Durability of Anti-Infective Effect of Long-Term Silicone Sheath Catheters Impregnated with Antimicrobial Agents

ROBERT K. TCHOLAKIAN1* AND ISSAM I. RAAD2

Department of Interdisciplinary Research, University of Texas—Houston Medical School,1 and The Department of Infectious Diseases, Infection Control and Employee Health, University of Texas M. D. Anderson Cancer Center,2 Houston, Texas

Received 4 January 2001/Returned for modification 7 February 2001/Accepted 10 April 2001

This study was performed to test the long-term antimicrobial efficacy of impregnated silicone catheters comprising an antimicrobial layer sandwiched between an external surface sheath and a luminal surface silicone sheath. The design of the catheter permits the introduction of various antimicrobials in addition to anticoagulants or antifibrins in the antimicrobial layer and allows their gradual release over a period of months after insertion. The in vitro data presented show that the catheter can provide antimicrobial activity for 90 days, after being replated for 15 7-day cycles of replating. When the catheters were immersed in human serum and incubated at 37°C, they demonstrated significant antimicrobial activity after more than 325 days of incubation. The significant long-term in vitro antimicrobial activity observed may imply effective in vivo activity for almost 1 year after insertion and could serve as a cost-effective alternative to surgically implantable silicone catheters.

The use of central venous catheters (CVC) is important in the treatment of critically and chronically ill patients. However, these catheters are the leading source of bloodstream infection (6, 14). The incidence of infections is significantly higher in immunocompromised patients requiring long-term CVC for total parenteral nutrition maintenance and for chemotherapy treatment.

It has been shown that catheter infection starts right after insertion, with the formation of a thrombin film covering the external and luminal surfaces of the catheter (3, 23). This forms the matrix that enhances microorganism colonization on the catheter surfaces (2, 4, 8, 9, 11, 13, 17, 21, 22, 28). Several studies have shown that medical devices with antimicrobial activity decrease the risk of colonization and infection (1, 7, 10, 12, 15, 16, 18–20, 24–26, 27). Various attempts have been made to incorporate an antimicrobial delivery system into catheters, including those directed at adhering a pharmacologically active ingredient to the catheter surface (12, 24, 26).

Several surface-coated catheters depended on the use of cationic surfactants such as tri-iododecylmethyl ammonium chloride to facilitate bonding between the pharmacologically active ingredient and the catheter surface (10, 27). Such surface coatings have limited effectiveness due to their limited capacity for adsorbing antimicrobials, short binding duration, and possible toxicity at the catheter-tissue interfaces. Various other attempts to impregnate long-term silicone catheters have not been satisfactory and have failed to provide long-term catheter infection control.

One procedure for controlling catheter infection is to design antimicrobial catheters whereby the antimicrobial agent and the silicone compound are mixed prior to catheter extrusion.

In addition to providing antimicrobial agents to combat catheter-related infections, it is desirable for the catheters to be able to incorporate other agents, such as anticoagulants and antifibrins, as adjuncts to the antimicrobial agents to prevent thrombotic occlusions and microbial colonization of the external and luminal surfaces of the catheter. Furthermore, in addition to the above characteristics of the catheter, it is desirable for the design to be adaptable to a variety of catheter sizes from simple single to multiple lumen. The present study is directed toward testing the long-term antimicrobial durability of such a catheter design.

MATERIALS AND METHODS

Pharmacological agents were micronized in a mortar and pestle. Known concentrations of these agents were mixed with silicone sealant RTV-732 (Dow Corning, Midland, Mich.) until a homogeneous matrix was formed. This matrix was embedded as a sandwich between an external thin silicone sheath and an internal thin-walled silicone tube (Fig. 1). Each time a catheter segment was made, two different-size molds of solid Teflon 2.5 cm long were used. The smaller mold was used to construct the jacket containing the pharmacologically active agent over the inner luminal surface consisting of a specified thin-walled silicone tube, and the larger mold was used to construct the external silicone sheath to exact specifications over the jacket containing the pharmacologic agent. The bores in the Teflon molds used above were precisely drilled to specifications, honed, and polished to prevent the silicone from adhering to them. The molding process involved placing the silicone matrix on each half of the Teflon mold and then placing the silicone tube horizontally in the middle of the matrix-filled bore. The two aligned halves of the mold were pressed together and clamped until the catalysis was complete (about 30 to 60 min). The two halves of the mold were separated, and the catheter was released. The catheter now consisted of a jacket containing the pharmacologically active ingredients bonded to the silicone inner tube. Excess material was trimmed, and the catheter segment was horizontally placed in the bore of the larger mold containing RTV sealant only. The two halves were pressed and clamped. After catalysis, the completed catheter was removed, trimmed, and left to ventilate for several days prior to use. Cross-sectional segments, each 2.5 cm long, of the above design were tested in vitro for their long-term antimicrobial activity. Two experiments were performed.

In experiment 1, the long-term antimicrobial activity of the catheter was tested with a combination of minocycline and rifampin (2:1 by weight) at a 5% concentration compounded with silicone. The catheters and similarly prepared controls were sterilized in ethylene oxide, allowed to totally ventilate, and embedded
in agar with *Staphylococcus epidermidis* as follows. On day 1, *S. epidermidis* 5667 was subcultured to a blood agar plate from frozen stock. On day 2, 500 ml of Mueller-Hinton agar was prepared, and 5 ml of a 0.5 McFarland turbidity standard was added when the agar was cool to the touch. A 10-ml layer of agar was poured into each plate and allowed to harden. The silicone catheter was placed in the center of the dish, and a small amount of agar was poured to partially cover the catheter. This agar was allowed to harden, and then more agar was poured to completely cover the catheter. There was a total of 40 ml of medium per 100- by 15-mm plate. The plate was incubated for 24 h at 35°C. On day 3, the zones of inhibition (in millimeters) were measured after 24 h and recorded. On day 7, the catheter was aseptically transferred to a new plate for an additional 7-day incubation (one cycle). The zones of inhibition were recorded, and the cycle was repeated weekly for the specified period. To accommodate holidays and weekends, some cycles varied by 1 day.

In this experiment, the catheter was challenged with 15 agar plate transfers over a 90-day period. In some studies, polyurethane catheters coated with chlorhexidine gluconate and silver sulfadiazine were used and the zones of inhibition were measured daily, and on day 7, at which time the catheters were transferred to a new plate.

In experiment 2, the long-term antimicrobial efficacy of silicone-sheathed antimicrobial catheters was investigated after they had been submerged in serum for the specified time. Cross-sectional segments of the catheters 2.5 cm long with 0.014-in.-thick silicone sheaths were sterilized in ethylene oxide, submerged in human serum, covered, and incubated at 37°C for the specified time. Similarly handled sham-impregnated catheters were also prepared. The samples were left incubating in the serum for 7, 14, 21, 28, 42, 56, 70, 90, 120, 160, 190, 250, 275, 300, or 325 days. At each specified time, the samples were removed from the serum and implanted in agar plates containing *S. epidermidis* 5667 organisms, similar to the procedure in experiment 1. The 24-h zones of inhibition were measured as a measure of the efficacy of the catheters to control microbial growth.

In addition to the above experiments, the efficacy of catheter segments in inhibiting bacterial growth within 24 h was tested against *Pseudomonas aeruginosa* MF and MA strains and *Candida albicans* G and R strains. All these strains, including *S. epidermidis* 5667, were clinical strains causing catheter-related bloodstream infections at M. D. Anderson Cancer Center.

RESULTS

The results of testing the long-term antimicrobial activity of the catheter (experiment 1) are shown in Fig. 2. The silicone-sheathed antimicrobial device maintained significant antimicrobial activity for at least 90 days, as indicated by zones of inhibition of >15 mm (Fig. 2), even though the catheter was challenged repeatedly by reimplantation for 15 consecutive 7-day cycles. The significant antimicrobial activity of polyurethane catheters coated with chlorhexidine gluconate and silver sulfadiazine diminished to nonsignificant levels (<15 mm) within 8 days of implantation. Further reimplantations were discontinued after the zones of inhibition reached zero.

The results of testing the long-term efficacy of silicone-sheathed antimicrobial catheters are shown in Table 1. The results demonstrate the significant long-term antimicrobial activity of the catheter design where significant long-term antimicrobial activity in vitro (zone of inhibition, >15 mm) was demonstrated continuously for over 325 days.

In addition, duplicate 24-h zones of inhibition were 19 and 19 mm for *P. aeruginosa* MF strain, 22 and 19 mm for *P. aeruginosa* MA strain, 32 and 31 mm for *C. albicans* G strain, and 27 and 27 mm for *C. albicans* R strain.

DISCUSSION

Coating of short-term CVC with antimicrobial agents has been shown to decrease the risk of catheter-related infections (10, 12, 15, 18, 19, 24–26, 27). However, so far long-term antimicrobial silicone CVC are not available. Our unique cath-
eter design of incorporating antimicrobial agents into the body of the long-term silicone catheter and controlling the release of the agents from such a depot through a very thin silicone sheath (26a) serves four purposes. First, it provides a long-term antimicrobial preservative of the silicone catheter surfaces; second, it allows slow diffusion of the antimicrobial agents, thus prolonging the life of the depot in the catheter, third, it protects adjacent tissues from toxicity due to the high local concentrations of the antimicrobial depot; and fourth, it significantly minimizes the risk of contribution of the catheter antibiotics to systemic levels of the agents. The catheter design is applicable to mono- and multilumen catheters and does not compromise the diameter of the catheter since the antimicrobial layer is part of the catheter structure.

The decision to test the catheters by totally embedding them in the agar media was made to demonstrate in vitro more accurately the efficacy of the catheter design by simulating in vivo-like conditions. The antimicrobial agents diffuse circumferentially on the surface of the catheter and diffuse luminally along the whole surface of the catheter lumen. Protection of the external and luminal surfaces of the catheter is essential in inhibiting the attachment of organisms to the fibrin sheath (3, 23), thus, eliminating the formation of the biofilm matrix in which a variety of infective organisms embed themselves (2, 8, 9, 11, 13, 22, 28).

We used a combination of minocycline and rifampin because it was demonstrated that when combined the agents have significant antimicrobial activity (5, 18, 19). The zones of inhibition demonstrated in Fig. 1 were significant and continued to be so after 15 replatings of the same catheter. The zones of inhibition at the end of 90 days were significantly larger than 15 mm. Sherertz et al. reported that in vitro zones of inhibition of $\geq$15 mm were efficacious in vivo (25). We used this criterion of in vivo effectiveness since their design, using the modified Kirby-Bauer method, was quite comparable to the one used in our study. In contrast, polyurethane catheters coated with chlorhexidine gluconate and silver sulfadiazine (shown in clinical tests by others to reduce infection rates at least fourfold over the rates associated with uncoated controls [15]) lost significant antimicrobial activity within one 7-day cycle and lost all antimicrobial activity by the second 7-day cycle.

We used a combination of minocycline and rifampin because it was demonstrated that when combined the agents have significant antimicrobial activity (5, 18, 19). The zones of inhibition demonstrated in Fig. 1 were significant and continued to be so after 15 replatings of the same catheter. The zones of inhibition at the end of 90 days were significantly larger than 15 mm. Sherertz et al. reported that in vitro zones of inhibition of $\geq$15 mm were efficacious in vivo (25). We used this criterion of in vivo effectiveness since their design, using the modified Kirby-Bauer method, was quite comparable to the one used in our study. In contrast, polyurethane catheters coated with chlorhexidine gluconate and silver sulfadiazine (shown in clinical tests by others to reduce infection rates at least fourfold over the rates associated with uncoated controls [15]) lost significant antimicrobial activity within one 7-day cycle and lost all antimicrobial activity by the second 7-day cycle.

The significant zones of inhibition obtained 325 days after the catheters were first embedded in serum and then plated at specified intervals attests to the potential contribution of the

### TABLE 1. Zones of inhibition of antimicrobial agent-impregnated silicone catheters immersed in human serum for specified periods

<table>
<thead>
<tr>
<th>Time (days) in serum</th>
<th>Zone of inhibition (mm)(^a) in:</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>37</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>14</td>
<td>30</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>21</td>
<td>36</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>28</td>
<td>36</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>42</td>
<td>37</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>56</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>70</td>
<td>38</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>90</td>
<td>35</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>120</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>160</td>
<td>27</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>190</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>250</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>275</td>
<td>18</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>300</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>325</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

\(^a\) Data represent 24-h zones of inhibition after each implantation. Baseline data represent catheters not immersed in human serum before implantation. Two independent samples were used. Controls were treated identically to experimental catheters and sterilized using ethylene oxide; they had no zones of inhibition (0.0 mm).
silicone sheath catheters to providing efficacious long-term antimicrobial activity. This is the first time it has been shown that a silicone catheter design has the potential of providing in vivo antimicrobial protection for about 1 year.

Furthermore, it is important to state that the antimicrobial agents used in these studies, namely, minocycline and rifampin, are not the only pharmacological agents available. Antibiotics in general might not be the best choice for long-term catheters because of concern about the emergence of resistant organisms. The design can be adapted to a host of pharmacologically active ingredients or combinations thereof, such as anticoagulants, antibifibrin agents, anti-inflammatory agents, and antiseptics. Anticoagulants can include EDTA, heparin, urokinase, and streptokinase. Anti-inflammatory agents can include nonsteroid anti-inflammatory agents and salicylates. Antimicrobials can include a host of antibacterial, antifungal, and broad-spectrum antiseptic agents. These pharmacologically active ingredients can be introduced into the antimicrobial layer of the catheter singly or in combination as desired. Further studies should be performed to determine whether this technology is clinically useful in the prevention of long-term catheter-related infections and whether it represents a potential cost-effective alternative to the surgically implantable devices (22).

ACKNOWLEDGMENTS

There was no industrial or pharmaceutical funding for this study. The source of funding was academic. R.K.T. and I.I.R. are coinventors. There was no industrial or pharmaceutical funding for this study. R.K.T. and I.I.R. are coinventors. Furthermore, it is important to state that the antimicrobial activity. This is the first time it has been shown that a silicone catheter design has the potential of providing in vivo antimicrobial protection for about 1 year.

REFERENCES