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Prolonged Antimicrobial Activity of a Catheter Containing Chlorhexidine-Silver Sulfadiazine Extends Protection against Catheter Infections In Vivo

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The present study evaluated in vitro and in vivo a new chlorhexidine (C)-silver sulfadiazine (S) vascular catheter (the CS2 catheter) characterized by a higher C content and by the extended release of the surface-bound antimicrobials. The CS2 catheter was compared with a first-generation, commercially available CS catheter (the CS1 catheter). The CS2 catheter produced slightly smaller zones of inhibition (mean difference, 0.9 mm [$P < 0.001$]) at 24 h against *Staphylococcus aureus* and five other microorganisms by several different methodologies. However, in a rabbit model, both CS catheters were similarly efficacious in preventing a catheter infection when the rabbits were inoculated with 10^4 to 10^7 CFU of *S. aureus* at the time of catheter insertion. The CS2 catheter retained its antimicrobial activity significantly longer in vitro and in vivo (half-lifes exceeded 34 and 7 days, respectively) and was also significantly more efficacious in preventing a catheter infection when 10^6 CFU of *S. aureus* was inoculated 2 days after catheter implantation ($P < 0.001$). These results suggest that prolonged anti-infective activity on the external catheter surface provides improved efficacy in the prevention of infection.

Vascular catheters coated with antiseptic or antimicrobial agents have been shown to significantly reduce the risk of catheter-related bloodstream infection (6, 8, 14), which is an important cause of morbidity and mortality, with a significant economic burden (10). Vascular catheters impregnated with chlorhexidine (C) and silver sulfadiazine (S) (CS1 catheters) were efficacious in preventing catheter-related bloodstream infections in a large randomized clinical trial (6), and their efficacy has been demonstrated in a meta-analysis (14) for patients with a mean duration of catheterization of less than 12 days. However, other studies, and in particular, another large randomized trial (5) with patients with a mean duration of catheterization of 20 days, found no benefit in the use of CS1 catheters, raising the possibility that the anti-infective protection offered by these catheters lasts only for a relatively short period of time (about 10 days). The present study evaluated whether a new catheter with increased C content and extended release of the surface-bound antimicrobials (the CS2 catheter) has improved anti-infective properties.

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MATERIALS AND METHODS

Catheters. Sterile segments of Arrowgard Blue central venous catheters (CS1 catheters; Arrow International, Reading, Pa.) and an experimental catheter (a CS2 catheter) impregnated with a three times larger amount of C and the same amount of S as the CS1 catheter Arrowgard Blue were studied. The average amounts of C acetate, silver, and sulfadiazine applied to the experimental catheter (the CS2 catheter) were 425, 24, and 56 μg/cm, respectively. The experimental catheter (manufactured by Arrow International) was impregnated with an improved surface treatment, which allows extended release of the surface-bound antimicrobials. Noncoated central venous catheters (Arrow International) were used as controls. All catheters were 7-French, triple-lumen polyurethane catheters. Both CS catheters were impregnated only on the external surface. For the studies with rabbits, each catheter segment was heat sealed on both ends.

In vitro antimicrobial activities of catheters. A modified Kirby-Bauer technique was used to assess catheter antimicrobial activity (13). Six clinical isolates that caused catheter-related bloodstream infections (*Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus faecalis, Enterobacter cloacae, Pseudomonas aeruginosa*, and *Candida albicans*) were studied. Each organism was grown overnight in Trypticase soy broth to a suspension of 10^6 or 10^8 CFU/ml. A cotton swab placed in this suspension was rubbed across the surface of a Trypticase soy agar plate (150 by 15 mm; BBL, Cockeysville, Md.). Individual catheters were cut into 12-mm (for the vertical placement) and 24-mm (for the horizontal placement) segments. For each agar plate, one vertical segment was inserted perpendicular to the surface and one horizontal segment was inserted parallel to the surface streaked with the suspension of the organism tested. The plates were then incubated at 35°C for 24 h. Zone sizes were assessed by measuring the diameter perpendicular to the long axis of the catheter. Six horizontal and six vertical segments of each catheter type were tested in parallel against each microorganism. Each experiment was performed twice, and the mean diameter of the zones produced by the 12 horizontal segments and the 12 vertical segments was calculated.

In vitro retention of antimicrobial activities of catheters. Sterile 12-mm catheter segments were each embedded vertically into a Trypticase soy agar plate (100 by 15 mm; Difco Laboratories, Detroit, Mich.) inoculated with a suspension containing 10^8 CFU/ml of *S. aureus* P1 (five plates for each catheter type) (12). After 24 h of incubation at 35°C, zones of inhibition were measured. Each day for 34 days the segments were transferred to freshly inoculated plates.

Rabbit model of *S. aureus* infection: 7-day efficacy. A previously described rabbit model of *S. aureus* infection (12) was used. The protocol for animal studies was approved by the Animal Care and Use Committee, Wake Forest University Baptist Medical Center; animals were housed in facilities approved by the American Association for Accreditation of Laboratory Animal Care. Briefly, the P1 strain of *S. aureus* was grown overnight and diluted serially in phosphate-buffered saline (pH 7.3) to achieve the desired inoculum in 25 μl. Each rabbit's back was shaved, depilated, and prepared with povidone iodine. A pair of incisions (0.5 to 1 cm apart and 4 cm from the spine) was made for each catheter segment. A

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Jimshidi bone marrow needle was used to create two 3-cm tunnels through these two incisions. A catheter segment was inserted into both of these tunnels so that both ends of the segment were in the subcutaneous space and the middle of the segment rested on the skin. Inoculations were delivered subcutaneously next to the lateral catheter segment by use of a micropipette. At 7 days after catheter inoculation, the animals were killed. Each catheter was carefully removed and observed for gross purulence, and the lateral intracutaneous segment was placed into 4 ml of Trypticase soy broth. Quantitative culturing was done with an ultrasonic water bath (3 min, 47 kHz, 130 W; Bransonic B2200R-1; Branson Ultrasonic Corp., Danbury, Conn.) and serial dilutions and by blood agar surface plating (12). For each inoculum 10 segments of the CS2 catheter, 10 segments of the CS1 catheter, and 10 noncoated control catheters were studied.

Rabbit model: catheter harvesting for determination of retention of antimicrobial activity in vivo. Catheters were inserted into the subcutaneous space at time zero and were removed at specified intervals (range, 3 h to 7 days). Then the catheter segments were placed vertically in a Trypticase soy agar plate (Difco Laboratories) that had been streaked with a swab dipped in a suspension of 10^8 CFU of *S. aureus* P1 per ml, and zones of inhibition were measured at 24 h, as described above. Four catheters were evaluated for each time period.

Rabbit model: efficacy of catheters with delayed inoculation. Forty-five CS2 catheters and 44 CS1 catheters were implanted in the subcutaneous space. Two days later, an inoculum of 10^6 CFU of *S. aureus* P1 was delivered subcutaneously next to the lateral catheter segment. At seven days after inoculation the catheters were processed as described above for the 7-day efficacy studies.

Data analysis. Categorical variables were compared by chi-square tests, and continuous variables were compared by two-sample *t* test. An analysis of covariance (ANCOVA) model was fit to determine whether there was a difference between the CS1 and CS2 catheters on the zone size outcome after adjustment for the microorganism, the microorganism concentration of the suspension used to inoculate the agar plates (plate inoculum), and the position of the catheter segment in the agar plate (horizontal or vertical). The model fit considered each of the four independent variables (microorganism, plate inoculum, segment position, and catheter type) as class variables and the outcome (zone size) as a continuous variable. In all analyses we adjusted for all four independent variables.

RESULTS

In vitro antimicrobial activities of catheters. At 24 h the CS2 catheter produced slightly smaller zones of inhibition than the CS1 catheter against the microorganisms tested (Table 1), with a mean zone size difference under the different conditions of 0.9 mm ($P < 0.001$; ANCOVA). When the difference between catheters was examined by stratification by microorganism (adjusting for plate inoculum and segment position), the CS2 catheter always had smaller zones of inhibition than the CS1 catheter. The results were as follows: for *C. albicans*, 11.7 versus 12.4 mm ($P = 0.002$); for *E. cloacae*, 8.3 versus 9.3 mm ($P < 0.001$); for *E. faecalis*, 11.7 versus 12.8 mm ($P < 0.001$); for *P. aeruginosa*, 13.4 versus 14.3 mm ($P = 0.019$); for *S. aureus*, 15.7 versus 16.5 mm ($P = 0.006$); and for *S. epidermidis*, 16.6 versus 17.4 mm ($P = 0.011$). The zones of inhibition obtained with catheter segments placed horizontally on the agar plate were larger than the zones obtained with vertical segments (mean difference, 0.7 mm [$P < 0.001$; ANCOVA]). Also larger were the zone sizes obtained when the agar plate was inoculated with 10^6 instead of 10^8 microorganisms/ml (mean difference, 1.2 mm [$P < 0.001$; ANCOVA]).

In vivo antimicrobial activities of catheters. Both catheters were similarly efficacious in preventing colonization or catheter infection (purulence) in the rabbit model if the catheter segments were inoculated with increasing inocula of *S. aureus* (10^4 to 10^7 CFU) on the same day as insertion (Table 2).

In vitro retention of antimicrobial activity. Beyond day 6, the zones of inhibition obtained with the CS2 catheter segments were significantly larger ($P < 0.05$) than the zones obtained with the CS1 catheter segments. After 34 days, the CS2 catheter segments still produced zones of inhibition greater than half the initial diameter. In contrast, the diameters of the zones of inhibition of the CS1 catheters were reduced by 50% on day 6, and the in vitro antimicrobial activities of these catheters were completely lost after 20 days (Fig. 1A).

TABLE 1. In vitro antimicrobial activities of catheters[a]

Microorganism	Inoculum	Segment	Zone of inhibition (mm)		P^b
			CS1 catheter	CS2 catheter	
S. aureus	10^6	H	17.7 ± 2.21	16.3 ± 0.14	**0.047**
	10^6	V	16.3 ± 1.40	16.4 ± 1.67	0.906
	10^8	H	16.5 ± 1.60	15.2 ± 0.33	**0.019**
	10^8	V	15.5 ± 1.10	15.0 ± 0.77	0.239
S. epidermidis	10^6	H	18.4 ± 2.24	17.9 ± 1.81	0.497
	10^6	V	17.4 ± 1.80	16.7 ± 1.14	0.311
	10^8	H	17.7 ± 1.93	16.4 ± 1.06	0.073
	10^8	V	16.3 ± 1.62	15.4 ± 0.80	0.087
E. faecalis	10^6	H	14.4 ± 1.10	12.3 ± 1.44	**0.0003**
	10^6	V	13.5 ± 0.87	12.7 ± 0.73	**0.014**
	10^8	H	11.8 ± 0.92	11.1 ± 1.44	0.209
	10^8	V	11.7 ± 0.51	10.8 ± 0.70	**0.003**
P. aeruginosa	10^6	H	16.0 ± 0.71	14.4 ± 1.77	**0.012**
	10^6	V	14.2 ± 1.23	13.8 ± 1.79	0.473
	10^8	H	13.9 ± 2.0	13.1 ± 2.30	0.353
	10^8	V	13.0 ± 2.0	12.3 ± 2.61	0.451
E. cloacae	10^6	H	9.6 ± 2.13	8.2 ± 0.75	**0.050**
	10^6	V	9.2 ± 1.05	8.1 ± 0.55	**0.006**
	10^8	H	9.4 ± 1.83	8.4 ± 0.80	0.084
	10^8	V	9.2 ± 1.10	8.6 ± 0.55	0.102
C. albicans	10^6	H	13.5 ± 1.29	13.0 ± 1.33	0.323
	10^6	V	12.8 ± 0.94	12.4 ± 1.35	0.441
	10^8	H	12.2 ± 0.61	11.2 ± 1.04	**0.011**
	10^8	V	11.0 ± 0.53	10.3 ± 0.67	**0.006**

[a] The activities are presented as those produced after 24 h by horizontal (H) or vertical (V) segments of the commercially available and the experimental catheter on agar plates inoculated with a suspension of 10^6 or 10^8 microorganisms/ml. Each value represents the mean ± standard deviation for 12 segments.
[b] Two-sample *t* test. Boldface numbers indicate statistically significant differences.

In vivo retention of antimicrobial activity. The zones of inhibition produced by the CS2 catheter after ≥48 h of catheterization were significantly larger ($P < 0.05$) than the zones of inhibition produced by the CS1 catheters. After 2 days, the diameters of the inhibition zones of the commercial catheter were already reduced by more than 50% of the initial value, while the half-life of antimicrobial activity for the experimental catheter in vivo exceeded 7 days (Fig. 1B).

In vivo efficacies of catheters with delayed inoculation. When the two catheters containing CS were challenged with 10^6 CFU of *S. aureus* 2 days after implantation, the CS2 catheter demonstrated superior efficacy ($P < 0.001$) in comparison to that of the CS1 catheter (Table 3).

DISCUSSION

The use of CS1 central venous catheters has been shown to significantly reduce the incidence of catheter-related bloodstream infections when the mean duration of catheterization

TABLE 2. In vivo efficacies of catheters[a]

Inoculum (CFU) and catheter type	Log_{10} CFU removed (mean ± SD)	% of catheters with purulence at insertion site
0		
Nonimpregnated	0.6 ± 2.0	0
CS1	0.2 ± 0.6	0
CS2	No CFU removed	0
10^4		
Nonimpregnated	6.5 ± 0.4	100
CS1	1.4 ± 2.6[b]	0[b]
CS2	No CFU removed[b]	0[b]
10^5		
Nonimpregnated	5.7 ± 0.5	100
CS1	0.5 ± 1.2[b]	0[b]
CS2	0.8 ± 1.3[b]	0[b]
10^6		
Nonimpregnated	5.7 ± 0.3	100
CS1	0.1 ± 0.4[b]	0[b]
CS2	No CFU removed[b]	0[b]
10^7		
Nonimpregnated	5.8 ± 0.7	100
CS1	0.6 ± 1.3[b]	0[b]
CS2	No CFU removed[b]	0[b]

[a] *S. aureus* was inoculated on the same day as catheter insertion. Efficacy at preventing *S. aureus* infection was evaluated 7 days later. Ten catheters of each type were tested for each inoculum.
[b] Statistically different from the value for the nonimpregnated catheters in the same experiment ($P < 0.05$).

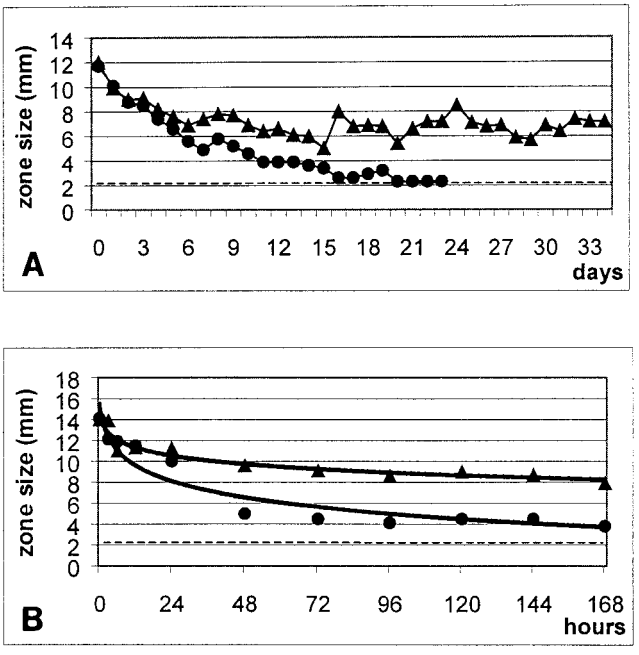

FIG. 1. In vitro (A) and in vivo (B) retention of antimicrobial activities of catheters. Each point represents the average of four (in vivo experiments) or five (in vitro experiments) catheter segments. ▲, CS2 catheter; ●, CS1 catheter. The dashed line represents the diameter of the catheter. Logarithmic regression lines were fitted for the representation of retention of antimicrobial activity in vivo (B).

was between 5 and 11 days (6, 14). However, no benefit from the use of CS1 catheters could be demonstrated in a large study with a longer duration of catheterization (mean duration, 20 days) (5). Also, a prospective randomized clinical trial demonstrated that catheters impregnated with minocycline and rifampin were significantly more efficacious in preventing catheter-related bloodstream infections than the currently available CS1 catheters (4).

There are at least three reasons that may explain the better efficacy of catheters containing minocycline-rifampin than CS1 catheters. First, catheters containing minocycline-rifampin produce larger zones of inhibition in vitro against various microorganisms (9), suggesting a more potent antimicrobial activity. Second, they are coated on both the external and the internal surfaces, offering protection against endoluminal infections, which are particularly important in the setting of long-term catheterization (>8 days) (11). Finally, they have longer half-lives of antimicrobial activity in vitro (9) and in vivo (2, 7). The relative importance of these considerations is unknown.

The present study examined whether the higher C content and the extended release of C and S from a CS2 catheter would prolong its antimicrobial activity and improve its efficacy in preventing catheter infections. The zones of inhibition against different microorganisms around CS2 catheters were slightly smaller than the zones of inhibition produced by the CS1 catheters. Yet, these small differences (maximum, 2.1 mm) are probably not clinically relevant (3), and the in vivo efficacies of the two catheters in the rabbit model were similar when *S. aureus* was inoculated immediately after the insertion of the catheter. The smaller zones of inhibition obtained at 24 h with the CS2 catheter, despite the higher C content and the same S content compared with those for the CS1 catheter, may be explained by a slower release of the antiseptics from the CS2 catheter. However, the CS2 catheter had a much longer half-life of antimicrobial activity than the current CS1 catheter both in vitro (≥34 versus 6 days) and in vivo (≥7 versus 2 days), and the more prolonged activity of the CS2 catheter was associated with much greater efficacy than that of the CS1 catheter when *S. aureus* inoculation was delayed by 2 days. To our knowledge, this is the first time that extension of the duration of activity of an anti-infective coating has been shown to protect against bacteria whose inoculation was delayed. Interestingly, the protection conferred by the CS2 catheter 2 days after insertion was associated with a zone size of only 9 mm (Fig. 1). Previous studies with this animal model with *S. aureus* inoculation at the time of catheter insertion have suggested that zones sizes of ≥18 mm are required to confer 100% protection against infection (3). Conversely, other studies with this model have shown that infections are harder to produce by delayed inoc-

TABLE 3. In vivo anti-infective efficacy with delayed inoculation[a]

Catheter type	Total no. of catheters	Log_{10} CFU *S. aureus* removed (mean ± SD)	% of catheters with purulence at insertion site
CS1	44	4.3 ± 2.8[b]	64[b]
CS2	45	0.3 ± 0.9[b]	0[b]

[a] The inoculation occurred 2 days after catheter implantation.
[b] $P < 0.001$.

ulation and require larger inocula (12, 13). It therefore seems consistent that a smaller amount of anti-infective activity may be required to prevent such infections than infections caused by inoculation of bacteria at the time of catheter insertion.

Catheter segments inserted into the agar vertically produced slightly smaller, circular zones of inhibition, while horizontally inserted segments produced slightly larger, elliptic zones. This is probably because the different positions of the segments and the different distances of the segments from the bottom of the plate change the dynamics of diffusion of the antiseptics into the agar (1).

In conclusion, the present study shows that prolonged anti-infective activity on the external catheter surface provides improved efficacy in preventing infections. The use of a new CS catheter (the CS2 catheter) with a larger amount of C and an extended release of the surface-bound antimicrobials is likely to further reduce the rate of catheter-related infections, especially if it is combined with antimicrobial protection of the catheter lumen and hub. Further studies are necessary to see how the efficacy of the CS2 catheter compares with those of other existing anti-infective catheters.

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