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Prevention of the adhesion of *Pseudomonas aeruginosa* to human buccal epithelial cells

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Summary

The influence of protamine on the adhesion of *Pseudomonas aeruginosa* to human buccal cells was investigated using 2 collection strains and 5 other strains isolated from the hospital environment. The serotype and pyocin type of the strains was established and their antibiotic sensitivity determined. For concentrations lower than 100 μg/ml there was a decrease in adhesion correlating with protamine concentration. Maximum inhibition was reached at about 100 μg/ml.

Introduction

Pseudomonas aeruginosa is well known for its extreme resistance to antibacterials. The harbouring of plasmids does not explain totally the phenomenon as the permeability of the bacteria seems to be a determinant factor (Cullman et al., 1987; Kubesch et al., 1987), the outer membrane acting as a barrier (Leive, 1974; Russel, 1986).

In a study of factors which could modify the outer membrane permeability and therefore promote the penetration of antibacterials, we showed that *Pseudomonas aeruginosa* is much more sensitive to the action of chloroxylenol when the strain is grown in brain heart infusion broth (BHI; Difco) rather than in tryptic soy broth (TSB; Difco) (Dony et al., 1984; Devleeschouwer et al., 1986). We then investigated under the same conditions the sensitivity of this organism to other antibacterial agents and antibiotics. Although some antibiotics are more effective on the bacteria cultured in brain heart infusion, in contrast the bactericidal action of polymyxin B is greater when the cells are grown in TSB. This latter antibiotic is lethal to *Pseudomonas aeruginosa* only when fixed to the KDO residues of the lipopolysaccharides (Peterson et al., 1985). It seems that an ingredient of the BHI is fixed either at the same site as polymyxin B or in the immediate vicinity.

This fact led us to investigate molecules which, when added to the TSB, could restore the brain heart sensitive conditions. This medium being rich in basic proteins we took first molecules of this type such as protamine and poly-lysine. These substances added to TSB make *Pseudomonas aeruginosa* sensitive to chloroxylenol as when grown in BHI (Boussard et al., 1986). The addition of protamine also sensitises *Pseudomonas*

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aeruginosa to antibiotics such as sulphadimethoxin, cefotaxime, carbenicillin, ticarcillin and piperacillin. Moreover, the addition of protamine makes the organism resistant to the bactericidal action of polymyxin B (Boussard and Dony, 1988).

It therefore seemed interesting to investigate the influence of protamine on the adhesion of *Pseudomonas aeruginosa* to human buccal cells. Indeed, the adhesion of a bacteria to the host cell is the first step leading to the colonization of the organisms and infection.

Material and Methods

We used two collection strains of *Pseudomonas aeruginosa* namely ATCC 15442 and ATCC 27853, and five 0:12 (HABS) strains from the hospital environment. They were collected in sink traps of an intensive care unit of the Academic Hospital Erasme (ULB) and listed as the following: S137, S143, S156, S173 and S190.

The strains were grown in TSB (Difco) for 16 h at 37°C. The homogeneized culture was then centrifuged at 1500 × *g* for 10 min. The cells were suspended in PBS (pH 7.2) and washed 3 times in the same conditions. The last suspension was diluted in PBS to obtain a cell concentration of 10^9/ml.

Human epithelial buccal cells were harvested by swabbing the inner part of the cheeks of healthy, non-smoking donors. The first suspension of 10 ml in PBS was centrifuged at 1000 × *g* for 10 min. After the third washing, one drop of erythrosine B (C145430, 0.4% in PBS pH 7.2) was added to 4 drops of the cell suspension. The number of living cells was estimated with a toma cell and the suspension diluted to 10^5 cells/ml.

250 µl of the suspension of the human buccal cells were mixed with the same volume of the bacterial suspension. At this mixture 50 µl of a solution of protamine in PBS was added to obtain final concentrations of protamine of 0, 25, 50, 75, 100, 250 and 500 µg/ml. Contact was allowed for 2 h at 35°C under agitation at 100 rpm. The mixture was then centrifuged for 10 min at 1000 rpm. After the third washing two drops of erythrosine B were added to each vial and 3 additional washings were performed under the same conditions. The final clot was suspended in 500 µl of PBS at pH 7.2. This suspension was spread out on slides and dried for one night at room temperature. After counterstaining with Methylene blue, the slides were examined under immersion. The adherence was expressed as the mean value of the adherent bacteria by counting 50 living epithelial cells. The mean value obtained without protamine was taken in these experiments as 100% adhesion.

In addition to the adhesion experiments, we determined the serotype (HABS) and pyocin type (Gillies and Govan) of all the strains and investi-

TABLE 1

Sero and pyocin types and antibiotic susceptibility of the strains

Strains	Serotype (HABS)	Pyocin type	Antibiotic susceptibility							
			IMP	AN	CAZ	CL	GM	NOR	PIP	NN
ATCC 15442	1	UT/f	S	S	S	–	S	S	S	S
ATCC 27853	6	3/e	S	S	S	–	S	S	S	S
S 137	12	45/h	–	S	R	S	R	S	R	R
S 143	12	45/h	–	S	S	S	R	S	R	R
S 156	12	45/h	–	S	R	S	R	S	R	R
S 173	12	45/h	S	S	S	–	R	R	R	R
S 190	12	45/h	I	S	S	–	R	S	S	R

IMP = Imipenem; AN = Amikacin; CAZ = Ceftazidin; CL = Colimicin; GM = Gentamicin; NOR = Norfloxacin; PIP = Piperacillin; NN = Tobramycin; S = sensitive; I = intermediate; R = resistant.

TABLE 2

Adhesion percentage of the Pseudomonas aeruginosa strains according to the protamine concentration

Strains	Protamine concentration (μg/ml)					
	25	50	75	100	250	500
ATCC 27853				6	2	3
				7	8	9
ATCC 15442	74	46	24	13		
				12	13	14
S 137	109	72	45	23		
				10	8	11
S 143	76	57	49	27		
				7	7	9
				13	13	12
S 156	81	100	64	10		
				12	12	9
S 173	89	73	25	20		
				13	12	14
S 190	102	26	17	12		
	83			16		
				17	12	17

gated the antibiotic susceptibility by the classical antibiogram method of Bauer and Kirby using Rosco tablets.

Results

Table 1 shows the sero and pyocin patterns of the *Pseudomonas aeruginosa* strains as well as their antibiotic susceptibility. It is to be noted that the studied 0:12 strains exhibit a regular resistance to gentamicin and tobramycin and with some this resistance extends to piperacillin.

As the adhesion is being investigated, the results of Table 2 show a concentration-dependent action of the protamine. Indeed for the concentrations lower than 100 μg/ml there is a decrease of adhesion correlated with the protamine concentration. The maximum inhibition is reached at about 100 μg/ml and a further increase in protamine concentration does not improve the phenomenon.

The maximum values of inhibition recorded were for all the strains tested equal to or greater than 80%. The presence of protamine never led to an increase of the adherence of the strains.

Discussion

In a previous work we showed that protamine probably modified the structure of the lipopolysaccharides of the outer membrane of *Pseudomonas aeruginosa* (Boussard and Dony, 1988). Indeed the sensitivity to antibacterials such as antibiotics and disinfectants is modified in the presence of protamine by increasing the permeability probably by promoting the hydrophobic pathway. This promoting effect could result in the attachment of the molecule by means of electrostatic bonds between the negative charges of the outer membrane and the positive ones of this basic molecule. As a consequence the neutralization of some external negative charges and a possible reorganization of the outer membrane could pro-

mote the passage of some molecules such as phenols, some β-lactam antibiotics and partly reject antibiotics such as polymyxin B.

As external structures of the bacteria are involved in the adhesion, it was predictable that protamine could interfere with the adhesive sites. This action is concentration dependent and seems to reach a saturation level at 100 μg/ml of protamine. This interference acts either at the bacterial level or at the surface of the epithelial cell. We already recorded the same action of protamine on the adhesion of *Escherichia coli* 07 : K1(L)NM on human urinary cells (Ibwala et al., unpublished results).

We now intend to study the interference of protamine in the adhesion of other bacteria such as cocci and try to develop the possible therapeutic use of these observations.

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