An increased concentration of rifampicin bonded to gelatin-sealed Dacron reduces the incidence of subsequent graft infections following a staphylococcal challenge

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The purpose of this study was to determine if 10 mg/ml rifampicin bonded to gelatin-sealed Dacron (Gelsept) reduced staphylococcal infection. Grafts soaked in rifampicin were interposed in the left carotid artery of 20 merino sheep and then inoculated with $10^6$ colony-forming units of MRSA (10 sheep) or a slime producing Staphylococcus epidermidis (10 sheep). Grafts were harvested at 3 weeks, and perigraft abscess, anastomotic disruption and graft occlusion recorded. Swabs were taken to assess bacterial growth of the perigraft tissues, and external and internal graft surface. Graft segments were incubated in broth medium. Results were compared with previously published results that used graft that were not soaked in rifampicin (control) and grafts soaked in 1.2 mg/ml rifampicin. A total of 4/50 cultures were positive and significantly reduced for S. epidermidis compared with the control group of 30/50 ($P < 0.05$) and the 1.2 mg/ml group of 13/45 ($P < 0.05$). For the methicillin resistant staphylococcus aureus (MRSA) group, 6/40 cultures were positive, which was significantly reduced compared with the control group (38/40, $P < 0.05$) and the 1.2-mg/ml group (19/32, $P < 0.05$). In conclusion an increased concentration of rifampicin significantly reduced the incidence of prosthetic vascular graft infection following a challenge of MRSA or S. epidermidis. © 1998 Published by Elsevier Science Ltd. All rights reserved

Keywords: graft infection, Staphylococcus aureus, epidermidis, gelatin-sealed Dacron, rifampicin

Introduction

Prosthetic vascular graft infections are a relatively uncommon complication for the vascular surgeon; they have a reported incidence of 2–6% [1, 2]. Despite their infrequency, vascular graft infections are associated with significant morbidity and mortality [1, 2]. The causative organisms are predominantly staphylococci [1–6], but Escherichia coli, Pseudomonas aeruginosa and Enterobacteriaceae are also recognized pathogens [7–9]. There is general agreement that the usual time of entry of microorganisms occurs at the time of operation [7], although they may be haematogenous [4, 7] and lymphatic [2], and may be present in the arterial wall [10], which may contribute to subsequent graft infection.

Previous work has demonstrated that gelatin-sealed Dacron grafts soaked at a concentration of 1.2 mg/ml in a sheep carotid artery model reduced subsequent graft infection following challenge with high concentrations of Staphylococcus epidermidis [11] and Staphylococcus aureus [12]. In view of these results, the intention was to see if increasing the concentration of rifampicin to 10 mg/ml would further reduce the incidence of graft infection for both methicillin resistant S. aureus and S. epidermidis.
Methods and materials

Animal husbandry

Adult merino sheep, weighing 30–35 kg, were used for all experimental work. This experiment was approved by the Animal Care and Ethics Committee at Westmead Hospital. Sheep were allowed to graze normally up to the day of operation and were housed and fasted 18 h prior to the surgical procedure.

Perioperative care

All surgical procedures were performed under general anaesthesia. A premedication of thiazine 0.5 mg/kg (Xylazine, Nature Vet Pty, Sydney, Australia) and atropine sulphate (Atropine Sulphate, Parnell Labs Aust Pty, Australia) 0.03 mg/kg was given intramuscularly 30 min prior to induction of anaesthesia. Following premedication, the sheep were induced intravenously by sodium thiopentone (15 mg/kg; Boehringer Ingelheim Pty, Sydney, Australia) and maintained with oxygen and 1–2% Halothane (ICI Pharmaceutical Division, Melbourne, Australia). No antibiotics were given during or following the surgical procedure. The sheep were allowed to recover indoors overnight and were administered a single intramuscular injection of buprenorphine analgesia (Temgesic, Reckitt and Colman, Hull, UK) at a dose of 0.005 mg/kg. Following inspection of the wound, the sheep were allowed to pasture the following morning.

Operative procedure

The sheep were placed on the right side and the left neck region was shaved and prepared with chlorhexidine (0.5%) in alcohol (70%) and povidone iodine (10%). The prepared area was isolated with sterile drapes to ensure no bacterial contamination. A longitudinal neck incision was performed to expose and mobilize the left jugular vein and common carotid artery. Intravenous heparin (5000 units) was given via the jugular vein. The artery was then clamped proximally and distally and a 2-cm segment excised. The lumen of the remaining carotid artery and vagus nerve were both irrigated with lignocaine to prevent arterial spasm. The excised artery was replaced with a 2 cm x 5 mm gelatin-sealed Dacron (Gelstof, Vascutek, Scotland) interposition graft, which was sutured to the native artery using interrupted 6/0 polypropylene. Grafts were soaked in 10 mg/ml rifampicin (Rifadin, Merrell Dow, Australia) for 15 min at room temperature. On completion of the anastomosis, the graft surface was directly inoculated with either 10^6 colony-forming units of MRSA (obtained from a positive blood culture in a haematological patient with an infected central line) or 10^6 colony-forming units of a known slime that produced S. epidermidis (strain D336). Both organisms were resistant to methicillin but sensitive to rifampicin and vancomycin. On completion of the anastomosis, the subcutaneous tissues were closed with 2/0 Vicryl and the skin closed with 2/0 polypropylene suture. The heparin was not reversed.

Bacterial preparation

Aliquots of 1 ml of freeze-stored bacteria were thawed 24 h prior to the experiment. A 1-ml inoculum of bacteria was streaked onto a horse blood agar plate and incubated at 37°C overnight. On the morning of the experiment, colonies of bacteria were added to 3 ml of 0.9% normal saline solution to obtain a concentration of bacteria of 10^6 colony-forming units/ml as determined by comparison with McFarland standards [13]. The concentration of the inoculum was confirmed by 10-fold serial dilutions of the inocula. Aliquots of 10 μl of the fifth such dilution were cultured onto a horse blood agar plate and incubated at 37°C overnight. Resultant colony counts were performed the following morning.

Harvest and graft analysis

Sheep were grazed for 3 weeks and then killed for graft harvest. Perigraft abscess, anastomotic disruption and graft occlusion were recorded. Swabs were taken for both MRSA and S. epidermidis to assess any bacterial growth about the perigraft tissues, and the external and internal graft surface. A 3 to 5-mm segment of the graft was incubated in a brain–heart infusion. Grafts from S. epidermidis had an additional segment of graft that was ground for 5 min and then incubated in a brain–heart infusion. Therefore, from each sheep a possible four MRSA or five S. epidermidis cultures were taken. All organisms were identified by Gram staining and coagulase testing.

Statistical analysis

Results were compared with previous studies [11, 12], from the authors unit, with identical methodology that investigated a control group that were not soaked in rifampicin and a lower concentration of rifampicin of 1.2 mg/ml for the prevention of subsequent MRSA and S. epidermidis infection. The total number of infected grafts and number of infected specimens per culture type were analysed statistically with χ² analysis and Fisher's exact test, respectively, between treatment groups for each inoculated organism.

Results

Twenty sheep were successfully grafted. All received Gelstof grafts soaked in 10 mg/ml of rifampicin with
MRSA and *S. epidermidis* that were directly inoculated onto the graft surface of 10 sheep for each group. There was no morbidity or mortality arising from the procedures in either of the study groups. All grafts were harvested at 3 weeks as intended.

The rate of abscess formation, anastomotic disruption or thrombosis between control and treatment arms for *S. epidermidis* is outlined in Table 1. There was an association, although not significant, between graft thrombosis, perigraft abscess and anastomotic disruption for *S. epidermidis*.

For MRSA, graft thrombosis was significantly reduced in the higher concentration group compared with both lower concentration and control groups (*P* = 0.002 and *P* = 0.002, respectively). Abscess formation and anastomotic disruption were significantly reduced in the 10-mg/ml group (*P* = 0.0004 and *P* = 0.04, respectively) when compared with the control group (Table 2).

In the 10-mg/ml rifampin group, only four of 50 cultures were positive for *S. epidermidis* (Table 3). These positive cultures were obtained from one sheep. This finding was statistically significant when compared with the control group (30/50, χ² = 30.1, *P* < 0.05) and the 1.2-mg/ml group (13/45, χ² = 8.0, *P* < 0.05).

In the 10-mg/ml treatment group, six of a 40 cultures were positive for MRSA (Table 4). The reduction in the total number of cultures with the higher concentration group was significant when compared with the control group (38/40, χ² = 45.5, *P* < 0.05) and the 1.2-mg/ml group (19/32, χ² = 13.8, *P* < 0.05). Five grafts had no infection on any of the cultures. There was significant reduction any of the culture types with the higher concentration compared with the control group. When compared with the 1.2-mg/ml rifampin group there was a statistically significant reduction in graft segments that were culture positive, but not in the ‘indirect’ cultures, i.e. perigraft tissues, external surface and internal washings.

### Discussion

Infection of vascular prosthetic grafts, especially of the aorta, is an uncommon but dreaded occurrence [1, 2]. The exact etiology of vascular graft infections is not completely established and is likely to be multifactorial. The majority of graft infections are believed to occur at the time of graft insertion [10, 14] and are commonly caused by skin commensal staphylococci [1, 8]. This was demonstrated by Levy *et al.* [15], who showed that the majority of patients undergoing arterial revascularization were colonized with mucin-producing strains of coagulase-negative staphylococci. In an attempt to offer local protection and overcome the risk of infection at the time of surgery, and in the early postoperative period, many groups over several decades have developed methods of incorporating antibiotics or protein carrier molecules into grafts [16–26]. Rifampicin is a hydrophobic semi-synthetic substance with a high affinity for gelatin-coated grafts [25] and is active against the commonly causative microorganisms involved in graft infections, namely staphylococci [2, 3, 5, 26]. Passive incorporation of rifampicin into the Gelseal

### Table 1

Comparison of operative findings between control and treatment arms for *S. epidermidis*

<table>
<thead>
<tr>
<th>Operative Finding</th>
<th>Control (no rifampicin)</th>
<th>1.2 mg/ml rifampicin</th>
<th>10 mg/ml rifampicin</th>
<th>10 mg/ml versus control</th>
<th>10 mg/ml versus 1.2 mg/ml</th>
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</thead>
<tbody>
<tr>
<td>Number of sheep</td>
<td>10</td>
<td>9</td>
<td>10</td>
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<td></td>
</tr>
<tr>
<td>Abscess</td>
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<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anastomotic</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Disruption</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td></td>
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</table>

### Table 2

Comparison of operative findings between control and treatment arms for MRSA

<table>
<thead>
<tr>
<th>Operative Finding</th>
<th>Control (no rifampicin)</th>
<th>1.2 mg/ml rifampicin</th>
<th>10 mg/ml rifampicin</th>
<th>10 mg/ml versus control</th>
<th>10 mg/ml versus 1.2 mg/ml</th>
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<tr>
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<td></td>
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</tr>
<tr>
<td>Abscess</td>
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<td>0</td>
<td>0</td>
<td>0.0004</td>
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<tr>
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<td>1</td>
<td>0</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Disruption</td>
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<td>6</td>
<td>0</td>
<td>0.002</td>
<td>0.002</td>
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</table>

*Fisher’s exact test; NS = not significant.*
Table 3  Comparison of microbiological findings between control and treatment arms for *S. epidermidis*

<table>
<thead>
<tr>
<th>Type of culture</th>
<th>Control (no rifampicin)</th>
<th>1.2 g/ml rifampicin</th>
<th>10 mg/ml rifampicin</th>
<th>10 mg/ml versus control</th>
<th>10 mg/ml versus 1.2 mg/ml</th>
<th>P &lt;</th>
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</thead>
<tbody>
<tr>
<td>Periannular swabs</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0.01*</td>
<td>NS</td>
<td>NS</td>
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<td>External surface</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Internal surface</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Graft segment</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Ground graft segment</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>NS</td>
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<td>NS</td>
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<tr>
<td>Total positive cultures</td>
<td>30</td>
<td>13</td>
<td>4</td>
<td>0.05*</td>
<td>0.05*</td>
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</table>

*Fisher exact test; 'χ²' test; NS = not significant.

Table 4  Number of positive cultures in the control and treatment arms for MRSA

<table>
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<tr>
<th>Type of culture</th>
<th>Control (no rifampicin)</th>
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<th>10 mg/ml rifampicin</th>
<th>10 mg/ml versus control</th>
<th>10 mg/ml versus 1.2 mg/ml</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periannular swabs</td>
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<td>4</td>
<td>1</td>
<td>0.001*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>External surface</td>
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<td>4</td>
<td>1</td>
<td>0.05*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Surface</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>0.001*</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Internal surface</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>0.05*</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>38</td>
<td>19</td>
<td>6</td>
<td>0.05*</td>
<td>0.001*</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher exact test; 'χ²' test; NS = not significant.

graft has been shown to increase resistance to experimental bacterial contamination [21, 22, 24]. Additionally, Chernu and colleagues [18] demonstrated *in vitro* activity against *S. aureus* for 22.4 days in Dacron grafts bound with rifampicin with a collagen release system. Other studies have demonstrated that the *in vivo* graft bioactivity is significantly less and lasts up to only 4 days [23, 27, 28].

Rifampicin at both 1.2 mg/ml and 10 mg/ml resulted in a reduction in the incidence of abscess formation, anastomotic disruption and thrombosis when the inoculating organism was *S. epidermidis* (Table 3), although this was not statistically significant. With the MRSA group, both 10 mg/ml and 1.2 mg/ml of rifampicin reduced the rate of abscess formation, anastomotic disruption and graft thrombosis when compared with the control group. Additionally, the rate of graft thrombosis in the 10-mg/ml group was reduced compared with the 1.2-mg/ml group. Though the usual presentation of *S. epidermidis* graft infection is with perigraft abscess and/or graft-cutaneous sinus formation [29], this was not evident in this study, in which low numbers of perigraft abscesses were encountered. This may be in keeping with the pathogenicity of *S. epidermidis*, which is regarded as a low virulence pathogen. Alternatively, the bacterial load/cm² of graft may have been insufficient to cause an invasive graft infection.

The single perigraft abscess that was encountered was associated with graft thrombosis. Sardelic et al. [30] established the *S. epidermidis* vascular graft infection model, and similarly found an association with perigraft abscess formation and graft thrombosis, although this association was not significant. Avramovic and Fletcher, in a previous study that utilized the described ovine model, but with MRSA as the inoculating organism, suggested that vascular graft infections in sheep inevitably lead to graft thrombosis [22]. This observation may explain the reduction in graft thrombosis in MRSA-infected grafts with increasing rifampicin concentration and the subsequent reduction in infected grafts. Marsen et al. [31], in a small clinical series, speculated that thrombosed prostatic grafts may be more susceptible to infection. In previous studies of *S. epidermidis* infection, anastomotic pseudoneurysm formation or disruption was usually associated with contaminants, namely *Escherichia Coli*, *S. aureus* and *Pseudomonas aeruginosa* [32–34]. These findings are in concordance with the findings here, which showed that the reduction of MRSA, and not *S. epidermidis* graft infection, was associated with a reduction in anastomotic disruption.

The total number of infected cultures was significantly reduced for both *S. epidermidis* and MRSA when compared with both the control and the group.
An increased concentration of rifampicin produced a significant reduction in the incidence of prosthetic vascular graft infection following a challenge of MRSA or slime producing S. epidermidis.

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References


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