38 strains of *K. pneumoniae*, and 20 strains of *P. aeruginosa* isolated from patient specimens were studied. In a second set of experi-

ments, the effect of 15 μg of clindamycin per ml on amikacin and carbenicillin in combination was examined at 2, 4, and 18 h of incubation with 9 strains of *E. coli*, 19 strains of *K. pneumoniae*, and 18 strains of *P. aeruginosa*. All strains were resistant to clindamycin.

For this study, a modification of the checkerboard technique in microtiter plates was used (4). Organisms previously stored on corked agar slants were grown overnight in Mueller-Hinton broth and then were diluted 1:100 for use in the microtiter wells. The final concentration of antibiotics also was diluted in Mueller-Hinton broth. The final volume in the microtiter wells was 200 μl , consisting of 25 μl of the appropriate dilutions of each antibiotic, 125 μl of diluent broth, and 25 μl of the bacterial suspension. When three antibiotics were studied in combination, the checkerboard technique was used for the aminoglycoside and carbenicillin concentrations. Clindamycin, to provide a concentration of 15 $\mu\text{g/ml}$, was then added to each well. To keep all final volumes identical when an antibiotic was studied alone, an additional 25 μl of diluent broth was added to these wells. A sterile, 4-mm magnetic stirring rod was placed in each well prior to the addition of the appropriate dilutions. Placement of the microtiter plate on a magnetic stirrer results in rapid and simultaneous spinning of the rods and vigorous mixing of the well contents.

The microtiter plate with antibiotic-bacterial mixtures was incubated at 37 C and was withdrawn for sampling at 2, 4, and 18 h. The wells were mixed and sampled in duplicate with a replicator-type in-

oculator, specially constructed to fit the microtiter wells. At each sampling time, duplicate, antibiotic-free Trypticase soy agar plates were inoculated with approximately 2 μ l from each well. These agar plates were subsequently incubated at 37 C for 18 h (Fig. 1) to determine the MBC. (A 99.9% killing was represented by two or fewer colonies at the inoculation site, based on the calculated number of organisms inoculated in each well.) Direct inspection of the microtiter wells for turbidity after 18 h of incubation was used to determine the MIC.

The reproducibility of this microtiter method was studied as follows. The MIC (after 18 h of incubation) and MBC (at 4 and 18 h of incubation) were determined for the same antibiotic-bacterial combination in 10 replicate samples. The synergistic combination (amikacin and carbenicillin), as well as the antagonistic combination (amikacin and clindamycin), was studied in this fashion. The MIC, 4-h MBC, and 18-h MBC determinations agreed within one dilution for each of the 10 replications. The reproducibility of this method compares favorably with the tube dilution-pour plate technique. The definitions of synergy and antagonism for the purpose of this paper are those of Sabath (4).

The rate of the antibiotic killing by amikacin

alone and in the presence of clindamycin (15 μ g/ml) was determined with a spot plate method, which compares favorably with the standard pour plate technique and allows multiple organism-antibiotic combinations to be studied simultaneously. In brief, quantitative samples (5 μ l) of the antibiotic-bacterial mixture are removed from the incubator at timed intervals and spot plated on an antibiotic-free agar plate. To prevent spreading of the colonies, an additional drop of agar is placed over the spot inoculum. This plate is incubated for 4 h and the microcolonies are counted with the aid of a microfiche viewer. The number of organisms present in the mixture is determined by multiplication by the dilution factor. Analysis of variance for this method and the standard pour plate method for bacterial killing curves showed that the two methods are equally variable (Zinner et al., Proc. 9th Int. Congr. Chemother., London, England, in press).

RESULTS

The 18-h inhibitory and the early and late bactericidal activities of increasing concentrations of amikacin and of clindamycin are plotted as isobolograms in Fig. 2. Twenty strains of

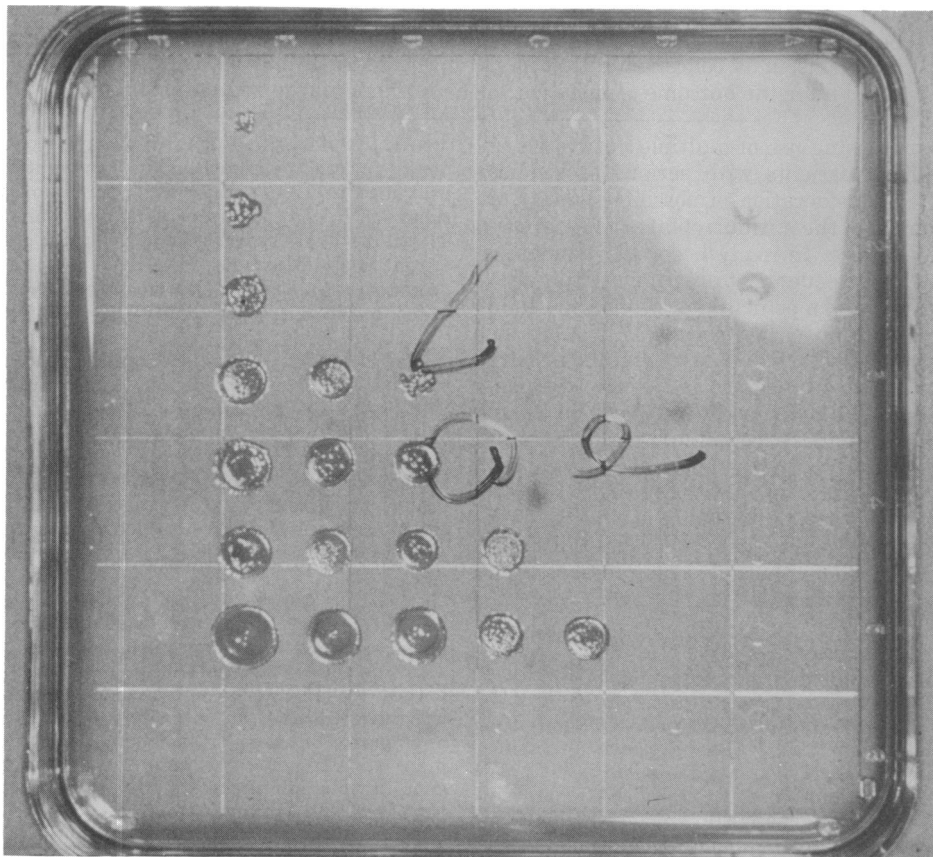


FIG. 1. Inoculated agar plate after 18 h of incubation at 37 C. The MBC of the antibiotic combinations are determined by visual inspection of bacterial growth (see text).

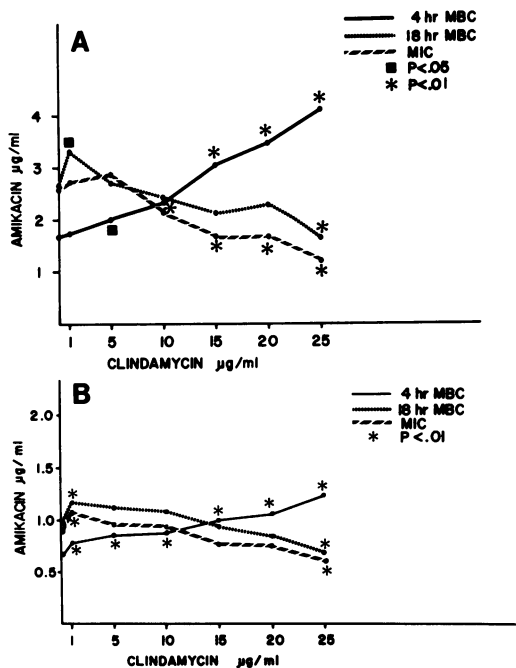


FIG. 2. Isobolograms representing the MBC at 4 and 18 h of incubation and the MIC at 18 h of incubation for amikacin and clindamycin against (A) 20 strains of *E. coli* and (B) 38 strains of *K. pneumoniae*. Symbols describing *P* values obtained with the paired-*t* test refer to significant differences between results obtained with and without the presence of clindamycin. Results obtained without clindamycin are plotted on the ordinate.

E. coli and 38 strains of *K. pneumoniae* were studied. Each point plotted represents the geometric mean concentration of each antibiotic in the combination required to produce a given biological effect (i.e., inhibition or killing after 4 or 18 h). The isobol representing bactericidal activity at 4 h of incubation demonstrates the antagonistic effect of clindamycin on the activity of the aminoglycoside against *E. coli* and *Klebsiellae*, respectively. Although the strains tested were extremely sensitive to amikacin, the inhibitory effect is seen at concentrations of clindamycin equal to or greater than 5 µg/ml. This effect is most pronounced at higher concentrations. However, after 18 h of incubation, no in vitro antagonism is seen and, in fact, very high concentrations of clindamycin (20 to 25 µg/ml) decrease both the MIC and MBC for these organisms. Similar results were obtained with clindamycin and gentamicin for *E. coli* but not for *Klebsiellae*. Clindamycin had no inhibiting effect on the killing of 20 strains of *P. aeruginosa* by either amikacin or gentamicin. The synergistic bactericidal effect of amika-

cin and carbenicillin against 9 strains of *E. coli* at 4 h was significantly inhibited by clindamycin at 15 µg/ml (Fig. 3). This inhibition was not seen at 18 h of incubation (not shown). The synergistic bactericidal and inhibitory activity of amikacin and carbenicillin for *P. aeruginosa* was not affected by this concentration of clindamycin at any incubation time.

As presented in Fig. 4, clindamycin (15 µg/ml) significantly slowed the early killing of 10 strains each of *E. coli* and *Klebsiellae*. No effect was found on the rate of killing of 10 strains of *Pseudomonas* (not shown). A slight increase in viable count at 0.5 h was seen in the *E. coli* treated with amikacin alone but not with amikacin plus clindamycin.

DISCUSSION

These data suggest that clindamycin over a wide range of clinically achievable concentrations may interfere with the early in vitro killing of *E. coli* and *Klebsiellae* by the aminoglycoside antibiotics amikacin and gentamicin. Clindamycin may reach 15 to 20 µg/ml in the serum of patients treated with intravenous clindamycin phosphate (5). At concentrations of 20 to 25 µg/ml, however, clindamycin has a slight enhancing effect on the activity of these agents at later periods of incubation. Clindamycin did not interfere with the activity of either aminoglycoside against *Pseudomonas* at any phase of the incubation period. Clindamycin at 15 µg/ml slowed slightly the rate of bacterial killing by amikacin against strains of *E. coli* and *Klebsiellae* but not against *P. aeruginosa*. Similar data have been presented by Steigbigel et al. (N. H. Steigbigel, J. I. Casey, and M. Wexler, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 286, 1974), who showed a decrease in early killing rates for *Staphylo-*

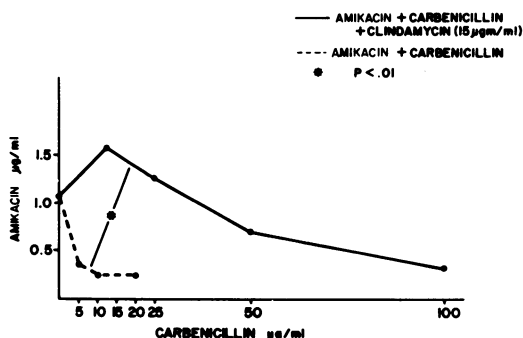


FIG. 3. Inhibition of bactericidal synergy of amikacin and carbenicillin by clindamycin (15 µg/ml) against nine strains of *E. coli* at 4 h of incubation.

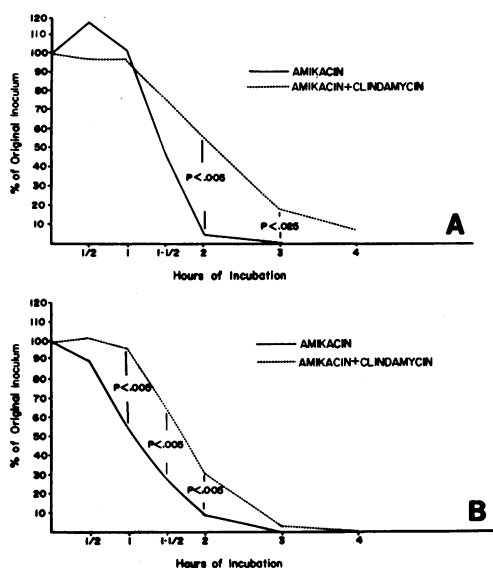


FIG. 4. Rates of bacterial killing by amikacin (2 $\mu\text{g/ml}$) with and without clindamycin (15 $\mu\text{g/ml}$) against (A) 10 strains of *E. coli* and (B) 10 strains of *K. pneumoniae*.

coccus aureus with clindamycin plus gentamicin when compared with gentamicin alone. Similar results were obtained with *E. coli*. Riff and Matulionis (Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 291, 1974) also have observed that clindamycin inhibited the early bacterial killing of several species of gram-negative and gram-positive bacteria by gentamicin.

Sabath and Toftegaard (6) were unable to demonstrate either synergy or antagonism of clindamycin and gentamicin against a variety of gram-negative and gram-positive organisms. Similarly, Fass et al. (1) did not find antagonism of gentamicin by clindamycin against a large number of organisms. Both of these studies tested only inhibitory activity at 18 h. Leng et al. (3) demonstrated synergy for clindamycin and gentamicin against *E. coli*, some strains of *E. aerogenes*, *P. mirabilis*, and one strain each of *Klebsiellae* and *Serratia marcescens*. These results were based on MIC determinations after overnight incubation. In addition, increased bactericidal activity against *E. coli* by this combination was demonstrated with kinetic studies only after 6 h of incubation with a very low inoculum. The present study demonstrates the

need to examine early bactericidal activity, as well as inhibitory activity, in studying the effect of antibiotic combinations.

The mechanism of this inhibition is not known. Both aminoglycosides and clindamycin inhibit protein synthesis but at different ribosomal sites (7). Clindamycin is thought to act at the 50S subunit site but might conceivably bind reversibly to the 30S subunit and interfere with early aminoglycoside killing. Alternatively, clindamycin may reversibly interfere with an effect of the aminoglycosides on the cell wall. Iida and Koike (2) have shown recently that chloramphenicol, tetracycline, and erythromycin blocked the formation in *E. coli* of cell wall blebs and the associated killing caused by several aminoglycosides.

The clinical significance of these in vitro findings remains to be determined. Although the in vitro effects presented with clinically achievable levels of clindamycin have not been studied in animal or clinical infection, the slight interference with early bacterial killing might alter the outcome of infection in the immunosuppressed host. Further studies of gram-negative bacterial infection in animal models may help to determine the clinical significance of these in vitro results.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant CA 13943 from the National Cancer Institute.

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