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Influence of protamine on the susceptibility of *Pseudomonas aeruginosa* to cefotaxime, sulphadimethoxine, polymyxin B and some β-lactam antibiotics

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Summary

Protamine, a basic protein, was shown to influence the activity of antibacterial agents. When added to tryptic soy broth it enhanced the bactericidal activity of cefotaxime, sulphadimethoxine, ticarcillin, piperacillin and carbenicillin against *Pseudomonas aeruginosa*. In contrast, the activity of polymyxin B against this species was markedly reduced in the presence of protamine.

Introduction

Pseudomonas aeruginosa is outstandingly resistant to antimicrobials (Wiedemann et al., 1985). This resistance cannot be completely explained by the presence of plasmids (Godfrey and Bryan, 1984). It is known that the growth medium can influence the permeability of the microbial cell to antimicrobials (Leive, 1974; Hancock, 1985; Nikaido and Vaara, 1985). We demonstrated changes in permeability of cells as a result of adsorption of medium components to the cell wall, and notable variations in sensitivity of *Pseudomonas aeruginosa* to chloroxylenol, phenol and Crystal violet were observed in different culture media (Dony et al., 1984; Devleeschouwer et al., 1986).

As regards the hypothesis of a modification occurring during the growth, it seemed important to investigate the influence of a basic protein, namely protamine, on the activity of antibacterials on *Pseudomonas aeruginosa* (Boussard et al., 1986). We first studied the influence of protamine by our classical method for the evaluation of the effectiveness of disinfectants for carboxy- and ureidopenicillin and cefotaxime on *Pseudomonas aeruginosa* in comparison with sulphadimethoxine and polymyxin B (Devleeschouwer and Dony, 1981).

We then studied the influence of protamine on the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for β-lactams on *Pseudomonas aeruginosa*.

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Materials and Methods

Pseudomonas aeruginosa ATCC 27853 was used.

Tryptic soy broth (Difco) was used for the determination of MIC.

Antimicrobial agents. The antibiotics tested were ticarcillin and carbenicillin (Beecham Pharmaceuticals), piperacillin (Lederle/Cyanamid), cefotaxime (Hoechst-Roussel), sulphadimethoxine (Roche) and polymyxin B (R.I.T.). Protamine sulphate (Serva Feinbiochemica Heidelberg) was used at subinhibitory concentrations. We obtained with this molecule alone an MIC of 1 mg/ml; the highest concentration used here was 500 μg/ml.

We studied the influence of protamine on the bactericidal activity of antibiotics by employing the method used in our laboratory for the study of disinfectants (Devleeschouwer and Dony, 1981). It allows one to follow the kinetics of the antimicrobial action. To a solution of 10 ml containing 1 mg/ml of antibiotic and 0, 100, 250 or 500 μg/ml of protamine we added 0.4 ml of an overnight broth culture of *Pseudomonas aeruginosa.*

The number of viable micro-organisms in this broth culture was determined by plate count. After 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 18 and 24 h we evaluated the number of viable bacteria contained in 1 ml of the solution for each time by plate count. The bactericidal activity of the antibiotics was determined by measuring the logarithmic reduction of the initial number of organisms after different contact times in solutions containing antibacterials with or without protamine.

MIC tests (Chabbert, 1972) were performed in the presence and absence of varying concentrations, 100–500 μg/ml, of protamine and antibiotic ranged from 0.25 μg/ml to 4 mg/ml. To 5 ml of each antibiotic concentration, either containing or not containing protamine, 50 μl of an overnight broth culture of the strain corresponding to an inoculum of 10^9 cfu/ml was added. The MIC of the antibiotic was the lowest concentration that inhibited visible growth, by comparison to controls, after incubation at 37 °C for 24 h.

The MBC was the lowest concentration of antibiotic that decreased the viable count by 3 logarithms. Each dilution was plated on tryptic soy agar and examined for growth after 24 h at 37 °C.

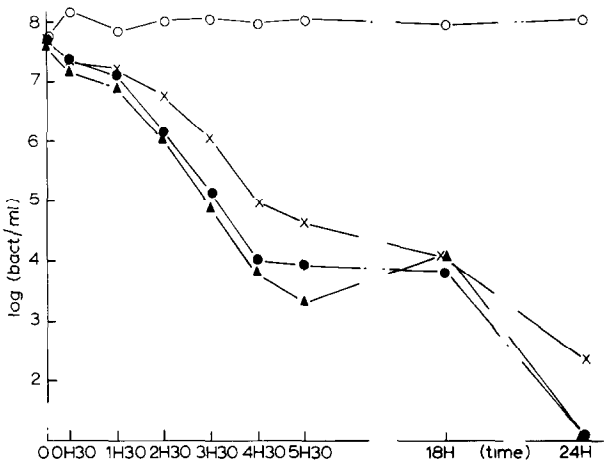

Fig. 1. Study of piperacillin on *Pseudomonas aeruginosa* ATCC 27853. ○, contact between bacteria and piperacillin at 1 mg/ml; ×, contact between bacteria and the mixture containing protamine at 100 μg/ml and piperacillin at 1 mg/ml; ●, contact between bacteria and the mixture containing protamine at 250 μg/ml and piperacillin at 1 mg/ml; ▲, contact between bacteria and the mixture containing protamine at 500 μg/ml and piperacillin at 1 mg/ml.

Using the method for the evaluation of the in vitro bactericidal activity, we observed that the

TABLE 1

Influence of protamine on MIC and MBC values obtained for Pseudomonas aeruginosa ATCC 27853 with β-lactam antibiotics

Antibiotic	Protamine (μg/ml)	MIC (24 h) (μg/ml)	MBC (μg/ml)
Cefotaxim	0	125	500
	100	32	63
	250	8	32
	500	2	8
Piperacillin	0	4000	4000
	100	32	250
	250	4	125
	500	1	125
Carbenicillin	0	250	1000
	100	16	32
	250	8	16
	500	4	8
Ticarcillin	0	250	1000
	100	16	32
	250	4	8
	500	1	2

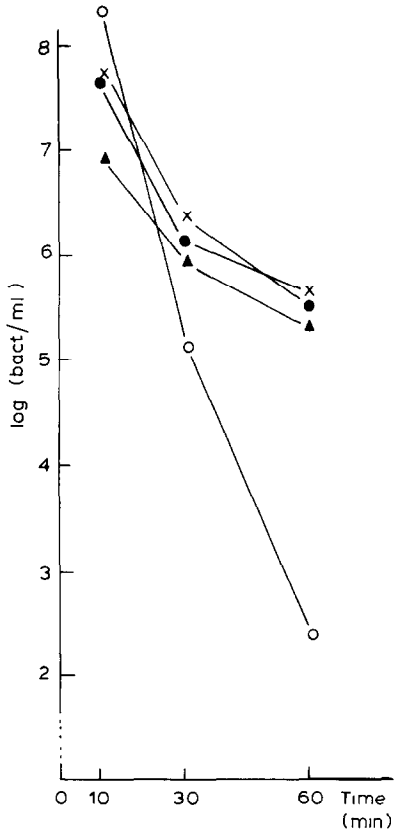

Fig. 2. Study of polymyxin B on *Pseudomonas aeruginosa* ATCC 27853: ○, contact between bacteria and polymyxin B at 1 mg/ml; ×, contact between bacteria and the mixture containing protamine at 100 μg/ml and polymyxin B at 1 mg/ml; ●, contact between bacteria and the mixture containing protamine at 250 μg/ml and polymyxin B at 1 mg/ml; ▲, contact between bacteria and the mixture containing protamine at 500 μg/ml and polymyxin B at 1 mg/ml.

direct association of protamine with sulphadimethoxine gave a reduction of 3 log phases of the initial number of *Pseudomonas aeruginosa* after a contact time of 4 h.

After a contact time of 4.5 h between cefotaxime and the bacteria we observed a reduction of about 4 logarithms in the presence of protamine at a final concentration of 100 μg/ml. The influence on this cephalosporin for 250 and 500 μg/ml in protamine on the sensitivity of *Pseudomonas aeruginosa* was nearly the same, namely 5 logarithms reduction for the same contact time. After 18 h a regrowth of bacteria was observed.

With the same method we compared the influence of the mixture of 100, 250 and 500 μg/ml of protamine on the activity of 3 penicillin molecules – ticarcillin, carbenicillin and piperacillin showed the same behaviour. The effect began after 2 h and was prolonged for one day. We observed a 3 logarithm reduction after 4.5 h for the 3 antibiotics studied (see the Fig. 1, given for piperacillin). On the other hand, protamine decreased the bactericidal activity of polymyxin B even for the addition of 100 μg/ml of protamine (Fig. 2). This antibiotic alone gave a reduction of 5 logarithms in 1 h whereas the addition of protamine decreased the polymyxin activity to only two log phases in the same time.

We also studied the influence of protamine on the MIC and MBC values for *Pseudomonas aeruginosa* with β-lactams (see Table 1). For all the β-lactams tested, protamine induced a decrease of their values. When the concentration increased from 100 to 250 and from 250 to 500 μg/ml the MIC and MBC values decreased by a factor of two or more for cefotaxime, carbenicillin and ticarcillin. For piperacillin MIC and MBC for the antibiotic alone was greater than 4 mg/ml; the association with protamine at 100 μg/ml gave a MIC of 32 μg/ml and a MBC of 250 μg/ml.

Discussion

Direct mixture of protamine with the antibiotics, except for polymyxin B, increased their bactericidal activity against *Pseudomonas aeruginosa.* For cefotaxime only, we observed a regrowth of the bacteria, showing the probable existence of a phenotypic adaptation.

In contrast, protamine decreased the bactericidal activity of polymyxin B. This antibiotic, which is a polypeptidic molecule, acts first through its fixation on the structure of the outer membrane of the bacteria, the site which is probably located in the deep core region and includes part of the lipid A of the lipopolysaccharides (Vaara and Vaara, 1983; Kropinski et al., 1985). The interaction between protamine and polymyxin B seems to be a competition for the same site of fixation, so the affinity between protamine and

the outer membrane should be greater than for the polymyxin B.

The influence of protamine on the other antibiotics studied here is not the same. The penetration of these agents through the outer membrane may occur in a different way, which will be investigated in a further study. For these antimicrobials, the interaction with protamine could be an electrostatic attraction between the molecule and the outer membrane. In conclusion, protamine could act through adsorption on the bacterial structure of *Pseudomonas aeruginosa* by a specific linkage but also through an electrostatic interaction.

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