ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1979, p. 580-586
0066-4804/79/04-0580/07$02.00/0

Vol. 15, No. 4

Bactericidal Effect of Combinations of Antimicrobial Drugs and Antineoplastic Antibiotics Against *Staphylococcus aureus*

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Received for publication 29 January 1979

Six antineoplastic antibiotics were tested against ten strains of *Staphylococcus aureus*. Four showed bacteriostatic and/or bactericidal activity against each of the ten strains, and two were only bacteriostatic for seven and nine strains, respectively. Using the cellophane transfer technique, combinations of these antineoplastic antibiotics with 16 antibacterial drugs were screened for combined bactericidal activity. Synergism or antagonism was demonstrated in about one-third of the combinations. Checkerboard titrations and killing curves confirmed these findings and indicated that the effective concentrations of the antibacterial agents were similar to those attainable in the serum after therapeutic doses of these drugs. Although the pharmacokinetics of the six antineoplastic antibiotics in humans are not fully known, at least one of them has a peak serum level corresponding to those values at which a bactericidal effect was produced in vitro.

Synergism and antagonism between combinations of antibacterial antibiotics has been well documented. Little attention, however, has been paid to possible synergism and antagonism between antibacterial drugs and antineoplastic antibiotics. Most antineoplastic antibiotics have measurable antibacterial activity but are not used to treat bacterial infections because of a low therapeutic index.

In 1967, Manten and Terra (13) described antagonism and indifference, but no synergism, between combinations of two antineoplastic antibiotics, dactinomycin and mitomycin C, and ampicillin and penicillin G when tested on *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. Since then, many new antimicrobial agents and antineoplastics have been introduced into clinical use, but there has been no systematic study of possible synergism or antagonism between these two groups of agents. Such a study might be of clinical importance and possible theoretical interest.

Most patients receiving antineoplastic drugs are potential recipients of antibacterial therapy because of their increased susceptibility to infections, and they frequently receive both types of agents simultaneously. We therefore decided to investigate a variety of combinations of antibacterial and antineoplastic agents against a series of different bacteria. This paper reports the results with strains of *S. aureus*.

(This work has been 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

Ten strains of *S. aureus* were examined. Of these, nine were strains isolated from pus, blood, or urine cultures in the Department of Clinical Microbiology of the Hadassah-Hebrew University Hospital. (The minimal bactericidal concentrations [MBC] of penicillin G for two of these nine strains were 16 and 64 μg/ml, respectively, and for seven strains MBCs were 256 μg/ml or higher. Strain 10 was *S. aureus* [Oxford] NCTC 6571, a non-penicillinase producer. The MBC of penicillin G was 1.0 μg/ml.) Minimum inhibitory concentrations/MBCs were determined by using serial dilutions of both the antibacterial and the antineoplastic drugs inoculated with an overnight culture of the organism to give a final concentration of 10^6 bacteria per ml.

Each of 16 antibacterial drugs was combined with each of 6 antineoplastic antibiotics, all of which are in routine clinical use, and the 96 combinations were screened against 10 strains of *S. aureus*, using the cellophane transfer technique (3) as modified by Cluzel et al. (6).

Full details of the technique have been reported (15). Commercially available cellophane (PO 300, 0.019 mm thick) was used for the tambours. The impregnated strips were prepared by immersing strips of Whatman no. 3 chromatographic paper in solutions of the drugs shown in Table 1 and drying at 37°C. These concentrations of the antibacterial drugs have been previously described as giving suitable test diffusion levels (5), but these authors did not examine the antineoplastic antibiotics. To select the concentrations of antineoplastic antibiotics shown in Table 1, we initially prepared several strips for each antineoplastic

TABLE 1. *Concentrations used to impregnate paper strips for the cellophane transfer technique*

Drug	Symbol	Concn (μg/ml)
Antibacterial		
Ampicillin	AM	500
Carbenicillin	CN	2,000
Cephaloridine	CD	500
Chloramphenicol	C	1,000
Clindamycin	CL	500
Colistin	CO	650
Erythromycin	E	200
Gentamicin	GE	500
Kanamycin	K	500
Methicillin	ME	1,000
Nalidixic acid	NA	500
Penicillin G	P	50
Rifampin	R	10
Streptomycin	S	500
Tetracycline	T	200
Vancomycin	V	400
Antineoplastic		
Bleomycin	B	100
Dactinomycin	AD	50
Daunorubicin	DN	200
Doxorubicin	DX	100
Mithramycin	MT	50
Mitomycin C	MC	100

drug, using varying concentrations of the drug in the impregnating solutions. The strip chosen for the tambour test was one that gave a zone of inhibition of 4 to 5 mm with strains of *S. aureus* seeded on agar plates. Care was taken to avoid "carryover" (5). Oxoid DST agar was used for the diffusion and transfer plates. The inoculum contained approximately 10^6 organisms per ml and was obtained by dilution of an overnight culture in Trypticase soy broth (Difco). All the results were classified as synergism, indifference, or antagonism by two of the authors independently, using previously published criteria (4, 10). Doubtful results were duplicated or even triplicated. On the basis of the cellophane transfer results, 11 combinations were chosen from groups of drugs that frequently showed synergism or antagonism, and these combinations were subjected to quantitative evaluation on several strains, using the checkerboard technique. Combinations which rarely showed synergism or antagonism were not chosen for quantitative analysis. Box titrations were performed, with doubling dilutions in Trypticase soy broth of the antimicrobial drug, the antineoplastic antibiotic, and every combination of each dilution of each agent. An inoculum of 0.05 ml of a 1:100 dilution of an overnight culture was added to each tube to give a final concentration of 10^5 to 10^6 organisms per ml. Minimum inhibitory concentrations were read after incubation at 37°C overnight. Quantitative subcultures were made of those tubes not showing growth by plating 0.01 ml on DST agar supplemented with 2% whole blood. Colonies were counted, and MBCs (99.9% kill) were recorded after overnight incubation of the plates at 37°C.

Fractional bactericidal concentrations (FBC) 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 (ΣFBC). By analogy with criteria defined by the Study Group (17) for the index of fractional inhibitory concentrations, a ΣFBC of <0.6, which is equivalent to reducing the concentration of each agent in the mixture to one-fourth of its MBC, was classified as significant synergy. An FBC index of >1.3 was regarded as significant antagonism.

Seven examples of synergism or antagonism were examined to follow the kinetic action of the drugs individually and in combination during 24 h, using concentrations of each drug selected from checkerboard titrations. One-tenth milliliter of a suitably diluted culture (prepared as above) was added to 2 ml of Trypticase soy broth containing the selected concentrations of the drugs alone or in combination. The tubes were incubated at 37°C, and samples were taken for viable counts at time intervals from 0 to 24 h.

RESULTS

Table 2 shows the antibacterial activity detected by the cellophane transfer technique of the antineoplastic agents acting alone. On removal of the tambour from the antibiotic-treated agar medium and its transfer to antibiotic-free medium, growth occurred on the tambour where the drug had been bacteriostatic and not where it had been bactericidal.

Dactinomycin was bacteriostatic and mitomycin C was bactericidal for all 10 strains. Daunorubicin was usually bactericidal, whereas its 14-hydroxy derivative, doxorubicin, was more frequently bacteriostatic. Bleomycin and mithramycin were either bacteriostatic or inactive. Minimum inhibitory concentrations/MBCs of the antimicrobial drugs and the antineoplastic antibiotics, determined by the serial dilution method, are shown in Table 3.

Ninety-six combinations were tested by the cellophane transfer technique on each strain, and the synergistic and antagonistic results are shown in Table 4. Although indifference was the most common outcome, synergism and antagonism occurred in 16.9 and 15.1% of the combinations, respectively.

The highest frequency of synergistic combinations was seen with mitomycin C (39%) and dactinomycin (35%). Antagonism was most fre-

TABLE 2. *Qualitative anti-staphylococcal activity of the antineoplastic antibiotics as determined by the cellophane transfer technique*

Activity on no. of strains indicated	No. of strains					
	AD[a]	MC	DN	DX	B	MT
Inactive	0	0	0	0	3	1
Bacteriostatic	10	0	2	7	7	9
Bactericidal	0	10	8	3	0	0

[a] See Table 1 for key to symbols.

TABLE 3. *Minimum inhibitory concentration (MIC) and MBCs (in micrograms per milliter) of antibiotics against nine clinical isolates of S. aureus* and *S. aureus (Oxford strain)*

Agent	Nine clinical isolates				Oxford strain	
	MIC		MBC		MIC	MBC
	Range	Median	Range	Median		
Antibacterial						
Penicillin G	16->256	256	16->256	256	0.03	1
Ampicillin	4->256	>256	4->256	>256	0.03	0.25
Carbenicillin	4-256	8	4->256	256	0.5	0.5
Methicillin	1-128	2	8->512	>512	2	2
Cephaloridine	0.03-1	0.12	32->512	128	<0.12	2
Vancomycin	1-4	2	1-4	2	1	1
Streptomycin	16->256	32	16->256	64	62	125
Kanamycin	4->256	16	4->256	64	16	16
Gentamicin	0.06-4	1	0.25-4	2	1	1
Erythromycin	<0.06->64	0.5	0.1->64	0.5	1	4
Clindamycin	<0.06->64	0.1	<0.06->64	1	<0.06	0.5
Chloramphenicol	4-32	4	4->512	64	2	4
Tetracycline	<0.01-32	16	<0.01-64	32	<0.01	0.25
Rifampin	<0.06-<0.06	<0.06	<0.06-1	<0.06	<0.06	<0.06
Colistin	256->512	>512	256->512	>512	>512	>512
Nalidixic acid	32-125	64	32->512	>512	32	>512
Antineoplastic	128-256	256	128->256	256	256	256
Bleomycin	0.25-8	4	2-3 2	16	0.12	32
Dactinomycin	4-16	8	4-32	8	4	4
Daunorubicin	8-32	16	8-32	32	16	16
Doxorubicin	0.12-0.5	0.25	16->16	16	0.06	0.06
Mithramycin	0.12-0.5	0.25	0.5->16	1	0.06	0.12
Mitomycin C						

quent with dactinomycin (24%) and mithramycin (25%). With the latter drug antagonism was 10 times more frequent than synergism. With daunorubicin, doxorubicin, and bleomycin, synergism and antagonism were rarely seen.

The high percentage of synergism seen with dactinomycin resulted mainly from its combination with chloramphenicol, tetracycline, colistin, or nalidixic acid. The combination of dactinomycin with aminoglycosides gave a high frequency of synergism with streptomycin and kanamycin, whereas with gentamicin the most frequently observed result was antagonism. Antagonism was frequent with the penicillinase-resistant β-lactam antibiotics (methicillin and cephaloridine) and also with clindamycin.

Mitomycin C was often synergistic with the penicillinase-resistant β-lactam antibiotics and with gentamicin, clindamycin, chloramphenicol, and rifampin. Antagonism was infrequent except with colistin and nalidixic acid, where it represented the most frequent outcome of the combinations.

With daunorubicin and doxorubicin, synergism was rarely seen except with erythromycin, but antagonism was relatively frequent with vancomycin, gentamicin, and clindamycin. Bleomycin was generally indifferent in combination but gave antagonism with clindamycin, chloramphenicol, and in half of the combinations with rifampin.

Combinations of mithramycin showed antagonism with the β-lactam antibiotics streptomycin and clindamycin.

The results of checkerboard titrations confirmed those obtained in the qualitative screening tests for synergism and antagonism using the cellophane transfer technique. They showed that both synergism and antagonism occurred at low concentrations of each drug.

FBCs were calculated and are shown, together with ΣFBC, in Table 5 (synergistic combinations) and Table 6 (antagonistic combinations).

The results of the kinetic studies confirmed the synergism or antagonism found with these combinations in the cellophane transfer screening.

The most dramatic results were seen with the

TABLE 4. *Number of strains showing synergism (S) or antagonism (A) in combinations of antineoplastic and antibacterial drugs*

	AD[a]		MC		DN		DX		B		MT		Total	
	S	A	S	A	S	A	S	A	S	A	S	A	S(%)[b]	A(%)[b]
P[a]	2	1[c]	1	1	1[c]	0	1[c]	0	0	0	0	2[c]	5 (8.3)	4 (6.6)
AM	2	1[c]	3[c]	1	1[c]	0	1[c]	0	0	0	0	2[c]	7 (11.6)	4 (6.6)
CN	3	3[c]	4[c]	1	1[c]	0	0	0	1	0	0	4[c]	9 (15)	8 (13.3)
ME	1	6[c]	8[c]	1	1	0	1	0	1	0	0	5	12 (20)	12 (20)
CD	1	7[c]	6[c]	2	0	1	0	1	1	0	0	6[c]	8 (13.3)	17 (27.4)
V	1	0	1	0	0	3	1[c]	3	1	0	0	1	4 (6.6)	
S	5	2	2	2	1	1	0	1	0	0	0	4	8 (13.3)	10 (16.7)
K	4	2	2	1	1	1	3	1	1	0	1	1	12 (20)	6 (10)
GE	0	6	7	0	0	2	2	4	1	1	1	2	11 (18.4)	15 (25)
E	3	0	4	3	3	0	4	0	1	0	1	1	16 (26.6)	4 (6.6)
CL	1	7	6	0	1	3	0	4	0	3	0	6	8 (13.3)	23 (38.4)
C	9	0	6	1	1	2	1	0	0	3	0	2	17 (27.4)	8 (13.3)
T	8	0	4	2	1	1	2	0	0	1	0	0	15 (25)	4 (6.6)
R	4	0	6	2	0	1	1	2	0	5	0	0	11 (18.4)	10 (16.7)
CO	7	0	3	4	0	1	1	0	0	0	0	0	1 (18.4)	5 (8.3)
NA	6	0	0	6	0	2	1	0	0	0	0	0	7 (11.6)	8 (13.3)
Total	57	35	63	27	12	18	19	16	7	13	3	36	161 (16.9)	145 (15.1)
%[d]	(35)	(24)	(39)	(19)	(8)	(12)	(12)	(11)	(4)	(9)	(2)	(25)		

[a] See Table 1 for key to symbols.
[b] Numbers in parentheses represent the percentage of 60 combinations.
[c] Includes *S. aureus* (Oxford).
[d] Numbers in parentheses represent the percentage of 160 combinations.

TABLE 5. *FBC indexes of synergistic combinations of antimicrobial and antineoplastic agents*

Strain	Antineoplastic agent	MBC (μg/ml)		FBC	Antimicrobial agent	MBC (μg/ml)		FBC	ΣFBC
		Alone	In combination			Alone	In combination		
10	Dactinomycin	4	0.5	0.12	Methicillin	2	0.25	0.12	0.24
4	Dactinomycin	8	4	0.5	Chloramphenicol	>512	1	<0.01	<0.51
7	Dactinomycin	>32	16	<0.5	Chloramphenicol	>512	2	<0.01	<0.51
4	Dactinomycin	16	4	0.25	Tetracycline	64	16	0.25	0.50
2	Mitomycin C	0.5	0.12	0.25	Methicillin	>512	8	<0.01	<0.26
4	Mitomycin C	0.5	0.12	0.25	Methicillin	8	1	0.12	0.37
7	Mitomycin C	0.5	0.06	0.12	Methicillin	>512	2	<0.01	<0.13
4	Mitomycin C	0.5	0.06	0.12	Cephaloridine	128	2	0.02	0.14
7	Mitomycin C	0.5	0.25	0.5	Cephaloridine	64	0.12	0.02	0.52
2	Mitomycin C	0.5	0.12	0.25	Gentamicin	4	0.5	0.12	0.37
4	Mitomycin C	1	0.25	0.25	Gentamicin	4	0.12	0.03	0.28
7	Mitomycin C	0.5	0.25	0.5	Gentamicin	4	0.12	?.03	0.53
2	Mitomycin C	1	0.25	0.25	Chloramphenicol	128	2	0.02	0.27
4	Mitomycin C	0.5	0.06	0.12	Chloramphenicol	32	4	0.12	0.24
7	Mitomycin C	0.5	0.12	0.25	Choloramphenicol	128	2	0.02	0.27
2	Mitomycin C	0.5	0.25	0.5	Nalidixic acid	>512	16	<0.03	<0.53
4	Mitomycin C	0.5	0.25	0.5	Nalidixic acid	>512	16	<0.03	<0.53
10	Mitomycin C	0.12	0.03	0.25	Nalidixic acid	>512	32	<0.06	<0.31
8	Mithramycin	>16	0.12	<0.01	Methicillin	2	1	0.5	<0.51
5	Mithramycin	>16	0.06	<0.01	Cephaloridine	512	0.12	0.01	<0.02

combination of 1/4 MBC of mitomycin C and 1/8 MBC of methicillin, which was completely bactericidal within 4 h, and the combination of mitomycin C (1/4 MBC) and chloramphenicol (1/64 MBC) which was also completely bactericidal within 4 h (see Fig. 1a). Mitomycin C (1/4 MBC) and gentamicin (1/8 MBC) were bactericidal after 8 h.

TABLE 6. *FBC indexes of antagonistic combinations of antimicrobial and antineoplastic agents*

Strain	Antineoplastic agent	MBC (μg/ml)		FBC	Antimicrobial agent	MBC (μg/ml)		FBC	ΣFBC
		Alone	In combination			Alone	In combination		
4	Dactinomycin	8	8	1	Methicillin	8	8	1	2
10	Dactinomycin	4	1	0.25	Methicillin	2	>8	>4	>4.25
4	Dactinomycin	16	6	1	Cephaloridine	16	16	1	2
10	Dactinomycin	32	8	0.25	Cephaloridine	2	>8	>4	>4.25
4	Mithramycin	16	1	0.06	Methicillin	8	>32	>4	>4.06
10	Mithramycin	>16	1	<0.06	Cephaloridine	0.06	8	>100	>100

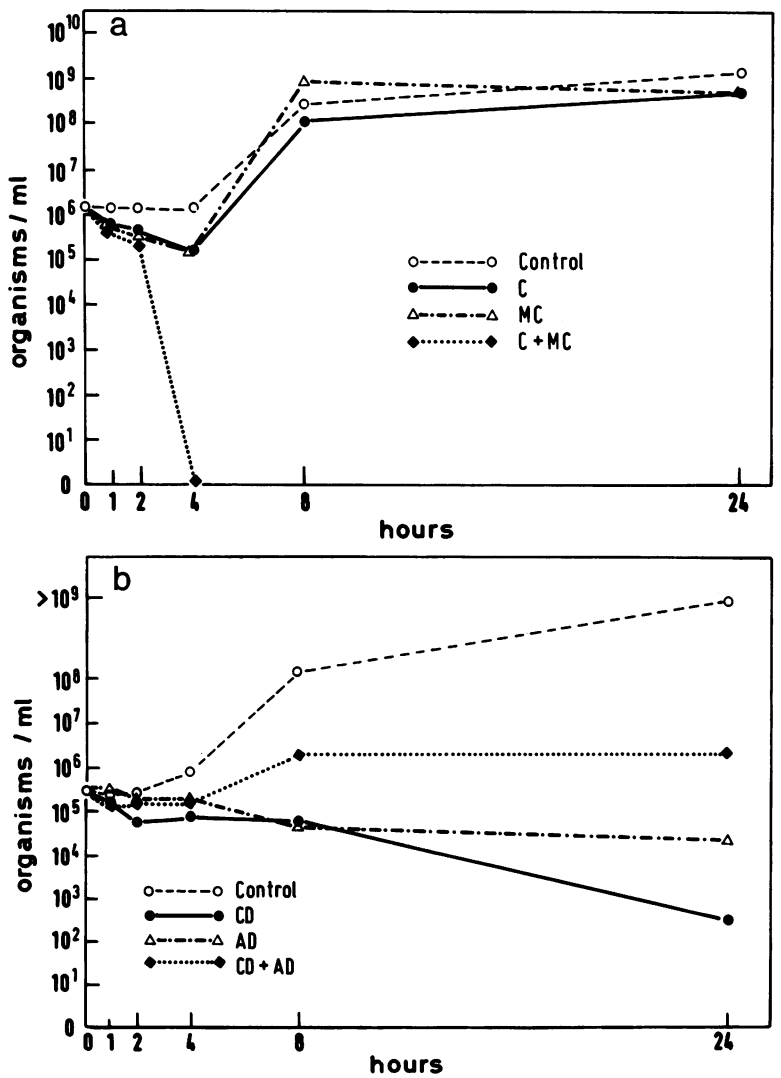

FIG. 1. *(a) Activity of mitomycin and chloramphenicol individually and in combination on S. aureus strain 7. C, Chloramphenicol, 2 μg/ml; MC, mitomycin 0.125 μg/ml; C+MC, chloramphenicol, 2 μg/ml, plus mitomycin, 0.125 μg/ml. (MBC of chloramphenicol, 128 μg/ml; MBC of mitomycin, 0.5 μg/ml.) (b) Activity of dactinomycin and cephaloridine individually and in combination on S. aureus strain 10. CD, Cephaloridine, 8 μg/ml; AD, dactinomycin, 0.5 μg/ml; CD+AD, cephaloridine, 8 μg/ml, plus dactinomycin, 0.5 μg/ml. (MBC of cephaloridine, 2 μg/ml; MBC of dactinomycin, >32 μg/ml.)*

The antagonism of dactinomycin and cephaloridine was confirmed by kinetic studies, and Fig. 1b shows that 1/64 MBC of dactinomycin with 4× the MBC of cephaloridine failed to sterilize the culture.

DISCUSSION

Our results show that synergism and antagonism are not phenomena restricted to combinations of common antimicrobial drugs but also occur with combinations of antineoplastic antibiotics and antimicrobial agents. With all the antibacterial drugs these concentrations are below those which can be achieved in the serum on standard therapeutic doses (2), and with at least one of the antineoplastic agents, mitomycin C, the serum concentrations (0.52 to 2.7 μg/ml) (8) are well above those at which synergism was shown.

Some agreement was seen with the general scheme of combined antibiotic action of Jawetz and Gunnison (12) as modified by Manten and Wisse (14) in that combinations of two bactericidal antibiotics were either synergistic or indifferent but rarely antagonistic; combinations of two bacteriostatic antibiotics were generally synergistic or indifferent; and combinations of a bactericidal with a bacteriostatic antibiotic resulted in antagonism or occasionally synergism or indifference.

We found that the combination of the bacteriostatic agents dactinomycin and chloramphenicol was synergistic on nine out of ten strains of *S. aureus*. Dactinomycin specifically inhibit deoxyribonucleic acid-directed ribonucleic acid synthesis by binding to the deoxyribonucleic acid to form a fairly stable complex; Acs et al. (1) have shown that dactinomycin also depolymerizes ribonucleic acid, especially that which accumulates in the presence of chloramphenicol. Dactinomycin showed a high degree of synergism with other bacteriostatic drugs blocking protein synthesis (erythromycin and tetracycline).

With chloramphenicol and tetracycline, the high level of synergism with mitomycin C, which is bactericidal, was not in accordance with the general scheme (12, 14). A kinetic study of the combination of mitomycin C with chloramphenicol showed a killing action similar to that of two bactericidal drugs in combination. The enhanced breakdown of bacterial deoxyribonucleic acid by mitomycin C when combined with chloramphenicol has previously been described by Constantopoulos and Tchen (7), who stated that all compounds which inhibit protein synthesis were found to enhance mitomycin C-induced DNA breakdown in *E. coli* B3. In strains of *S. aureus* we found that erythromycin and tetracycline also showed a high level of synergism in combination with mitomycin C.

Bleomycin, which was largely bacteriostatic, showed antagonism with clindamycin (bactericidal in all strains), chloramphenicol, and also with rifampin, which was bacteriostatic for three strains and bactericidal for two others. Garrod et al. (9) noted that the rifamycins do not obey the law on combined action.

Unexpected results were obtained with combinations of colistin and nalidixic acid, neither of which was active against the test strains. Dactinomycin showed synergism not only with colistin, which increases membrane permeability (16), but also with nalidixic acid, which inhibits the synthesis of nuclear deoxyribonucleic acid in susceptible bacteria (11). Both nalidixic acid and colistrin antagonized the bactericidal effect of mitomycin C, a drug which inhibits deoxyribonucleic acid synthesis. These results suggest that widely different mechanisms of synergism and antagonism are involved in these cases.

Combinations of the antineoplastic antibiotics with aminoglycosides were infrequently antagonistic except for the combinations of gentamicin with dactinomycin and doxorubicin and of streptomycin with mithramycin. Since the mode of action of the aminoglycosides is similar, these differences in the outcome of combinations of antineoplastic antibiotics with different aminoglycosides are quite inexplicable, but such differences have been reported in the combination of aminoglycosides with nalidixic acid (15).

This work shows that combinations of antineoplastic antibiotics and antimicrobial agents frequently demonstrated synergism or antagonism in vitro. The relevance of these findings to clinical therapy is unknown, but since individual patients often receive combinations of antineoplastic antibiotics with antibacterial drugs, the possibility of advantageous or unfavorable interactions should be considered.

ACKNOWLEDGMENTS

Thanks are due to the following for gifts of antibiotics: Beecham Research Laboratories (semisynthetic penicillins), Lepetit SpA (rifampin), E. Lilly (vancomycin), Merck Sharp and Dohme Inc. (dactinomycin), Nippon Kayaku Co. (bleomycin), Nissen Preminger, Tel Aviv (mithramycin), UCB-Smith SpA (colistin), Upjohn Ltd. (clindamycin), Winthrop Laboratories (nalidixic acid).

This work was supported in part by a study grant from the Hadassah Medical Organisation.

LITERATURE CITED

1. **Acs, G., E. Reich, and S. Valanju.** 1963. RNA metabolism of B. subtilis. Effects of actinomycin. Biochim. Biophys. Acta **76**:68-79.
2. **Barker, B. M., and F. Prescott.** 1973. Antimicrobial agents in medicine, p. 152-158. Blackwell Scientific Publications, Oxford.
3. **Chabbert, Y. A.** 1957. Une technique nouvelle d'etude de l'action bactericide d'antibiotiques: le transfert sur cel-

lophane. Ann. Inst. Pasteur Paris **93**:289–299.
4. **Chabbert, Y. A., and J. C. Patte.** 1960. Cellophane transfer: application to the study of activity of combinations of antibiotics. Appl. Microbiol. **8**:193–199.
5. **Chabbert, Y. A., and P. M. Waterworth.** 1965. Studies on the "carry-over" of antibiotics using the cellophane transfer technique. J. Clin. Pathol. **18**:314–316.
6. **Cluzel, R. R., R. Vaurs, M. Cluzel-Nigay, and M. Verner.** 1960. Une nouvelle technique d'etude du pouvoir bactericide des associations d'antibiotiques derivee du "transfert sur cellophane:" la disposition en croix. Ann. Inst. Pasteur Paris **98**:928–932.
7. **Constantopoulos, G., and T. T. Tchen.** 1964. Enhancement of mitomycin-C breakdown of DNA by inhibitors of protein synthesis. Biochim. Biophys. Acta **80**:456–462.
8. **Crooke, S. T., and W. T. Bradner.** 1976. Mitomycin-C: a review. Cancer Treat. Rev. **3**:121–139.
9. **Garrod, L. P., H. P. Lambert, and F. O'Grady.** 1973. Antibiotics and chemotherapy, 4th ed., p. 221. Churchill-Livingstone, Edinburgh.
10. **Garrod, L. P., and P. M. Waterworth.** 1962. Methods of testing combined antibiotic bactericidal action and the significance of the results. J. Clin. Pathol. **15**:328–338.
11. **Goss, W. A., W. H. Deitz, and T. M. Cook.** 1965. Mechanism of action of nalidixic acid on *Escherichia coli*. II. Inhibition of deoxyribonucleic acid and synthesis. J. Bacteriol. **89**:1068–1074.
12. **Jawetz, E., and J. B. Gunnison.** 1952. Studies on antibiotic synergism and antagonism: a scheme of combined antibiotic action. Antibiot. Chemother. **2**:243–248.
13. **Manten, A., and J. I. Terra.** 1967. Some observations on antagonism between penicillin and antineoplastic antibiotics. Acta Physiol. Pharmacol. Neerl. **14**:250–258.
14. **Manten, A., and M. J. Wisse.** 1961. Antagonism between antibacterial drugs. Nature (London) **192**:671–672.
15. **Michel, J., R. Luboshitsky, and T. Sacks.** 1973. Bactericidal effect of combinations of nalidixic acid and various antibiotics on *Enterobacteriaceae*. Antimicrob. Agents Chemother. **4**:201–204.
16. **Sebek, O. K.** 1967. Polymyxins and circulin, p. 142–152. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics, vol. 1, Mechanism of action. Springer-Verlag, Berlin.
17. **Study Group.** Japan Cooperative Bacteriological Study Group for Cotrimoxazole. 1973. Analysis of in-vitro antibacterial activities of the combination of trimethoprim and sulfamethoxazole on clinical isolates in Japan. J. Infect. Dis. **128**(Suppl.):502–507.