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Control of Staphylococcal Adhesion to Polymethylmethacrylate and Enhancement of Susceptibility to Antibiotics by Poloxamer 407

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We studied the antiadhesive effect of Poloxamer 407 (P407), together with modifications in the antimicrobial susceptibility of residual adherent staphylococci. Bacterial adherence was markedly inhibited (77% to more than 99.9%) whether polymethylmethacrylate was exposed to P407 before or during the adherence assay. Furthermore, residual adherent staphylococci appeared to be more susceptible to antibiotic activity, suggesting that combination of P407 with antibiotics could be a promising approach to the prevention of infection of foreign material.

Medical implants frequently have to be removed because of bacterial infection. Bacterial adherence, a prerequisite in these infections (7, 10, 29), is initially reversible but later becomes irreversible (18). The biomaterial surface is colonized, and bacteria develop an environment that protects them from host defenses and antibiotics (14, 27). The best prophylactic approach would be to prevent bacterial adhesion or to kill bacteria shortly after adhesion, during the reversible phase. Poloxamers, nontoxic, inert surfactants, are a broad group of compounds that were introduced commercially in the early 1950s as food additives and in pharmaceutical preparations. Some of these compounds have been reported to have unusual rheological characteristics (26) which permit them to be administered in liquid form and to gel in situ upon warming to body temperature. Investigations have mainly focused on Poloxamer 407 (P407), which forms a gel at the lowest concentration, is the least toxic, and is the most stable in solution (9). This material has already been proposed as a matrix for drug delivery (11, 21). Some poloxamers have been shown to have abhesive (antiadhesive) activity, which makes them useful as detergents in contact lens solutions (23). The prevention of fibrinogen adsorption and platelet adhesion to surfaces (1, 17) could be useful in the prevention of medical-implant infection. These poloxamers also prevent postsurgical tissue adhesion (4, 24).

Polymethylmethacrylate (PMMA) is an acrylic cement widely used in orthopedic surgery. It has the major disadvantage of promoting bacterial adherence (15). Antibiotics have been incorporated into this material for the prophylaxis of bone infections, but adherence studies have shown that bacteria can adhere to such pretreated PMMA and survive despite high antibiotic concentrations that would be lethal to bacteria in suspension (15, 22).

The aim of this study was to assess (i) the in vitro abhesive effect of P407 using PMMA coverslips as a support for bacterial adherence and (ii) changes in the susceptibility of residual adherent bacteria to antimicrobial agents.

Three staphylococcal strains known to adhere to PMMA were used: *Staphylococcus aureus* MRGR3 was a generous gift from D. Lew (Geneva, Switzerland), and *S. aureus* A970862 and *S. epidermidis* RP62A (ATCC 35984) were kindly provided by J. Etienne (Lyon, France). MRGR3 is a methicillin-resistant bloodstream isolate from a patient with an infected indwelling device. A970862 was isolated from a patient with septicemia. RP62A, a prolific slime producer, was first isolated by Christensen from an infected peritoneal dialysis catheter (2). Aliquots of each bacterial suspension were maintained at −80°C in Trypticase soy broth (TSB) (Difco, Detroit, Mich.) with 10% glycerol (Difco). For each experiment, aliquots of 1 ml were cultured twice until the early stationary phase in TSB medium at 37°C for 18 to 24 h. Cells were harvested by centrifugation, washed twice, and resuspended in pH 7.3 phosphate-buffered saline (PBS) with Ca, Mg, and 0.25% glucose (Bio-Mérieux, Campronne, France). The density of bacterial suspensions was adjusted by optical density measurement (Unicam SP 1800 Ultraviolet Spectrophotometer; Pye Unicam Ltd., Cambridge, England) and checked by inoculation on Trypticase soy agar (TSA) (Difco).

MICs were determined by the broth dilution method using geometric twofold dilutions in Mueller-Hinton broth (Difco)

TABLE 1. Inhibition of *S. aureus* and *S. epidermidis* adherence to PMMA in the presence of P407

Strain and treatment (no. of isolates)	Mean surface bacterial density ($\log_{10}$ CFU/cm^2) ± SD	Residual adherent bacteria (%)	Student test *P* value vs control
S. aureus MRGR3			
Control (17)	3.55 ± 0.13		
4% P407 (6)	2.20 ± 0.60	4.6	<0.001
15% P407 (6)	<1	<0.28	<0.001
S. aureus A970862			
Control (12)	4.77 ± 0.38		
4% P407 (6)	3.89 ± 0.71	13.3	<0.01
15% P407 (12)	1.73 ± 0.31	0.09	<0.001
S. epidermidis RP62A			
Control (25)	4.41 ± 0.15		
4% P407 (19)	3.28 ± 0.69	7.4	<0.001
15% P407 (6)	<1	<0.04	<0.001

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TABLE 2. Effect of 24-h PMMA pretreatment with P407 and/or vancomycin on *S. aureus* and *S. epidermidis* adherence to PMMA

Strain and pretreatment (no. of isolates)	Mean surface bacterial density ($\log_{10}$ CFU/cm^2) ± SD	Residual adherent bacteria (%)	Student test *P* value vs control
S. aureus MRGR3			
PBS control (15)	3.65 ± 0.17		
Vancomycin at 20 mg · ml^{-1} (9)	3.62 ± 0.27	95	NS[a]
4% P407 (6)	3.01 ± 0.48	23	<0.01
25% P407 (9)	2.64 ± 0.28[b]	9.9	<0.001
25% P407 + vancomycin at 20 mg · ml^{-1} (9)	2.56 ± 0.32[c]	8.2	<0.001
S. epidermidis RP62A			
PBS control (10)	4.55 ± 0.18		
4% P407 (6)	3.38 ± 0.41	6.7	<0.001

[a] NS, no significant difference.
[b] No significant difference versus pretreatment with 4% P407.
[c] No significant difference versus pretreatment with 25% P407.

in the presence or absence of P407 at two different concentrations (4 and 8%) for the three strains and for each antibiotic tested. MICs were determined after incubation at 37°C for 18 h. The antimicrobial agents were vancomycin hydrochloride (Lilly, Saint-Cloud, France) and gentamicin sulfate (Schering-Plough, Levallois-Perret, France). MICs in the presence of 15% P407 were determined by the E-test method because of marked viscosity at 37°C. A final inoculum of 10^7 CFU · ml^{-1} was prepared in Mueller-Hinton broth containing 15% P407 and was inoculated by swabbing the surfaces of Mueller-Hinton agar plates in three directions at 20°C. Strips of vancomycin and gentamicin were placed in the plates and incubated at 37°C for 18 h.

P407 (Pluronic F-127) was a gift from BASF (Levallois-Perret, France). P407 solutions were prepared in sterile PBS by the cold method described by Schmolka (26). Freshly prepared solutions were sterilized by filtration through a 0.22-μm-pore-size Millipore filter.

Adherence assay. PMMA coverslips (total surface area, 1.6 cm^2) were used as adherence supports. They were placed in glass tubes containing PBS with bacteria at 10^7 CFU · ml^{-1} and 0, 4, or 15% (wt/wt) P407. The tubes were then statically incubated at 37°C for 4 h. To remove nonadherent bacteria after incubation, each specimen was washed eight times by gentle shaking in distilled water (eight washes eliminated the majority of nonadherent bacteria).

Quantification of adherence. After the adherence assay and rinses, each specimen was placed in a glass tube containing 1 ml of filtered EDTA-trypsin solution (0.05%; GIBCO, Cergy Pontoise, France) and incubated for 15 min at 37°C. A 4-ml volume of sterile saline was then added to the trypsin solution, and the coverslips were sonicated for 30 min in a Transsonic TP 690 (ELMA) ultrasonic cleaner. The absence of any effect of trypsin and sonication on bacterial viability was checked first.

Quantitative cultures were then performed by plating 100 μl of the solution, either undiluted or serially diluted 10-fold on TSA. The results were expressed as numbers of CFU per square centimeter for an initial suspension of 10^7 CFU · ml^{-1}. When the number of adherent bacteria was 0 or below 10 CFU · cm^{-2} (our detection limit), we recorded it as <1 $\log_{10}$ CFU · cm^{-2}. The rate of residual bacterial adhesion, expressed as a percentage, is given as the number of adherent bacteria in treated samples divided by the number in control samples.

To count bacteria not detached by trypsin and sonication, after three rinses in distilled water, coverslips were pressed on TSA (10 times per side) and TSA was incubated for 24 h at 37°C.

Interactions between P407 and antimicrobial agents. No difference was noted in the MICs of gentamicin and vancomycin against the three strains when tested in the presence and absence of P407 at various concentrations. The MICs of gentamicin and vancomycin were 12 and 1.5 mg · liter^{-1} for MRGR3, 0.25 and 1 mg · liter^{-1} for A970862, and 6 and 1.5 mg · liter^{-1} for RP62A.

A possible P407 effect on the antibiotic susceptibility of adherent staphylococci was evaluated. Following the adherence assay in the presence of 4% P407, PMMA coverslips (with residual adherent bacteria) were incubated with an antimicrobial agent in brain heart infusion broth (Difco). The vancomycin concentration studied was the MIC, and the incubation time was 24 or 48 h. Gentamicin concentrations were the MIC and twice the MIC, and the incubation time was 24 h. Coverslips were rinsed three times before quantification of adherence was done as described above.

Preincubation assay. To determine whether P407 preferentially anchors to PMMA or to bacterial surfaces, they were pre-exposed to P407 for 24 h before the adherence assay. PMMA coverslips were incubated at room temperature with PBS and 0 (controls), 4, or 25% P407 and/or vancomycin at 20 mg · ml^{-1} and then rinsed three times in sterile water. For bacterial pre-exposure, P407 was added to TSB. The adherence assay was then performed as usual with PBS alone.

To identify a possible antibacterial effect of P407, bacterial densities after 4 and 24 h of exposure to 4 and 15% P407 were compared to those of control suspensions.

The groups treated with P407 were compared with the controls by using Student's *t* test.

In every case, the number of bacteria not detached by trypsin and sonication was negligible in comparison with the number of detached bacteria. The mean inoculum size was 10^7 CFU · ml^{-1}.

S. aureus and *S. epidermidis* adherence was strongly inhibited by P407, whatever the strain's capacity for adherence (Table 1). P407 comprises a central hydrophobic nucleus of polyoxypropylene surrounded by hydrophilic sequences of polyoxyethylene (POE) (8). A proposed mechanism of action is that the hydrophobic central block of the poloxamer anchors to the material and/or the bacterial surface, its protruding POE chains forming a sterically stabilized barrier to adhesion. The POE chains must be of sufficient length and mobility in aqueous solution to ensure an effective barrier to adhesion. The polyoxypropylene block size may also be a key factor in firm anchorage of the molecule to the material and/or the bacterial

TABLE 3. Density of *S. aureus* and *S. epidermidis* after 4 and 24 h of exposure to P407 at 4 and 15%

Strain and incubation time (h)	Mean bacterial density ($\log_{10}$ CFU/ml) ± SD[a]		
	Control	4% P407	15% P407
S. aureus MRGR3			
4	7.08 ± 0.15	7.07 ± 0.25	7.24 ± 0.37
24	8.29 ± 0.79	8.21 ± 0.89	7.69 ± 0.74
S. epidermidis RP62A			
4	7.09 ± 0.22	7.36 ± 0.36	7.32 ± 0.69
24	8.41 ± 0.67	8.36 ± 0.32	7.75 ± 0.97

[a] Numbers of isolates tested: control, 16; 4 and 15% P407, 8.

TABLE 4. Enhancement of vancomycin activity at 24 and 48 h on residual adherent *S. aureus* MRGR3 after 4 h of PMMA incubation with bacteria and 4% P407

Duration (h) and treatments (adherence assay, treatment)	Surface bacterial density (log_{10} CFU/cm^2)		% Residual adherent bacteria (*P* value)		Bacterial density evolution[b]
	Preantibiotic	Final[a]	Vs control, control	Vs control, vancomycin	
24					
Control, control		7.19 ± 0.19			
Control, vancomycin at MIC	3.55	4.73 ± 0.72	0.35 (<0.001)		15.2
P407, control		6.78 ± 0.21	40.0 (<0.01)		
P407, vancomycin at MIC	2.20	1.14 ± 0.89	$<10^{-4}$ (<0.001)	0.03 (<0.001)	0.086
48					
Control, control		7.36 ± 0.83			
Control, vancomycin at MIC	3.55	5.88 ± 1.15	3.31 (<0.05)		212
P407, control		6.68 ± 0.32	20.9 (NS)[c]		
P407, vancomycin at MIC	2.20	1.17 ± 1.15	$<10^{-4}$ (<0.001)	<0.01 (<0.001)	0.092

[a] The data are means ± standard deviations of nine isolates.
[b] Surface bacterial density change during exposure to vancomycin is the final surface bacterial density divided by the preantibiotic surface bacterial density.
[c] NS, no significant difference.

surface (3). This powerful nonspecific abhesive effect could result from an increase in surface hydrophilicity (3, 12), as hydrophobicity and adherence generally correlate (16, 25). In keeping with the hypothesis that poloxamers create a hydrated layer around bacteria and /or biomaterials, it is interesting that, as for the adherence of *Pseudomonas aeruginosa* (23), we found that increasing concentrations of P407 enhanced the inhibition of staphylococcal adherence (Table 1).

The nonspecific nature of the abhesive effect is a great advantage, as more than one type of bacterium is commonly isolated from infected biomaterials (20). Moreover, prevention of the adherence of some microorganisms, e.g., some strains of slime-producing *S. epidermidis* that are able to promote the adherence of organisms that would otherwise be nonadherent (5, 6), is of particular interest.

PMMA and bacterial surfaces were exposed to P407 before the adherence assay in order to determine if P407 preferentially anchors to one or the other. At similar P407 concentrations, pretreatment of PMMA was less effective against *S. aureus* adhesion than was the presence of P407 during the adherence assay (Table 2). The addition of vancomycin seemed to increase the abhesive effect, but the difference was not significant. Adherence of *S. epidermidis* was inhibited equally by pretreatment of PMMA and by the presence of P407 in the adherence assay, but it was less inhibited by pre-exposure of *S. epidermidis* to P407 (residual adherence, 52.78 and 28.49% at 4 and 15% P407, respectively). This suggested a stronger interaction with the PMMA surface than with the surface of *S. epidermidis*. Nevertheless, Portolés et al. (23) observed that the adherence of *P. aeruginosa* to contact lenses was inhibited (99%) when bacteria were pre-exposed to P407 but not when the contact lenses were pretreated. Conversely, poloxamer-treated and POE-grafted surfaces were shown to be protein or *S. epidermidis* resistant (3, 17). The differences in the above results may be explained by the characteristics of the strains used, the different supporting materials, and different methods of treating these materials.

Poloxamers have been shown to be active against mycobacteria (12, 13). In our work, P407 did not have significant intrinsic activity against staphylococci when it was present in the broth, even if this was not a favorable medium for microbial growth (Table 3).

We have previously shown that combination of vancomycin with P407 does not impair antimicrobial activity (28a). This observation is interesting, as the two components might act synergistically against implant colonization and infection. This was confirmed by our results, as vancomycin and gentamicin were far more effective on residual adherent staphylococci when P407 was present during the adherence assay (Tables 4 and 5). While the surface bacterial density on PMMA continued to increase in control specimens when they were exposed to the antibiotic, it decreased when bacteria had adhered in the presence of P407. Synergism between P407 and antibiotics appeared to be a two-step phenomenon: first, a decrease in the

TABLE 5. Enhancement of gentamicin activity at 24 h on residual adherent *S. aureus* A970862 after 4 h of PMMA incubation with bacteria and 4% P407

Treatments (adherence assay, treatment)	Surface bacterial density (log_{10} CFU/cm^2)		% Residual adherent bacteria (*P* value)		Bacterial density evolution[c]
	Preantibiotic	Final[a]	Vs control, control	Vs control, gentamicin[b]	
Control, control		6.89 ± 0.10			
Control, gentamicin (1 × MIC)	4.77	6.78 ± 0.40	76.54 (NS)[d]		102
Control, gentamicin (2 × MIC)	4.77	5.62 ± 0.15	5.36 (<0.001)		7.14
P407, control		5.74 ± 0.90	7.12 (<0.02)		
P407, gentamicin (1 × MIC)	3.89	4.71 ± 0.22	0.66 (<0.001)	0.86 (<0.01)	6.54
P407, gentamicin (2 × MIC)	3.89	<1	$<10^{-4}$ (<0.001)	$<10^{-3}$ (<0.001)	0.001

[a] The data are means ± standard deviations of six isolates.
[b] The data were compared at the same concentration of gentamicin.
[c] Surface bacterial density change during exposure to gentamicin is the final surface bacterial density divided by the preantibiotic surface bacterial density.
[d] NS, no significant difference.

number of adherent bacteria, second, for residual adherent bacteria, a qualitative modification of binding, resulting in restoration of susceptibility to antibiotic activity. The P407 barrier to adhesion may thus prevent the colonization of PMMA and the resulting modifications of infecting bacteria, leaving them more susceptible to antibiotic action.

This study illustrates the potential of P407 for inhibiting the attachment of *S. aureus* and *S. epidermidis* and for increasing their susceptibility to antibiotics once they are adherent. Moreover, poloxamer-coated surfaces have been shown to be resistant to fibrinogen and platelet adhesion (1, 17). As these two components are present in vivo and cover foreign-body surfaces, thereby promoting bacterial adhesion (19, 28), this last property should reinforce the inhibitory effect of P407 on bacterial adherence. To validate this hypothesis, studies with a guinea pig model of foreign-body infection which unites all of the in vivo conditions are now under way in our laboratory. An effective way of decreasing infection of biomaterials is to reduce surface adhesion and rapidly kill bacteria that still adhere before biofilm formation. The nonspecificity of the abhesive effect of P407, together with its inhibition of antibiotic resistance mechanisms, suggests that the combination of P407 with an antimicrobial agent could be a promising approach to prevent infection of foreign materials in vivo. Antibiotic-loaded poloxamer could be used to coat prosthetic material at the time of surgery in order to protect it against primary infection and early hematogenous infection.

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