

Efficacy of Dicloxacillin-Coated Polyurethane Catheters in Preventing Subcutaneous *Staphylococcus aureus* Infection in Mice

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In a mouse model, dicloxacillin-coated polyurethane catheters or control (uncoated) catheters were placed subcutaneously and then *Staphylococcus aureus* was inoculated at the time of insertion, 24 or 48 h later. The in vivo half-life of the antibiotic was 11 to 16 h. When 10^5 CFU of *S. aureus* were inoculated at the time of catheter insertion, dicloxacillin-coated catheters kept the number of *S. aureus* removed from catheters by sonication below 10^2 CFU at 12, 24, 48, and 96 h after inoculation compared with titers greater than $10^{3.5}$ CFU for control catheters ($P < 0.05$). When *S. aureus* was inoculated 24 h after catheter insertion, control catheters averaged greater than 10^2 CFU of *S. aureus* removed compared with less than $10^{1.5}$ CFU for the dicloxacillin-coated catheters ($P < 0.05$). No difference was found between coated and control catheters when *S. aureus* was inoculated 48 h after catheter insertion, but *S. aureus* titers averaged less than 10^2 CFU for all experimental groups. Our data suggest that in mice, regional prophylaxis of *S. aureus* subcutaneous space infection is feasible with catheters coated with dicloxacillin and that the presence of antibiotic is only necessary for the first 24 to 48 h.

Catheter-related vascular infection continues to be an important cause of morbidity and mortality for patients requiring vascular access. These infections are most commonly caused by staphylococci—either coagulase-negative staphylococci or *Staphylococcus aureus* (7, 8). Infections caused by coagulase-negative staphylococci are frequently mild and easily treated by either removing the catheter or a course of antibiotics with the catheter in place (2, 21). *S. aureus* infections are usually more serious and require removal of the catheter and extended antibiotic therapy (9, 20). Because of the greater clinical impact of *S. aureus* infections, we became interested in whether such infections were preventable.

S. aureus produces vascular access infection primarily by multiplication of the organism on the skin at the catheter insertion site followed by growth of the organism along the subcutaneous tunnel of the catheter (1, 13). This information suggests that a reduction in the number of *S. aureus* at the insertion site can reduce the likelihood of infection. Zinner et al. (22) have demonstrated in humans that it is possible to reduce the incidence of insertion site colonization with *S. aureus* with a topical polymyxin-neomycin-bacitracin ointment. More to the point, Trooskin et al. (19) were able to prevent *S. aureus* infection in rats by the use of polyethylene catheters coated with penicillin G.

These reports encouraged us to investigate the effect of catheter-bound antibiotic on *S. aureus* infection in a mouse model of foreign body infection. Using ionic binding techniques, it was possible to bind dicloxacillin to polyurethane catheters, resulting in an in vivo half-life of 11 to 16 h. These catheters were able to prevent infection from occurring if *S. aureus* was inoculated at the time of catheter insertion or 24 or 48 h later. Our data provide additional evidence that

catheter-bound antibiotic has the potential to reduce the incidence of *S. aureus* vascular access infection.

MATERIALS AND METHODS

Binding of antibiotics. Three methods were employed by Becton Dickinson Polymer Research (Dayton, Ohio) to bind dicloxacillin (Bristol Laboratories, Syracuse, N.Y.) to 16-gauge Vialon polyurethane catheters (18). In the first method, dicloxacillin was complexed with a quaternary ammonium (QA), and two concentrations of this complex (D-QA) were used to coat catheters (type 1, low; type 2, high). In the second method, a low-molecular-weight polyurethane plus the D-QA complex were used in solution to coat catheters. Two concentrations of the low-molecular-weight polyurethane were employed (type 3, low; type 4, high). In the third method (type 5), catheters were first coated with a solution containing the D-QA complex plus the low-molecular-weight polyurethane followed by a second coating with the D-QA complex. After preparation, each catheter was placed in an individual packet, sterilized, and stored for later use.

In vitro catheter antibiotic activity. Catheter antibiotic activity was assayed in vitro by a modified Kirby-Bauer technique. *S. aureus* (ATCC 25923, dicloxacillin MIC/MBC = 0.07/0.15 $\mu\text{g/ml}$) was grown for 18 h in Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy broth and diluted to 10^6 CFU/ml in phosphate-buffered saline, and a cotton swab placed in this suspension was rubbed across the surface of a Trypticase soy agar plate. Individual catheters were cut into 20-mm lengths, pressed into the agar overlaid with *S. aureus*, and incubated overnight at 37°C. Zone sizes were assessed by measuring the diameter perpendicular to the long axis of the catheter.

In vivo catheter antibiotic half-life. The animal model employed was a modified version of that described by Christensen et al. (3). ICR Swiss mice (30 g; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were anesthetized with a combination of ether and ketamine. The back

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of each mouse was shaved followed by the liberal use of a depilatory (Nair; Carter Wallace, Inc., New York, N.Y.) to produce an area of skin devoid of hair. Then a catheter segment (1 cm) was inserted through a scalpel incision into the subcutaneous space. The incision was closed with a single silk stitch.

The half-life of catheter antibiotic activity was evaluated by placing dicloxacillin-coated or control catheters subcutaneously in mice with or without an inoculum of 10^5 CFU of *S. aureus*. Catheters were removed at intervals of 6, 12, 24, 48 or 96 h. Each catheter was then placed in an *S. aureus* plate, and the zone size was determined as described above for the in vitro studies. Four catheters (one per mouse) were evaluated for each time period.

Half-lives were calculated by the relationship: $t_{1/2} = -0.301/\text{slope}$. The slope in this equation is generated by first converting the agar zone sizes to log 10 and then performing linear regression with zone size as the y coordinate and time in vivo as the x coordinate.

Mouse model of *S. aureus* infection. Mice were prepared as in the in vivo half-life experiments and then inoculated (50 or 100 μ l) with either phosphate-buffered saline alone or phosphate-buffered saline plus 10^5 to 10^6 CFU of *S. aureus* subcutaneously next to the catheter. In the first experiment, dicloxacillin-coated catheters were compared with control catheters for their effect on *S. aureus* inoculated at the time of catheter insertion. In the second experiment, two types of coated catheters (type 1 and 5) were compared with control catheters for their effect on *S. aureus* inoculated 24 h after the catheters were inserted. In the final experiment, the same two coated catheters and control catheters were used with *S. aureus* inoculated 48 h after the catheters were inserted. In all three experiments, 8 to 10 mice were evaluated for each catheter type at each time interval.

Catheter harvesting. Mice were sacrificed by cervical dislocation, and the catheters were aseptically removed. Each catheter was placed in 4 ml of Trypticase soy broth and sonicated for 3 min in a water bath sonicator (100 W, 80,000 Hz; Ultrasonic Industries, Inc., Plainview, N.Y.). The broth was serially diluted and surface plated on Trypticase soy agar to determine the number of bacteria removed from each catheter. Each catheter was then placed in Trypticase soy broth and incubated overnight at 37°C to assess whether any bacteria remained on the catheter. A swab of the subcutaneous pocket was plated on blood agar, and the percentage of hemolytic colonies was used to correct the total organism counts obtained from the sonication method to give a more accurate representation of the number of *S. aureus*. For example, if 90% of the colonies were hemolytic and looked like *S. aureus*, then the final count would be multiplied by 0.9 to correct for possible contamination. In addition, the counts were used to provide another measure of the number of *S. aureus* in the subcutaneous catheter pocket.

Statistics. Owing to a wide range in the number of organisms removed from catheters placed subcutaneously, all organism counts were converted to log 10 so that the means of these numbers would be geometric means. The numbers of *S. aureus* removed from each type of catheter at each time interval were compared by the Student *t* test or the Mann-Whitney rank sum test.

RESULTS

In vitro catheter antibiotic activity. Dicloxacillin-coated catheters produced 29- to 37-mm zones of inhibition (44 type

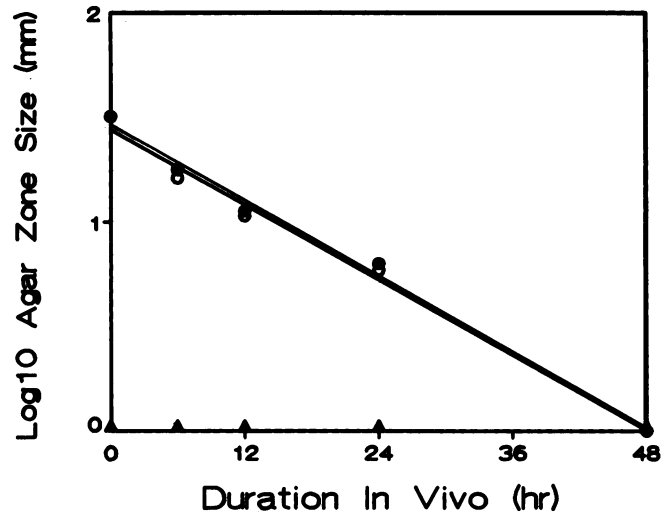


FIG. 1. Effect of *S. aureus* infection on the in vivo half-life of dicloxacillin bonded to type 1 polyurethane catheters. Symbols: ●, dicloxacillin-coated catheters; ○, dicloxacillin-coated catheters plus *S. aureus* infection; ▲, control catheters with or without *S. aureus* infection.

1 catheters, $34.3 \text{ mm} \pm 5.3$ (standard deviation); 8 type 5 catheters, 34.3 ± 2.2) when placed in *S. aureus* Kirby-Bauer plates. We have previously shown that this zone size corresponds to 28 μg of dicloxacillin per cm^2 of catheter surface area or 15 $\mu\text{g}/1\text{-cm}$ segment (18). For a 30-g mouse, this is equivalent to a total body dose of 0.5 mg/kg. This dicloxacillin activity was stable for greater than 1 month when the catheters were stored in desiccated individual plastic packets. No zone was produced when either the QA or the low-molecular-weight polyurethane or both were placed on the catheters without dicloxacillin.

In vivo catheter antibiotic half-life. The in vivo antibiotic half-lives of type 1, 2, and 5 dicloxacillin-coated catheters have been estimated graphically and reported previously as 12, 12, and 24 h, respectively (18). The actual calculated half-lives based on the slopes of the regression lines for all five catheter types studied were as follows: type 1, 11.4 ± 0.5 (standard deviation) h; type 2, 12.8 ± 1.1 h; type 3, 12.3 ± 0.6 h; type 4, 12.7 ± 0.8 h; and type 5, 16.2 ± 1.8 . The correlation coefficients (*r*) calculated from the regression analyses were quite high, ranging from 0.92 to 0.98. Type 1 through 4 coated catheters had no measurable activity after 48 h. Type 5 had significantly greater zone sizes than all four of the other catheters at 12, 24, 48, and 96 h ($P < 0.05$).

S. aureus had no effect on in vivo half-life with type 1 catheters (Fig. 1). Noncoated control catheters did not produce zones of inhibition.

Effect of dicloxacillin-coated catheters on *S. aureus* infection. Overall, 17% of the catheters inserted were not found in the subcutaneous pocket at the time of harvesting. There was no difference between the control group catheters and either type 1 or type 5 catheters in this respect. This resulted in data on 6 to 10 catheters being available for analysis at each time interval. Over 95% of the catheter cultures showed no evidence of contamination based on colony morphology and the presence of hemolysis on blood agar plates.

With *S. aureus* inoculation at the time of catheter insertion (Fig. 2), the geometric mean titers for type 1 catheters were less than those for control catheters at all five sample intervals ($P < 0.001$). At 96 h, 86% (six of seven) of the

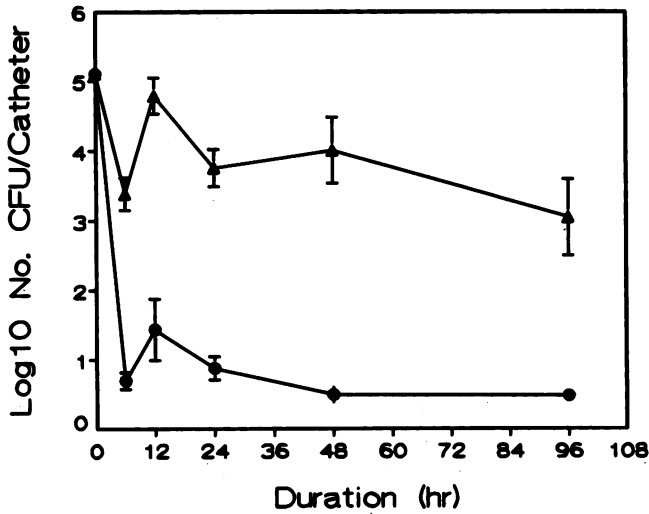


FIG. 2. Effect of dicloxacin-coated catheters on *S. aureus* inoculated at the time of catheter insertion. Bars represent standard error of the geometric mean; where no bar is seen, the standard error was smaller than the symbol. Symbols: ●, dicloxacin-coated catheters (type 1); ▲, control catheters.

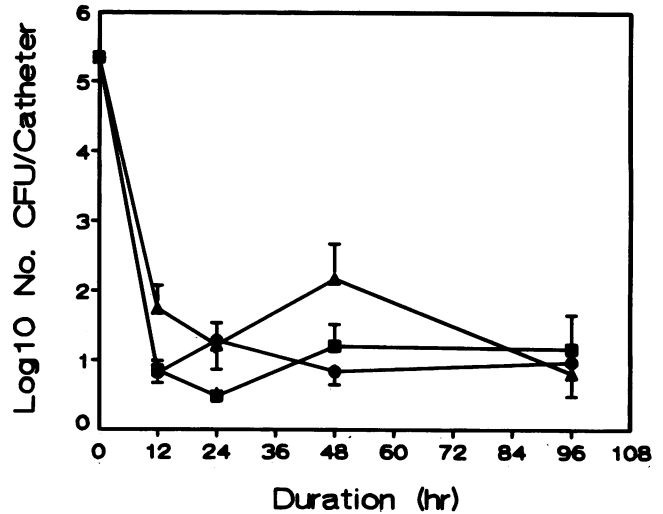


FIG. 4. Effect of dicloxacin-coated catheters on *S. aureus* inoculated 48 h after catheter insertion. Bars represent standard error of the geometric mean; where no bar is seen, the standard error was smaller than the symbol. Symbols: ●, dicloxacin-coated catheters (type 1); ■, dicloxacin-coated catheters (type 5); ▲, control catheters.

coated catheters were culture negative for *S. aureus* in broth and had no growth with the swab culture of the subcutaneous catheter pocket compared with 0% (zero of nine) of control catheters.

When *S. aureus* was inoculated 24 h after the catheters were implanted (Fig. 3), the bacterial titers seen with the control catheters were significantly lower ($P < 0.05$ at 12, 24, and 48 h; Mann-Whitney) than the bacterial titers found with control catheters after the zero hour inoculation. Under these circumstances, the two antibiotic-coated catheters still had lower numbers of *S. aureus* removed from the catheters at 12, 48 and 96 h ($P < 0.05$) in comparison with control catheters. At 96 h after *S. aureus* inoculation, 50% (5 of 10)

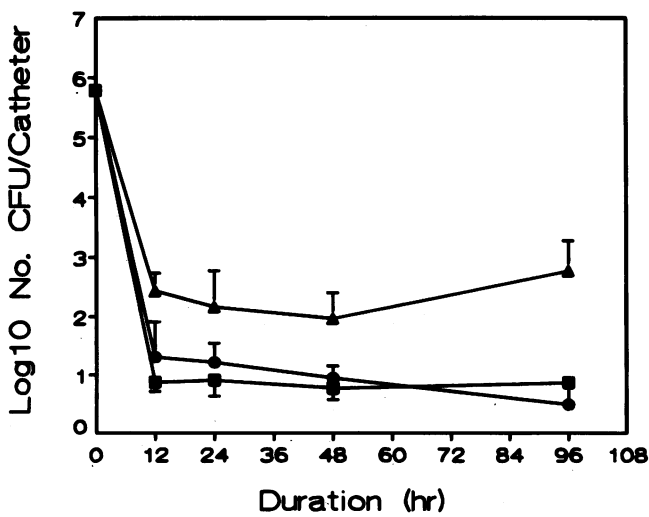


FIG. 3. Effect of dicloxacin-coated catheters on *S. aureus* inoculated 24 h after catheter insertion. Bars represent standard error of the geometric mean; where no bar is seen, the standard error was smaller than the symbol. Symbols: ●, dicloxacin-coated catheters (type 1); ■, dicloxacin-coated catheters (type 5); ▲, control catheters.

of type 1 catheters and 0% (0 of 6) of type 5 catheters were culture negative by broth testing and had associated negative swab cultures versus 0% (0 of 10) of control catheters.

In the final experimental group, *S. aureus* was inoculated 48 h after the catheters were implanted (Fig. 4). Control catheter *S. aureus* titers were significantly lower than those obtained with a zero hour inoculation (at 12, 24, 48, and 96 h, $P < 0.05$; Mann-Whitney), and a significant proportion of these animals had sterile catheters without exposure to antibiotics (Table 1). At 96 h after *S. aureus* inoculation, 43% (3 of 7) of type 1 catheters and 63% (5 of 8) of type 5 catheters were culture negative by broth culture and had associated negative swab cultures versus 30% (3 of 10) of control catheters. In this group, it was not possible to show any clear differences between the control catheters and the antibiotic-coated catheters. This may be due more to the difficulty in producing an infection than to a lack of antibiotic activity.

No abscesses were seen around the catheters whether the *S. aureus* was inoculated at 0, 24, or 48 h after the catheters were inserted.

DISCUSSION

S. aureus catheter-related vascular infections continue to be an important problem for patients requiring vascular

TABLE 1. Relationship among time of catheter insertion, *S. aureus* inoculation, and frequency of culture-negative control catheters by broth culture

<i>S. aureus</i> inoculation time (h)	Log 10 starting inoculum	% of culture-negative catheters after:			
		12 h	24 h	48 h	96 h
0	5.1	0	0	0	0
24	5.8	0	0	11	11
48	5.3	0	16	30	40

access. In certain patient populations such as those requiring acute hemodialysis via the subclavian vein, *S. aureus* infection rates of 5% are routine and rates as high as 10% have been reported (4, 12, 17). These infections may require prolonged treatment owing to the associated occurrence of endocarditis (14, 20). Although uncommon, death may occur related to these infections (14, 17).

Catheter insertion for vascular access can be considered analogous to a surgical procedure. High infection rates associated with surgical procedures can usually be lowered by using prophylactic antibiotics (15). Since existing vascular access infection rates occur in the setting of topical antibiotic use, any further decrease in infection rates will most likely result from some additional route of antibiotic delivery. While systemic prophylactic antibiotics have been shown to work with vascular graft infections, no one has investigated the use of systemic antibiotics to prevent vascular catheter infection (11, 16). However, there are data suggesting that the use of regionally delivered antibiotics (i.e., catheter-bound antibiotic) is effective at decreasing vascular infection (6, 19).

Trooskin et al. (19) have demonstrated that penicillin G can be bound to polyethylene catheters by using the quaternary amine tridodecylmethylammonium chloride. When inserted in the jugular veins of rats, these catheters were capable of preventing catheter colonization for 5 days after challenge with 10^8 *S. aureus* (0 of 10 animals). In contrast, untreated control catheters were significantly more likely to be colonized (7 of 11 animals, $P < 0.005$). Greco and Harvey (6) have demonstrated a similar prophylactic effect using oxacillin bound to tetrafluoroethylene vascular grafts via benzalkonium chloride. In a dog model, they found that after an inoculum of 10^7 *S. aureus*, 12 of 15 control grafts were culture positive after 6 weeks versus 5 of 15 grafts coated with oxacillin.

Our study confirms and extends these animal model findings. Using catheter-bound dicloxacillin, it was possible to kill all or most of 10^5 to 10^6 *S. aureus* inoculated at the time of catheter insertion or 24 h later. This effect persisted for at least 96 h after *S. aureus* inoculation even though type 1 catheters had no detectable antibiotic activity after 48 h in vivo. Although these results seem exciting, one important consideration suggests that any conclusions should still be tempered. In this mouse model no abscesses formed despite 10^6 *S. aureus* plus a foreign body. This was also true for several other *S. aureus* strains (clinical isolates from vascular catheter infections) that we used subsequently in the same model (data not shown). In comparison, in humans only 10^2 *S. aureus* inoculated subcutaneously along with a silk suture are necessary to produce an abscess (5). Similar results have been shown by James and MacLeod (10) with silk and cotton sutures in a mouse model of *S. aureus* stitch infection. However, in this same model nylon sutures were 1,000-fold less likely to produce a purulent infection. Given the marked differences between suture materials in their predisposition to purulent infection, it is very possible that polyurethane catheters have a low risk of infection associated with them analogous to nylon sutures. Further work is necessary to determine whether dicloxacillin will have an effect in a more virulent model of *S. aureus* infection.

A new concept that is suggested by this study is that the longer the catheter resided in the mouse prior to organism inoculation, the more difficult it was to produce an infection. When *S. aureus* was inoculated 48 h after a control catheter was inserted, 40% of the catheters removed 96 h later were sterile versus 0% if the organisms were inoculated at the time

of insertion (Table 1). If this consideration applies to human vascular access sites, it implies that preventing the multiplication of *S. aureus* in the catheter-created wound during the first few days after insertion could reduce the likelihood of *S. aureus* infection. Substantiation of this finding would provide a very strong theoretical argument for the use of prophylactic antibiotics with vascular catheters. Our data in mice indicate that catheter-bound antibiotic is one way to accomplish this prophylaxis.

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