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Clinical Significance of In Vitro Synergism Between Antibiotics in Gram-Negative Infections

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Five combinations of antibiotics (ampicillin/gentamicin, cephalothin/gentamicin, carbenicillin/gentamicin, polymyxin/carbenicillin, and carbenicillin/cephalothin) were investigated in vitro and in 148 severe infectious episodes caused by gram-negative bacilli in patients with disseminated cancer. The use of combinations that were synergistic in vitro against the offending microorganism (synergy was defined as occurring when the minimal inhibitory concentration of each of the drugs in the combination was one-quarter or less of the minimal inhibitory concentration of each drug alone) was associated with a significantly better response to antibacterial therapy ($P < 0.01$) than the use of combinations that were not synergistic against the causative agent.

Patients with advanced neoplasma, receiving chemotherapy with immunosuppressive agents, are highly susceptible to infection and often die from it. The infecting microorganisms are usually hospital-acquired and antibiotic-resistant. Therefore, many investigators in such situations have resorted to the use of drug combinations.

The rationale for the use of combinations of antibiotics in the treatment of severe infection presumed to be caused by gram-negative bacilli is the possible synergistic effect that may exist between the antibiotics used (6); to date, clinical documentation of the merit of synergistic antibiotic combinations has been limited to penicillin plus streptomycin in endocarditis due to Viridans group streptococci and in enterococcal infections, and to other antibiotics against gram-negative bacilli in some instances of urinary tract infections (6, 10). However, the clinical significance of in vitro synergism has not yet been defined in the treatment of other infections. Therefore, this study was undertaken to investigate the effectiveness in severe gram-negative infections of combinations of antibiotics which are synergistic in vitro. It will be shown that the use of two antibiotics that are synergistic in vitro against the microorganism responsible for the infection may be associated with a significantly better outcome than that achieved with a combination which is not synergistic for the offending microorganism.

A preliminary report of this study was presented at the 11th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, N.J., 19-22 October 1971.

MATERIALS AND METHODS

The patients studied were hospitalized on the medical and surgical wards of the Institut Jules Bordet between January 1970 and March 1972. Most of them had received radiation therapy or cytotoxic drugs, or had undergone extensive surgery; most of them had been hospitalized for 15 days or longer when the gram-negative infection was diagnosed and had been treated during the 4 weeks preceding the infection with various antibiotics, especially ampicillin and tetracyclines. Only patients with a bacteriologically proven infection were considered for this report. To be considered as pathogenic, the microorganisms had to have been isolated from at least two consecutive blood or urine cultures in pure growth, or from two consecutive sputum or wound cultures as the only or the predominant organisms. Characterization of bacteria was accomplished by standard methods (2).

Therapy with antibiotics was started in all patients as soon as the infection was suspected. Five combinations of antibiotics were employed consecutively: ampicillin plus gentamicin, cephalothin plus gentamicin, carbenicillin plus gentamicin, carbenicillin plus polymyxin B, and carbenicillin plus cephalothin. The penicillins and cephalothin were given as rapid intravenous infusions in the following daily doses: ampicillin, 100 to 200 mg/kg; cephalothin, 100 to 200 mg/kg; and carbenicillin, 300 to 500 mg/kg. Gentamicin and polymyxin B were given intramuscularly except in a few thrombocytopenic patients in whom intravenous administration was employed; the daily dose for gentamicin was 4 to 6 mg/kg and for polymyxin B was 3 mg/kg. The weight ratios of the combination of antibiotics was chosen as a reflection of the minimal inhibitory concentration (MIC) considered to indicate susceptibility. All antibiotics were administered in three daily divided doses.

The organisms causing infection in this series were tested for susceptibility to ampicillin, cephalothin, carbenicillin, gentamicin, and polymyxin B by the inocula-replicating method (11) on Muller-Hinton agar plates containing serial twofold dilutions of antibiotics. Bacteria to be tested were grown overnight in Trypticase soy broth and diluted 10 times with Trypticase soy broth before use; at this dilution, the inoculum contained approximately 10^8 viable cells per ml.

Microorganisms were considered to be susceptible to ampicillin, cephalothin, carbenicillin, gentamicin, and polymyxin B if their MIC was equal to or lower than 25, 25, 50, 3, and 3 μg ml, respectively. The bacteriostatic tests for synergistic action were performed with the same technique. The various antibiotics were mixed in the following weight proportions: ampicillin/gentamicin, 8/1; cephalothin/gentamicin, 8/1, carbenicillin/gentamicin, 16/1; carbenicillin/polymyxin B, 16/1; carbenicillin/cephalothin, 3/1. We evaluated antibiotic combinations by classifying them as synergistic when the combination was at least four times as effective as each drug alone and as not synergistic in all other cases, since antagonism was only found for five strains by the agar dilution tests, and in these instances could not be confirmed by standard killing curve studies.

Bactericidal studies were performed by exposing the microorganisms to be tested (10^7 cells/ml) to the antibiotics alone or in combination in Trypticase soy broth (ampicillin, 25 μg/ml; cephalothin, 25 μg/ml; carbenicillin, 50 μg/ml; gentamicin, 3 μg/ml; polymyxin B, 3 μg/ml). At 1, 2, 3, and 4 hr, the number of viable bacteria was ascertained by doing plate counts. These studies were performed with representative strains for which synergism or indifferent action had been shown by the agar dilution tests and for all five strains for which antagonism had been suggested. Antagonism could not be verified by the bactericidal studies, but the presence or the absence of synergism was usually confirmed.

For 116 patients, the bactericidal activity of serum obtained on the 3rd day of therapy immediately prior to the administration of the combination (through concentration) and 1 hr after the injection (peak concentration) was determined. Sera were diluted with Trypticase soy broth and inoculated with the microorganism responsible for the infection in the patient under consideration at a concentration of 10^4 viable cells per ml. The highest dilution that failed to show macroscopic growth was considered as the maximal bacteriostatic dilution. Samples from clear tubes were plated on sheep blood-agar plates to determine the maximal bactericidal dilution.

The clinical results of antimicrobial therapy were determined as follows: patients were considered to have had a favorable response to antimicrobial therapy if they became afebrile and if clinical or radiological signs of infection (or both) disappeared. More precisely, in patients with urinary tract infections bacteriuria had to clear. Disappearance of purulent sputum, rales, and pulmonary infiltrates was required in pulmonary infections, and disappearance of pain, redness, and purulent discharge in wound infection was required for therapy to be considered successful. Patients who died of their underlying malignancy were considered to have had a good response if the original infection resolved. In patients who did not respond, or who died before the initial infection resolved, and in those in whom the infection recurred within 1 week of cessation of therapy, the antibiotic treatment was considered to have failed. It is recognized that patients such as those studied in this series are extremely susceptible to bacterial infection, and that it is difficult to define, in such patients, the severity of the infection and the clinical effectiveness of any antimicrobial therapy in any standard fashion; however, all of them presented a severe underlying disease which has been shown to be a major determinant for the outcome of serious gram-negative infections (3, 7).

RESULTS

Population. A total of 148 patients were available for this study; 113 of them had a disseminated malignant solid tumor and 35 presented with either acute leukemia or lymphoma. Failure of antimicrobial therapy was observed in 28% of the patients with solid tumors and in 45% of the patients who had leukemia or lymphoma. This is not a statistically significant difference at the 0.05 level. No significant differences in the rate of failure of the therapy were observed in relationship to the site of the tumor. Antimicrobial therapy failed in 44% of the patients who were over 60 years old and in 42% of the patients who were younger. The sex of the patient had no influence on the rate of failure of antibiotics.

Leucopenic patients (less than 3,000 granulocytes per mm^3), who were all receiving cytotoxic therapy, had a failure rate of antibacterial therapy of 34%, not significantly different from that of 32% found in nonleucopenic patients.

The response to antimicrobial therapy was not influenced by prior administration of antibiotics.

Site and bacteriological nature of the infection. In this series, 35 infections were caused by coliforms (33 *Escherichia coli* and 2 *Citrobacter freundii*), 30 were due to organisms belonging to the *Klebsiella-Enterobacter* group (26 *Klebsiella* and 4 *Enterobacter* species), 41 were due to *Proteus* organisms (29 *P. mirabilis* and 12 *P. vulgaris*), and 42 to *Pseudomonas aeruginosa*. Infections due to *E. coli* were associated with a rate of failure of antimicrobial therapy of 29%, which was lower than that in infections caused by the *Klebsiella-Enterobacter* (40%) or *Proteus* organisms (39%). The rate of failure observed in infections caused by *P. aeruginosa* was 26%. These differences were not statistically significant.

Respiratory tract infections represented the most common type of sepsis in this series; this

was presumably due to the very large number of patients with neoplasms of the respiratory tract. Hospital-acquired pulmonary infections caused by gram-negative rods are frequent in debilitated patients and often follow antibiotic treatment of pneumonia in hospitalized patients (12). Moreover, neoplasia of the respiratory tract is a major predisposing factor to gram-negative pneumonia (7). It is recognized, however, that the distinction between colonization and infection of the respiratory tract may be difficult to make. In all patients in this series, purulent

TABLE 1. *Relationship between in vitro synergism and clinical outcome*[a]

Drug combination, clinical outcome, and in vitro synergism	Leukopenia[b]		Type of tumor[c]		Site of infection[d]				Microorganisms[e]				Total
	+	−	L	S	S	P	U	W	C	K-E	P	PS	
Carbenicillin + gentamicin													
Clinical success													
Synergism	8	20	7	21	7	11	2	8	4	2	6	16	28
No synergism	5	10	2	13	3	9	1	2	4	5	4	2	15
Clinical failure													
Synergism	0	5	1	4	1	3	0	1	1	0	1	3	5
No synergism	3	6	4	5	0	5	3	1	1	2	4	2	9
Cephalothin + gentamicin													
Clinical success													
Synergism	0	4	1	3	0	4	0	0	0	2	2	0	4
No synergism	0	5	1	4	1	4	0	0	1	1	1	2	5
Clinical failure													
Synergism	0	1	0	1	0	1	0	0	0	0	1	0	1
No synergism	1	7	4	4	2	4	1	1	2	1	3	2	8
Ampicillin + gentamicin													
Clinical success													
Synergism	1	7	2	6	0	4	2	2	5	0	3	0	8
No synergism	1	4	2	3	1	2	1	1	1	0	1	3	5
Clinical failure													
Synergism	0	5	0	5	1	4	0	0	2	0	3	0	5
No synergism	0	5	1	4	0	4	0	1	2	1	0	2	5
Carbenicillin + polymyxin B													
Clinical success													
Synergism	1	2	0	3	0	2	1	0	1	0	2	0	3
No synergism	2	5	0	7	1	2	3	1	2	2	2	1	7
Clinical failure													
Synergism	0	1	0	1	0	1	0	0	1	0	0	0	1
No synergism	5	6	3	8	6	1	3	1	1	6	3	1	11
Carbenicillin + cephalothin													
Clinical success													
Synergism	1	14	3	12	10	2	0	3	5	5	2	3	15
No synergism	2	7	1	8	1	2	2	4	2	1	2	4	9
Clinical failure													
Synergism	1	0	1	0	1	0	0	0	0	1	0	0	1
No synergism	1	2	2	1	2	0	1	0	0	1	1	1	3
Total													
Clinical success													
Synergism	11	47	13	45	17	23	5	13	15	9	15	19	58
No synergism	10	31	6	35	7	19	7	8	10	9	10	12	41
Clinical failure													
Synergism	1	12	2	11	3	9	0	1	4	1	5	3	13
No synergism	10	26	14	22	10	14	8	4	6	11	11	8	36

[a] Data are displayed to show the role of various factors such as leucopenia, type of tumor, infecting organism, and site of infection. The number of patients in each group is indicated.

[b] Leucopenia: +, present; −, absent.

[c] Type of tumor: L, lymphoma or leukemia; S, solid tumor.

[d] Site of infection: S, septicemia; P, pulmonary; U, urinary; W, wound or cellulitis.

[e] Microorganisms: C, *E. coli*; K-E, *Klebsiella-Enterobacter*; P, *Proteus*; PS, *P. aeruginosa*.

sputum containing gram-negative rods was observed. In many patients, it has been obtained through a transtracheal aspiration.

The rate of failure of antibacterial therapy in septicemias was 35%, not significantly different from that observed in respiratory tract infections (35%), urinary tract infections (40%), or cellulitis (19%). Septicemia was found predominantly in patients with acute leukemia and gastrointestinal neoplasms, and originated most often from neoplastic or chemotherapy-induced lesions of the gastrointestinal tract. Urinary tract infections occurred predominantly, as could be expected, in patients with urinary or genital neoplasms. No specific organism was found to be associated with one type of infection or one type of tumor.

Antimicrobial therapy. As shown in Table 1, the use of the carbenicillin/gentamicin combination and that of the carbenicillin/cephalothin combination was associated with a lower rate of failure of antimicrobial therapy (24 and 17%, respectively) than that of carbenicillin/polymyxin B (54%), ampicillin/gentamicin (43%), or cephalothin/gentamicin (50%).

However, these latter series are small, and only a very large randomized study could establish whether one of these combinations of antibiotics is really superior to another.

More than 50% of the bacteria responsible for infection in this series were resistant to ampicillin, cephalothin, and carbenicillin, as defined above. The median MIC of gentamicin for these organisms was 3 μg/ml, and that of polymyxin B was 2.6 μg/ml. These concentrations of gentamicin and polymyxin B may be reached in the blood after administration of usual doses, but much higher levels are difficult to obtain because both antibiotics are toxic. Thus, most of the microorganisms responsible for infections here can be considered as relatively resistant to the antimicrobial agents employed. This might explain why no significant differences could be found between the clinical results observed in infections due to microorganisms susceptible or resistant to a single antibiotic.

When synergism was present in vitro between the two antibiotics used for the treatment of infection, the rate of failure was 18%; this contrasts to the rate of failure found in infections caused by organisms against which the antibiotics were not synergistic (47%). The difference is statistically highly significant ($P < 0.01$), and a similar trend was observed for all of the five antibiotic regimens tested here with the exception of the ampicillin/gentamicin combination, as shown in Table 2.

Since a serious objection to these studies might be the heterogeneity of the population (different types of infection, various antimicrobial regimens), we have studied the role of in vitro synergism in the group which received carbenicillin and gentamicin. The rate of failure was 15% when synergism had been documented in vitro and 37% when no synergism was present ($P < 0.05$). A similar favorable influence of the synergistic effect of the antibiotics used on the mortality rate was observed in the group of patients who presented with septicemia ($P < 0.01$).

Table 3 makes it appear that there are two groups of patients with regard to failure, those showing synergism between the two antibiotics with failure rates of 10 to 27% and those without synergism with failure rates from 34 to 71%. However, synergism, if demonstrated, was beneficial. It was most important to the outcome when the organism was resistant to both antibiotics.

These observations are understandable since, in synergistic combinations, the potentiation of one antibiotic by the other may be considerable; Fig. 1 shows that synergistic combinations render the microorganisms extremely susceptible to concentrations of both antimicrobial agents which can be easily obtained or exceeded in the serum after administration of usual doses. That in patients treated with a synergistic combination of antibiotics the bactericidal activity of the serum was higher than that observed in patients treated with nonsynergistic combinations is shown in Table 4. The geometric mean observed in this study for the peak bactericidal activity of diluted serum from patients treated with synergistic combinations was 1/16 whereas that in the other patients was 1/4.

TABLE 2. *Relationship between the use of a synergistic combination of antibiotics and the clinical outcome*

Type of antibiotic therapy	No. of patients	No. of patients in whom therapy failed		
		Total	Synergism in vitro	No synergism in vitro
Carbenicillin + gentamicin	57	14	5[a]	9
Carbenicillin + polymyxin B	22	12	1	11
Ampicillin + gentamicin	23	10	5	5
Cephalothin + gentamicin	18	9	1	8
Carbenicillin + cephalothin	28	4	1	3
Total	148	49	13[b]	36

[a] $P < 0.05$.
[b] $P < 0.01$.

TABLE 3. *In vitro tests of antibacterial activity and clinical effectiveness of various combinations of antibiotics*[a]

Carbenicillin (50 μg/ml), ampicillin (25 μg/ml), cephalothin (25 μg/ml)	Synergism[b]	Gentamicin (3.1 μg/ml), polymyxin (3.1 μg/ml), cephalothin (25 μg/ml)[c]	No. of patients	Failures	
				No.	Percent
S	+	S	29	3	10
S	+	R	13	2	15
R	+	S	19	5	26
R	+	R	11	3	27
S	−	S	23	8	34
S	−	R	12	4	33
R	−	S	26	14	53
R	−	R	14	10	71

[a] R, resistant; S, susceptible.
[b] Synergism, +; no synergism, −.
[c] In cases where carbenicillin plus cephalothin were used.

DISCUSSION

The best known example of a synergistic combination is that of benzylpenicillin (or other penicillins) plus streptomycin (or other aminoglycoside antibiotics) for the treatment of enterococcal infections (5). The various combinations of antibiotics used here have also been shown to be synergistic occasionally in vitro against gram-

TABLE 4. *Relationship between synergism as demonstrated in vitro by bacteriostatic tests and the bactericidal activity of sera of patients treated with synergistic and nonsynergistic combinations*

Results of agar dilution tests	No.	Bactericial activity of serum	
		Through concn	Peak concn
Synergism	52	1/8 (1/2–1/16)	1/16 (1/2–1/64)
No synergism	64	1/2 (1/2–1/8)	1/4 (1/2–1/32)

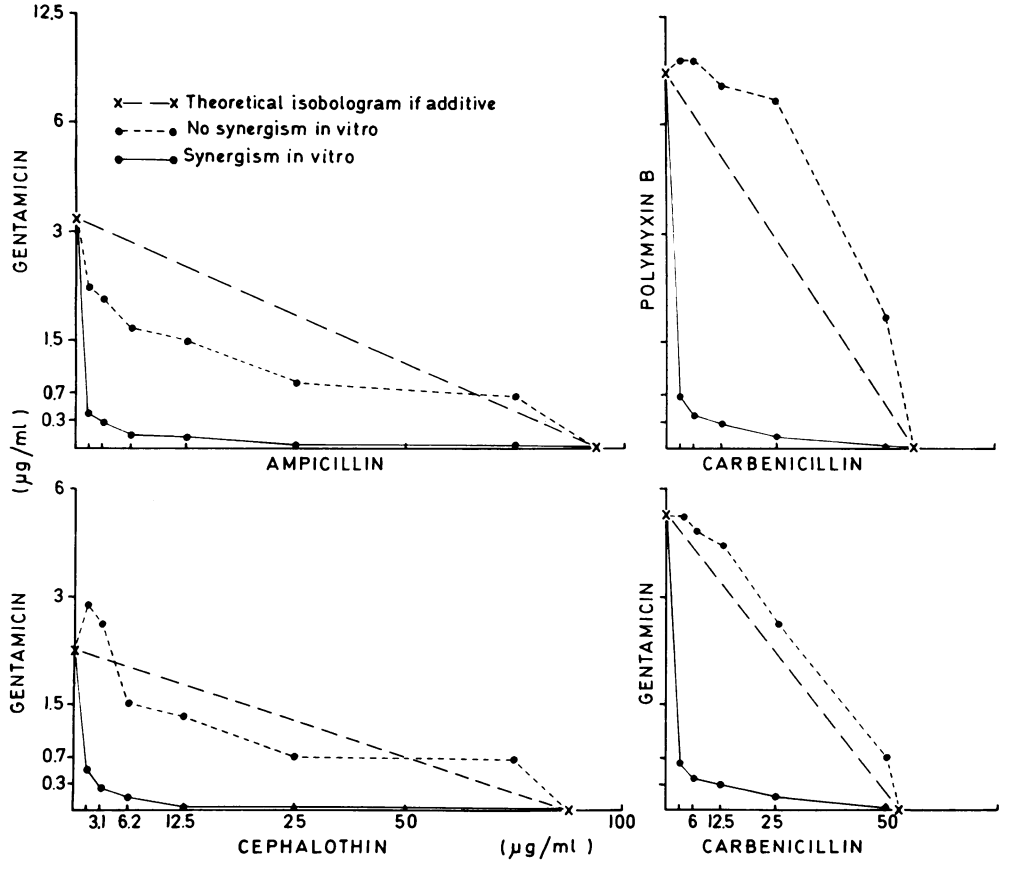

FIG. 1. *Isobolograms of combined action of the various combinations of antibiotics tested against the pathogens isolated in this study.*

negative bacilli (1, 3, 8). A number of possible mechanisms have been suggested to explain this effect (6).

The potentiation of one antibiotic by the other may be considerable for synergistic combinations when tested in vitro. In addition, our data suggest that the inhibitory activity of sera from patients treated with such synergistic combinations is higher than that observed in patients treated with associations of antibiotics which are merely additive.

Failure to show antagonism is not especially surprising in view of the use of fixed ratio combinations.

Seven negative correlates with clinical (bacteriological) outcome were noted. Of these, three—age, sex, and previous antibiotic treatment—have been found to be minor by other investigators. The other four—leukopenia, site of infection, the infecting bacterial species, and susceptibility of the strain—were not found to be significant in this series. However, the relatively small number of patients studied here does not allow one to consider them as unimportant; although the importance of these factors may be overshadowed by the importance of the underlying disease of the host, they should probably be taken into consideration.

On the other hand, our data clearly suggest that the use of synergistic combinations for therapy of gram-negative infections is associated with a better clinical response than the use of nonsynergistic combinations.

Each situation in which it seems advisable or necessary to use antibiotics in combination must be carefully evaluated because the dangers of toxicity, sensitization, superinfection, or increase in resistant organisms which may follow the use of an additional antibiotic should not be minimized. However, the mortality in serious gram-negative infections is extremely high, especially in debilitated patients, and the use of synergistic combinations of antibiotics might prove to be a valuable means for reducing this high mortality rate. Therefore, when one is dealing with a serious infection presumably caused by gram-negative rods, it might be useful to test routinely combinations of antibiotics for a potential synergistic action. Routine use of combinations of antibiotics should, however, be practiced with caution because only a part of the organisms, half in the present study, demonstrate synergism.

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