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Bactericidal Effect of Combinations of Antimicrobial Drugs and Antineoplastic Antibiotics Against Gram-Negative Bacilli

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Six antineoplastic antibiotics showed little antibacterial activity against 28 strains of four species of gram-negative enteric bacteria. By using the cellophane transfer technique, combinations of these agents with 16 antibacterial drugs usually showed indifference. However, combinations of mitomycin C, especially with the aminoglycosides, were synergistic on strains of *Escherichia coli, Proteus*, and *Klebsiella pneumoniae*. Bleomycin, on the other hand, often showed antagonism on strains of *E. coli* and *K. pneumoniae* with the β-lactams, aminoglycosides, and other antibacterial agents. Checkerboard titrations and kinetic killing curves confirmed these findings.

We have recently described (14) both synergism and antagonism with combinations of each of six antineoplastic antibiotics and each of 16 antibacterial drugs when tested against 10 strains of *Staphylococcus aureus*. The present paper records the bactericidal activity of the same drug combinations on four species of gram-negative bacilli which are commonly implicated in infections occurring in patients with malignant neoplastic disease (2, 10, 12).

(This work will be submitted to the Hebrew University of Jerusalem by J. Y. J. in partial fulfillment of the requirements for a Ph.D. degree.)

MATERIALS AND METHODS

The 28 strains of gram-negative bacilli used were isolated from blood, urine, and pus samples in the Department of Clinical Microbiology of the Hadassah University Hospital and consisted of 6 strains of *Escherichia coli*, 3 strains of *Proteus mirabilis*, 2 of *Proteus morganii*, 1 of *Proteus rettgeri*, 6 of *Klebsiella pneumoniae* and 10 strains of *Pseudomonas aeruginosa*.

The combinations of antibacterial and antineoplastic agents were initially screened by the cellophane transfer technique (3-5, 7). Details of this method and the other methods used to determine minimal inhibitory concentrations and minimal bactericidal concentrations (MBCs) and for the quantitative evaluation of combined activity were described in our previous paper (14).

The antibacterial drugs, antineoplastic antibiotics, and the concentrations of the drugs employed to impregnate the test strips used in the cellophane transfer technique were identical to those described by Jacobs et al. (14) except for penicillin G (600 μg/ml) and rifampin (1,000 μg/ml). The results of cellophane transfer screening were classified as synergism, indifference, or antagonism by two of the authors independently according to published criteria (4, 11). Doubtful results were duplicated or triplicated.

Checkerboard titrations (14) were used to confirm the synergistic and antagonistic combinations indicated by the cellophane transfer technique.

Fractional bactericidal concentrations (FBCs) of each drug were calculated by dividing the MBC of the drug in combination by the MBC of the drug alone. The total of the FBCs of the two drugs tested is the FBC index (∑FBC) (14). Kinetic killing curves were prepared after following the action of the drug combination during 24 h, in examples selected according to results of the cellophane transfer test and checkerboard titrations.

RESULTS

The antibacterial activity of the antineoplastic antibiotics acting alone, as detected by the cellophane transfer technique, showed that mitomycin C was bacteriostatic on three strains and bactericidal on three strains of *E. coli* and bactericidal on two strains of *Proteus morganii* and one strain each of *P. rettgeri* and *P. mirabilis*; it was bacteriostatic on two strains and bactericidal on three strains of *K. pneumoniae* and bacteriostatic on seven strains and bactericidal on one strain of *P. aeruginosa*. Bleomycin was bactericidal on all six strains of *E. coli* and on five strains of *K. pneumoniae* and bacteriostatic on the sixth strain; it was inactive on the strains of *Proteus* and *P. aeruginosa*. Daunorubicin was bacteriostatic on two strains of *E. coli* and on one strain of *P. aeruginosa*, and its derivative, doxorubicin, was bacteriostatic on only one strain of *E. coli* but inactive on the remaining strains and species. Dactinomycin and mithramycin showed minimal or no activity on any of the 28 strains examined.

A total of 96 combinations of an antineoplastic antibiotic with an antibacterial drug were tested on each of the 28 strains by the cellophane transfer technique, i.e., 448 combinations for each antineoplastic antibiotic, a total of 2,688 combinations. Generally, synergism and antagonism were infrequent (5.8 and 7.1%, respectively), most of the combinations showing indifference.

Synergism and antagonism were rare with dactinomycin (1.1 and 1.6% of the combinations, respectively) and with mithramycin (1.1 and 2.0% respectively). The combination of dactinomycin with chloramphenicol was synergistic on one strain of *P. rettgeri*, for which dactinomycin was bactericidal, and one strain of *P. mirabilis*, against which it showed no activity. The combination of dactinomycin with tetracycline was synergistic on one strain of *E. coli*. Synergism and antagonism were seen slightly more frequently in combinations of daunorubicin (4.9 and 3.3%) and of doxorubicin (3.3 and 4.2%, respectively). With daunorubicin, antagonism was seen with gentamicin on three strains, with kanamycin on two strains, and with streptomycin on one strain of *K. pneumoniae*. Doxorubicin produced antagonism with gentamicin and with kanamycin on three strains and with streptomycin on one strain of *K. pneumoniae*.

Table 1 shows the detailed results of the cellophane transfer technique with combinations of mitomycin C. Eighty-eight combinations (19.6%) demonstrated synergism, whereas 31 combinations (6.9%) were antagonistic. Synergism was frequently seen with carbenicillin (but not with the other β-lactam antibiotics) on strains of *E. coli* and *P. aeruginosa*, and also with the aminoglycosides, with chloramphenicol (on *E. coli* and *Proteus* spp.) and with nalidixic acid (except on *P. aeruginosa*).

Bleomycin was antagonistic in 112 combinations (25%), and it was rarely synergistic (4.2%). Table 2 summarizes the results of combinations of the antibacterial drugs with bleomycin and shows the synergism and antagonism found on strains of *E. coli* and *K. pneumoniae*. These combinations almost always showed indifference on *Proteus* and on *P. aeruginosa* and details are therefore not shown. On *E. coli* and *K. pneumoniae* bleomycin generally showed antagonism when combined with the β-lactam antibiotics, the aminoglycosides, rifampin, erythromycin and clindamycin, chloramphenicol and tetracycline, and colistin. It did however show synergism with nalidixic acid on some strains of *E. coli* but antagonism on *K. pneumoniae*. Occasionally, both synergism and antagonism were seen in the same combination; these phenomena were concentration dependent. Table 3 shows the synergism demonstrated by bleomycin with gentamicin (subinhibitory concentrations of the aminoglycoside) and antagonism (inhibitory concentrations of aminoglycoside).

Checkerboard titration results confirmed those data obtained in the qualitative screening tests for synergism and antagonism by the cellophane transfer technique. FBCs for synergistic and antagonistic combinations were calculated and are shown together with ∑FBC in Table 3. Similarly, kinetic studies on selected combinations confirmed the synergism results previously obtained, although in some quantitative experiments previously demonstrated antagonism could not be confirmed. With *E. coli*, a combination of gentamicin (one fourth MBC) with mitomycin C (one fourth MBC) was completely bactericidal within 4 h (see Fig. 1). Figure 2 shows the bactericidal effect of nalidixic acid (one fourth MBC) in combination with bleomycin (one fourth MBC) on *E. coli*. With *P. aeruginosa* a combination of carbenicillin (one fourth MBC) with one fourth of mitomycin C was shown to be synergistic and bactericidal within 8 h (Fig. 3).

DISCUSSION

We have previously demonstrated (14) that dactinomycin showed bacteriostatic activity on all 10 strains of *S. aureus* tested and that the combination of this antineoplastic with the bacteriostatic drugs chloramphenicol and tetracycline and with erythromycin was frequently synergistic. In contrast, 27 of the 28 strains of gram-negative bacilli which we tested were resistant to dactinomycin and failed to show synergism with bacteriostatic drugs. With the one strain of

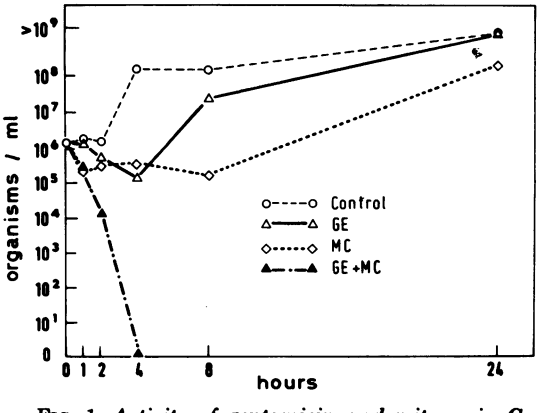

FIG. 1. *Activity of gentamicin and mitomycin C individually and in combination on a strain of E. coli. GE, Gentamicin, 2 μg/ml; MC, mitomycin C (1 μg/ml); GE + MC, gentamicin (2 μg/ml) plus mitomycin C (1 μg/ml). (MBC of gentamicin, 8 μg/ml; MBC of mitomycin C, 4 μg/ml).*

TABLE 1. *Number of strains showing synergism or antagonism in combinations of mitomycin C with 16 antibacterial drugs*

Drug[a]	*E. coli* (6 strains)		*Proteus* sp. (6 strains)		*K. pneumoniae* (6 strains)		*P. aeruginosa* (10 strains)		Total (%) (28 strains)	
	S[b]	A[b]	S	A	S	A	S	A	S	A
P	1	0	0	0	0	0	0	0	1 (3.6)[c]	0 (0)[c]
AM	0	0	0	1	0	0	0	0	0 (0)	1 (3.6)
CN	3	0	1	1	0	0	6	0	10 (35.7)	1 (3.6)
ME	1	2	0	2	0	0	0	0	1 (3.6)	4 (14.3)
CD	2	2	0	3	0	0	0	0	2 (7.1)	5 (17.9)
V	1	0	0	2	0	0	0	0	1 (3.6)	2 (7.1)
S	3	0	2	2	3	0	2	0	10 (35.7)	0 (0)
K	6	0	4	0	4	0	1	0	15 (53.6)	0 (0)
GE	5	0	5	0	5	0	2	0	17 (60.7)	0 (0)
E	0	1	0	1	0	0	0	0	0 (0)	2 (7.1)
CL	0	1	0	1	0	0	0	0	0 (0)	2 (7.1)
C	4	0	3	2	1	0	0	0	8 (28.6)	2 (7.1)
T	2	0	0	2	0	0	0	0	2 (7.1)	2 (7.1)
R	1	1	3	0	0	0	0	7	4 (14.3)	8 (28.6)
CO	3	0	1	1	1	1	0	0	5 (17.9)	2 (7.1)
NA	4	0	5	0	3	0	0	0	12 (42.8)	0 (0)
Total	36 (37.5)[d]	7 (7.3)[d]	24 (25)[d]	18 (18.8)[d]	17 (17.7)[d]	1 (1.0)[d]	11 (6.9)[e]	7 (4.4)[e]	88 (19.6)	31 (6.9)

[a] P, Penicillin G; AM, ampicillin; CN, carbenicillin; ME, methicillin; CD, cephaloridine; V, vancomycin; S, streptomycin; K, kanamycin; GE, gentamicin; E, erythromycin; CL, clindamycin; C, chloramphenicol; T, tetracycline; R, rifampin; CO, colistin; and NA, nalidixic acid.

[b] S, Synergism; A, antagonism.

[c] Numbers in parentheses represent the percentage of 28 combinations.

[d] Numbers in parentheses represent the percentage of 96 combinations.

[e] Numbers in parentheses represent the percentage of 160 combinations.

TABLE 2. *Number of strains showing synergism or antagonism in combinations of bleomycin with 16 antibacterial drugs*[a]

Drug	*E. coli* (6 strains)		*K. pneumoniae* (6 strains)		Total (%) (28 strains)	
	S	A	S	A	S	A
P	0	3	0	6	0 (0)[b]	9 (32.1)[b]
AM	1	4	0	6	1 (3.6)	10 (35.7)
CN	0	2	0	4	1 (3.6)	7 (25.0)
ME	0	4	0	4	0 (0)	8 (28.6)
CD	1	4	1	3	2 (7.1)	7 (25.0)
V	0	4	0	4	0 (0)	8 (28.6)
S	0	3	1	1	1 (3.6)	4 (14.3)
K	1	3	0	4	1 (3.6)	7 (25.9)
GE	0	4	1	3	1 (3.6)	8 (28.6)
E	0	2	0	5	0 (0)	7 (25.0)
CL	0	3	0	4	0 (0)	7 (25.0)
C	1	2	0	5	1 (3.6)	7 (25.0)
T	0	3	3	2	3 (10.7)	5 (17.9)
R	0	4	2	3	2 (7.1)	7 (25.0)
CO	2	2	0	5	2 (7.1)	7 (25.0)
NA	3	0	0	4	4 (14.3)	4 (14.3)
Total (%)	9 (9.4)[c]	47 (49)[c]	8 (8.3)[c]	63 (65.6)[c]	19 (4.2)[d]	112 (25.0)[d]

[a] See footnote to Table 1 for key to abbreviations. For the sake of brevity, results for *Proteus* spp. (six strains) and *P. aeruginosa* (10 strains) are not tabulated.

[b] Numbers in parentheses represent the percentage of 28 combinations.

[c] Numbers in parentheses represent the percentage of 96 combinations.

[d] Totals (and percentages) include two cases of synergism (CN, NA) and one of antagonism (GE) with *Proteus* species, and one case of synergism (R) and one case of antagonism (CN) with *P. aeruginosa*.

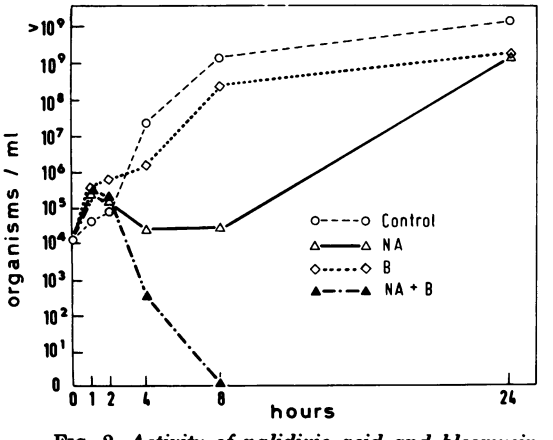

FIG. 2. *Activity of nalidixic acid and bleomycin individually and in combination on a strain of E. coli. NA, Nalidixic acid (2 μg/ml); B, bleomycin (2 μg/ml); NA + B, nalidixic acid (2 μg/ml) plus bleomycin (2 μg/ml). (MBC of nalidixic acid, 8 μg/ml; MBC of bleomycin, 8 μg/ml).*

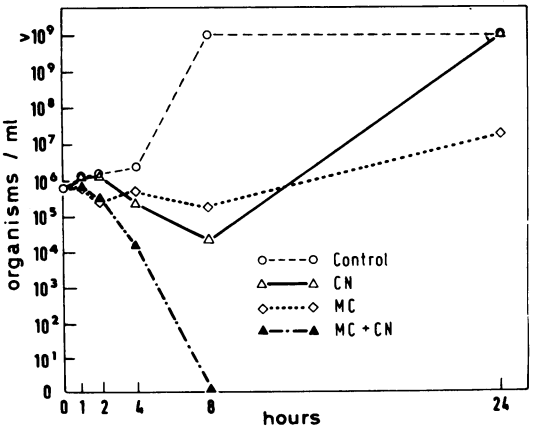

FIG. 3. *Activity of carbenicillin and mitomycin C individually and in combination on a strain of P. aeruginosa. CN, Carbenicillin (32 μg/ml); MC, mitomycin C (2 μg/ml); CN + MC, carbenicillin (32 μg/ml) plus mitomycin C (2 μg/ml). (MBC of carbenicillin, 128 μg/ml; MBC of mitomycin C, 8 μg/ml).*

P. rettgeri for which dactinomycin was bactericidal, synergism was demonstrated with chloramphenicol. The resistance of gram-negative bacilli to dactinomycin has been attributed to the impermeability of the cell wall or cytoplasmic membrane to the antibiotic (6, 15). Nakajima and Kawamata (19) demonstrated that dactinomycin inhibited the growth of *E. coli* in the presence of subinhibitory concentrations of col-

TABLE 3. *FBC indices of antagonistic and synergistic combinations of antimicrobial and antineoplastic drugs*

Organism/ strain no.	Antineoplastic agent	MBC (μg/ml)		FBC	Antimicrobial agent	MBC (μg/ml)		FBC	ΣFBC
		Alone	In combination			Alone	In combination		
E. coli									
2	Bleomycin	8	>2	0.25	Gentamicin	4	>8	>2	>2.25
2	Bleomycin	8	2	0.25	Gentamicin	4	1	0.25	0.50
2	Bleomycin	8	2	0.25	Nalidixic acid	8	2	0.25	0.50
3	Bleomycin	16	2	0.12	Nalidixic acid	16	4	0.25	0.37
4	Bleomycin	16	2	0.12	Nalidixic acid	>32	1	<0.03	<0.15
2	Mitomycin C	4	0.25	0.06	Chloramphenicol	>125	4	<0.03	<0.09
3	Mitomycin C	4	0.25	0.06	Chloramphenicol	>500	16	<0.03	<0.09
5	Mitomycin C	8	1	0.12	Chloramphenicol	250	16	0.06	0.18
2	Mitomycin C	4	1	0.25	Nalidixic acid	8	2	0.25	0.50
4	Mitomycin C	4	0.5	0.12	Nalidixic acid	16	8	0.50	0.62
2	Mitomycin C	4	0.25	0.06	Gentamicin	4	1	0.25	0.31
3	Mitomycin C	4	1	0.25	Gentamicin	8	1	0.12	0.37
5	Mitomycin C	16	4	0.25	Gentamicin	8	1	0.12	0.37
P. aeruginosa									
4	Mitomycin C	8	2	0.25	Carbenicillin	128	32	0.25	0.50
8	Mitomycin C	8	2	0.25	Carbenicillin	128	32	0.25	0.50
9	Mitomycin C	16	4	0.25	Carbenicillin	64	16	0.25	0.50
K. pneumoniae									
3	Doxorubicin	64	8	0.12	Rifampin	>256	32	<0.12	<0.24

istin and suggested that this was due to the effect of colistin on the bacterial cell surface. With the cellophane transfer technique, colistin was bactericidal for 21 of the 28 strains tested and showed no synergism with dactinomycin on these strains. With the 7 strains resistant to colistin we failed to show synergism, in contrast to our previous findings (14) with staphylococci, which are inherently resistant to colistin, and in which 7 of 10 strains showed synergism with the dactinomycin-colistin combination.

Mitomycin C demonstrated frequent synergism, and this was prominent with the aminoglycosides in all species. In addition to its inhibition of DNA synthesis, Coles and Gross (9) observed that mitomycin C inhibited penicillinase synthesis in *S. aureus*. We have shown (14) that combinations of β-lactam antibiotics with mitomycin C were often synergistic on *S. aureus*, but with the gram-negative bacilli synergism was mainly noted in combinations with carbenicillin on *E. coli* and *P. aeruginosa*.

Bleomycin rarely showed synergism. Antagonism was frequently demonstrated in combinations with chloramphenicol and with rifampin. This is in accordance with our previous findings with *S. aureus* (14). Cohen and I (8) reported that chloramphenicol, which blocks protein synthesis and relaxes ribonucleic acid synthesis in stringent strains of *E. coli*, significantly inhibited the lethal action of bleomycin. Bleomycin has been shown (13) to damage bacterial DNA and induce production of protein X, an inhibitor of cell septation and cell division. This induction can be blocked by either chloramphenicol or rifampin (13), and Satta and Pardee (20) showed that rifampin improved the recovery of *E. coli* whose deoxyribonucleic acid had been damaged by treatment with another drug. Bleomycin-gentamicin combinations showed both synergism and antagonism on *E. coli*. Such concentration-dependent synergism-antagonism in the bleomycin-gentamicin combination (and in mithramycin-cephaloridine and mithramycin-methicillin combinations) has also been shown (14) in *S. aureus*. This phenomenon has also been described in combinations of two antibacterial antibiotics (17).

The antagonism seen between daunorubicin and doxorubicin and the aminoglycosides has also been described by Moody et al. (18), who observed antagonism on strains of *K. pneumoniae* and *P. aeruginosa* between daunorubicin and gentamicin with concentrations equivalent

to those achievable in the blood. They suggested that such antagonism might interfere with effective therapy against *K. pneumoniae* and that lack of response to therapy with gentamicin during concurrent daunorubicin treatment should lead to re-evaluation of antimicrobial therapy. Both the anthracyclines showed antagonism with carbenicillin on two strains of *E. coli* and one strain of *P. aeruginosa*. Moody et al. (18), however, found no evidence of antagonism between daunorubicin and ticarcillin, an analog of carbenicillin.

The pharmacokinetics of the antineoplastic antibiotics are incompletely known, but we found that the MBCs of the antibacterial drugs required in the combinations tested were in general similar to those concentrations attainable in the serum after therapeutic doses (1), except for rifampin (16).

Previous work has shown that an in vitro synergistic bactericidal effect and also antagonism occurred in combinations of antineoplastic antibiotics with antibacterial drugs in *S. aureus* (14). The present investigation shows that these phenomena also occur in four species of gram-negative bacilli, but the frequency of these reactions was lower than those found with *S. aureus* (14).

Although synergism and antagonism between these groups of drugs do not seem to have occurred in humans (21), the present in vitro findings indicate that the possibility should be considered.

The clinical significance of these results is not known, and may be minimal, since the antineoplastic drugs are rarely continued after the patient has developed signs of infection. Moreover, these drugs are not usually given in multiple daily dosage at the present time, but this information should be available since treatment protocols may change in the future.

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