THE PREVENTION OF PROSTHETIC INFECTION USING A CROSS-LINKED ALBUMIN COATING IN A RABBIT MODEL

YUEHUEI H. AN, JAY BRADLEY, DENNIS L. POWERS, RICHARD J. FRIEDMAN

From the Medical University of South Carolina, Charleston and Clemson University, Clemson, USA

We evaluated the effects of a serum protein coating on prosthetic infection in 29 adult male rabbits divided into three groups: control, albumin-coated and uncoated. We used 34 grit-blasted, commercially pure titanium implants. Eleven were coated with cross-linked albumin. All the implants were exposed to a suspension of Staphylococcus epidermidis before implantation.

Our findings showed that albumin-coated implants had a much lower infection rate (27%) than the uncoated implants (62%). This may be a useful method of reducing the infection of prostheses.

MATERIALS AND METHODS

We used 29 male New Zealand White rabbits with an average weight of 4.0 ± 0.5 kg. All were free from infection and were kept in individual cages. They were divided randomly into three groups: control, BSA-coated, and uncoated (Table I).

Implants. We used 34 cp-Ti cylindrical implants measuring 7.5 × 5.5 mm. All had been grit-blasted and passivated by sonication in a detergent solution for 15 minutes, then in 100% acetone for 15 minutes and finally in 30% nitric acid for 30 minutes. After each sonication, the samples were rinsed gently three times with deionised water and then dried on filter paper with desiccated nitrogen.

Eleven implants were coated with BSA using a cross-linking method with carbodi-imide. They were soaked in a mixture of 11 ml of 20% BSA (Fraction V; Sigma, St Louis, Missouri) and 10 ml of 0.2 M CDI (Sigma, St Louis, Missouri) for 10 minutes. Excess solution was removed by draining on filter paper for one minute before drying in a petri dish for 24 hours. The implants were subsequently gas-sterilised using ethylene oxide. Before implantation, they were exposed to a bacterial PBS suspension of Staph. epidermidis (RP-62A, 10^6 CFU/ml) at 37°C for one hour. The

Table I. Details of the experimental design and results

<table>
<thead>
<tr>
<th>Group</th>
<th>Staph. epidermidis incubation</th>
<th>Number of animals</th>
<th>Number of infections</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No</td>
<td>5 (10 implants)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coated</td>
<td>Yes</td>
<td>11</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>Uncoated</td>
<td>Yes</td>
<td>13</td>
<td>8</td>
<td>62</td>
</tr>
</tbody>
</table>
control implants were not exposed to bacteria (Table I).

**Implantation surgery.** The animals were anaesthetised using ketamine 30 mg/kg and Rompun (Miles Laboratories, Shawnee Mission, Kansas) 5 mg/kg intramuscularly, and were maintained with Surital (Parke-Davis Inc., Morris Plains, New Jersey) during surgery. Under strict sterile conditions, through a direct lateral approach, a transverse hole was made 5 mm proximal to the articular surface of the femorotibial joint and 5 mm posterior to the patellofemoral joint. Care was taken not to penetrate the medial cortex of the femur. The implants were then inserted according to the experimental plan (Fig. 1). Bilateral implantation was performed for the control group. The wound was closed in layers with absorbable sutures.

After surgery the animals were housed free in the cages. Those showing discomfort received buprenorphine (0.02 to 0.05 mg/kg) every 12 hours as required.

**Evaluation.** The clinical characteristics of this animal model in that it had no bulky soft tissue around the knee area, allowed any local changes to be seen easily.
Infection was diagnosed if one or more of the following criteria were fulfilled:

**Clinical infection.** Animals with an obvious local soft-tissue abscess containing pus verified at postmortem were considered to be clinically infected.

**Histological infection.** There are many histological signs of possible prosthetic infection such as abscess formation, acute or chronic inflammation, bone sequestrae, bone resorption or bone formation, fibrinous exudate, haemorrhage, fibrosis of bone-marrow space and bacteria in inflammatory cells. In our study, only samples with abscess formation were considered to be infected (Fig. 2). A positive bacterial Gram stain strengthens the diagnosis but is not in itself definite evidence of infection, especially if no abscess is found (Fig. 3).

**Positive coagulase-negative staphylococcus (CNS) culture.** This also strengthens the diagnosis, but again is not in itself definite evidence of infection unless there is clinical or histological evidence of infection present at the same time.

The temperature was recorded daily and the wounds inspected for signs of infection. The rabbits were killed humanely when a clinical infection was seen or at the end of the fourth week. We performed swab cultures or pus cultures when obvious pus was found.

All of the distal femora were decalcified and the implants retrieved. The tissue was embedded in paraffin. Sections 6 μm thick were cut and stained with haematoxylin and eosin and a Gram stain (Bacto Gram Stain set; Difco, Detroit, Michigan). They were analysed for signs of soft-tissue or bony infection under light microscopy.

**RESULTS AND DISCUSSION**

There were no infected rabbits in the control group which had no adherent bacteria on the implants. The BSA-coated implants had a lower rate of infection (3/11 animals, 27%) than uncoated implants (8/13 animals, 62%) (Table I). Of the 11 infections, seven were diagnosed by the presence of a clinical abscess and confirmed by histology, one was by clinical abscess and confirmed by histology and a positive CNS culture, one was by histological abscess and a positive CNS culture, and two by histology alone (Table II).

We used chi-squared analysis to compare the infection rate between coated and uncoated groups. The chi-squared value of 2.82 has a p value between 0.05 and 0.1, indicating a possible trend due to the small sample size.

Regarding the criteria for the diagnosis of infection, a clinical soft-tissue abscess containing pus is sound evidence of infection, and this is also the most reliable sign of clinical infection in man. Bone sequestrae are very significant for the diagnosis of chronic clinical infection but those in our study are believed to have resulted from drilling. A few were found in the area of bone resorption in which the pus was located.

On the histological sections positive Gram-stained bacteria could be seen in the cytoplasm of inflammatory cells (neutrophils, macrophages, or occasionally fibroblasts) within an abscess, in the wall of an abscess, or in an infiltrated area (Fig. 3). In our study, bacteria were found in all cases of a diagnosed infection and also in four animals without signs of clinical infection, histological abscess, and a positive culture.

Although bacterial culture of a wound exudate or pus is routinely used clinically, a positive culture by itself is not strong enough evidence for the diagnosis of infection if there is no other clinical or histological evidence to support this diagnosis. A false-positive result may be due to contamination of the sample, and a false-negative result to a sample of pus in which all of the bacteria are dead. The latter may have occurred in our study, since only two CNS cultures in the 11 infections were positive.

Our findings show that there was a decreased infection rate in the BSA-coated prostheses, indicating the effective-
ness of the cross-linked albumin coating. This may prove to be useful in preventing clinical prosthetic infection.

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REFERENCES