Comparison of the resistance to infection of rifampin-bonded gelatin-sealed and silver/collagen-coated polyester prostheses

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Purpose: The purpose of this study was to compare the efficacy of rifampin-bonded gelatin-sealed and silver acetate/collagen-coated knitted polyester prostheses for the prevention of bacteremic graft infection in an animal model.

Methods: Eighteen 6.0-mm polyester grafts (length, 5.0 cm) were implanted in dogs end-to-end into the infrarenal aorta. The dogs were divided into four groups as a function of type of prosthesis implanted. The dogs in groups I (n = 3) and II (n = 3) received control gelatin-sealed or collagen-coated polyester prostheses, respectively. In group III (n = 6), the dogs received rifampin-bonded gelatin-sealed polyester prostheses. In group IV (n = 6), the dogs received silver/collagen-coated polyester prostheses. Two days after implantation, the grafts were challenged with 6 × 10^7 Staphylococcus aureus intravenously. One week after implantation, the grafts were harvested with sterile technique. Quantitative cultures were obtained from all the harvested grafts. The results were expressed as colony-forming units per cm^2 of graft material. Bacteriologic study was also performed on various tissue samples. The chi-square test was used to compare the culture proven infection of control and antimicrobial grafts.

Results: All the control grafts were infected with S. aureus at the time of removal. Five of the six silver/collagen-coated grafts were infected, whereas none of the six rifampin-bonded gelatin-sealed grafts grew S. aureus (P < .01). There was no significant difference in the number of positive culture results of organ samples between the different groups of dogs.

Conclusion: These results indicate that rifampin-bonded gelatin-sealed polyester grafts are significantly more resistant to bacteremic infection than are silver/collagen-coated polyester grafts in a highly challenging model. (J Vasc Surg 2002; 35:1260-3.)

Despite refinements in graft fabrication, implantation techniques, and routine antibiotic prophylaxis, vascular prosthetic infections remain a serious complication of reconstructive vascular surgery. Graft infection is always difficult to eradicate and, if not recognized or adequately treated, eventually causes prosthesis failure, hemorrhage, or sepsis. Even in experienced centers and in recent series, mortality and amputation rates associated with infected grafts remain significant. 1-5

Our laboratory has had a long interest in the development of a prosthesis resistant to infection. The introduction of sealed grafts has enabled the use of the scalant as an antibiotic bonding carrier. In previous studies, we showed the efficacy of rifampin-bonded gelatin-sealed polyester prostheses in the prevention of bacterial graft infection6 and in the treatment of aortic graft infections with in situ replacement. 7 We also showed the efficacy of rifampin-bonded prostheses in the prevention of graft reinfection after in situ replacement of a prosthesis infected by a highly rifampin resistant Staphylococcus epidermidis. 8

More recently, silver-coated knitted polyester prostheses have been proposed as an alternative for prevention or treatment of prosthetic graft infections. The purpose of this experimental study was to compare the efficacy of rifampin-bonded gelatin-sealed knitted and silver-coated polyester prostheses in resisting infection after a bacteremic challenge.

MATERIALS AND METHODS

Protocol. The infrarenal aortas of 18 dogs were replaced with four types of prostheses. In groups I and II (n = 3 in each group), the animals received a conventional sealed knitted polyester prosthesis. In groups III and IV, the animals received a rifampin-bonded (n = 6) or silver-coated (n = 6) knitted polyester prosthesis. Two days after implantation, a bacteremia was produced with an intravenous injection of 10^7 S. aureus. One week after implantation, the prostheses were removed and submitted to quantitative bacteriologic studies.

Materials. Four types of vascular prostheses were used in this study: collagen-coated knitted polyester prostheses (Intergard, InterVascular, La Ciotat, France), silver acetate/collagen-coated knitted polyester prostheses (Intergard Silver, InterVascular), gelatin-sealed knitted polyester prostheses (Gelsoft Huls, Sulzer Vascutek, Inchinnan, Scotland), and the same gelatin-sealed prostheses soaked in rifampin (60 mg/L)
grafts). All the grafts were 6 mm-diameter tubes. Rifampin was Rifadin (Laboratoires Roussel Diamant, Paris, France).

**Implantation of prostheses.** Eighteen female mongrel dogs that weighed 12 to 20 kg underwent anesthesia with intravenous injection of pentobarbital at a dose of 30 mg/kg and were mechanically ventilated. Hydration was maintained with continuous infusion of Ringer’s lactate at the rate of 15 mL/kg/h. After the dogs were placed in dorsal decubitus position, laparotomy was performed with sterile surgical conditions and a 3-cm to 4-cm segment of the infrarenal abdominal aorta was exposed. The lumbar and the inferior mesenteric arteries were ligated and sectioned to allow better mobilization of the exposed arterial segment. After intravenous administration of a 0.5 mg/kg bolus of heparin, the aorta was clamped just below the renal arteries and at its end. A 5-mm to 8-mm aortic segment then was removed. Resection resulted in a 30-mm to 40-mm gap after elastic recoil of the sectioned artery. The prosthesis was implanted with two end-to-end anastomoses with the use of 6-0 polypropylene sutures. After the anastomoses were completed and hemostasis checked, the prosthesis was covered with direct closure of the posterior parietal peritoneum. The abdominal wall was closed in layers with a standard surgical technique. Mechanical ventilation was maintained until awakening. Dogs did not receive perioperative antibiotic prophylaxis. All animals received humane care in compliance with the Principles of Laboratory Animal Care and Guide for the Care and Use of Laboratory Animals. The dogs were placed in individual cages and examined daily.

**Bacterial strain and bacteremic challenge.** The bacterial strain used in this study was S aureus A980142, from the French National Reference Center. S aureus A980142 is sensitive to rifampin but resistant to methicillin (heterogeneous type). Bacteria were stored at −20°C. When necessary, the strain was plated on tryptic soy agar (TSA) for 18 hours at 37°C. Four colonies then were transferred with a sterile loop to 40 mL of tryptic soy broth and incubated for 18 hours at 37°C. The resulting suspension was centrifuged (10 minutes, 3000 rpm), and the pellet was resuspended in 4 mL of normal saline solution. This obtained suspension was the inoculating solution. The number of viable organisms in the inoculating solution was verified with backplating on TSA. Forty-eight hours after graft implantation, transient bacteremia was produced in the dogs with intravenous injection of 1 mL S aureus suspension over a period of 1 minute.

**Implantation of prostheses.** The animals were killed 5 days after the bacteremic challenge. Death was performed with sterile surgical conditions after anesthetization as previously described. Macroscopic signs of infection were noted. In addition to the prosthesis, samples for bacteriologic studies were collected from various organs (liver, spleen, left kidney, and lung).

**Bacteriologic studies.** Explanted prostheses were split lengthwise to facilitate the extraction of bacteria. The whole prosthesis was placed in a sterile mortar with 2 mL of saline solution and hand-crushed for 2 minutes. One milliliter of the crushing effluent and 0.1 mL aliquots of serial dilutions ranging from 10⁻¹ to 10⁻⁴ were cultured on TSA. Dishes were incubated at 37°C with aerobic conditions for 48 hours. Colony counts were estimated in colony-formation units (CFU) and then extrapolated to the 2-mL initial suspension. Final quantitative estimation of contamination on the surface of the prosthesis was expressed as the number of CFU/cm².

The tissue specimens were placed in trypticase soy broth supplemented with glucose and incubated for as much as 7 days at 37°C. Broth content was vortexed and plated on TSA. Plates were incubated at 37°C with aerobic conditions for 48 hours. Broth culture results were classified as positive if the study strain of S aureus was recovered.

**Statistical analysis.** The number of culture-positive infected grafts observed in the different groups was compared with the χ² test. Statistical significance was assigned when P values were less than .05.

**RESULTS**

The actual inoculum size (mean ± standard deviation) was 6.3 ± 2.8 × 10⁹ CFU/mL, without significant differences between the four groups of dogs. One dog in group IV died 3 days after bacterial infusion with evidence of graft infection at autopsy and was included in further analysis. All the grafts were patent at the time of explantation. Macroscopic signs of infection were observed with all control grafts and with five of the silver-coated grafts. All the control grafts were infected with S aureus. Five of the six silver/collagen-coated grafts in group IV were infected with S aureus. None of the gelatin-sealed rifampin-bonded grafts (0/6) grew bacteria (Table I). This difference was statistically significant (P < .01) when compared with the control groups and the silver-coated group. Bacteriologic study results on samples taken from organs were positive in four of 12 samples in group I, five of 12 samples in group II, in seven of 24 samples in group III, and in nine of 24 samples in group IV, without statistically significant difference (Table II).

**DISCUSSION**

This experimental study confirms the efficacy of rifampin-bonded gelatin-sealed prostheses and shows the inability of silver/collagen-coated prostheses to resist infection from S aureus bacteremia. In view of the increasing bacterial resistance to antibiotics, silver coating was an appealing concept for the prevention of graft infections. Different forms of silver molecules have been bonded to a large variety of medical devices, including central vascular, urinary, and peritoneal catheters, prosthetic heart valve sewing rings, sutures, fracture fixation devices, and vascular prostheses. Despite their large number, the antiinfective activity of these silver-coated medical devices has not been collectively addressed. Even when they showed an antimicrobial activity in vitro, implanted medical prostheses that were coated with silver alone have not been proven to be infection-resistant in most clinical studies. Randomized clinical studies that show the efficacy of
Table I. Bacteriologic evaluation of prostheses

<table>
<thead>
<tr>
<th>Group</th>
<th>Injected grafts</th>
<th>Viable counts of infected grafts, median (range) (CFU/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3/3</td>
<td>2 (2.75 × 10⁶)</td>
</tr>
<tr>
<td>Group II</td>
<td>3/3</td>
<td>12 (5 × 10⁶-9.9 × 10⁵)</td>
</tr>
<tr>
<td>Group III</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>5/6*</td>
<td>4.1 × 10⁴ (2.5 × 10³-5.6 × 10⁴)</td>
</tr>
</tbody>
</table>

*P < .01 versus gelatin-scaled rifampin-bonded prostheses.

Table II. Bacteriologic study of organ samples

<table>
<thead>
<tr>
<th></th>
<th>Kidney</th>
<th>Lung</th>
<th>Spleen</th>
<th>Liver</th>
<th>All samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0/3</td>
<td>1/3</td>
<td>2/3</td>
<td>1/3</td>
<td>4/12</td>
</tr>
<tr>
<td>Group II</td>
<td>1/3</td>
<td>2/3</td>
<td>0/3</td>
<td>2/3</td>
<td>5/12</td>
</tr>
<tr>
<td>Group III</td>
<td>2/6</td>
<td>2/6</td>
<td>1/6</td>
<td>2/6</td>
<td>7/24</td>
</tr>
<tr>
<td>Group IV</td>
<td>2/6</td>
<td>2/6</td>
<td>5/6</td>
<td>2/6</td>
<td>9/24</td>
</tr>
</tbody>
</table>

silver-coated prostheses are rare and have dealt with small numbers of patients or are controversial. The differences observed between in vitro experiments and clinical studies can be the consequence of minimal leaching or nonleaching silver-coated surfaces and limitations caused by potential silver toxicity. Different techniques have been used to incorporate silver alone onto the surface of prostheses. When the adherence of silver molecules is tight, as observed in silver-coated prosthetic heart valve sewing rings, despite the durability of the antimicrobial surface, the minimal leaching of silver off the device may be a disadvantage, with small zones of bacterial inhibition around the coated material in vitro and a limited clinical efficacy. When the technique used to incorporate silver onto the surface of a prosthesis allows leaching of silver off the surface or when the silver is complexed with antibiotics or other antimicrobial agents, larger zones of inhibition are obtained and a better clinical antimicrobial efficacy can be expected.

Silver ions are active against a broad spectrum of bacteria, and their antibacterial activity is proportional to their concentration. On the contrary, metallic silver has only slight antibacterial efficacy because of its chemical stability. Silver ions, often released from inorganic salt solutions, act by displacing other essential metal ions such as Ca²⁺ or Zn²⁺.

The binding of silver ions to the bacterial DNA interacts with cellular oxidation processes and inhibits the respiratory chain. This activity is proportional to the silver ion concentration, and the minimum inhibitory concentration of silver to staphylococci is between 0.5 mg/L and 10 mg/L. Pseudomonas species are more susceptible to silver ions than are staphylococci. Bacterialidal effects are achieved through silver-DNA chelate complexes. Nevertheless, the biologic environment may influence and decrease the antimicrobial activity of silver ions. In particular, blood proteins deposited onto the surface of implants, such as albumin, may bind silver ions that no longer have antimicrobial activity. In addition, the conversion of silver acetate to silver chloride in the presence of the high chloride ion content of the body will also reduce the availability of silver ions. Although silver acetate is fairly soluble in water (1.02 g/L/100 mL), silver chloride is almost insoluble (0.000689 g/L/100 mL). These factors may have contributed to the lack of antimicrobial efficacy of the silver acetate added to the collagen coating of the prostheses implanted in dogs of group IV.

The efficacy of complexing silver with antibiotics on the surface of polytetrafluoroethylene grafts has also been evaluated in several studies. The complexing of silver and norfloxacin increased the antibacterial activity of polytetrafluoroethylene grafts compared with grafts coated with norfloxacin alone and offered significant protection against infection from local bacterial contamination in a dog model. In another in vivo experimental study, a complex of silver and ciprofloxacin increased the elution and prolonged the duration of ciprofloxacin release from the coated polytetrafluoroethylene compared with grafts coated with ciprofloxacin alone. In a dog model of S. aureus infection of aortic polytetrafluoroethylene grafts, the mean number of bacteria retrieved from grafts coated with silver and oxacillin or silver and amikacin was significantly lower than that observed with uncoated grafts. In these experiments, the large zones of inhibition measured in vitro with the silver-antibiotic-coated grafts suggested that the antibiotic is largely responsible for the antimicrobial efficacy in vivo.

The potential toxicity of silver-containing medical devices suggests the use of other techniques to bind antimicrobial agents onto vascular prostheses, and many experimental studies have shown the efficacy of antibiotic-bonded grafts to reduce the incidence rate of graft infection. The advent of protein-sealed polyester prostheses has raised the possibility of the sealant as a vehicle for antibiotic delivery. However, an infection-resistant prosthesis should comply with the following prerequisites. First, the antimicrobial agent bonded to the prosthesis should have a bactericidal effect against the bacteria involved in graft infections. Second, it should be nonallergenic and have a minimal risk of toxicity. Third, the duration of the antibacterial activity of the bonded graft should be as long as possible to allow a satisfactory healing without infection.
Finally, the technique used to bind the antimicrobial agent should be easy to accomplish in the usual clinical setting.

With consideration of these prerequisites, rifampin was a good candidate for bonding, with a strong affinity for gelatin-sealed polymer grafts. Rifampin shows a wide antibacterial activity against most aerobic gram-positive cocci, notably with remarkable antistaphylococcal potency, and against many aerobic gram-negative organisms that cause vascular graft infection. It is, overall, a well-tolerated drug, especially after the parenteral administration of a single dose slowly released in the bloodstream. With a dog model of bactereemia, we showed the resistance of thoracoabdominal aortic rifampin-bonded grafts to an early postoperative methicillin-resistant Staphylococcus aureus bacteremia. These experimental results were the rationale for a prospective randomized trial that evaluated the efficacy of rifampin in the prevention of early wound and graft infection after prosthetic aortoiliacometal reconstruction. In this trial, conducted in 260 patients in 90 centers, the incidence rate of wound infection was significantly reduced in patients who received a rifampin-bonded graft in association with perioperative systemic antibiotics.36

Rifampin-bonded grafts may also be a promising alternative for in situ replacement of infected grafts. With the use of a dog model, we showed that rifampin-bonded gelatin-sealed grafts were resistant to infection when used for in situ replacement of a graft infected with Staphylococcus epidermidis. Because of the high local concentrations of rifampin obtained with this technique, we also recently showed its efficacy when the challenging Staphylococcus epidermidis was resistant to rifampin. Early clinical results observed after in situ replacement of infected grafts with rifampin-bonded polymer appear favorable,20 which justifies further investigations of antibiotic-bonded vascular prostheses.

In conclusion, silver acetate does not appear to afford knitted collagen-coated polyester prostheses a significant antimicrobial activity in vivo. Rifampin soaking of gelatin-coated prostheses is a technique that has been shown to be highly effective in vivo and that can be used in patients at risk of infection.

REFERENCES


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