Possible Role of Capillary Action in Pathogenesis of Experimental Catheter-Associated Dermal Tunnel Infections

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An animal model of vascular-catheter-associated dermal tunnel infections was developed to study the pathogenesis of such infections. Bacteria inoculated onto entry sites of catheters into skin could be identified by culture and Gram stain on the tips of plastic catheters (4 cm from the entry site) within 1 h of inoculation, whether the animal was inoculated at the time of insertion of the catheter or 1 week afterwards. Histological examination of dermal tunnels revealed that the introduction of bacteria preceded the development of tissue inflammation. Bacteria on entry sites of percutaneous catheters moved rapidly from the entry site into the dermal tunnel along the external catheter surface, perhaps suspended in a fluid phase and propelled by capillary action.

Despite extensive clinical and laboratory investigation, the pathogenesis of intravascular-catheter-associated infection remains poorly understood. In some instances microorganisms may be introduced directly into the lumen of the catheter via a contaminated infusate (9) or colonized catheter hubs (6) and may be carried in the infused solution into the blood. The prevailing opinion, however, is derived from studies which have correlated catheter-associated infection with colonization of the dermal entry site (12), inflammation (11), and the presence of microorganisms on the external surfaces of catheters (10, 11). These studies suggest that most infections originate locally at the intradermal cannula wound. Subsequently, microorganisms are thought to migrate along the external catheter surface through the dermal tunnel into the cannulated blood vessel (7).

However, the mode of microbial spread along an external catheter surface through a dermal tunnel has not been elucidated. Furthermore, many of the physical and biological interactions which occur between host, microbe, and catheter remain undetermined. Yet, definition of these interactions is critical to the formulation of strategies to prevent the development of catheter-associated infection. To study the development and progression of such infections, we have developed a small-animal model of catheter-associated dermal tunnel infections. The results of this microbiological and histological study suggest that a potentially important pathogenic mechanism in these infections may be the phenomenon of capillary action, whereby microorganisms suspended in a fluid phase are passively transported along the solid external surface of a plastic cannula.

MATERIALS AND METHODS

Microorganisms. One strain of *Staphylococcus aureus* and one strain of coagulase-negative staphylococcus were used in this study. Each strain was isolated from blood and catheter tips of patients hospitalized at Massachusetts General Hospital. The *S. aureus* was methicillin susceptible; the coagulase-negative staphylococcus was methicillin resistant. It was determined by the method of Christensen et al. (1) that the coagulase-negative staphylococcus was a slime-

producing strain. Bacteria were maintained on brucella agar supplemented with 5% horse blood. After overnight incubation in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), a 0.5-ml suspension of each organism was inoculated onto the surface of a brucella agar plate (BAP) supplemented with 5% horse blood. The suspension was evenly spread over the agar surface with a sterile loop. BAPs were incubated at 37°C for 24 h, and a dense lawn of microbial growth was produced on the agar surface. To obtain the inocula for the animal catheter sites, a cotton-tipped swab was lightly dragged over one radius of these BAPs, providing a small volume of thick, pastelike material on the cotton swab.

Production of dermal tunnel infections. We used CD-1 female white mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) weighing approximately 20 g. General anesthesia was induced with intraperitoneal chloral hydrate. Dorsal hair was shaved, and skin was vigorously swabbed with ethanol. Sterile, 16-gauge, 2-inch (5.08-cm) Teflon (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) Angiocaths (provided by Deseret Medical, Inc., Sandy, Utah) were used. Skin overlying the lower spine was grasped with sterile forceps, and the catheter was inserted percutaneously over the needle stylet. The catheter was threaded subcutaneously along the spine until the tip of the catheter lodged in the subcutaneous tissue of the neck. The stylet was withdrawn, and the catheter hub was removed with sterile scissors; a 1-cm length of catheter remained external to the skin. The catheter was anchored with a single silk suture, which pierced the skin and the external portion of the catheter tube. The suture was placed approximately 1 cm from the catheter entry site (Fig. 1). The external end of the catheter was sealed with a clay plug to prevent contamination of the lumen.

The experimental design of the study is summarized in Table 1. There were three groups of mice. Group 1 was not inoculated with bacteria; group 2 was inoculated with bacteria immediately after catheterization; group 3 was inoculated with bacteria 7 days after catheterization. An attempt was made to roughly standardize the bacterial inoculum from one animal to another as described above. The contents of the swab were immediately painted onto the catheter entry site in three strokes directed caudad. Mice were kept in individual cages.

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FIG. 1. Location of dorsal subdermal catheter and retaining suture.

Bacterial cultures. Group 1 mice had skin cultures performed before catheter insertion. A sterile shaving head was used for each animal. Before being cleansed with alcohol, the skin over the future entry site was cultured by being swabbed with sterile broth. The swab was evenly streaked over the surface of a BAP.

After overnight incubation at 37°C, plates were examined and colonies (CFU) were counted by visual inspection. The following grading system was used: 4+, CFU too numerous to count; 3+, 100 to 1,000 CFU; 2+, 30 to 99 CFU; 1+, <30 CFU; 0, no growth. Individual CFU were identified by standard microbiological methods.

Immediately after mice were sacrificed, the catheter entry site of all animals was cultured with a premoistened sterile cotton swab. Cultures were processed and graded 0 to 4+ by the method described above for skin cultures.

Dermal tunnels and catheters were recovered in the following manner. A rectangle of dorsal skin extending from the entry site to the neck was excised with sterile scissors. Skin and catheter were removed en bloc. The external portion and a few millimeters of catheter below the skin were cut away and discarded. The remaining 4 cm of catheter was separated from the dermal tunnel with sterile forceps. Care was taken to avoid catheter contact with the entry site portion of the tunnel.

Catheters were cut with sterile scissors into two 2-cm segments, designated the entry site and tip segments. Each segment was immediately cultured semiquantitatively by the method of Maki et al. (11).

Dermal tunnels were cultured by firmly pressing the underside of the rectangle of skin directly onto the surface of a BAP. After incubation of plates, colonial growth was graded 0 to 4+ by using criteria similar to those described for entry site cultures.

Catheter Gram stains. After semiquantitative culture, each catheter segment was Gram stained and examined under oil immersion light microscopy (2). Each catheter was exam-

ined for 15 min and graded as follows: 4+, organisms too numerous to count; 3+, approximately one organism per 5 oil immersion fields; 2+, approximately one organism per 20 oil immersion fields; 1+, one to five organisms on entire catheter; and 0, no organisms seen (2).

Histology. Dermal tunnels were fixed in Formalin. Some tunnels were sectioned longitudinally, and others were sectioned transversely. Transverse sections were cut from entry site and tip areas of dermal tunnels. A few catheters were sectioned transversely with catheter segments in place. Sections were stained with hematoxylin and eosin and Brown and Brenn tissue Gram stain.

RESULTS

Bacteriology. (i) Uninoculated catheters. Bacterial skin cultures on mice before catheterization showed mainly coagulase-negative staphylococci with 1+ cultures. After catheterization, dermal tunnels were negative for five mice, but mice sacrificed at 1, 16, and 23 days gave 1+ cultures for coagulase-negative staphylococci. Catheter cultures and Gram stains remained negative throughout the study.

(ii) Catheters inoculated at insertion. Entry site and dermal tunnel cultures were generally 4+ for either coagulase-negative staphylococci or *S. aureus* up to 12 h after catheterization. However, at later periods cultures were less strongly positive. This finding was particularly prominent in the dermal tunnels of animals inoculated with coagulase-negative staphylococci. At 48 h, these dermal tunnels were only 1+ to 2+ (Table 2).

Results of semiquantitative catheter cultures revealed that catheters became colonized within 1 h of entry site inoculation. Catheter cultures also diminished in positivity the longer the catheter was in place. In each instance, the organism isolated on catheter cultures was identical to the inoculated strains.

Whereas catheter cultures became less positive with duration of catheterization, Gram stains increased in positivity with time and were uniformly 4+ for gram-positive cocci at 24 and 48 h.

(iii) Catheters inoculated after insertion. Three group 3 mice were inoculated with coagulase-negative staphylococci, and three were inoculated with *S. aureus*. Results of semiquantitative catheter cultures were similar to those for group 2 animals which were sacrificed at 24 h. For animals inoculated with coagulase-negative staphylococci, there were an average of 30 CFU per BAP of coagulase-negative staphylococci on entry site segments and 17 CFU per BAP for tip segments. Animals inoculated with *S. aureus* gave an average of 90 CFU per BAP of *S. aureus* for entry site segments and 17 CFU per BAP for tip segments.

Histology. Histological sections of dermal tunnels revealed that catheters were positioned between a thin layer of fat under the platysma muscle and paravertebral fascia. The

TABLE 1. Experimental design^a

Group	No. of mice	Time of bacterial inoculation	Strain of bacteria	Time of animal sacrifice	Histology of dermal tunnel examined
1	8	Not inoculated	None	12 h; 1, 5, 7, 16, 23, 24 days	Yes
2	24	Immediately postcatheterization	Coagulase-negative staphylococcus or <i>S. aureus</i>	Coagulase-negative staphylococcus: 1, 3, 6, 12, 24, 48 h; <i>S. aureus</i> : 12, 24, 48 h	Yes
3	6	7 days postcatheterization	Coagulase-negative staphylococcus or <i>S. aureus</i>	24 h	No

^a For all three groups, catheter Gram stains and cultures of entry site, tunnel, and catheter were done.

TABLE 2. Culture and Gram stains of catheter and sites (group 2 animals) with entry site inoculated immediately after catheterization

Organism	No. of mice	Duration of catheterization (h)	Culture score		Culture of catheter segment ^a		Gram stain of catheter segment	
			Entry site ^b	Dermal tunnel ^b	Entry site ^b	Tip ^b	Entry site	Tip
Coagulase-negative staphylococcus	2	1	4+	4+	151	32	3+	3+
	2	3	4+	4+	116	110	3+	3+
	2	6	4+	4+	>500	153	3+	3+
	3	12	4+	3+, 4+	68	27	3+	3+
	3	24	3+	2+, 3+	19	1	4+	4+
	3	48	2+, 3+	1+, 2+	2	7	4+	4+
<i>S. aureus</i>	3	12	4+	4+	138	46	4+	4+
	3	24	4+	3+, 4+	115	5	4+	4+
	3	48	3+, 4+	2+, 3+	7	6	4+	4+

^a Number of CFU per semiquantitative agar plate; average for two or three animals.

^b In all cases, bacteria isolated were identical to the organism inoculated on the entry site.

results of histological examinations of uninoculated and inoculated animals are summarized in Table 3. Group 1 mice had marked inflammation of subplatysmal fat at 12 h despite lack of bacterial contamination. Inflammatory changes increased over time. At 24 and 48 h a ring of fibrin and inflammatory cells surrounded the catheter (Fig. 2).

Group 2 mice had very minimal histologic changes 1 h postinoculation. However, tissue Gram stains from these animals revealed gram-positive cocci in singles, pairs, and small clusters in subplatysmal fat. Inflammatory changes became progressively more severe later. A catheter tract was defined by fibrin and inflammatory cells at 24 h. By 48 h a dense coagulum and crust of pus had formed along the

catheter tract (Fig. 3). Gram-positive cocci infiltrated the fat and extended into the platysma.

The histological sections of group 1 animals and group 2 animals were indistinguishable at 12 and 24 h after insertion, except that tissue Gram stains of the dermal tunnels of the uninoculated animals failed to reveal bacteria. At 48 h there were greater inflammatory changes in group 2 animals. Group 1 animals did not have the same degree of coagulum and crust of pus along the catheter tract.

There were only marginal differences between dermal tunnels colonized with coagulase-negative staphylococci and those colonized with *S. aureus*. At each time when comparison could be made, there appeared to be slightly more

FIG. 2. Uninoculated catheter tract, 24 h after catheter insertion. CS, Catheter space. Magnification, $\times 50$. Inset: Fibrin and inflammatory cells surround the catheter space. Magnification, $\times 788$.

FIG. 3. Inoculated catheter tract, 48 h after insertion and inoculation with *S. epidermidis* (transverse section). Magnification, $\times 31$. Inset: A dense purulent infiltrate with coagulum has formed along the catheter tract. Magnification, $\times 788$.

inflammation in tunnels colonized with *S. aureus*. There also did not appear to be a significant difference in the histology of sections taken from entry site portions as opposed to tip portions of the tunnels.

DISCUSSION

This study demonstrates that bacteria introduced onto the dermal entry site of a catheter inserted into the dorsum of a mouse can be recovered from the catheter tip and dermal tunnel within 1 h of inoculation of freshly inserted catheters.

Catheters became colonized when staphylococci were painted onto either fresh catheter puncture sites or mature puncture sites which were 7 days old. Histological examination of these dermal tunnels revealed that, soon after inoculation, bacteria were present without significant tissue inflammation. Thus, local pyogenic infection of the entry site with subsequent spread of infection through tissue planes was not a prerequisite for penetration of bacteria into the dermal tunnel. An alternative explanation for the rapid movement of nonmotile bacteria like staphylococci through a dermal tunnel must be sought.

We believe that the most reasonable explanation for such

TABLE 3. Dermal tunnel histology

Time (h) postcatheterization	Dermal tunnel histology of mice ^a	
	Uninoculated (group 1)	Inoculated (group 2)
1	Not examined	Slight edema of subplatysmal fat, minimal increase in mast cells; Gram stain: positive
3	Not examined	Slight edema of fat with mild PMN infiltration; Gram stain: positive
12	Moderate edema of subplatysmal fat with PMN, mononuclear, and mast cell infiltration; Gram stain: negative	Moderate edema of subplatysmal fat with PMN, mononuclear, and mast cell infiltration; Gram stain: positive
24	Ring of fibrin and inflammatory cells surrounding catheter; Gram stain: negative	Ring of fibrin and inflammatory cells surrounding catheter; Gram stain: positive
48	Ring of fibrin and inflammatory cells surrounding catheter; Gram stain: negative	Dense coagulum and crust of pus around catheter; Gram stain: positive

^a Hematoxylin and eosin and Bram and Brenn tissue Gram stains. PMN, Polymorphonuclear leukocytes.

rapid bacterial movement through noninflamed dermal tunnels is capillary action. The speed of colonization of a 4-cm catheter strongly suggests that capillarity played a role in the colonization of catheter tips and dermal tunnels in this animal model. We hypothesize that bacteria suspended in a fluid phase were passively carried by capillary action over the surface of the plastic catheter. Further studies regarding similar movement of other materials, including soluble dyes, or fluids of various viscosities, may help to define this mechanism.

Irregularities in the catheter surface (as demonstrated by scanning electron microscopy [4]) may serve as a nidus for bacteria to settle out of suspension onto the catheter surface. Once bacteria come into contact with the plastic surface, electrostatic forces such as hydrophobic bonding, hydrogen bonding, and van der Waals forces may produce adhesion between microbe and polymer. Whether our model will adequately define the more complex problem of catheters inserted through the subcutaneous tissue into the intravascular space, carrying a free-flowing fluid, remains to be determined.

In this model, a significant inflammatory reaction followed insertion of the catheters, even when catheters were not contaminated with bacteria. It appears that sterile Teflon catheters act as irritants to the subcutaneous tissue of a mouse. When the exit site was contaminated, however, the colonization of catheter surface and dermal tunnels appeared even before the histologic inflammatory response occurred. It was difficult to distinguish inflammatory changes caused by the presence of bacteria from inflammation caused by the catheter alone, but it was clear that the inflammatory change was not required for the spread of organisms along the tunnel.

It is also noteworthy that mice which were not artificially inoculated with bacteria failed to spontaneously develop catheter colonization, even after 24 days of catheterization. It is likely that there were insufficient numbers of skin flora and perhaps insufficient moisture at the catheter entry site to produce conditions favorable for catheter colonization.

The viability of bacteria within dermal tunnels diminished rapidly. Microbial colony counts of coagulase-negative staphylococci and *S. aureus* isolated from tissue and catheter surfaces declined within 24 h of colonization, suggesting that the dermal tunnel was a relatively hostile environment for bacterial proliferation. Poor survival of microorganisms within dermal tunnels may be a factor in the prevention of catheter-associated infections in subcutaneously tunnelled lines.

There is no direct evidence in humans that capillary action contributes to the pathogenesis of catheter-associated infections. However, some recent observations may provide indirect support for our hypothesis. Craven et al. (3) found that the incidence of catheter colonization was greater in patients with transparent polyurethane entry site dressings than in patients with dry sterile gauze dressings. Katich and Band (5) noted a cluster of *S. aureus* catheter-associated infections in their hospital when transparent polyurethane catheter site dressings were introduced. These infections declined when dry, sterile gauze dressings were substituted. By way of explanation, we suggest that moisture may accumulate under plastic occlusive dressings to a larger extent than under gauze dressings and so increase the likelihood that skin flora will enter into suspension with

droplets of moisture. Once suspended, skin flora around the entry site may invade the tunnel and move along the catheter surface by capillary action.

A number of clinical implications arise if capillary action is, indeed, a factor in the development of catheter-associated infections. First, the importance of maintaining asepsis of the catheter entry site by periodic application of antimicrobial compounds such as iodophors would be highlighted (8). Furthermore, it might be important to keep the entry site as free of moisture as possible by means of dry, sterile gauze dressings.

Plastic catheter polymers, such as Teflon, which are less wettable than others, like polyvinyl chloride, may in theory diminish capillary spread of liquids, but it is doubtful that this would be clinically significant. At the present, the use of subcutaneously tunnelled catheters seems to offer the greatest protection against capillary movement of microorganisms from the catheter entry site to the intravascular compartment.

ACKNOWLEDGMENT

This work was supported in part by Public Health Service Training Grant 5-T32-A107061-09.

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