In Vitro Synergy of Clindamycin and Aminoglycosides against Chlamydia trachomatis

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The importance of Chlamydia trachomatis as an etiologic agent in the development of pelvic inflammatory disease (PID) is well documented. Although there are numerous antimicrobial agents that are effective against C. trachomatis, one of the most frequent combinations that is used to treat PID is clindamycin and gentamicin. The efficacy of clindamycin as the sole treatment for chlamydial infections has been questioned. In fact, the Centers for Disease Control (Atlanta, Ga.) has recommended the use of doxycycline following clindamycin and gentamicin treatment of PID confirmed or suspected to be caused by C. trachomatis. This study was designed to determine whether there is any synergistic in vitro activity between clindamycin and gentamicin or tobramycin on inhibition of C. trachomatis replication. In this experiment, the MIC of clindamycin decreased two- to threefold when an aminoglycoside was added. This occurred even though aminoglycosides by themselves had essentially no effect against C. trachomatis. The mechanism of this interaction is uncertain.

The incidence of pelvic inflammatory disease (PID) is significant. Each year in the United States, 5.3 of every 1,000 females between the ages of 15 and 44 are hospitalized (15). The bacteriology of upper genital tract disease has changed substantially as well. Numerous studies have demonstrated that Chlamydia trachomatis has emerged as one of the major pathogens responsible for the development of PID (6, 11, 14). Currently, the Centers for Disease Control (Atlanta, Ga.) recommends clindamycin and gentamicin as an acceptable regimen for the treatment of PID (3). The efficacy of clindamycin in eradicating C. trachomatis in clinical trials has not been sufficiently characterized, such that the Centers for Disease Control has advised that doxycycline be administered orally following inpatient treatment specifically to eradicate C. trachomatis (3). In vitro susceptibility data with clindamycin have shown moderate activity, with MICs and MBCs being in the range of 1.0 to 2.0 μg/ml (7). The ability of the aminoglycosides to inhibit chlamydial growth in vitro is essentially zero at any clinically relevant concentration (12). Despite this, the clinical cure rate for PID when clindamycin is used in combination with an aminoglycoside is in excess of 85 to 90%, with most failures being a result of tubo-ovarian abscesses (8, 16). Because thousands of women in the United States are being treated with clindamycin and gentamicin or tobramycin annually for acute PID, we undertook a study to determine whether there is evidence of in vitro synergy between these two drugs that could account for its admirable clinical success.

MATERIALS AND METHODS

Three clinical isolates obtained from the endocervixes of three patients with PID admitted to the Ben Taub gynecology service were used. Criteria for the diagnosis of PID included (i) temperature of >100.4°F (38.0°C), (ii) lower abdominal pain and tenderness, (iii) cervical motion tenderness, (iv) adnexal tenderness, and (v) leukocytosis of >12,000/mm³ with a "left shift" (>11 band forms). Isolates were collected on Dacron swabs and transported to the laboratory in chlamydia transport medium (Bartels Immunodiagnostics, Seattle, Wash.). Primary isolation and subsequent passages were performed on McCoy cells in 1-dram (3.7-ml) vials and 1 ml of feeding medium (Bartels Immunodiagnostics), and confirmation of C. trachomatis was performed with a chlamydia-specific immunofluorescence stain (Ortho Diagnostics, Inc., Raritan, N.J.). The specimens were passed three times in antibiotic-containing medium followed by three passages in antibiotic-free medium (minimal essential medium with 5% fetal bovine serum [Sigma Chemical Co., St. Louis, Mo.]). The organisms were then diluted such that 0.1 ml would deliver approximately 2 × 10⁸ organisms. The organisms were added to McCoy cells that were incubated in antibiotic-free medium. The antibiotic susceptibility portion of the experiment was performed as described above for the primary isolation, except that antibiotic-free medium was used and the study antibiotic was added following inoculation and centrifugation at the concentrations described below. Inclusions were considered abnormal and not counted if they were less than 50% of the size of the control inclusions, stained less intensely, and were irregular in shape. These criteria were used because the abnormal inclusions did not reproduce on subsequent passages.

An experimental checkerboard design was used. Clindamycin (The Upjohn Co., Kalamazoo, Mich.) was used at concentrations of 0.25, 0.5, 1.0, and 2.0 μg/ml. Gentamicin (Sigma) was used at concentrations of 2, 4, 8, and 16 μg/ml. Each antibiotic was filtered through a 0.45-nm-pore-size filter prior to use. Each vial was run in duplicate, and the specimens were passed three times. The MIC was considered the lowest concentration at which no inclusion bodies (or only abnormal inclusion bodies as defined above) could be seen after the first passage.

The most frequent method of reporting synergy in chlamydial growth involves the calculation of fractional inhibitory concentration (1). However, when one of the drugs was not active against the organism, no MIC could be calculated and this method could not be used. An alternative method
was the use of an isobologram to demonstrate graphically the relationship between the two drugs (13). When the antibiotic concentrations were plotted against each other with each datum point representing an outcome (in this case, the MIC), a concave upward line represented synergy, a concave downward line illustrated antagonism, and a straight line indicated indifference between the two drugs (see Fig. 1 and 2).

RESULTS

When used alone, clindamycin eradicated 90 to 100% of the organisms consistently at doses of 0.5 to 1.0 mcg/ml with an MIC of 1 mcg/ml. Gentamicin or tobramycin alone had essentially no effect on the number of inclusions seen at any dose up to 16 mcg/ml. Figures 1 and 2 are isobolograms with clindamycin-gentamicin and clindamycin-tobramycin, respectively. When either of the aminoglycosides was added to clindamycin, the MIC of clindamycin decreased by two- to threefold, depending on the dose of aminoglycoside added. This synergistic effect was sustained through all three passages for both sets of drugs; i.e., there was no late emergence of additional inclusions with subsequent passages (the so-called MBC). The dashed lines in Fig. 1 and 2 represent a theoretical condition in which the two drugs had no combined effect on C. trachomatis (i.e., indifference). Interestingly, in the combined drug groups there was a further diminution in the number of inclusions seen with subsequent passages which was not seen with either drug alone.

DISCUSSION

The effect of combined antibiotics on the growth of C. trachomatis in vitro has been tested infrequently (4, 9). Synergy can be considered to be present if the effect observed with a combination of antibiotics is greater than the sum of the effects observed with the two drugs independently. In the current experiment, there was a definite diminution in the number of organisms when gentamicin or tobramycin was added to clindamycin. The total eradication of organisms (MIC) was also seen at lower concentrations of clindamycin when an aminoglycoside was added. Interestingly, we found that subsequent passing of the organism in the combined drug group resulted in a progressive decrease in the number of inclusion bodies seen. As this effect was not seen with either drug alone, one can surmise that there is some combined effect of the drugs which reduces the reproductive potential of the reticulate body. The mechanism of action of gentamicin is mainly due to interference with the 30S ribosomal unit function and its ability to form an initiation complex with mRNA. Clindamycin binds (reversibly) to the 50S ribosomal subunit and also interferes with translational expression of proteins at the level of mRNA. We are uncertain why C. trachomatis is more susceptible to a combination of clindamycin and an aminoglycoside when the organism is apparently resistant to the aminoglycoside alone. The finding of synergy and the continued diminution of inclusions seen with passing might be explained by enhanced alteration of protein synthesis, resulting in abnormal receptors for adherence to host cells, abnormal cell wall permeability allowing for increased intracellular antibiotic levels, or altered translation of other proteins that are necessary for cell reproduction. Further study is necessary to determine why synergy is present with a drug that apparently has no effect on C. trachomatis replication alone.

In a study evaluating the efficacy of clindamycin alone, Gjøen and colleagues (5) have shown that in 45% of males with C. trachomatis-cultured nongonococcal urethritis, the organism was still present at the completion of therapy, questioning the ability of clindamycin alone to eradicate C. trachomatis. Bowie et al. (2) reisolated C. trachomatis in 30% of males with nongonococcal urethritis.
after treatment with clindamycin, although over 50% of these males had resumed sexual activity. Other studies with small numbers of females have refuted this, however, asserting the effective elimination of *C. trachomatis* following treatment with clindamycin alone (H. A. Hammill, L. L. Hollins, and A. D. Heggie, Abstr. Annu. Meet. Infect. Dis. Soc. Obstet. Gynecol. 1987). These studies suggest that chlamydia carriage may be more readily eliminated in females compared with that in males, although there is no direct experimental evidence to support this. When tobramycin was added to clindamycin, Landers and associates (10) have shown that *C. trachomatis* was eradicated in 15 of 15 patients with chlamydia-positive PID. Numerous other studies have shown that the combination of clindamycin with an aminoglycoside is at least as successful as any tetracycline-based regimen in effecting clinical cures in patients with PID (8, 16). Results of those studies suggest that either the chlamydia organism is eradicated by the antibiotics, or the antibiotic-induced change in the microbiology of the upper genital tract diminishes the ability of chlamydia to be either pathogenic or to reproduce effectively. Results of the current study suggest that the in vitro synergy seen with the combination of clindamycin and an aminoglycoside may explain the excellent clinical successes seen with these drugs in the treatment of PID. Even though there appears to be in vitro synergy against *C. trachomatis* with clindamycin and an aminoglycoside, until clinical studies demonstrate acceptable eradication of this organism from patients with chlamydia-positive PID, doxycycline remains the treatment of choice for patients with chlamydia disease. The administration of doxycycline following treatment with parenteral clindamycin-gentamicin as recommended by the Centers for Disease Control is compatible with the findings of this study.

LITERATURE CITED