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Modulation of Biofilms of *Pseudomonas aeruginosa* by Quinolones

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The interaction between four fluoroquinolones (ciprofloxacin, norfloxacin, pefloxacin, and ofloxacin) and biofilms of *Pseudomonas aeruginosa* in wells of microtiter plates and on segments of vascular catheters was studied in an in vitro model of vascular catheter colonization. Subinhibitory concentrations (one-half, one-fourth, and one-eighth of the MIC) of the fluoroquinolones reduced the adherence of *P. aeruginosa* to 30 to 33, 44 to 47, and 61 to 67% of that of controls, respectively. The addition of high concentrations of the fluoroquinolones (12.5 and 400 μg/ml) to preformed biofilms (grown for 48 h at 37°C) decreased the adherence of *P. aeruginosa* to 69 to 77 and 39 to 60% of that of controls, respectively. In an in vitro model of vascular catheter colonization, subinhibitory concentrations (one-half, one-fourth, and one-eighth of the MIC) of fluoroquinolones reduced the number of adherent bacteria to 21 to 23, 40 to 46, and 55 to 70% of that of the controls, respectively. Scanning electron microscopy demonstrated a significant reduction in glycocalyx formation and adherent bacteria in the presence of pefloxacin at one-half to one-eighth of the MIC. Vascular catheter segments precolonized with *P. aeruginosa* for 24 h and exposed to the fluoroquinolones at 4 to 25 times the MIC (50 μg/ml) for 2 h showed <5% growth of adherent cells compared with controls. No adherent organisms were cultured in the presence of 8 to 50 times the MIC (100 μg/ml). Scanning electron microscopy studies of preformed biofilms exposed to pefloxacin verified the results obtained by culture. These data show that subinhibitory concentrations of ciprofloxacin, norfloxacin, pefloxacin, and ofloxacin inhibit the adherence of *P. aeruginosa* to plastic surfaces and vascular catheters. Clinically achievable concentrations of fluoroquinolones (50 to 100 μg/ml) were able to eradicate preformed biofilms on vascular catheters.

The use of various medical devices such as indwelling vascular catheters, cardiac pacemakers, prosthetic heart valves, chronic ambulatory peritoneal dialysis catheters, and prosthetic joints has greatly facilitated the medical and surgical management of serious illnesses. Bacterial infections following colonization and biofilm formation on these devices are a major cause of morbidity in patients receiving prosthetic devices (5). Biofilms have also been demonstrated on tissues in chronic bacterial diseases that are characterized by resistance to antimicrobial chemotherapy (27). Extracellular slime or glycocalyx production is an important factor in the adherence of bacteria and their protection from host defense mechanisms. Extensive glycocalyx production has been demonstrated on cultures of *Staphylococcus epidermidis* (14), *S. aureus* (4), *Pseudomonas aeruginosa* (15), *Escherichia coli* (3), and *Bacteroides* species (16).

Different approaches (including the use of various antimicrobial agents) to the eradication of biofilm bacteria have been studied. The combination of tobramycin and piperacillin (1), the combination of tosufloxacin and erythromycin (12), clindamycin (16), and clarithromycin (27, 28) have been reported to be effective in eradication of biofilm-associated bacteria. However, the clinical usefulness of these experimentally effective strategies remains to be established (27).

In this study, we investigated the effects of subinhibitory concentrations of fluoroquinolones on glycocalyx production and adherence of *P. aeruginosa* to plastic surfaces and to vascular catheters in an in vitro model of vascular catheter colonization. The interaction between preformed biofilms of *P. aeruginosa* and the fluoroquinolones was also studied.

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MATERIALS AND METHODS

Organisms. A total of 50 *P. aeruginosa* isolates from clinical specimens (mainly from blood) recently submitted to the Clinical Pathology Department, Kasr El-Aini Hospital, Cairo University, Cairo, Egypt, were used. Isolation, purification, and maintenance of the isolates were done with brain heart infusion agar or Mueller-Hinton agar medium. The organisms were stored in skim milk at −70°C. Before use, the organisms were subcultured in Mueller-Hinton broth for 18 to 24 h at 37°C. The inoculum was standardized to contain 10^5 to 10^6 CFU/ml by McFarland standards and verified by viable counts.

Antimicrobial agents. Antimicrobial agents were kindly provided by Miles Laboratories Inc., West Haven, Conn. (ciprofloxacin); Merck Sharp & Dohme, Rahway, N.J. (norfloxacin); Roger Bellon Laboratories, Neuilly-sur-Seine, France (pefloxacin); and Ortho Pharmaceuticals, Raritan, N.J. (ofloxacin). The fluoroquinolones were stored and diluted in accordance with the manufacturers' instructions. All four fluoroquinolones were used in all of the experiments except scanning electron microscopy, in which only pefloxacin was used.

Adherence assay. (i) Tube method. Glycocalyx production was determined as described by Christensen et al. (7). Briefly, two to three colonies of *P. aeruginosa* were inoculated into 5 ml of Trypticase soy broth (TSB) in plastic conical tubes (Falcon, Oxnard, Calif.) in triplicate. Saccharide-free basal medium (TSB without glucose) that lacks the substrate for polysaccharides was used as a control. Cultures were incubated at 37°C for 18 to 20 h. The contents were aspirated, and one unstained tube and one each stained with trypan blue and safranin were examined. Slime positivity was judged by the presence of a visible unstained or stained film lining the wall of the tube. Formation of a ring at the liquid-air interface was not considered a positive result. The tubes were also examined by phase-contrast microscopy to detect the presence of adherent microcolonies.

(ii) Spectrophotometric method. Adherence was determined by using a spectrophotometric method described by Christensen et al. (8). Briefly, stationary 18-h cultures of *P. aeruginosa* in 5 ml of TSB were washed, diluted with fresh TSB, and standardized to contain 5×10^5 to 1×10^6 CFU/ml. Aliquots (0.2 ml) of the diluted cultures were added to the wells of sterile flat-bottom polystyrene tissue culture plates. The plates used were Microtest III (Falcon no. 3072; Becton Dickinson). The manufacturer sterilizes these plates with gamma radiation and electrically charges the surfaces to diminish the hydrophobicity of the polystyrene. Following 18 h of incubation at 37°C, the contents of the tissue culture plates were gently aspirated with a Pasteur pipette connected to a low vacuum.

The plates were then washed four times with sterile phosphate-buffered saline (pH 7.2). Slime and adherent organisms were fixed overnight with Bouin fixative. The fixative was removed by washing the wells three or four times with 50% ethanol. The wells were stained with Hucker crystal violet. The excess stain was removed by placing the plates under running distilled water, and then the plates were air dried. The optical density of the stained adherent films was read with a Microplate Reader (Titertek Plus; ICN Biomedicals, Inc., Costa Mesa, Calif.) at a wavelength of 550 nm. Measurements were performed in triplicate and repeated three times.

Effects of subinhibitory concentrations of fluoroquinolones on adherence of *P. aeruginosa*. MICs of the fluoroquinolones (ciprofloxacin, norfloxacin, pefloxacin, and ofloxacin) were determined by a broth microdilution technique (19). The concentrations of the drugs in the medium ranged from 0.125 to 128 μg/ml. The MIC was defined as the lowest concentration of a drug which inhibited visible growth at 37°C after overnight incubation. Each fluoroquinolone was used at one-half, one-fourth, and one-eighth of the MIC to study its effect on the adherence of *P. aeruginosa* as described above (spectrophotometric method). Various concentrations of antimicrobial agents (0.1 ml in TSB) were added to triplicate wells containing 0.1 ml of the culture. Drug-free medium was used in control experiments.

Interaction between preformed biofilms of *P. aeruginosa* and fluoroquinolones. Biofilm formation by *P. aeruginosa* was carried out in 96-well flat-bottom tissue culture plates as described above (spectrophotometric method). After 48 h of incubation at 37°C, the supernatant from each well was aspirated and the wells were washed twice with normal saline (0.9% sodium chloride in water) without disturbing the biofilms at the bottom of the wells. Various fluoroquinolone concentrations (12.5 to 400 μg/ml) in 200 μl of normal saline were then added to the wells. Normal saline with no antimicrobial agents was added to the control wells. The plates were incubated at 37°C for 48 h.

After incubation, the contents of each well were aspirated and the wells were washed twice with normal saline. The adherent cells were fixed in place with Bouin's fixative solution. The wells were stained with Hucker crystal violet, and the excess stain was removed by washing under running distilled water. After air drying, the optical density of the stained film was read with a Microplate Reader at a wavelength of 550 nm.

In vitro model of vascular catheter colonization. (i) Preparation of inoculum. An 18-h culture, grown in TSB with 0.3% yeast extract, was harvested by centrifugation and washed twice in normal saline. The suspension was standardized by using McFarland standards. The inoculum size was confirmed by viable count on Trypticase soy agar plates. A 500-ml bag of sterile normal saline containing 5% glucose (D_5NS) was used as the reservoir for colonization of catheter segments. Inocula ranging in size from 10^5 to 10^9 CFU were added to the infusate.

(ii) Colonization of catheters. A modified Robbins device was used to study the formation of biofilms and colonization of catheters by *P. aeruginosa* (14). The device is constructed of an acrylic block 42 cm long with a lumen of 2 by 10 mm. Twenty-five evenly spaced sampling ports are designed in such a way that the catheter material (0.5 cm^2) attached to sampling plugs lies flush with the inner surface without disturbing flow characteristics. The design prevents the occurrence of artifacts of cell sedimentation on the biomaterial and allows salient fluid dynamic features to be defined. The device was modified so that 10-mm segments of vascular catheters would fit across the lips of the sampling ports. We have demonstrated colonization of catheter segments in this model on both the external and the luminal surfaces. After placement of the catheter segments in the sampling inserts, the entire apparatus was sterilized with ethylene oxide.

(iii) Biofilm formation. To study the formation of biofilms of *P. aeruginosa* on vascular catheters, the D_5NS containing the organism was infused through the modified Robbins device. An intravenous infusion pump (Gemini PC2; IMED, San Diego, Calif.) and an infusion set (Travenol Laboratories, Deerfield, Ill.) was used to infuse 30 ml of solution per h.

After infusion of 500 ml of D_5NS solution, six vascular catheter segments were removed with sterile forceps and two of these were cultured on agar plates by embedding them slightly in agar. Four segments were sonicated in precooled saline for 30 s at a 30% cycle and an output of 3.5 to dislodge the sessile (adherent) cells. Two of these segments were cultured on agar plates, while the others were washed in normal saline three times by vortexing them for 10 s before culturing them on agar plates. Quantitative cultures revealed organisms (*P. aeruginosa*) from all three of the normal saline washes.

The rest of the catheter segments, remaining in the device, were flushed by running 250 ml of normal saline through the device over 1 h to remove planktonic (free-floating) bacteria. After flushing, the segments were divided into five groups. Group one (three segments) was cultured on agar plates without further processing. Groups two and the three (three segments each) were sonicated for 30 s at a 30% cycle and an output of 3.5. Segments from group 2 were cultured on agar without further processing. Group 3 segments were washed with normal saline once by vortexing for 10 s. Group four segments (five segments) were sonicated and washed twice. Group 5 (five segments) was sonicated for 2 min and vortexed for 1 min, and the number of cells released from the segments into normal saline was determined by viable counts. Segments from groups 1 and 3 were also used for scanning electron microscopy.

(iv) Catheter cultures. The presence of viable cells on the catheter segments was evaluated by embedding the catheter segments into Mueller-Hinton agar plates. The plates were incubated at 37°C and examined for growth around the segments after 24 and 48 h.

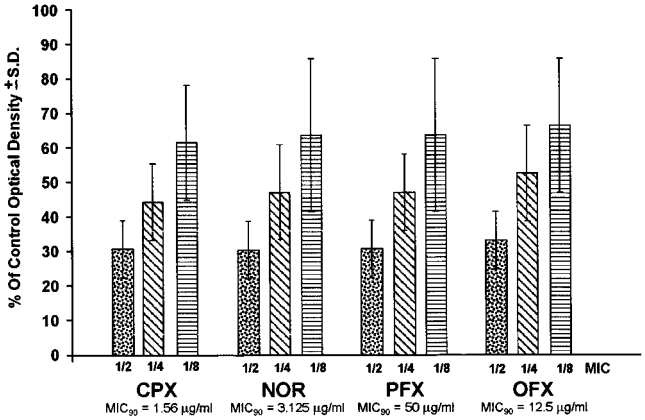

FIG. 1. Relative optical densities of adherent biofilms of 20 isolates of *P. aeruginosa* in the presence of ciprofloxacin (CPX), norfloxacin (NOR), pefloxacin (PFX), and ofloxacin (OFX) at one-half, one-fourth, and one-eighth of the MIC. MIC_{90}, MIC for 90% of the isolates tested. S.D., standard deviation.

(v) Scanning electron microscopy. Determination of the presence of organisms (dispersed or in microcolonies) and semiquantitative analysis of biofilm, including glycocalyx, formation were done by scanning electron microscopy (17). Catheter segments were prepared for scanning electron microscopy by fixation for 3 h at 4°C in 2.5% glutaraldehyde in 0.1 M cacodylate buffer containing 0.15% ruthenium red. The segments were rinsed in fresh 0.1 M cacodylate buffer for 10 min (repeated three times). This was followed by refixation for 60 min at 4°C in 1.5% osmium tetroxide in the same buffer. The catheters were washed in 0.1 M cacodylate buffer for 10 min (repeated three times). The segments were dehydrated in a series of aqueous ethanol solutions (30 to 100%) and dried with a critical-point dryer (Autosamdri 814; Tousimis, Rockville, Md.) with carbon dioxide. The specimens were mounted on aluminum stubs with silver paste, allowed to dry for 3 h, and then coated with gold-palladium with a Polaron E5100 II cool-sputter coater. The segments were examined in a scanning electron microscope (S-500; Hitachi, Mountain view, Calif.) at 20 kV.

(vi) Effects of subinhibitory concentrations of fluoroquinolones on adherence of *P. aeruginosa*. To study the effects of subinhibitory concentrations of fluoroquinolones on bacterial adherence in this in vitro model of vascular catheter colonization, fluoroquinolones were added to the inoculated infusate bags (500 ml of D_5NS) during colonization at one-half, one-fourth, and one-eighth of the MIC. Drug-free infusate was used as a control. For scanning electron microscopy experiments, only pefloxacin was used.

(vii) Interaction between preformed biofilms of *P. aeruginosa* and fluoroquinolones. To study the effects of fluoroquinolones on preformed biofilms of *P. aeruginosa*, 500-ml bags of D_5NS containing the organism were infused through the modified Robbins device (30 ml/h). Bags of saline solution (250 ml) containing the fluoroquinolones (50 and 100 μg/ml) were then infused through the modified Robbins device (125 ml/h) for 2 h.

(viii) Control. Drug-free saline bags were used as a control.

Statistical analysis. The data were converted to percentage of baseline, and a one-way analysis of variance was performed for repeated measures. Follow-up tests were performed with a paired *t* test using a conservative *P* value of 0.01 for statistical significance.

RESULTS

Adherence assay. The results of the tube method showed that 20 of the 50 *P. aeruginosa* isolates were positive for glycocalyx production. The results of the spectrophotometric method revealed that the optical densities of the formed biofilms of these 20 isolates were 0.12 to 0.56. The isolates that were negative for glycocalyx production by the tube test had optical densities of 0.045 to 0.09. The 20 isolates that were positive for glycocalyx production were selected for further studies.

Effects of subinhibitory concentrations of fluoroquinolones on glycocalyx production and adherence of *P. aeruginosa*. The MICs of the fluoroquinolones for the 20 adherent isolates were 1.0 to 80 μg/ml, and the MICs for 90% of the isolates tested were 1.56 to 50 μg/ml. The MICs for the two isolates used for

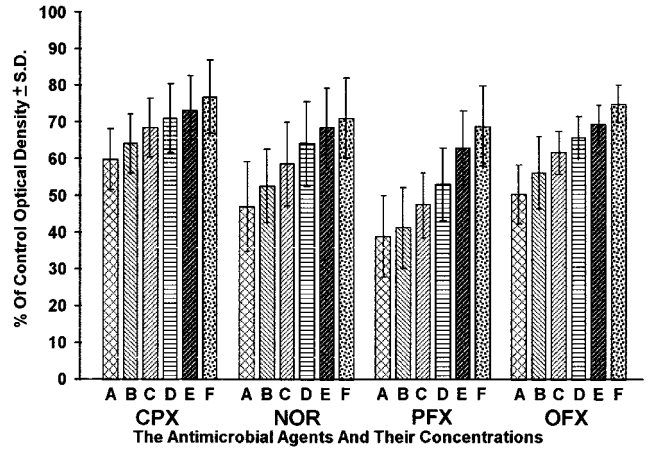

FIG. 2. Relative optical densities of preformed biofilms of 20 isolates of *P. aeruginosa* after treatment with ciprofloxacin (CPX), norfloxacin (NOR), pefloxacin (PFX), and ofloxacin (OFX) for 48 h. A, 400 μg/ml; B, 200 μg/ml; C, 100 μg/ml; D, 50 μg/ml; E, 25 μg/ml; F, 12.5 μg/ml. S.D., standard deviation.

in vitro colonization of vascular catheters ranged from 2 to 12 μg/ml. Figure 1 shows that all four fluoroquinolones at the subinhibitory concentrations tested significantly reduced the optical density of the biofilms of *P. aeruginosa* ($P < 0.01$). The optical density of the biofilms was reduced to 30 to 33% of that of the control in the presence of the fluoroquinolones at one-half of the MIC and to 61 to 67% in the presence of one-eighth of the MIC. In the presence of one-fourth and one-eighth of the MIC, no inhibitory effect on growth was observed. In the presence of one-half of the MIC, the log number of CFU of the organisms was reduced by 0.1 to 0.8 (range of log numbers of CFU of the untreated samples, 8.1 to 8.9). These reductions in the numbers of organisms correlated with 3 to 10% reductions in the optical densities of biofilms formed by the same organisms.

Interaction between preformed biofilms of *P. aeruginosa* and fluoroquinolones. As shown in Fig. 2, treatment of preformed biofilms of *P. aeruginosa* with quinolone concentrations ranging from 12.5 to 400 μg/ml caused significant reductions in the optical densities of the preformed biofilms ($P < 0.01$). Treatment with the test drugs at 400 μg/ml (approximately 5 to 400 times the MIC) caused reductions in optical density to 39 to 60% of that of the controls, whereas treatment with 12.5 μg/ml reduced the optical density to 69 to 77% of that of the controls. Pefloxacin was the most effective fluoroquinolone in eradicating the preformed biofilms, while ciprofloxacin had the least activity ($P < 0.01$) (Fig. 2).

In vitro model of vascular catheter colonization. Two isolates of glycocalyx-producing *P. aeruginosa* were used in these experiments. The preliminary studies showed that the optimal inoculum size for colonization of catheter segments was 10^6 to 10^8 CFU. The organisms were grown from all segments that were unflushed, unultrasonicated, and unwashed, indicating the presence of both free-floating (planktonic) and adherent (sessile) bacteria (Table 1). When the catheters were flushed only (unsonicated, unwashed), growth was detected in untreated catheters, indicating the presence of adherent bacteria. Catheters that were flushed and ultrasonicated but not washed following ultrasonication also showed bacterial growth, representing dislodged adherent organisms. No growth was detected in the segments that were flushed, ultrasonicated, and then washed. This sequence led to loss of planktonic bacteria by flushing and dislodgement by ultrasonication of sessile bacteria that were subsequently washed off.

TABLE 1. Effects of subinhibitory concentrations of fluoroquinolones on adherence of *P. aeruginosa* in an in vitro model of vascular catheter colonization

Catheter treatment[a]	Organisms expected[b]	Untreated (control)[c]	Treated[c]		
			One-half of MIC	One-fourth of MIC	One-eighth of MIC
UNF, UNS, UNW	FR + AD	+++	+	++	++
FL, UNS, UNW	AD	++	±	+	+
FL, SN, UNW	DG	+	±	±	±
FL, SN, WS	None	−	−	−	−

[a] UNF, unflushed; UNS, unsonicated; UNW, unwashed; FL, flushed; SN, sonicated; WS, washed.
[b] FR, free; AD, adherent; DG, dislodged.
[c] +++, heavy growth; ++, moderate growth; +, slight growth; −, no growth; ±, very little or no growth.

Effects of subinhibitory concentrations of fluoroquinolones on glycocalyx production and adherence of *P. aeruginosa* in the in vitro model of vascular catheter colonization. Catheters colonized in the presence of subinhibitory concentrations of fluoroquinolones also showed growth when cultured unflushed, unsonicated, and unwashed (Table 1). Growth was less than that in untreated catheters, especially in segments treated with fluoroquinolones at one-half of the MIC. Growth was further reduced in treated segments that were flushed but not ultrasonicated or washed. In the presence of one-half of the MIC, many of the catheter segments showed no growth. Treated catheters that were flushed and ultrasonicated but not washed following ultrasonication showed decreased or no growth. No growth was detected in segments that were flushed, ultrasonicated, and then washed. During the infusion period, fluoroquinolones at subinhibitory concentrations had no antibacterial activity on the organisms. The results in Fig. 3 show that the presence of fluoroquinolones at subinhibitory concentrations inhibited the adherence of *P. aeruginosa* to vascular catheters. The range of the mean numbers of CFU per cath-

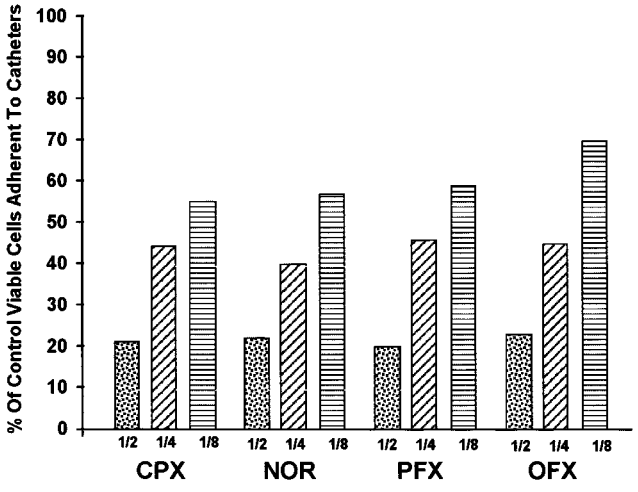

FIG. 3. Relative inhibition of adherence of two *P. aeruginosa* isolates to vascular catheter segments by ciprofloxacin (CPX), norfloxacin (NOR), pefloxacin (PFX), and ofloxacin (OFX) in an in vitro model of vascular catheter colonization. Flushed segments had been sonicated and vortexed as described in Materials and Methods to release adherent bacteria.

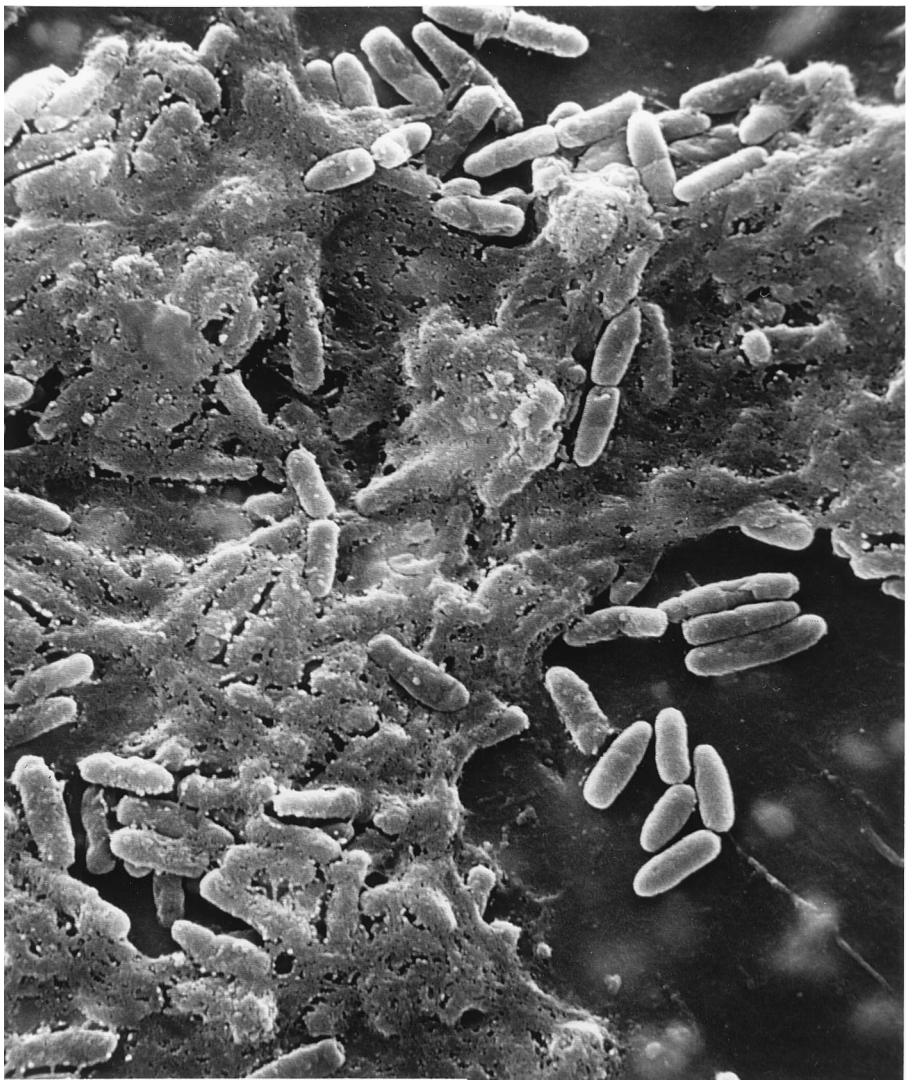

FIG. 4. Scanning electron micrograph showing a large number of microcolonies of *P. aeruginosa* covered with glycocalyx adherent to the surface of an untreated catheter segment after flushing and before sonication (magnification, ×6,200).

eter segment was 32 to 48 for untreated catheter segments. For segments treated with fluoroquinolones at one-half, one-fourth, and one-eighth of the MIC, the ranges of the mean numbers of CFU per catheter segment were 7 to 10, 14 to 22, and 20 to 28, respectively. There was a correlation between this inhibitory effect and the fluoroquinolone concentration (Fig. 3).

Scanning electron microscopy showed a large amount of glycocalyx and adherent microcolonies on untreated, flushed, unsonicated, and unwashed segments (Fig. 4). Complete removal of the adherent bacteria and traces of glycocalyx was observed on untreated, flushed, sonicated, and washed segments. Similar experiments were done with different concentrations of pefloxacin. With subinhibitory concentrations of pefloxacin, a small amount of glycocalyx was present and a significant reduction in the number of adherent bacteria was observed (Fig. 5). This effect increased as the concentration of pefloxacin increased, i.e., the lowest number of adherent cells was observed in the presence of one-half of the MIC (Fig. 5).

Interaction between preformed biofilms of *P. aeruginosa* and fluoroquinolones in the in vitro model of vascular catheter colonization. Flushed, unsonicated, and unwashed catheter segments showed no growth when preformed biofilms were treated with fluoroquinolones at 100 μg/ml (8 to 50 times the MIC). With 50 μg/ml (4 to 25 times the MIC), a few organisms were cultured on some of the segments. As shown in Fig. 6, scanning electron microscopy revealed significant eradication of the biofilms on segments treated with pefloxacin at 50 μg/ml (five times the MIC) for 2 h. Complete eradication of the biofilms was achieved by treatment with pefloxacin at 100 μg/ml (10 times the MIC).

DISCUSSION

Adherence of microorganisms to specific substrates is considered to be a crucial step in the initiation of colonization and subsequent infection (22). Extracellular slime or glycocalyx produced by bacteria plays an important role in the development and persistence of biomaterial-associated infections. The matrix of bacteria and endogenous factors (collectively termed a biofilm layer) which envelops the surface of a colonized implant anchors the bacteria firmly to the surface of the device (11). The adherent bacteria are protected from host defense

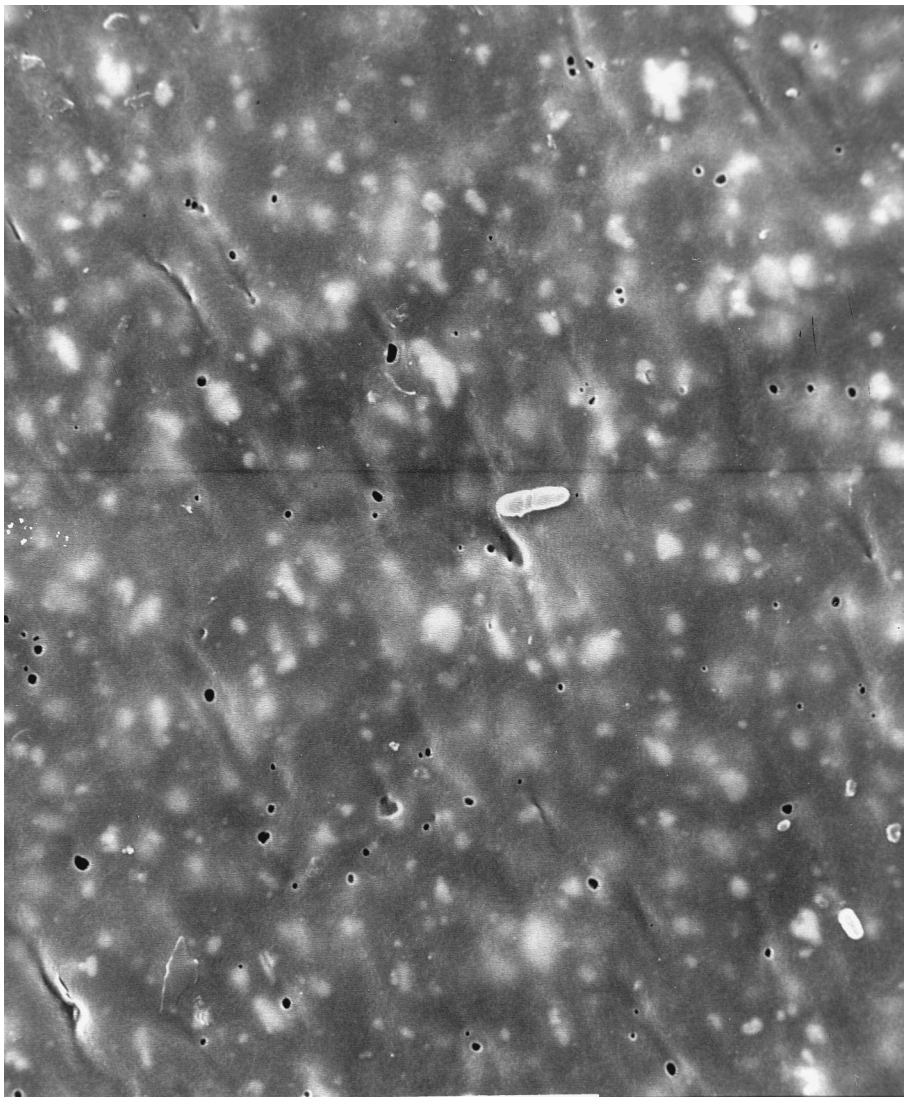

FIG. 5. Scanning electron micrograph showing reduction in the amount of glycocalyx and number of adherent microcolonies of *P. aeruginosa* in the presence of pefloxacin at one-half of the MIC (magnification, ×4,000).

mechanisms and the activity of antimicrobial agents (13). Biofilm-associated bacteria are a major concern in the treatment of device-related infections because of their resistance to a wide range of antimicrobial agents (20, 24, 25).

The effect of subinhibitory concentrations of some antimicrobial agents on glycocalyx formation and adherence of bacteria has been studied by various investigators. Clindamycin and trospectomycin have been used in a previous study. Both antimicrobial agents at concentrations of less than 2 μg/ml significantly reduced *S. epidermidis* glycocalyx formation and adherence to vascular catheters (14). As reported by Lamb et al. (16), subinhibitory concentrations of clindamycin inhibit the adherence of *Bacteroides* species and *E. coli* to bone surfaces in vitro.

Our results show that subinhibitory concentrations of the four fluoroquinolones tested significantly inhibited the glycocalyx production of *P. aeruginosa* and its adherence to inert surfaces of microtiter plates. This inhibitory effect was dependent on the concentration of the agent. Among the concentrations tested, the maximum inhibitory effect of the fluoroquinolones was achieved by one-half of the MIC. With the fluoroquinolones at one-fourth or one-eighth of the MIC, no inhibitory effect on the growth of the organisms was observed. With the fluoroquinolones at one-half of the MIC, the log numbers of CFU were reduced by 0.1 to 0.8 (range of log numbers of CFU of drug-free samples, 8.1 to 8.9). The reduction in the log numbers of CFU by 0.1 to 0.8 decreased the optical density by only 3.0 to 10.0% as determined in control experiments with different inocula in the absence of fluoroquinolones. Therefore, reduction of the optical density of the formed biofilms in the presence of the fluoroquinolones at one-half of the MIC was essentially due to their inhibitory effect on glycocalyx formation and bacterial adherence rather than to their effect on viable counts. Among the fluoroquinolones used, there was no significant difference in the inhibitory effects on bacterial glycocalyx production and adherence.

It has been documented that many chronic infections involve colonization by bacteria growing as adherent biofilms within an extended polysaccharide glycocalyx (27). Despite some efforts, eradication of biofilm-associated bacteria has been found to be

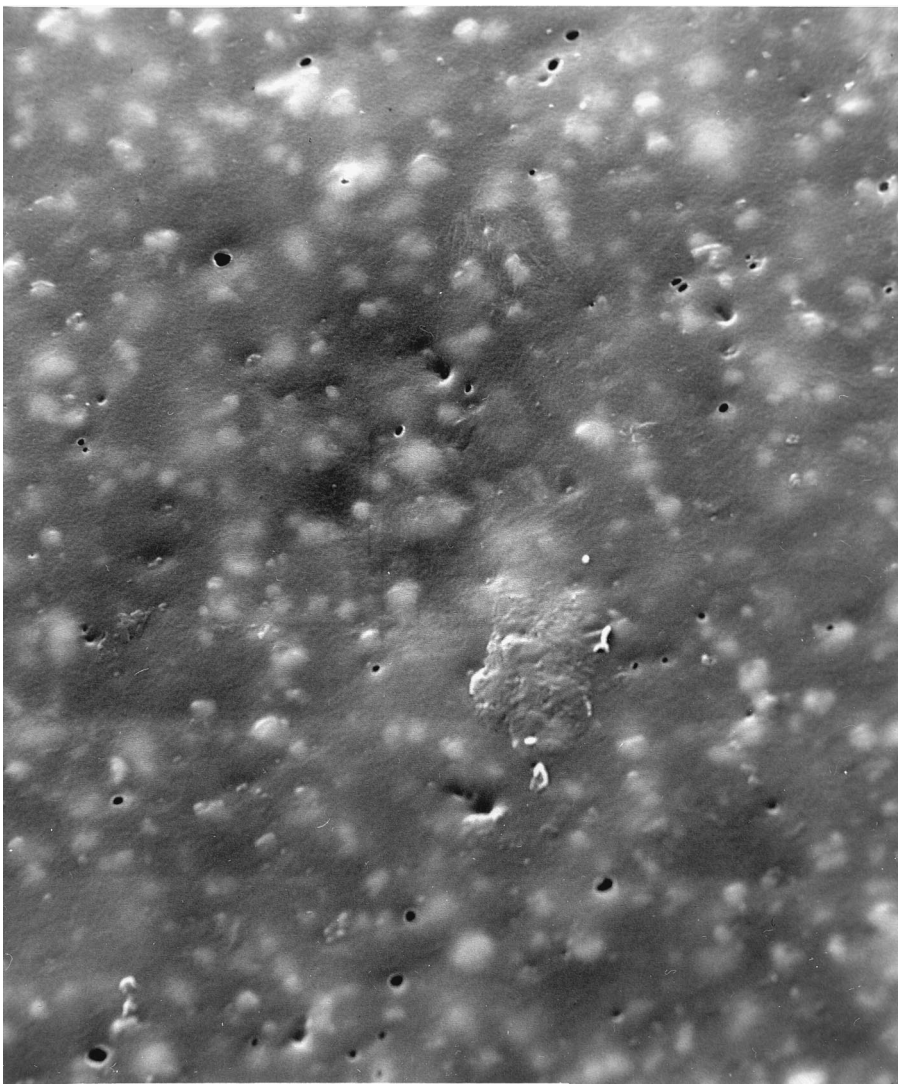

FIG. 6. Scanning electron micrograph showing traces of glycocalyx without adherent bacteria after treatment with pefloxacin at 100 μg/ml for 2 h (magnification, ×4,000).

difficult (1, 2, 9, 21). The slow growth rate and the presence of a glycocalyx matrix in the biofilm structure are considered to be important mechanisms for resistance of biofilm-associated bacteria to antimicrobial agents. Therefore, reduction of glycocalyx formation can potentially render these bacteria more susceptible to antimicrobial agents (6, 10, 26). Yasuda et al. (27) reported that treatment of biofilms of *P. aeruginosa* with clarithromycin (for 5 days) caused a decrease in the quantity of hexose and alginate and also in the quantity of polysaccharides. Clarithromycin eradicated glycocalyx production by *P. aeruginosa* by an unknown mechanism (27).

In our studies, the treatment of 2-day-old biofilms of *P. aeruginosa* in the wells of microtiter plates with high concentrations of fluoroquinolones (12.5 to 400 μg/ml) resulted in reduction of the optical density of the adherent films ($P < 0.01$). Also, the results revealed that pefloxacin was the most effective of the four fluoroquinolones in eradicating the biofilm produced by *P. aeruginosa* ($P < 0.01$), although it was not the most effective antimicrobial agent as determined by in vitro susceptibility testing. The ability to eradicate the biofilm may be independent of antimicrobial activity. Clarithromycin efficiently eradicated biofilms formed by *P. aeruginosa*, although this organism is highly resistant to clarithromycin (27). Our results indicate that fluoroquinolones may be useful for treatment of chronic biofilm-associated infections. The concentrations of fluoroquinolones would be particularly high in areas such as the urinary tract and the vascular lines through which they are infused.

The two most common mechanisms for colonization of vascular catheters are contamination of the skin at the insertion site and contamination of the catheter hub. Gram-negative bacilli may also be involved in infections due to a contaminated infusate (18). The modified Robbins device and the delivery system are analogous to those used for intravenous therapy in humans. The same model was used previously by Khardori et al. (14) to study the effects of subinhibitory concentrations of clindamycin and trospectomycin on the adherence of *S. epidermidis* to vascular catheters.

When the catheters were flushed, ultrasonicated, and washed, no bacterial growth was seen in the presence or absence of antimicrobial agents. These three procedures remove adherent, as well as planktonic, bacteria. When catheters were col-

onized in the absence of antimicrobial agents, bacterial growth was observed in segments flushed but not sonicated. In the presence of antimicrobial agents (at one-half, one-fourth, and one-eighth of the MIC), the catheters showed no or slight growth after flushing and without sonication, indicating significant inhibition of the adherence of the tested organisms to the vascular catheters. These findings were further evaluated by observation of glycocalyx formation and adherent bacteria on the surface of the catheter segments by scanning electron microscopy. Significant reduction of the numbers of adherent bacteria in the presence of pefloxacin at one-half, one-fourth, and one-eighth of the MIC was observed. The maximum reduction effect was detected at one-half of the MIC. Reid et al. (23) reported that pretreatment of silicone latex urinary tract catheters in vitro with a subinhibitory concentration (0.1 μg/ml) of ciprofloxacin for 1, 24, and 48 h significantly reduced the adherence of *P. aeruginosa* AK1. Our data show that the subinhibitory concentrations of the four fluoroquinolones tested inhibit glycocalyx production and the adherence of *P. aeruginosa* to vascular catheters. One of the concerns in using these agents for prophylaxis is the potential for development of resistance; however, this potential should not be significant because of the very low number of organisms, if any, involved at the times of insertion.

The activity of the fluoroquinolones in eradicating a preformed biofilm and removal of adherent bacteria in the in vitro model of vascular catheter colonization was confirmed by scanning electron microscopy. Most of the adherent cells were removed from colonized catheters treated with pefloxacin at 50 μg/ml, and a very small amount of glycocalyx remained. No adherent cells were observed on the segments treated with 100 μg/ml for 2 h. The activity of ciprofloxacin in eradicating preformed biofilms of *P. aeruginosa* was also reported by Reid et al. (23). The adherent biofilms of *P. aeruginosa* were eradicated by 24 h of interaction with ciprofloxacin at 50 and 100 μg/ml. On the other hand, Nickel et al. (20) demonstrated significant resistance to tobramycin in 8-h-old biofilm-associated *P. aeruginosa* on urinary tract catheters.

The data presented here indicate that ciprofloxacin, norfloxacin, pefloxacin, and ofloxacin, at subinhibitory concentrations, inhibit *P. aeruginosa* glycocalyx formation and adherence to plastic surfaces and vascular catheters in a dose-dependent manner. Preformed biofilms of *P. aeruginosa* were eradicated by higher concentrations (5 to 400 times the MIC) of fluoroquinolones. These results support the findings of other investigators suggesting that fluoroquinolones may be useful in the prevention or treatment of biofilm-associated infection due to *P. aeruginosa*. A well-controlled clinical trial is needed to determine the clinical relevance of these experimental data.

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