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Inhibition of the adhesion of *Escherichia coli* to human urinary epithelial cells

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Summary

The influence of protamine and D-mannose on the adhesion of *Escherichia coli* to human urinary epithelial cells was investigated using one collection strain, two urinary and one fecal strain from hospital origin. At a concentration of 100 μg/ml of protamine, inhibition occurs after a brief period of contact of inhibitor with either the bacteria or epithelial cells. The same inhibition was observed with D-mannose at 1.25 mg%.

Introduction

Adhesion occurs widely throughout the microbial world; it has been described for environmental strains as well as for the commensal organisms, pathogens or opportunists. (Archimbaud and Chabanon, 1986).

Bacterial adhesion is the first step in the colonization of a host by a pathogen; furthermore, it also plays a role in the persistence of the commensal flora, for example, in the gastrointestinal tract. (Lee, 1980; Savage, 1980a, b, 1983, 1985; Virkola et al., 1988; Wold et al., 1988).

Adhesion results in an interaction between the bacteria and the receptor cell. This may be non-specific, resulting from ionic or electrostatic interactions, hydrogen bonding or hydrophobic bonding between lectins and membranes (Longer and Robinon, 1986). The bonds may be specific, through fimbriae or adhesins that are protein surface antigens and carbohydrate receptors on the host cell.

Adhesion can be inhibited in many ways, for example, the use of antisera directed against the surface antigens, or of proteases acting on the adhesins, via the addition of structural analogs or through the action of lectins on the receptor (Rutter et al., 1984). Structural analogy forms the basis for using simple carbohydrates or some of their derivatives such as D-mannose to cause inhibition (Old, 1972, 1985; Chabanon, 1984).

In our laboratory, Boussard and co-workers (1986) investigated the influence of protamine on

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the permeability of *Pseudomonas aeruginosa* as well as that on the adhesion of this organism on human buccal epithelial cells (Boussard and Dony, 1988, Boussard et al., 1989).

Since we were involved in the study of the hemagglutination and hemolytic patterns of urinary and intestinal *Escherichia coli* strains, we decided to investigate the influence of protamine on the adhesion of some *E. coli* strains to human urinary epithelial cells.

Materials and Methods

Bacterial strains

We used four human pathogenic *E. coli* strains: ATCC 23503, a 07 : Kl(L)NM serotype isolated from urine by Kauffman (1944); U930(31)1, isolated from the urine of a 74-year-old hospitalized female patient; U11261(12), isolated from the urine of an 82-year-old female patient with polyalkyuria and fever; S50/11, isolated from the faeces of a $2\frac{1}{2}$-year-old child with gastroenteritidis – its serotype (0142 : K86) is a rare EPEC serotype in western Europe.

The latter three strains were obtained from the Microbiological Laboratory, Moliere Longchamp Hospital, Brussels.

Strains were cultured in 10 ml brain heart infusion broth (BHI) and 10 ml colonization factor broth (CFB)(both from Difco) for 18 h at 37 ° C. Subsequently, cultures were centrifuged at $1500 \times g$ for 12 min, then suspended in PBS (pH 7.1) at a concentration of 10^8 bacteria/ml.

Urinary epithelial cells

Morning urine was collected from healthy young women between 19 and 40 years of age without previous urinary tract infectious episodes and who were not taking oral contraceptives. Samples containing too much mucus, amorphous sediments, red cells and residual bacteria were discarded.

After centrifugation at $250 \times g$ for 12 min, the cells were washed three times with PBS (pH 7.1). The clot was then suspended in 4 ml buffer. The number of viable cells was determined in a Neubauer cell with a mixture of the cell suspension and 1 drop of erythrosine B (C145430 0.4% in PBS, pH 7.1). The cell suspension was adjusted to 10^5 cells/ml.

Bacterial adhesion

A sample (500 μl) of the bacterial suspension, incubated at 37 ° C for 30 min to regenerate the fimbriae (Novotny et al., 1969), was mixed with an equal volume of uroepithelial cells. The mixture was then incubated at 37 ° C for 1 h with constant agitation at 80 rpm. After this period of contact, the mixture was centrifuged and washed (four times; 12 min per wash) with PBS buffer in order to eliminate non adhesive bacteria. Two drops of erythrosine were then added to the suspension that was again centrifuged (six times; 12 min per wash) in PBS at $250 \times g$ to eliminate excess dye. A volume of 0.5 ml of the final clot was spread onto a glass slide and dried at room temperature (Reid and Brooks, 1983). The slide was then stained with methylene blue (C1 52015 at 0.4% in PBS), followed by rinsing three times with distilled water and drying at room temperature.

Negative controls were performed to determine the amount of residual bacteria, cocci or rods, normally adherent to the urinary cells. This number must not exceed 5 per cell. In these controls, 500 μl of the cell suspension were mixed with an equal volume of buffer and the mixture treated as described for the the adhesion tests .

For inhibition studies, the potential inhibitors, D-mannose (Difco) and protamine sulphate (Serva), were dissolved in PBS (pH 7.1). Initially, the inhibitors were mixed with either the urinary cell suspension or the bacteria and allowed to stand for 15 min or 24 h at room temperature. Thereafter, 500 μl of these suspensions were mixed with an equal volume of the urinary cell suspension or bacterial suspension and the adhesion test performed as described above. Final concentrations were 100 μg/ml (protamine) and 1.25 mg% (D-mannose).

In order to verify whether the interaction of these products was only a juxtaposition or a real fixation, in some experiments, after a 15 min contact time between the potential inhibitors and urinary cells at room temperature, the mixtures

TABLE 1

Adhesion of E. coli strains on urinary cells (expressed as number of bacteria per cell)

Strain	Culture medium	
	BHI	CFB
ATCC 23503	31	32
U930(31)1	52	89
U11261(12)	12	16
S50/11	42	30

TABLE 2

Reproducibility of the method of adhesion as tested with strain ATCC 23503

Expt No.	Culture medium	
	BHI	CFB
1	33	32
2	28	29
3	33	36
Mean	31	32

were washed three times with PBS buffer, followed by the adherence trial being performed as described above.

Evaluation of adhesion

The slides were microscopically examined under immersion. Adhesion is expressed as the mean value of the number of adherent bacteria per urinary cell as counted on 40 viable urinary cells. Viable cells appear with a dark blue nucleus and a pale blue cytoplasm whereas the dead cells have a dark blue cytoplasm.

In the absence of inhibitors, the strains can be divided into four classes on the basis of adhesion according to Orskov et al. (1980): (1) non-adherent strains (0 bacteria/cell); (2) moderately adherent strains: (1–20 bacteria/cell); (3) adherent strains: (21–50 bacteria/cell); (4) very adherent strains (> 50 bacteria/cell).

Results

Table 1 lists the values of the adhesion expressed as the mean number of bacteria/cell for the four strains tested according to the medium used for the bacterial inoculum. With the exception of strain U930, these values are similar for strains grown in both media. Nevertheless, the four strains can be classified following the scheme of Orskov et al. as very adherent (U930(31)1), adherent (ATCC 23503 and S50/11) and mod-

TABLE 3

Influence of protamine and D-mannose on adhesion of E. coli ATCC 23503 on urinary cells

Test conditions	Culture medium					
	BHI			CFB		
	No. of organisms per cell	Adhesion (%)	Inhibition (%)	No. of organisms per cell	Adhesion (%)	Inhibition (%)
Without inhibition	31	100	–	32	100	–
Protamine (100 μg/ml)						
+ *E. coli*, 15 min	4	12.9	87.1	5	15.6	84.4
+ *E. coli*, 24 h	0	0	100	2	6.3	93.7
+ urinary cells, 15 min	2	6.5	93.5	1	3.1	96.9
+ urinary cells, 24 h	1	3.2	96.8	1	3.1	96.9
D-Mannose						
+ *E. coli*, 15 min	3	9.7	90.3	3	9.4	90.6
+ *E. coli*, 24 h	6	19.4	80.3	4	12.5	87.6
+ urinary cells, 15 min	2	6.5	93.5	1	3.1	96.9
+ urinary cells, 24 h	1	3.2	96.8	1	3.1	96.9

TABLE 4

Influence of protamine on the adhesion on urinary cells of three wild pathogenic E. coli strains

Strain	Culture medium					
	BHI			CFB		
	No. of bacteria/cell	Adhesion (%)	Inhibition (%)	No. of bacteria/cell	Adhesion (%)	Inhibition (%)
U930(31)1						
Normal	52	100	–	89	100	–
+ protamine, 15 min	9	17.3	82.7	10	11.2	88.8
U11261(12)						
Normal	12	100	–	16	100	–
+ protamine, 15 min	6	50	50	4	25	75
S50/11						
Normal	42	100	–	30	100	–
+ protamine, 15 min	5	11.9	88.1	7	23.3	76.7

erately adherent (U11261(12)). The data recorded for strain ATCC 23503 are similar to those collected in the literature (Davis et al., 1981). Reproducibility of the method was determined with the use of *E. coli* ATCC 23503 as shown in Table 2.

Table 3 shows the data concerning the influence of protamine (100 μg/ml) and D-mannose (1.25 mg%) on the adhesion of strain ATCC 23503 on urinary cells. The most consistent results are those for inhibitor being mixed first with urinary cells. Bacterial binding with the cells is complete after 15 min contact.

Contact of protamine with the bacteria first also leads to appreciable inhibition of adhesion; however, under these conditions, more extensive inhibition is obtained after a longer period of contact between the inhibitor and bacteria. In contrast, a long period of contact between D-mannose and bacteria could reduce the degree of inhibition of adhesion recorded. Nevertheless, under all test conditions, inhibition of adhesion was equal to or greater than 80%.

Table 4 shows data on the inhibition by protamine (100 μg/ml) of the adhesion for the other three strains tested. Inhibition appears to be strain dependent and varies, according to the strain tested, between 50 and 88%.

Table 5 illustrates the data on the influence on inhibition of adhesion by washing after the contact period between protamine or D-mannose and

TABLE 5

Influence of washing on the inhibition of adhesion of E. coli ATCC 23503 on urinary cells

Test conditions	Culture medium					
	BHI			CFB		
	No. of bacteria/cell	Adhesion (%)	Inhibition (%)	No. of bacteria/cell	Adhesion (%)	Inhibition (%)
Without inhibition	31	100	–	32	100	–
+ protamine + cells, 15 min	2	6.5	93.5	1	3.1	96.9
+ protamine + cells, 15 min and 3 washings	5	16.1	83.9	4	12.5	87.5
+ mannose + cells, 15 min	2	6.5	93.5	1	3.1	96.9
+ mannose + cells, 15 min and 3 washings	3	9.7	90.3	3	9.4	90.6

urinary cells. The inhibitory effect of protamine and D-mannose on adhesion is still significant even after three washes with PBS buffer, indicating that the link between the inhibitor and the epithelial cell is indeed strong only about 10% of the inhibitory power being lost during such treatment.

Discussion

The first point to be clarified concerns whether the method is effective as used under our experimental conditions. The fact that we obtained values close to those in the literature concerning strain ATCC 23503 (Davis et al., 1981) is a positive indication in favor of the use of this method to classify wild pathogenic strains according to their adhesion capacities.

Moreover, the reproducibility of the method is good, as indicated by the similarity between values obtained in repeated experiments.

When considering the inhibition tests, adhesion of the bacteria could be inhibited to a large extent. It appears that the most consistent data are obtained when the inhibitor is mixed with urinary cells; nevertheless, mixing initially with bacteria also yielded satisfactory results. This is an important observation in view of the potential therapeutic use of such inhibitors whereby in some circumstances we could envisage 'coating' human cells in vivo in order to prevent adhesion of organisms. The fact that washing does not give rise to significant reduction of the inhibitory power increases the possibility of the potential use of these inhibitors in therapeutics.

The strain ATCC 23503 [07:K1(L)NM] of urinary origin is able to express several types of pili, including those of type 1 (Avots-Avotins et al., 1981). The action of D-mannose on this kind of pili is well known and thus a decrease in adhesion of this strain under the influence of D-mannose could be predictable.

Regarding protamine, this compound has also been shown to inhibit adhesion on human epithelial bucal cells by several strains of *Ps. aeruginosa* (Boussard et al., 1989). The nature of the binding between protamine and bacteria would appear to be non-specific since the former binds to several different species of Gram-negative organisms. This phenomenon could be due to association between the negative charges of the bacterial outer membrane and the positive ones of protamine. A similar action may occur with epithelial cell carbohydrates as these structures are widely implicated to participate in the links between bacteria and host (Rutter et al., 1984; Longer and Robinon, 1986).

Conclusion

The model used in our experiments provides a reproducible system for the classification of *E. coli* strains according to their adhesion properties. Moreover, it allows the demonstration of inhibition of adhesion by protamine and D-mannose. Such inhibition occurs after a brief period of contact (not exceeding 15 min) which indicates promise in the possible therapeutic use of inhibitors.

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