The Broad-Spectrum Activity and Efficacy of Catheters Coated with Minocycline and Rifampin

Issam Raad, Rabih Darouiche, Ray Hachem, Mohammad Mansouri, and Gerald P. Bodey

The in vitro and in vivo activities of catheters coated with minocycline and rifampin and with chlorhexidine gluconate and silver sulfadiazine were evaluated. When incubated in serum at 37°C, the half-life of the inhibitory activity of catheters coated with minocycline and rifampin was 25 days compared with 3 days for catheters coated with chlorhexidine gluconate and silver sulfadiazine. In a rabbit model, catheters coated with minocycline and rifampin were significantly more efficacious than catheters coated with chlorhexidine and silver sulfadiazine in preventing colonization and infection with Staphylococcus aureus (P < .05). Catheters coated with minocycline and rifampin demonstrated broad-spectrum in vitro inhibitory activity against gram-positive bacteria, gram-negative bacteria, and Candida albicans that was significantly superior to the inhibitory activity of catheters coated with chlorhexidine gluconate and silver sulfadiazine (P < .01). Minocycline and rifampin were also highly efficacious in preventing colonization and infection in vivo.

Indwelling vascular catheters are a leading cause of primary nosocomial sepsis [1, 2], and urinary catheters are a leading cause of nosocomial urinary tract infection [3–5]. Despite their association with infectious complications, these devices are essential for the appropriate management of hospitalized and chronically ill patients.

Colonization of bacteria and fungi is a prelude to catheter infection [6] and is more common than initially thought. Recent data using scanning and transmission electron microscopy have suggested that most indwelling catheters are colonized with bacteria and fungi [7]. Organisms adhere to catheter surfaces and embed in a biofilm layer that consists of host factors such as fibrin, fibronectin, and a microbial exopolysaccharide material [8]. Microorganisms embedded in the biofilm layer are resistant to glycopeptide antibiotics and normal host defenses such as antibodies and phagocytic polymorphonuclear cells [9–12].

To limit catheter infections, several interventions have been studied. These include the use of maximal sterile barriers, topical antibiotics or antimicrobial flush solutions, tunneled catheters, subcutaneous ports, and subcutaneous silver cuffs [13–17]. Most of these interventions have resulted in a significant reduction of catheter infections; however, catheter-related infections continue to occur, often with more resistant organisms, resulting in serious morbidity and mortality [18].

The antimicrobial coating of catheters has resulted in a significant reduction in the colonization of these devices in vitro and in vivo [19, 20]. A common method of coating catheters involves the use of a cationic surfactant called tridodecylmethyl ammonium chloride (TDMAC) that binds to the catheter surfaces at one end and to anionic antimicrobials at the other. In a prospective randomized clinical trial, Kamal et al. [21] showed that when cefazolin was bonded to TDMAC-coated catheters the devices were significantly less prone to colonization than were uncoated control catheters. Maki et al. [22] found that when catheters were coated with chlorhexidine gluconate and silver sulfadiazine (CH-SS), the incidence of catheter-related sepsis was reduced >4-fold.

The issue remaining is which antimicrobial agents should be used to coat the catheter. As a result of the emergence of resistant gram-positive and gram-negative bacteria, β-lactam and glycopeptide antibiotics, which are considered the first-line drugs for treating established infections, should not be used prophylactically. In an in vitro model, minocycline combined with rifampin was more efficacious than other antibiotic combinations, such as vancomycin and rifampin, in preventing colonization of Staphylococcus epidermidis to silicone catheter surfaces [23]. In addition, the minocycline-rifampin combination has broad antistaphylococcal, anti-gram-negative bacillary, and antifungal activity [24]. This study assessed the in vitro and in vivo efficacy of catheters coated with minocycline and rifampin.

Materials and Methods

Antibiotic bonding of catheters. Polyurethane triple lumen catheters (20-cm long, 13 gauge [7 French]; Bio-guard AB Coating; Cook, Bloomington, IN) precoated with the cationic surfactant TDMAC were immersed for 15 min in a methanol solution that contained 60 mg/mL minocycline (Lederle Laboratories, Pearl River, NY) and 30 mg/mL rifampin (Ciba-Geigy, Summit, NJ). Bio-guard catheters coated with TDMAC alone (no antibiotics) were used as negative controls. Arrow Gard catheters (Arrow International, Reading, PA) of the same size as the triple lumen catheters and precoated with CH-SS were used as positive controls.
In vitro antimicrobial activity of catheters. The antimicrobial activity of catheters was assessed by a modified Kirby-Bauer technique. Five different septicemia strains of 11 organisms (S. epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pneumoniae, Enterobacter species, Proteus mirabilis, Acinetobacter baumannii, Escherichia coli, Candida albicans, and Stenotrophomas maltophilia) were independently grown for 18 h in trypticase soy broth (TSB; BBL Microbiology Systems, Cockeysville, MD) to a concentration of 0.5 McFarland U (10^6 cfu/mL). A cotton swab was placed in this suspension and rubbed across the surface of a Mueller-Hinton agar plate. Twenty-millimeter segments of individual catheters were pressed into the agar overlaid with the bacteria or Candida organisms tested and incubated overnight at 37°C. Zone sizes were assessed by measuring the distance perpendicular to the long axis of the catheter segment.

In vitro antimicrobial half-life. The half-life of antimicrobial activity of coated catheters was determined by immersing several catheter segments in human serum and incubating the serum at 37°C. Segments were removed for testing at 3, 7, 10, 15, and 30 days. The antimicrobial activity against S. epidermidis at the different intervals was determined by the modified Kirby-Bauer technique (described earlier) and compared with the baseline zones of inhibitions determined on day 1 before immersion in sera.

Levels of antibiotics on catheters. Individual 1-cm segments of catheters coated with minocycline-rifampin were extracted in 5 mL of methylene chloride. The segments were sonicated in methylene chloride for 15 min, left to sit for 45 min at room temperature, and vortexed for 5 s. After catheter segments were removed, the extracted solution was air-dried, and the retained antibiotics were suspended in 1 mL of the buffer used for high-performance liquid chromatography (HPLC) as described by Darouiche and Hamill [25]. Aliquots (10 mL) of the extracted antibiotic suspension were injected into the HPLC system (μBondapack C_{18} bond column [60 Å, 4 μm, 3.9 × 150 mm], model 510 solvent delivery pump, model 700 WISP satellite sample processor, 486 UV absorbance detector, and Maxima 820 operating station; Waters Chromatography Division, Millipore, Milford, MA).

Both minocycline and rifampin were detected under the same separation conditions [25]. The mobile phase consisted of KH₂PO₄ (60%) and acetonitrile (40%) with the final pH adjusted to ~3.25 using 85% O-phosphoric acid. The HPLC system was run under an isocratic reversed-phase condition with a flow rate of 1 mL/min. Detection was done at a wavelength of 339 nm. Minocycline was eluted at 1.5 min, and rifampin was detected at 13 min. In pilot trials, known amounts of antibiotics were added to 1-cm segments of uncoated catheters and extracted as outlined above. More than 90% of both antibiotics were recovered. After construction of standard curves, antibiotic controls were measured with a consistent accuracy of >85%.

Rabbit model of S. aureus infection. We used an established rabbit model (3- to 4-month-old New Zealand White rabbits; 3–4 kg) as described by Sherertz et al. [20]. S. aureus strain PI (ATCC 25923) was obtained from the laboratory of R. J. Sherertz (Wake Forest University, Bowman-Gray School of Medicine, Winston-Salem, NC). The S. aureus strain was grown overnight in TSB, washed twice in PBS, pH 7.3, and diluted serially in PBS to achieve an inoculum of 10^6 cfu/mL. By use of a tuberculin syringe with a blunt-tipped needle, 0.1-mL aliquots of bacterial suspension were delivered subcutaneously next to the lateral catheter segment.

Animals were anesthetized with a 1-mL combination of ketamine (80 mg/mL) and xylazine (20 mg/mL). The back of each animal was shaved, depilated with Nair (Carter Wallace, New York), and prepared with povidone iodine (Clinidine; Clinipad, Guilford, CT). We made a pair of incisions (0.5–1 cm apart and 4 cm from the spine) for each catheter segment. We used 7 French (13 gauge) triple lumen polyurethane vascular catheters, including some Bio-guard catheters coated with TDMAC only (negative controls), Arrow Gard catheters coated with CH-SS (positive controls), and some coated with minocycline-rifampin. A Jimshidle bone marrow needle (Pharmaseal; Baxter Healthcare, Valencia, CA) was used to create two 3-cm tunnels through two incisions. A 6-cm catheter segment was inserted into both tunnels so that both ends of the catheter were in the subcutaneous space and the middle of the catheter rested on the skin. Four pairs of catheter segments were inserted into each rabbit. The catheter segment closest to the spine (medial) was secured with a silk suture; only the catheter segment without the suture was inoculated. A 0.1-mL aliquot of the bacterial suspension of S. aureus PI (10^6 cfu/mL) was inoculated at the insertion site of the lateral segment.

Animals were sacrificed 7 days later. Each catheter segment was removed and observed for gross purulence; the intercutaneous segments were placed in sterile containers and transferred to the laboratory. Each segment was initially cultured semiquantitatively using the roll-plate technique [26]. Each segment was subsequently placed in 5 mL of TSB and cultured quantitatively by the sonication technique [27, 28].

Data analysis. The significance of differences in the frequencies of categorical variables was determined using the χ² or Fisher’s exact tests. Continuous variables with normal distribution were compared by Student’s t test; continuous variables that were not normally distributed were compared by the Mann-Whitney test. P ≤ .05 was considered to be statistically significant.

Results

In vitro antimicrobial activity of coated catheters. Catheter segments coated with minocycline-rifampin demonstrated a broad-spectrum of activity against bacteria and C. albicans (table 1). The largest zones of inhibition were associated with gram-positive bacteria. For S. epidermidis, the mean size of the zones of inhibition of catheters coated with minocycline-rifampin was 42.6 ± 1.7 compared with 15.4 ± 1.1 (P < .01) for catheters coated with CH-SS. Similar results were obtained for other gram-positive bacteria, S. aureus, E. faecalis, and Corynebacterium species. The activity of minocycline-rifampin–coated catheters against gram-negative bacilli, as measured by zones of inhibition, was highest for A. baumannii (previously named Acinetobacter calcoaceticus variant anitratus) and lowest for P. aeruginosa. However, for all gram-negative bacilli, the activity of minocycline-rifampin–coated catheters was 2–10 times greater than for catheters coated with CH-SS (P < .01). The mean zone of inhibition for C. albicans was 19.0 ± 2.6. The C. albicans zones of inhibition were uniquely characterized by an inner zone of complete inhibition and a
NOTE. 5 different bacteremic strains of each organism were tested. Zones of inhibition are expressed as mean of diameter of zone: ± SD. P < .01 for all comparisons.

Table 1. Comparative efficacy of catheters coated with minocycline and rifampin and catheters coated with chlorhexidine gluconate and silver sulfadiazine against various organisms.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Minocycline-rifampin</th>
<th>Chlorhexidine and silver sulfadiazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>14.8 ± 3.2</td>
<td>3.0 ± 3.0</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>26.6 ± 7.9</td>
<td>7.0 ± 6.8</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>18.8 ± 6.5</td>
<td>6.0 ± 2.2</td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>18.8 ± 5.1</td>
<td>5.2 ± 3.4</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>17.2 ± 4.4</td>
<td>6.2 ± 2.8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>19.6 ± 7.2</td>
<td>10.4 ± 2.7</td>
</tr>
<tr>
<td>Acinetobacter baumanii</td>
<td>31.0 ± 13.6</td>
<td>5.2 ± 5.4</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>29.0 ± 9.3</td>
<td>3.0 ± 5.2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>36.0 ± 1.3</td>
<td>13.2 ± 1.3</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>42.6 ± 1.7</td>
<td>15.4 ± 1.1</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>19.0 ± 2.6</td>
<td>7.4 ± 4.3</td>
</tr>
</tbody>
</table>

NOTE. 5 different bacteremic strains of each organism were tested. Zones of inhibition are expressed as mean of diameter of zone ± SD. P < .01 for all comparisons.

wider halo of presumed partial growth inhibition (figure 1). Diameters shown are for the inner zones. Although smaller than the zones of inhibition of minocycline-rifampin–coated catheters against gram-positive bacteria, the activity against C. albicans was significantly better (P < .01) than that of catheters coated with CH-SS (table 1).

Stability and half-life of the antimicrobial activity of coated catheters. Catheters coated with minocycline-rifampin or CH-SS did not show any significant difference in vitro activity against S. epidermidis or S. aureus before or after gas sterilization with ethylene oxide. In serum at 37°C, the half-life of the antimicrobial activity against S. epidermidis of catheters coated with CH-SS was 3 days versus 25 days for catheters coated with minocycline-rifampin. The zones of inhibition of minocycline-rifampin–coated catheters at baseline (day 1) was 3.2 times greater than those treated with CH-SS. This ratio increased to 4.9 at day 15 and 7.0 at day 30 (figure 2). Figure 3 shows shelf-life data for catheters coated with minocycline-rifampin while stored at 25°C. The zones of inhibition after 12 months were 90% of those at baseline.

The HPLC-determined concentration of minocycline and rifampin on catheters was 290 and 93 μg/cm, respectively; thus, the concentration of minocycline was >3-fold higher than that of rifampin.

In vivo activity of coated catheters. Seven days after insertion and inoculation, purulence was noted at the insertion site of all control catheters coated with TDMAC only. Semiquantitative cultures of control segments had > 10³ cfu per 2-cm segment from all 32 catheters (table 2). Most of the catheters coated with CH-SS (22 [65%] of 34) had insignificant results by semiquantitative cultures (< 15 cfu/segment and no purulence at the insertion site). However, breakthrough colonization occurred in 12 (35%) of the 34 CH-SS–treated catheter segments (> 15 cfu obtained by semiquantitative cultures). Heavy colonization (> 10⁶ cfu) by roll-plate culture technique was
Figure 2. Life potential (in days) of coated catheters after coating and inoculation in serum at 37°C. Antimicrobial activity was determined at different intervals by modified Kirby-Bauer technique. CH-SS*, catheters coated with chlorhexidine gluconate and silver sulfadiazine.

noted in 6 (18%) of the 34 CH-SS–treated catheter segments. All segments coated with minocycline-rifampin remained sterile 7 days after inoculation.

Quantitative sonication cultures showed a similar trend (table 3). All 40 segments coated with minocycline-rifampin remained sterile by the sonication culture technique. Most of the control catheter segments coated with TDMAC but no antimicrobials (25 [89%] of 28) were heavily colonized with >10² cfu. Most of the catheter segments coated with CH-SS (12/18) had negative cultures or insignificant numbers of colonizing bacteria (20–10² cfu). However, one-third of the CH-SS–treated catheters were heavily colonized (>10² cfu).

Discussion

Medical devices with antimicrobial activity decrease the risk of colonization and ultimately of infection [19–22]. Chlorhexidine gluconate is often favored as an antiseptic with broad-spectrum activity. Sherertz et al. [20] demonstrated the efficacy of coating catheters with chlorhexidine in vitro and in vivo. In a prospective, randomized clinical trial, Maki et al. [22] reported that catheters coated with TDMAC and CH-SS decreased the risk of catheter-related septicemia by >4-fold. Kamal et al. [21] used TDMAC as a binding surfactant and coated vascular catheters with cefazolin. They reported a significant
Table 2. Efficacy of antimicrobial-coated catheters against bacterial colonization in a rabbit model using the semiquantitative roll-plate culture technique.

<table>
<thead>
<tr>
<th>Catheter type</th>
<th>No. tested</th>
<th>0</th>
<th>1–14</th>
<th>15–10^3</th>
<th>&gt;10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>32 (100)</td>
</tr>
<tr>
<td>Coated with chlorhexidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sulfdiazine</td>
<td>34</td>
<td>7 (21)</td>
<td>15 (44)</td>
<td>6 (18)</td>
<td>6 (18)</td>
</tr>
<tr>
<td>Coated with minocycline + rifampin</td>
<td>38</td>
<td>38 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Per 2-cm catheter segment.

The minocycline-rifampin combination has unique characteristics that promote its consideration for incorporation onto the surface of vascular catheters. Both agents are active against methicillin-sensitive and -resistant staphylococci [32–36]. In addition, minocycline and rifampin are broad-spectrum agents with activity against gram-negative bacilli and candida [37–40]. In combination, these agents are not antagonistic and are occasionally synergistic [41–43]. Unlike glycopeptides, penicillins, cephalosporins, aminoglycosides, quinolones, amphoterin B, or the azoles, minocycline and rifampin are not routinely used as therapeutic agents to treat bloodstream infections. In contradistinction to glycopeptide antibiotics, rifampin has not been shown to be inhibited by the exopolysaccharide fibrous glycocalyx that is generated by the slime-producing S. epidermidis [11, 44].

The activities of minocycline-rifampin–treated catheters against gram-positive and -negative bacteria and C. albicans were significantly superior to the activities of CH-SS–treated catheters as judged by the sizes of the zones of inhibition. By using the same in vitro and in vivo rabbit model that we describe in the current study, Sherertz et al. [20] established a quantitative relationship between the size of the zone of inhibition in vitro and the concentration of S. aureus cultured from the tip of coated catheters 7 days after inoculation of the insertion site. Coated catheters with a zone size of ≥15 mm were highly predictive of in vivo efficacy, as reflected by prevention of colonization of the indwelling catheter [20]. The mean zone diameter of minocycline-rifampin–treated catheters against organisms commonly associated with vascular catheter infections (S. epidermidis, S. aureus, Corynebacterium species, and some gram-negative bacilli [e.g., S. maltophilia and A. baumannii]) was ≥31.0 mm (table 1). If the conclusions of the Sherertz model can be applied to organisms other than S. aureus, the findings of this study may predict protection of minocycline-rifampin–treated catheters against colonization with such organisms.

The average zone of inhibition of minocycline-rifampin–treated catheters against C. albicans and other gram-negative bacilli, including P. aeruginosa, was 14.8–19.6 mm, which also should be predictive of good in vivo and clinical outcome. For the CH-SS–treated catheters, the average zone of inhibition was 15.4 mm for S. epidermidis and borderline (10–15 mm) for S. aureus, Corynebacterium species, and E. coli. Based on the sizes of zones of inhibition, CH-SS–treated catheters did not possess adequate antimicrobial activity against the other...
organisms tested. However, it is possible that the catheters treated with CH-SS would have sufficient antimicrobial activity to inhibit most of the organisms associated with vascular catheter–related infections. A prospective randomized clinical trial would be necessary to compare the efficacy of coating catheters with CH-SS and minocycline-rifampin.

The long antimicrobial half-life (25 days) of minocycline-rifampin–treated catheters in serum at 37°C (zone \(\geq 15\) mm after 30 days of incubation) is predictive of long-term catheter protection against colonization with \(S.\) epidermidis. The data and the persistently large zones are consistent with the relatively high levels of minocycline (290 \(\mu g/cm\)) and rifampin (93 \(\mu g/cm\)) bonded to the catheters. In a susceptibility study of 197 catheter-related bacteremic strains of methicillin-resistant \(S.\) epidermidis and \(S.\) aureus from the University of Texas M. D. Anderson Cancer Center, the MIC \(_{50}\) of minocycline and rifampin were 2.0 and 0.06 \(\mu g/mL\), respectively [43]. Although the levels of the two antibiotics are high from an antimicrobial viewpoint, from a pharmacokinetic perspective, the levels would not be expected to result in any detectable levels in the sera of patients with indwelling catheters. Assuming an indwelling catheter segment of 15 cm, the overall amounts of minocycline and rifampin along the subcutaneous and intravascular segments would be \(\sim\)4.5 and 1.5 mg, respectively. Because these quantities would be released slowly into the serum over several weeks, it should be undetectable in serum at any time point (the detectability limit in our HPLC system was 0.05 \(\mu g/mL\) for both antibiotics).

The in vivo data from the rabbit experiments are consistent with the in vitro results and with reports from other animal and clinical studies. Sherertz et al. [20] demonstrated an inverse relationship between the in vitro zones of inhibition of catheters coated with antimicrobial agents and the number of \(S.\) aureus removed from a catheter indwelling an animal 7 days after inoculation of the insertion site with \(10^6\) cfu. In our animal experiments, we used the model described by Sherertz et al. (except that we inoculated the insertion site with \(10^5\) rather than \(10^4\) cfu of \(S.\) aureus PI). In CH-SS–treated catheters with 12- to 14-mm zones of inhibition against \(S.\) aureus, significant colonization of indwelling catheters was noted in about one-third of cases. For catheters treated with minocycline-rifampin and with zones of inhibition of 34–39 mm, all indwelling catheters remained sterile, and negative semiquantitative and quantitative cultures resulted in significant differences in efficacy.

We recently conducted a prospective randomized clinical study on 234 hospitalized patients in which we demonstrated the efficacy of minocycline-rifampin–coated catheters in significantly decreasing the risk of catheter colonization and in preventing catheter-related bacteraemia [45]. Quantitative skin-insertion-site cultures done at the time of catheter insertion and removal as well as susceptibility studies of all organisms that colonized the minocycline-rifampin–coated catheters failed to show development of resistance to minocycline or rifampin. HPLC studies on sera failed to detect either antimicrobial. The zones of inhibition of minocycline-rifampin–coated catheters removed from patients at various intervals showed that the antimicrobial protection provided by such catheters can last \(\geq 2\) weeks [46]. This finding is consistent with our results with minocycline-rifampin–coated catheters placed in serum, which maintained an optimal inhibitory antimicrobial activity for a similar time period.

In summary, antiinfective catheters coated with minocycline-rifampin had a broad spectrum of in vitro activity against staphylococci, other gram-positive bacteria, gram-negative bacillar organisms, and \(C.\) albicans. The efficacy of minocycline-rifampin–treated catheters remained stable at room temperature for 12 months, and the half-life of antimicrobial activity of minocycline-rifampin–treated catheters during inoculation in serum at 37°C was 25 days. Catheters treated with these agents also effectively prevented catheter infection in a rabbit model. The in vitro and in vivo (animal) data correlate with subsequent clinical data that demonstrated the efficacy of minocycline-rifampin–coated catheters in preventing colonization and catheter-related bacteraemia.

References


