Acta Oncologica 29 (1990) Fasc. 1

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INTERACTIONS BETWEEN ANTIBIOTICS AND ANTINEOPLASTIC DRUGS ON ANTIBACTERIAL ACTIVITY IN VITRO

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Abstract

The effects of various combinations of antibacterial (ampicillin, cephadroxil, doxycycline, imipenem, trimethoprimsulfadiazine) and antineoplastic (cisplatin, epirubicin, mitoxantrone) drugs were evaluated in vitro with regard to antibacterial activity on five clinical isolates from cancer patients of respectively *Escherichia coli, Staphylococcus aureus* and *Streptococcus faecalis*. With one exception no significant effects on the bacterial growth were observed in the presence of the antitumor drug alone. In contrast, all five strains of *Strept. faecalis* grew better when mitoxantrone was included in a concentration of 0.1 mg/l. A synergistic action between imipenem and mitoxantrone was seen for single strains of *Staph. aureus* and *E. coli*. Furthermore, a dose-effect related inhibition by cisplatin on the growth of *Strept. faecalis* in the presence of sub MIC levels of trimethoprimsulfadiazine was observed. The study indicates that interaction between antibacterial and antineoplastic drugs is an erratic phenomenon, which has to be dealt with separately for each combination of drugs as well as for each bacterial strain.

Key words: Antibiotics, antineoplastic drugs, antibacterial activity.

Bacterial infections remain a serious problem in patients with advanced cancer and hematologic malignancies, especially in those with granulocytopenia (1–3). Thus, most patients treated with antineoplastic drugs are potential recipients of antimicrobial drugs. Very often these drugs have to be given in combination. Although synergistic and antagonistic actions between antibacterial drugs have been well documented (e.g. 4–6) the influence of antineoplastic drugs on the antibacterial activity has only been considered by few authors (2, 7–12). Furthermore, the continuous addition to the therapeutic arsenal of new and chemically modified antibiotics with different modes of action makes it necessary to evaluate new antibacterial-antineoplastic combinations of interest in the clinical situation. The aim of the present study was therefore to elucidate in vitro the effects of various of these new combinations in clinical use in relation to bacterial species most commonly isolated in cancer patients in our hospital.

Material and Methods

Antibiotics and antineoplastic drugs. The following antibiotics, delivered as potency defined substances, were included in the study: ampicillin (Doktacillin, ASTRA, Sweden), imipenem (Thienamycin, MSD, USA), trimethoprimsulfadiazine (Trimin, ASTRA, Sweden), cephadroxil (Cefamox, Bristol-Myers, USA) and doxycycline (Vibramycin, Pfizer, USA). Stock solutions of each antibiotic containing 1 000 mg/l were prepared in distilled water. For trimethoprimsulfadiazine this was based on the sum of the two drugs assuming a trimethoprim/sulfadiazine of ratio 9/41.

The antitumor drugs mitoxantrone (Novanthron, Lederle, USA), epirubicin (Farmorubicin, Farmitalia, Italy) and cisplatin (Cisplatin, Nycomed, Norway) were prepared in aqueous stock solutions of 1 000 mg/l. All drugs were further diluted in Müller-Hinton broth to desired concentration.

Bacterial strains. Five clinical isolates of *Staphylococcus aureus, Escherichia coli* and *Streptococcus faecalis* were used in the present study.

Susceptibility testing. All strains were sensitive to the antibiotics included in the study except *Streptococcus faecalis,* which was not susceptible to cephadroxil. The MIC (the minimal inhibitory concentration) for each anti-

Accepted for publication 1 February 1989.

biotic on the individual strain was established in Müller-Hinton broth and confirmed by the standard agar dilution method using 2-fold dilutions of each antibiotic. The inoculum corresponded to 10^5 colony forming units/ml (cfu).

Experimental design. Based on the results of the MIC determinations of the individual strain for each of the antibiotics, concentrations corresponding to eight times below and above MIC for each strain were used in combination with each of three concentrations of the antineoplastic drugs (0, 0.1 and 10 mg/l). These last concentrations were chosen to represent anticipated levels at various times during cancer therapy. All drugs were also tested as a single substance at each concentration. The experiments were designed as time killing analyses allowing optical density reading of individual tubes after 2, 4, 6, 11 and 24 h. All experiments were performed in duplicates.

Definition of synergism and antagonism. Synergistic and antagonistic effects were defined as a decrease or increase respectively of the MIC of the strain versus the pertinent antibiotic in the presence of the antineoplastic drug differing more than two titer steps from that of the antibiotic drug alone.

Controls. A similar time killing experiment was performed using each strain in the presence of antibiotics but not antitumor drugs and in the presence of antitumor drugs and absence of antibiotics.

Results

Influence of antitumor drugs on bacterial growth. Up to a concentration of 10 mg/l no inhibition was noted in the presence of the antitumor drugs at any time of the 24-h growth experiments. On the contrary, as indicated in Table 1, all five *Streptococcus faecalis* strains grew better when mitoxantrone (0.1 mg/l) was included than in the absence of or at higher concentrations of this drug. This phenomenon was reproducible for these *Strept. faecalis* strains but was never demonstrated with the *Staph. aureus* or the *E. coli* strains. Nor was a significant concentration-dependent growth-stimulating activity noted with the other two antitumor drugs at any concentration.

Interactions between antitumor drugs and antibiotics on E. coli, Staph. aureus and Strept. faecalis strains. Using the definition for antagonistic and synergistic effects between antitumor and antibiotic drugs, no antagonistic effects were demonstrated on *Staph. aureus* and *E. coli* (Tables 2 and 3). A single strain each of *E. coli* and *Staph. aureus* showed a synergistic reaction, and in both instances this was confined to the combination imipenem and mitoxantrone. No interaction was observed between imipenem, doxycycline and the antitumor drugs in their antibacterial activity versus *Strept. faecalis*. Only with one of the four strains tested a slight antagonistic effect (MIC increased 3 titer steps) was noted when mitoxantrone was added to ampicillin. As mentioned above,

Table 1

Growth of Streptococcus faecalis strains in presence of mitoxantrone*

Strain	Concentrations of mitoxantrone (mg/l)		
	0	0.1	10
1	0.67	1.2	0.78
2	0.60	1.0	0.70
3	0.31	1.1	0.31
4	0.56	1.3	0.66
5	0.57	1.0	0.68

* Optical density readings at 500 nm after 24 h in Müller-Hinton broth.

Strept. faecalis grew better in Müller-Hinton broth in the presence of low concentrations (0.1 mg/l) of mitoxantrone than in the absence or at higher concentrations (10 mg/l) of this drug. This phenomenon disappeared when trimethoprimsulfadiazine was added in a concentration of 1.25 mg/l—a concentration that was below the MIC of the strains. These *Strept. faecalis* strains were also tested in the same trimethoprimsulfadiazine concentration in the presence of various concentrations of cisplatin. As can be seen in Table 4 a decrease in growth was noted in the presence of increasing concentrations of cisplatin over a 24-h period, thus demonstrating a dose dependent synergism between these two drugs.

Discussion

Various methods can be used to demonstrate interactions between antibiotics on bacterial growth and killing (5, 6, 13). The pros and cons of available methods were recently reviewed (4, 6). The choice of the time-killing method was founded on the fact that it allowed a continuous analysis during the interaction period of the growth or inhibition in the presence of various combinations of drugs. The selection of antitumor agents and antibiotics was based on the extent of use at the department of oncology in relation to the three bacterial species most commonly encountered in the cancer patients in our hospital. The presently time-consuming method used to determine interactions between antibiotics and antitumor drugs only allowed analysis of a limited number of antitumor concentrations. The two concentrations chosen corresponded to a rather high therapeutically desirable level and a low level representing the lower range during a treatment cycle.

Various definitions of synergism and antagonism have been proposed including a method for quantitative calculations (reviewed by 4). These definitions are based on determination of the MIC of the individual drug alone and in combinations. Since the presently used antitumor drugs had no antibacterial activity in therapeutically achievable

Table 2

Effects of antitumor drugs on the antibacterial activity of antibiotics on Escherichia coli (5 strains)*

	Mitoxantrone			Epirubicin			Cisplatin		
	A	I	S	A	I	S	A	I	S
Ampicillin	–	5	–	–	5	–	–	5	–
Trim/sulfa	–	5	–	–	5	–	–	5	–
Cephadroxil	–	5	–	–	5	–	–	5	–
Imipenem	–	4	1	–	5	–	–	5	–
Doxycycline	–	5	–	–	5	–	–	5	–

*A = antagonism; I = indifference; S = synergism.

Table 3

Effects of antitumor drugs on the antibacterial activity of antibiotics on Staphylococcus aureus (5 strains)*

	Mitoxantrone			Epirubicin			Cisplatin		
	A	I	S	A	I	S	A	I	S
Ampicillin	–	5	–	–	5	–	–	5	–
Trim/sulfa	–	5	–	–	5	–	–	5	–
Cephadroxil	–	5	–	–	5	–	–	5	–
Imipenem	–	4	1	–	5	–	–	5	–
Doxycycline	–	5	–	–	5	–	–	5	–

*A = antagonism; I = indifference; S = synergism.

Table 4

Growth of Streptococcus faecalis strains in the presence of sub-MIC concentrations of Trim/sulfa and increasing concentrations of cisplatin

Cisplatin (mg/l)		
0	0.1	10
1.1* (0.90–1.1)	0.65* (0.54–0.71)	0.39* (0.36–0.43)

*Average optical density at 500 nm of 5 strains.
The range is shown within parenthesis.

concentrations it seemed more logical to establish the difference in MICs for an antibiotic in the absence and presence of an antitumor drug. Thus, if MIC of the combination increased 4 times or more above that of the antibiotic alone it was assumed that the antitumor drug acted antagonistically. Synergism was defined accordingly.

During the preliminary testing of the sensitivity of the bacterial strains in the presence of the antitumor drugs, a strange phenomenon was registered for *Streptococcus faecalis*. The growth stimulating effect of mitoxantrone noted at low concentrations has to our knowledge not been recorded before. Available information on the mechanism of action of this drug on eucaryotic cells being a 'DNA-reactive reagent' does not indicate a possible mechanism of action. This phenomenon seems to be restricted to *Strept. faecalis* strains since it was not observed with the *Staph. aureus* and *E. coli* strains. The practical implication in the clinical situation is at present obscure, but a potential role for patients with enterococcal urinary tract infections should perhaps not be neglected.

Except for synergistic actions between mitoxantrone and imipenem on a single strain of *Staph. aureus* and *E. coli* respectively, no interaction was noted between the antitumor drugs and the antibiotics in the present study.

Interactions between antitumor drugs and antibiotics in various combinations against different microorganisms have earlier been reported (7–12). Thus, antagonistic effects have been demonstrated (8, 9) and antibacterial activity has been ascribed to some antineoplastic drugs (8, 10, 14). Among the latter, 5-fluorouracil, dactinomycin and doxorubicin have been shown also to interfere in bioassays of antibiotics in concentrations reached in human blood (14). This agrees with our finding of a dose-dependent inhibition by cisplatin of the growth of *Strept. faecalis* strains in the presence of sub-MIC levels of trimethoprimsulfadiazine.

The present results and those reported in the literature indicate that interactions between antitumor drugs and antibiotics are an erratic phenomenon which has to be dealt with for each combination separately. Furthermore, most antitumor drugs have a potentially toxic effect for instance on the renal tissue (15). This can influence the pharmacokinetics of the antibiotics as well as the antitumor drugs (16) resulting in concentration levels that have to be carefully monitored to avoid further side reactions. Also in this situation, knowledge of the interactions between antitumor drugs and antibiotics at different concentration levels is of clinical importance. Further studies on the multifacetted problem of interactions between these two types of drugs seem to be justified.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Society for Cancer Research, and the Lions Foundation in Umeå, Sweden.

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