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Interactions of β-Lactam Antibiotics and Antineoplastic Agents

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The in vitro interactions of four β-lactam antibiotics and five antineoplastic agents were examined with 100 clinically isolated strains of four species of gram-negative bacilli. Generally, by the checkerboard dilution method, β-lactam antibiotics, when tested in combination with mitomycin C, bleomycin, or 5-fluorouracil, showed synergistic action, whereas when tested in combination with carboquone, they showed antagonistic action. Almost no combinations of adriamycin showed the interactions. Among β-lactam antibiotics, piperacillin was more frequently synergistic than cefoperazone, cefazolin, or carbenicillin when tested in combination with each antineoplastic agent against various species.

The course of patients undergoing cancer therapy is apt to be complicated by infection; therefore, combination therapy with antimicrobial agents and antineoplastic agents is given in many cases. Manten and Terra (9), Jacobs et al. (7), and Michel et al. (10) reported that some combinations of antimicrobial agents and antineoplastic agents showed synergistic interactions in vitro.

In the present study, we investigated the in vitro interactions of two new β-lactam antibiotics, cefoperazone and piperacillin, with a broad antibacterial spectrum and antineoplastic agents (mitomycin C, bleomycin, adriamycin, 5-fluorouracil, and carboquone) against gram-negative bacilli, using cefazolin and carbenicillin as controls.

MATERIALS AND METHODS

Isolates. Twenty-five fresh clinical isolates each of *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris*, and *Pseudomonas aeruginosa* were used.

Drugs. The β-lactam antibiotics studied were cefoperazone (Toyama Chemical Co.), cefazolin (Fujisawa Pharmaceutical Co.), piperacillin (Toyama Chemical Co.), and carbenicillin (Fujisawa Pharmaceutical Co.). The antineoplastic agents studied were mitomycin C (Sankyo Co.), bleomycin (Nihon Kayaku Co.), adriamycin (Kyowa Hakko Co.), 5-fluorouracil (Fuji Chemical Co.), and carboquone (Sankyo Co.).

Antibacterial activity test. Checkerboard dilution tests were performed by twofold serial dilution (100 to 0.2 μg/ml) in Mueller-Hinton agar (Eiken Co.) of β-lactam antibiotics, antineoplastic agents, and every combination of each dilution of each drug.

The bacteria were cultured overnight in Mueller-Hinton broth (Difco Laboratories) at 37°C, and one loopful of a 1/100 dilution (approximately 10^4 CFU) was inoculated on the plate. After overnight culture, the minimum inhibitory concentration (MIC) was measured by the presence or absence of the growth of the bacteria.

Drug interaction was determined by the method described by Elion et al. (3) and Berenbaum (1). The fractional inhibitory concentrations (FICs) of the β-lactam antibiotics were calculated by dividing the inhibitory concentration of the β-lactam antibiotic in the combination for 50% of the isolates (MIC_{50}) by the MIC_{50} of the β-lactam antibiotic alone; the FICs of the antineoplastic agents were similarly calculated. The sum of the FICs of the two drugs tested was the FIC index. After determining the MIC_{50} for each β-lactam antibiotic alone and with various concentrations of an antineoplastic agent, we calculated the FIC indices by fixing the FICs for the antineoplastic agent within the range of 0.05 to 0.60. Similarly, the FIC indices were determined by fixing the FICs for the β-lactam antibiotic within the range of 0.05 to 0.60. The mean of selected four to eight FIC indices was then determined. According to the criteria defined by Jacobs et al. (7) for the fractional bactericidal concentrations, a mean FIC index of <0.60 was classified as synergy. A mean FIC index of >1.30 was regarded as antagonism.

Growth curves. An overnight culture of *P. aeruginosa* S68 was inoculated into fresh Mueller-Hinton broth. After preincubation, a log-phase culture was diluted to a density of 10^4 cells per ml, and the selected concentrations of the drugs alone or in combination were added. The number of viable cells was determined at 2, 4, 6, and 24 h after drug addition.

RESULTS

Antibacterial activity of individual drugs. (i) β-Lactam antibiotics. Cefoperazone and piperacillin showed much better antibacterial activity than did cefazolin and carbenicillin against four

TABLE 1. Comparative antibacterial activities of β-lactam antibiotics

Species (no. of isolates)	β-Lactam antibiotic	MIC (μg/ml)		
		Range	50%	90%
E. coli (25)	Cefoperazone	≤0.20–3.13	≤0.20	0.70
	Cefazolin	0.78–3.13	1.17	2.20
	Piperacillin	≤0.20–>100	1.22	>100
	Carbenicillin	1.56–>100	4.75	>100
K. pneumoniae (25)	Cefoperazone	≤0.20–>100	0.34	>100
	Cefazolin	1.56–>100	1.81	17.8
	Piperacillin	1.56–>100	14.8	>100
	Carbenicillin	100–>100	>100	>100
P. vulgaris (25)	Cefoperazone	0.39–>100	1.66	35.6
	Cefazolin	>100	>100	>100
	Piperacillin	≤0.20–>100	0.85	35.6
	Carbenicillin	0.78–>100	35.6	>100
P. aeruginosa (25)	Cefoperazone	0.39–25	2.79	14.0
	Cefazolin	>100	>100	>100
	Piperacillin	0.78–25	2.22	10.2
	Carbenicillin	1.56–>100	28.2	>100

species of *E. coli*, *K. pneumoniae*, *P. vulgaris*, and *P. aeruginosa*. Cefoperazone showed exceptional antibacterial activity against *E. coli*, with an MIC_{50} <0.2 μg/ml (Table 1).

(ii) Antineoplastic agents. Mitomycin C had the broadest spectrum of activity against the four species, followed by 5-fluorouracil and bleomycin. However, adriamycin and carboquone proved to be inactive (Table 2).

Interactions in the checkerboard dilution method. The interactions of antimicrobial and antineoplastic agents against *E. coli*, *K. pneumoniae*, *P. vulgaris*, and *P. aeruginosa* are shown in Tables 3 through 6.

When β-lactam antibiotics were combined with antineoplastic agents against 25 strains of *E. coli*, most combinations showed neither synergistic nor antagonistic interactions (Table 3).

TABLE 2. Comparative antibacterial activities of antineoplastic agents

Species (no. of isolates)	Antineoplastic agent	MIC (μg/ml)		
		Range	50%	90%
E. coli (25)	Mitomycin C	≤0.20–25	2.04	4.85
	Bleomycin	0.39–6.25	1.25	2.71
	Adriamycin	100–>100	>100	>100
	5-Fluorouracil	1.56–>100	8.65	70.0
	Carboquone	50–>100	>100	>100
K. pneumoniae (25)	Mitomycin C	0.78–25	2.50	14.0
	Bleomycin	0.39–6.25	1.91	4.10
	Adriamycin	>100	>100	>100
	5-Fluorouracil	1.56–>100	>100	>100
	Carboquone	>100	>100	>100
P. vulgaris (25)	Mitomycin C	0.39–25	2.34	5.90
	Bleomycin	0.78–>100	>100	>100
	Adriamycin	12.5–>100	>100	>100
	5-Fluorouracil	0.39–50	1.24	3.00
	Carboquone	>100	>100	>100
P. aeruginosa (25)	Mitomycin C	0.78–6.25	1.61	3.55
	Bleomycin	>100	>100	>100
	Adriamycin	12.5–>100	>100	>100
	5-Fluorouracil	0.39–>100	10.8	>100
	Carboquone	>100	>100	>100

TABLE 3. Mean FIC index of combinations of β-lactam antibiotics and antineoplastic agents against 25 strains of *E. coli*

Antineoplastic agent	Cefoperazone	Cefazolin	Piperacillin	Carbenicillin
Mitomycin C	ND[a]	1.12	0.96	0.99
Bleomycin	ND	0.94	1.08	0.82
Adriamycin	ND	0.83	1.11	1.11
5-Fluorouracil	ND	0.61	0.68	0.64
Carboquone	ND	1.21	1.24	1.18

[a] ND, Not detected.

However, when combined with 5-fluorouracil, cefazolin, piperacillin, and carbenicillin were slightly synergistic (mean FIC indices of 0.61 to 0.68).

When β-lactam antibiotics were combined with antineoplastic agents against 25 strains of *K. pneumoniae*, most combinations showed neither synergistic nor antagonistic interactions (Table 4). However, the combination of piperacillin with bleomycin was synergistic. On the other hand, the combinations of cefoperazone or cefazolin with carboquone were antagonistic. The effects of combinations of cefoperazone, cefazolin, or piperacillin with adriamycin approached antagonism.

With the exception of cefazolin, when β-lactam antibiotics were combined with antineoplastic agents against 25 strains of *P. vulgaris*, most combinations showed synergism (Table 5). Synergism was not observed in the combinations of cefazolin with antineoplastic agents or when carboquone was combined with cefoperazone or cefazolin.

When β-lactam antibiotics were combined with antineoplastic agents against 25 strains of *P. aeruginosa*, the effects were either synergistic or antagonistic (Table 6). Synergism was observed between piperacillin and mitomycin C or 5-fluorouracil. However, antagonism resulted when cefoperazone, piperacillin, or carbenicillin was combined with carboquone and when carbenicillin was combined with adriamycin.

The effects of cefoperazone, piperacillin, or carbenicillin when combined with concentrations of the antineoplastic agents approximating the maximum serum levels resulting from usual adult dosages (4, 5, 8) were tested against 25 strains of *P. aeruginosa*. The antibacterial activities of cefoperazone, piperacillin, and carbenicillin were increased by combination with fixed concentrations of mitomycin C or 5-fluorouracil (Table 7).

Growth curves. In combination with mitomycin C, an increase in the bactericidal activities of cefoperazone and piperacillin, but not of carbenicillin, against *P. aeruginosa* was observed for up to 6 h, but regrowth was observed at 24 h (Fig. 1).

DISCUSSION

We studied the interactions of β-lactam antibiotics and antineoplastic agents by the checkerboard dilution method with a total of 100 strains of *E. coli, K. pneumoniae, P. vulgaris*, and *P. aeruginosa*. β-Lactam antibiotics generally showed synergism in combination with mitomycin C, bleomycin, or 5-fluorouracil, but showed antagonism in combination with carboquone. Almost all combinations of β-lactam antibiotics with adriamycin showed neither synergistic nor antagonistic interactions. However, the types of interactions varied with each β-lactam antibiotic and species. Among β-lactam antibiotics, piperacillin was more frequently synergistic than were other antibiotics, and *P. vulgaris* tended to be more synergistically affected than other species.

These results suggested the possibility of different interactions if the test conditions were altered. For example, cefoperazone was highly active against *E. coli* and *K. pneumoniae*; consequently, the interactions between cefoperazone and antineoplastic agents against *E. coli* were difficult to determine. If the fixed concentration

TABLE 4. Mean FIC index of combinations of β-lactam antibiotics and antineoplastic agents against 25 strains of *K. pneumoniae*

Antineoplastic agent	Cefoperazone	Cefazolin	Piperacillin	Carbenicillin
Mitomycin C	1.02	1.04	0.70	1.13
Bleomycin	≤0.80	0.90	0.58	1.09
Adriamycin	1.23	1.22	1.20	1.17
5-Fluorouracil	≤0.74	0.87	1.19	1.08
Carboquone	1.41	1.41	1.16	1.21

TABLE 5. Mean FIC index of combinations of β-lactam antibiotics and antineoplastic agents against 25 strains of *P. vulgaris*

Antineoplastic agent	Cefoperazone	Cefazolin	Piperacillin	Carbenicillin
Mitomycin C	0.55	1.13	0.58	0.33
Bleomycin	0.59	1.13	0.49	0.29
Adriamycin	0.71	1.16	0.81	0.28
5-Fluorouracil	0.51	0.99	0.69	0.45
Carboquone	1.21	1.20	1.19	0.92

TABLE 6. Mean FIC index of combinations of β-lactam antibiotics and antineoplastic agents against 25 strains of *P. aeruginosa*

Antineoplastic agent	Cefoperazone	Cefazolin	Piperacillin	Carbenicillin
Mitomycin C	1.08	1.14	0.58	0.82
Bleomycin	1.25	1.21	1.20	1.22
Adriamycin	1.03	1.16	1.03	1.32
5-Fluorouracil	0.69	0.90	0.57	0.69
Carboquone	1.50	1.21	1.45	1.45

of cefoperazone were decreased, its combination with 5-fluorouracil would at least show synergism.

Michel et al. (10) reported that combinations of mitomycin C with aminoglycosides were synergistic against strains of *E. coli, Proteus* sp., and *K. pneumoniae*, but that bleomycin was often antagonistic against strains of *E. coli* and *K. pneumoniae* when tested with β-lactam antibiotics, aminoglycosides, and other antibacterial agents. Jacobs et al. (7) described synergism or antagonism in combinations of antimicrobial agents (β-lactam antibiotics and other agents) and antineoplastic agents (mitomycin C, bleomycin, etc.) against *Staphylococcus aureus*. Manten and Terra (9) reported antagonism and indifference, but no synergism, in combinations of mitomycin C or dactinomycin and β-lactam antibiotics when tested against *E. coli, Candida albicans*, and gram-positive species. Moody et al. (11) found no evidence of antagonism between daunorubicin and ticarcillin against *E. coli, K. pneumoniae*, or *P. aeruginosa*. Wright and Matsen (12) reported the effect on the antibacterial activities of 5-fluorouracil, doxorubicin, or dactinomycin of use with antimicrobial agents. These results did not always correlate with ours. Michel et al. (10), for example, used high concentrations of antineoplastic agents in their study. When we adjusted the concentrations of antineoplastic agents to those approximating the maximum serum levels resulting from the usual adult dosages, the antibacterial activities of the β-lactam antibiotics changed and correlated with the mean FIC indices.

Because the pharmacokinetics of antineoplastic agents in humans are not fully known, it is difficult to discuss whether the interactions of β-lactam antibiotics and antineoplastic agents are synergistic or antagonistic in body fluids; however, we believe that the interactions in humans approximate those in our studies.

Regarding the mechanisms of interactions, Coles and Gross (2) reported that mitomycin C has an inhibitory effect on β-lactamase induction by *S. aureus*. Gudas and Pardee (6) suggested that bleomycin has an inhibitory effect on DNA synthesis and the induction of protein X. The

TABLE 7. MIC_{50} of β-lactam antibiotics in the presence or absence of antineoplastic agents against 25 strains of *P. aeruginosa*

β-Lactam antibiotic	MIC_{50} (μg/ml) of β-lactam antibiotics			
	Alone	+ Mitomycin C[a] (0.2 μg/ml)	+ Bleomycin[b] (1.56 μg/ml)	+ 5-Fluorouracil[c] (3.13 μg/ml)
Cefoperazone	2.79	2.30	3.30	0.63
Piperacillin	2.35	1.38	2.45	0.37
Carbenicillin	30.0	26.1	39.2	8.00

[a] The maximum serum level after intravenous administration of mitomycin C was 2 mg/day in humans.
[b] The maximum serum level after intravenous administration of bleomycin was 7.5 mg/day in humans.
[c] The maximum serum level after oral administration of 5-fluorouracil was 500 mg/day in humans.

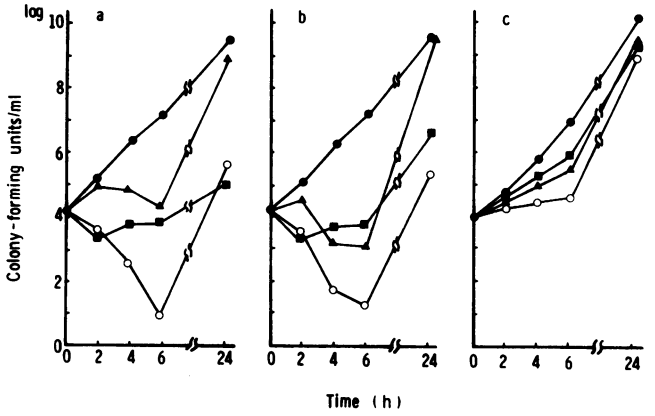

FIG. 1. Bactericidal effect of β-lactam antibiotics and mitomycin C alone or in combination against *P. aeruginosa* S68. Symbols: ●, control; ■, mitomycin C (0.78 μg/ml) alone; (a) ▲, cefoperazone (1.56 μg/ml) alone; ○, cefoperazone plus mitomycin C; (b) ▲, piperacillin (1.56 μg/ml) alone; ○, piperacillin plus mitomycin C; (c) ▲, carbenicillin (12.5 μg/ml) alone; ○, carbenicillin plus mitomycin C (MICs of cefoperazone, piperacillin, carbenicillin, and mitomycin C were 3.13, 3.13, 25, and 1.56 μg/ml, respectively).

difference in interactions between combinations of β-lactam antibiotics with antineoplastic agents may be caused by these differences in mechanisms of action.

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