Synergistic Action of Erythromycin and Cefamandole 
Against Bacteroides fragilis Subsp. fragilis 

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Erythromycin and cefamandole have exhibited synergistic activity against eight strains of Bacteroides fragilis subsp. fragilis. In concentrations of only 0.25 to 0.5 \( \mu \text{g} \) of erythromycin per ml (easily obtainable with oral therapy), less than 0.4 \( \mu \text{g} \) (0.015 to 1.0) of cefamandole per ml inhibited the B. fragilis strains. In the presence of the erythromycin, the potency of cefamandole was increased more than 100-fold. On the basis of the mechanism of action of these two antibiotics, the synergism may be related to inhibition of beta-lactamase formation by the erythromycin, removal of the bacterial cell wall by the cefamandole permitting erythromycin penetration to the ribosomal level, and decreasing inoculum effect. 

The dominant species of bacteria isolated from the intestinal tract of man is Bacteroides fragilis (1, 21). These same organisms have been isolated from patients with infection: 437 of 7,500 nonfecal specimens submitted to the laboratory for culture. Only 30 of these anaerobes were present as pure culture (5). In a review of 306 cases of anaerobic infection reported in the literature, 211 or 69% occurred in patients with malignancy. The remainder had some other compromising condition, such as diabetes, steroid therapy, etc. (7). It evolves, therefore, that in antimicrobial programs designed for prevention or treatment of infection, both in the compromised host and severe infections of undetermined etiology, it may be reasonable to include agents that are active against B. fragilis subsp. fragilis in addition to those antibiotics active against other potential pathogens (3). 

Several agents, when studied in combination, have shown synergistic activity against B. fragilis subsp. fragilis (3). This report describes the results of in vitro tests of eight clinical isolates of B. fragilis subsp. fragilis against two antimicrobial agents, erythromycin and cefamandole. 

MATERIALS AND METHODS 

Antibiotics. Stock solutions of 1,000 \( \mu \text{g/ml} \) were made by diluting standard powers of lithium cefamandole and erythromycin gluceptate (Eli Lilly and Co., Indianapolis, Ind.) in phosphate buffer, pH 7.2. The final working dilutions were made in supplemented brain heart infusion (Difco Laboratories, Detroit, Mich.) broth. 

Antibiotic dilution. The microdilutions for each antibiotic for determination of the minimal inhibitory concentration (MIC) of the anaerobic organisms were made outside the glove box (Coy Manufacturing, Ann Arbor, Mich.) with an Autotiter IV (Ames Co., Division of Miles Laboratory, Elkhart, Ind.), producing a typical "checkerboard" (2, 19). The plates were passed into the glove box and allowed to reduce prior to inoculation. The range of antibiotic concentrations in the checkerboard arrangement, after inoculation, was 32 to 0.015 \( \mu \text{g/ml} \) for each antibiotic. Growth and sterility controls were maintained for each assay. 

Medium. The medium in the microdilution plate wells consisted of brain heart infusion broth supplemented with yeast extract, 0.5%, plus 1 \( \mu \text{g} \) of heme and 5 \( \mu \text{g} \) of menadione per ml. L-Cysteine, as the hydrochloride (0.5% final concentration), was added to the medium as a reducing agent. Indigo carmine dye (colorless at an \( E_0 \) of less than -0.125 mV) was added as a redox indicator to control wells (20, 23). The pH of the medium was adjusted to 8.2 with NaOH before autoclaving. After autoclaving (the indicator dye is now colorless), the medium was stored in the glove box and allowed to cool. The pH stabilized at 7.2 in this controlled atmosphere (10% \( \text{H}_2, \) 10% \( \text{CO}_2, \) 80% \( \text{N}_2 \)). 

Organisms. The eight strains of B. fragilis subsp. fragilis were picked at random from 77 clinical isolates recovered from the Indiana University School of Medicine hospitals between 1972 and 1975. The criteria for identification were those described in the literature (13). 

Inoculum. The inoculum for the plates consisted of 50 \( \mu \text{l} \) per well of a 1:100 dilution of an overnight broth culture, approximately \( 10^6 \) organisms per ml. The plates were inoculated using 50-\( \mu \)l plastic, disposable pipettes (no. 22038, Cooke Laboratory Products, Alexandria, Va.). The final inoculum per well of \( 10^6 \) organisms per ml was verified by using a
du Pont Luminescence Biometer (E. I. du Pont Instruments, Wilmington, Del.). The adenosine 5'-triphosphate produced by bacteria is proportional to the number of bacteria present. Since the luminescence of luciferase enzyme can be quantitated to the amount of adenosine 5'-triphosphate present, luminescence can be used to measure bacteria counts. The Biometer is designed to convert luminescence directly to bacterial count (4, 15).

After inoculation, the plates were sealed with plastic tape and incubated in the anaerobic glove box at 37°C for 24 h. After incubation the plates were read with the Autotray Viewer (Ames Co.) (14, 17). The addition of L-cysteine, prereducing the medium by autoclaving, and storage in the controlled atmosphere of the glove box made it possible to read the results in 24 instead of 48 h.

RESULTS

The susceptibility of the eight Bacteroides strains to erythromycin and cefamandole alone is shown in the second and third columns of Table 1. When erythromycin was present in a concentration of 0.5 μg/ml, all eight strains were susceptible to less than 0.015 μg of cefamandole per ml. The lowest concentration for each antibiotic showing synergism, when studied in combination, is underlined in the table. The increase in activity of the erythromycin and cefamandole in combination is illustrated in the last column of Table 1. An average concentration of 0.2 μg of erythromycin and less than 0.4 μg of cefamandole per ml in combination inhibited B. fragilis subsp. fragilis. The range of concentrations showing activity against the eight strains was 0.125 to 0.25 μg of erythromycin and <0.015 to 2 μg of cefamandole per ml.

Drug interactions were considered to be synergistic when the strain of Bacteroides was inhibited by the combination of erythromycin and cefamandole, each at a concentration of less than or equal to 25% of its MIC when tested alone. As shown in the last column of Table 1, six of the eight strains exhibited this degree of synergism. The overall effect of the combination on the eight strains of Bacteroides was evaluated by constructing an isobologram (Fig. 1). The isobologram for the combination can be compared with the theoretical straight-line isobologram formed by connecting the points representing the mean MIC for each antibiotic. Bowing of the isobologram toward the

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<th>Table 1. Synergistic action of erythromycin and cefamandole against Bacteroides fragilis subsp. fragilis</th>
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<td><strong>Strain</strong></td>
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<td>Average</td>
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* Drug concentrations in micrograms per milliliter.

* Erythromycin/cefamandole.

* The underlined figures designate the lowest concentration of each antibiotic showing synergism.

* — , indicates no additional effect.
intersection of the vertical and horizontal axes is indicative of synergism (22). Maximal synergistic effect was observed when the average concentration of erythromycin was 0.2 μg/ml and that of cefamandole was 0.4 μg/ml.

**DISCUSSION**

The only combination of antimicrobial agents that has shown significant synergistic activity in vitro against B. fragilis subsp. fragilis is clindamycin with metronidazole (3). It would be reassuring to know that the antimicrobial agents being used clinically to treat infections are also effective against anaerobic Bacteroides, even though this is considered a relatively rare pathogen causing infection in humans. Our in vitro data, coupled with the blood levels achievable with erythromycin and cefamandole, suggest an alternate combination to use as therapy in selected Bacteroides infection. The average peak blood level after the usual oral dose of erythromycin has been shown to be 1.4 μg/ml (10). In concentrations of 0.5 μg/ml or greater of erythromycin, less than 0.015 μg of cefamandole per ml was needed for inhibition of the Bacteroides strains studied. Obviously, the peak level of 19 μg/ml obtained after 1 g of cefamandole injected intramuscularly (11) would be more than 1,000-fold greater than the MIC of cefamandole required to inhibit these same Bacteroides strains when erythromycin is present in concentrations of 0.5 μg/ml or greater.

It has been suggested that a knowledge of the mechanism of synergy permits antibacterial substances to be used effectively (19). The accepted antibacterial action of the cephalosporins is related to inhibition of cell wall synthesis (8). However, since B. fragilis subsp. fragilis produces a beta-lactamase that destroys susceptible cephalosporins, these agents may not be effective against these bacteria (5, 6). It has been shown that these antibiotics that lose their activity because of enzymatic degradation show a marked increase in activity as the inoculum is decreased (16). It has been suggested that beta-lactam antibiotics remove the cell wall and permit erythromycin to more easily penetrate the bacterial cell and reach the ribosome (9, 12). This, in turn, slows or stops beta-lactamase synthesis, allowing greater cephalosporin activity. As the viable inoculum is reduced, both agents show greater activity.

Although essentially all strains of B. fragilis subsp. fragilis can be inhibited by easily obtainable concentrations of several antimicrobial agents, combinations such as cefamandole plus erythromycin may prove useful in the treatment of selected infections, where this organism is implicated as a single or primary pathogen, and for infections due to this organism where maximal antibacterial activity is desired.

In summary, eight clinical isolates of B. fragilis subsp. fragilis were tested against cefamandole and erythromycin by the microdilution technique. A synergistic effect was observed. No antagonism was noted. For selected Bacteroides infections, the combination of cefamandole and erythromycin may be useful.

**LITERATURE CITED**


