Fibrin sheath enhances central venous catheter infection

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Objective: To determine whether fibrin-coated central venous catheters have a higher infection rate, and spawn more septic emboli, than uncoated catheters after exposure to bacteremia.

Design: Animal study comparing catheter infection and blood cultures of fibrin-coated and uncoated catheters exposed to bacteremia.

Setting: Animal laboratory.

Subjects: Adult male Sprague-Dawley rats.

Interventions: A total of 210 rats had catheters placed with the proximal end buried subcutaneously. Rats were divided into three groups: tail vein bacterial injection on day 0 (no fibrin group) or on day 10 (fibrin group), or no injection/saline injection (control, n = 40). Bacterial injections were $1 \times 10^8$ colony forming units of either Staphylococcus epidermidis ($n = 100$) or Enterobacter cloacae ($n = 60$). Animals were killed 3 days after injection. Blood cultures were obtained via cardiac puncture, and catheters were removed via the chest. Half of the catheter was rolled onto agar and the other half was placed in trypticase soy broth. Plates and broth were incubated at 37°C for 48 hrs. The presence of >15 colonies on roll plates, or growth in broth, was accepted as a positive sign of infection. Microscopy was performed on day 20-10 catheters. Thirty animals without catheters had bacterial injections and underwent blood culture 3 days after injection.

Measurements and Main Results: Catheter infection with S. epidermidis occurred in 32% of roll plates and 80% of broth from the fibrin group vs. 4% and 20% from the no fibrin group ($p < .01$ for each). Catheter infection with E. cloacae occurred in 50% of roll plates and 80% of broth from the fibrin group vs. 0% and 12% from the no fibrin group ($p < .01$ for each). Positive blood cultures occurred in 47 of 68 animals from the fibrin group vs. 8 of 68 from the no fibrin group ($p < .01$). Microscopy showed a fibrin sheath on 20 of 20 catheters. Without catheters, 30 of 30 blood cultures were negative.

Conclusion: The fibrin sheath significantly enhanced catheter-related infection and persistent bacteremia. (Crit Care Med 2002; 30:908–912)

Key Words: central venous catheter infection; fibrin sheath

Central venous catheter infection and subsequent catheter-related sepsis are well-studied complications of central venous catheter use. Catheter infection develops via several routes (1, 2): exogenously, i.e., spread directly from the exit site or hub of the catheter (3–13); from the infusate (14–16); or endogenously from bacteremia (13, 17–19). Exogenous catheter contamination has been studied extensively by many authors, who have shown correlation between infecting organisms and the flora of the skin surrounding the exit site (3–6); the hub (8, 7, 13, 14), and the hands of healthcare workers (20). Hematogenous seeding of catheters is believed to be less common than exogenous contamination, and less is known about this method of catheter infection.

Adherence of bacteria to the catheter is essential for the occurrence of catheter infection via bacteremia. An important factor affecting bacterial adherence is the presence of a fibrin sheath. When a biofilm is placed in the bloodstream, a delicate fibrin coating rapidly develops around the material (21–23). Once established, this fibrin sheath plays a pivotal role as the interface between the catheter and bacteria in the surrounding blood. For bacteria to adhere to and infect a catheter, they must first bind to this fibrin coating. However, the importance and effect of the fibrin sheath on bacterial adherence and subsequent catheter infection is unclear. Moreover, when infected, the fibrin sheath has been postulated to be a source of septic emboli. The purpose of this study was: 1) to develop an animal model to study bacterial adherence and catheter infection after bacteremia and to determine the incidence of catheter infection from bacteremia in the model; 2) to determine the effect of the fibrin sheath on catheter infection after bacteremia; and 3) to determine the incidence of septic emboli from infected fibrin-coated central venous catheters.

METHODS

This experimental protocol was approved by the Animal Welfare Committee of the University of Arkansas for Medical Sciences and Arkansas Children’s Hospital. A total of 210 adult male Sprague-Dawley rats (250–300 g) were acclimatized in an environmentally controlled barrier area for at least 5 days before beginning the experiment. The experiment was performed in two sets because of the number of animals that could be handled at one time. Animals were anesthetized with intra-peritoneal sodium pentobarbital (50 mg/kg) and the ventral neck was shaved and prepped using isopropyl alcohol and povidone solutions. A 1-cm longitudinal incision was made and the right internal jugular vein was isolated. A venotomy was made and a 1.0- to 1.5-cm segment of a 2.7-Fr silicone catheter (C.R. Bard, Salt Lake City, UT) was placed with the tip in the superior vena cava. A silk ligature was used to secure the catheter in place and ligate the internal jugular vein. Although the vein is not typically ligated in adult patients receiving central venous catheters, it is...
frequently ligated in pediatric patients. Moreover, ligation was necessary in this model to secure the catheter in place, preventing migration or dislodgement of the catheter. In addition, the length of occluded vein between the site of venotomy and catheter entrance and the confluence of the internal jugular vein with the subclavian vein in the 200 g rat is 1 mm or less. The suture was entirely extraluminal and never exposed to blood flow, and therefore, should not have influenced the culture results. The skin was closed with a running absorbable suture, with the proximal tip of the catheter left buried in the subcutaneous space to eliminate exit site and hub infection.

Staphylococcus epidermidis (M187SPII) or Enterobacter cloacae, both previously isolated from patients and kept frozen at −70°C, were used to inoculate the animals. The Staphylococcus species used is this experiment is a known biofilm producer and has been previously described by Rupp et al (24). Animals were given 1 ml of a suspension of 1 × 10^8 colony forming units (cfu)/mL of bacteria via tail vein injection on either the day of surgery (day 0, no fibrin group, n = 50 Staphylococcus, n = 30 Enterobacter) or on postoperative day 10 (day 10, fibrin group, n = 50 Staphylococcus, n = 30 Enterobacter). Pour plates determined final bacteria concentrations. As a control, 40 animals underwent catheter placement and received either sterile saline injection on day 0 (n = 10) or day 10 (n = 10), or no injection (n = 20). Three days after the bacterial inoculum, experimental animals were killed by CO_2 asphyxiation. Control animals were killed on day 3 after injection, or, for animals who did not receive injections, on postoperative day 7. The animal’s abdomen and chest were prepped using isopropyl alcohol and povidone-iodine solution. Blood (3 mL) was aspirated for culture by cardiac puncture to assess the animal’s ability to clear bacteremia, without central venous catheters were injected with 1 × 10^8 cfu of either Staphylococcus (n = 15) or Enterobacter (n = 15). Three days after injection, the animals were killed with CO_2 asphyxiation. The chest was prepped using isopropyl alcohol and povadone solution and blood cultures (3 mL) were drawn by cardiac puncture to assess the animal’s ability to clear bacteremia in the absence of a vascular foreign body. Blood cultures were incubated and identified as described above.

RESULTS

Two hundred animals completed the experiment. Ten animals, equally divided between the groups, died as a result of anesthetic complications. The incidence of catheter-related infection was significantly higher in the presence of a fibrin sheath. After exposure to S. epidermidis, 10 of 50 (20%) of the no fibrin group were broth culture positive, whereas 40 of 50 (80%) of the fibrin group animals were broth culture positive (p < .01) (Table 1). Roll plates showed >15 colonies in 8 of 50 (4%) no fibrin animals vs. 16 of 50 (32%) fibrin group animals (p < .01). All animals with positive roll plates had positive broth cultures. After exposure to E. cloacae, 4 of 30 (12%) no fibrin animals were broth culture positive, vs. 24 of 30 (80%) from the fibrin group (p < .01) (Table 2). Roll plates were positive for 15 of 30 (50%) fibrin group catheters vs. 0 of 30 no fibrin group catheters (p < .01). Random analytic profile index testing confirmed infection with the inoculating organism in 20 of 20 positive catheters. Catheters from saline injected (n = 20) and noninjected controls (n = 20) were all culture negative both in broth and on plates.

Blood cultures were positive in 8 of 38 (21%) of no fibrin animals and 32 of 38 (84%) fibrin group animals inoculated with Staphylococcus (p < .01). Twelve of the blood cultures were mishandled and were excluded. With Enterobacter, 0 of 30 no fibrin group blood cultures were positive vs. 17 of 30 (57%) fibrin group blood cultures (p < .01). All positive blood cultures were identified as the inoculating organism. Blood cultures were positive in 0 of 40 of control animals. Collectively, 47 of 68 positive blood cultures occurred in animals from the fibrin group vs. 8 of 68 from the no fibrin group (p < .01). Of 30 animals without catheters who were blood cultured 3 days after bacterial inoculation, 0 cultures were positive.

### Table 1. Catheter infection with Staphylococcus epidermidis

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<td>Roll plates</td>
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### Table 2. Catheter infection with Enterobacter cloacae

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positive (0/15 Staphylococcus, 0/15 Enterobacter). Light microscopy confirmed the presence of a fibrin sheath on 20 of 20 (100%) fibrin group catheters.

DISCUSSION

Central venous catheters account for over 90% of all catheter-related bloodstream infections (27). Catheter-related infections carry a 10% to 20% mortality risk, as well as additional morbidity, and prolong hospital stays (mean 7 days), with costs in excess of $6,000 per patient episode (28–32). Moreover, the use of catheters and the incidence of infections are both increasing (28, 30, 31). Contamination of catheters can occur during insertion, exogenously through the hub or exit site, or endogenously as a result of transient bacteremia from a remote source. According to the Centers for Disease Control and Prevention’s National Nosocomial Infection Surveillance System, S. epidermidis is the most frequently isolated organism in catheter-related infections and is responsible for 28% of all nosocomial bloodstream infections (1, 2). Whether bacteria migrate from exogenous sources or encounter the catheter via hematogenous spreading, the first step in colonization and infection is adherence and attachment to the catheter (33). Bacterial adherence is a complex process that is not fully understood. Known mechanisms of adherence include electrostatic, hydrophobic, or hydrophilic properties, receptor-mediated binding, and specific bacterial properties (34–36). The later phases of adherence are mediated by polysaccharide intercellular adhesive proteins. These proteins mediate binding between the bacteria, the biomaterials, and with other bacteria. Clinically, infectious S. epidermidis possesses the ability to produce a slime-like glyocalyx, known as biofilm. Once bacteria adhere and form macrocolonies, the adherence proteins signal the colony to begin biofilm production. Biofilm is a heterogeneous composite of simple and complex sugars combined with various proteins. The composition of the biofilm varies between species and varies with the environmental conditions in which the bacteria are growing (unpublished data). The presence of biofilm has been shown to aid in attachment and to protect against the cellular immune response (36, 37). Biofilm-producing S. epidermidis isolated from an infected patient’s catheter and previously described (38) was used for this study. Coagulase-negative Staphylococcus was used for this study because this is the organism that most commonly infects catheters. A Gram-negative organism was also utilized to broaden the scope of the findings inasmuch as Gram-negative bacteremia is a frequent problem in surgical patients.

This study examined central venous catheter infection in catheters exposed to transient bacteremia. This method was chosen because it isolates the process of bacterial adherence and attachment without potential contamination from exogenous sources and contact contamination. Moreover, the true incidence of catheter infection after bacteremia is not known. We successfully developed a model of catheter infection using bacteria administered via tail vein injection. This model demonstrates that catheter infection occurs after bacteremia to an appreciable extent. Whether the degree of bacteremia used in this experiment is often seen clinically is unknown, however, the fact that infection can occur via this route is clearly demonstrated. It remains to be seen what is the minimum amount of bacteremia needed to infect either fibrin coated or uncoated catheters. We used this model to investigate the role of the fibrin sheath in central venous catheter infection following bacteremia.

The fibrin sheath is a proteinaceous film composed of fibrin, laminin, collagen, fibronectin, and immunoglobulins (34, 36), and serves to isolate an artificial material from the surrounding native tissue or bloodstream. This fibrin sheath rapidly forms around intravenous catheters within 24 hrs, and then continues to evolve over weeks and months to contain collagen and even smooth muscle (21, 39). The effect of the fibrin sheath on catheter infection via bacteremia is not well studied. Some authors have shown the fibrin sheath to be protective against bacterial colonization and infection by preventing bacterial adherence (40), whereas others have shown that the fibrin sheath interferes with granulocyte and immune function (37, 41) and inhibits the action of glycopeptide antibiotics (42). In a model utilizing S. aureus, Lloyd et al. (40) found that the fibrin sheath protected against catheter colonization in animals given 1 $\times$ 10^7 cfu/mL of S. aureus via the tail vein. Conversely, other studies suggest that the fibrin sheath enhances bacterial attachment and replication of S. epidermidis (34, 43). In vitro evidence suggests that the fibrin sheath promotes adherence of coagulase-negative staphylococci to artificial surfaces (34, 44). Biofilm-producing staphylococci showed increased adherence to coverslips coated with fibronectin compared with uncoated coverslips (44), with fibronectin functioning as a ligand for receptor-mediated S. epidermidis binding (45–48).

In this study, catheter infection occurred more frequently in the presence of a fibrin sheath. Not only do these findings confirm that catheters can become infected from bacteremia, they support the hypothesis that the fibrin sheath promotes and mediates bacterial attachment and infection. Catheters exposed to bacteremia at the time of insertion had a significantly lower incidence of infection than catheters exposed to the same level of bacteremia in a delayed fashion after implantation. Because catheter placement is a minor procedure, surgical stress is minimal and cannot account solely for the difference in infection rate between the groups. The only difference between the two groups is the presence of a fibrin sheath on the delayed inoculation catheters. The presence of a fibrin sheath enhanced the infection of catheters as measured by roll plates, broth culture, and blood culture. Broth culture is more sensitive and detects smaller numbers of bacteria than roll plates by allowing bacteria to replicate before plating. Broth culture showed an even greater infection rate in the presence of fibrin than roll plates. Roll plates are commonly employed in the clinical setting to detect catheter infection. Using this technique, a statistically significant difference in the incidence of infection between fibrin-
coated and non-fibrin-coated catheters was also detected and is probably more reflective of clinically relevant catheter infection.

This study also demonstrated that infected catheters can be the source of positive blood cultures, and that the fibrin sheath enhances the propensity for persistent bacteremia. In the absence of a fibrin sheath, infected catheters produced positive blood cultures only 21% of the time, compared with 84% of infected fibrin-coated catheters. The high proportion of positive blood cultures in fibrin-coated catheters suggests that infected fibrin-coated catheters may be a source of persistent bacteremia. Future experiments may include organ culture to better define the degree of septicaemia from infected catheters.

Different catheter materials have different characteristics with regard to the incidence of infection and may develop differing fibrin sheaths. In our laboratory, we have found that silicone catheters have a significantly higher incidence of late blood-borne catheter infection after bacteremia than do polyurethane catheters (49). Although these differences exist, they were controlled for in this study by the use of a single catheter material throughout the experiment.

CONCLUSION

This study demonstrates that the fibrin sheath is an important component of bacterial adherence to catheters, and its presence enhances the attachment of both Gram-positive and Gram-negative bacteria to silicone catheters. Because bacteria adhere more readily to the fibrin sheath than the catheter itself, catheter infection after bacteremia is more likely to occur after the fibrin sheath has developed than as a result of bacteremia at the time of insertion. Infected fibrin-coated catheters must be considered as a source of positive blood cultures. Additional work needs to be done to investigate the significance of septicaemia from infected catheters, the effect of catheter material on composition of the fibrin sheath, and ways to modify or eliminate the fibrin sheath from catheters.

REFERENCES


