A new antibacterial carrier of hyaluronic acid gel

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Abstract

Background. Postoperative infection following total joint arthroplasty is thought to be one of the most serious and devastating complications. To develop an effective treatment for this complication, we tested a bioabsorbable antibacterial carrier that is made from novel cross-linked hyaluronic acid (HA) gel and gentamicin (GM).

Methods. Antibacterial activity of the carrier was evaluated by the agar diffusion test, direct contact test, in vivo mouse model, and in vivo rabbit osteomyelitis model.

Results. GM-containing HA gel suppressed bacterial growth both in vitro and in vivo. In the rabbit osteomyelitis model, beads coated with HA gel containing GM did not disturb bone ongrowth in the femoral stem.

Conclusions. Our bioabsorbable carrier of antibiotic-containing HA gel is effective for prophylactic treatment or treatment of an actual deep infection following total joint arthroplasty.

Introduction

Postoperative bacterial infection is one of the most serious complications after total joint replacement surgery. Unfortunately, for salvage of a failed total joint replacement in the presence of recurrent infection, alternative treatments such as amputation, arthrodesis, or resection arthroplasty should be considered.¹ In general, when deep infection has occurred following total joint arthroplasty, open debridement and intravenous antibiotic therapy have been performed. However, a high failure rate of infected total joint arthroplasties with debridement and component retention has been reported. Therefore, immediate component removal is recommended in the case of infected total joint arthroplasty, and a therapeutic antibiotic-containing bone cement is used as a spacer for the dead space.²,³ Such bone cement has an antibacterial effect, helps stabilize the limb, and facilitates reimplantation. Recently, the U.S. Food and Drug Administration has approved antibiotic bone cement, at least for use in infected total joint arthroplasties. Fortunately, the infection subsides with the use of antibiotic-containing bone cement, although the patients have to undergo surgery several times because removal of the bone cement is necessary.

On the other hand, an infected total joint arthroplasty leads to unhappy patients, surgeons with a tarnished reputation, and an event that is extremely high-cost for the treatment. Most surgeons would agree that prevention of infections is important. In fact, the prophylactic use of antibiotic bone cement is increasing gradually in Europe.⁴ Engesaeter et al. have reported that prophylactic antibiotic bone cement was significantly effective to prevent deep infections after cemented total hip arthroplasty.⁵ However, there is no prophylactic antibacterial carrier in use in uncemented total joint arthroplasty at this time.

Thus, we developed a novel antibacterial carrier that is made of an antibiotic-containing hyaluronic acid (HA) gel. HA does not provoke any allergic reactions but has an antiinflammatory effect. In addition, because HA undergoes spontaneous resorption inside the body with time, there is no need to perform an operation to remove it after the infection has subsided, if it is used as an antibacterial carrier. However, there is one critical problem for application of HA as an antibacterial carrier: HA is unable to maintain the antibacterial effect until all bacteria are eliminated. We solved this problem by developing a novel HA gel⁶ that is composed of a cross-linked HA containing no chemical reagents. The purpose of this study was to investigate whether this newly developed antibiotic-containing HA gel is effective as an antibacterial carrier in vitro and in vivo.
Materials and methods

Agar diffusion test

We investigated the antibacterial activity of orthopedic materials and those coated with gentamicin (GM)-containing HA gel. The following implant materials were used for antibacterial activity assays: CoCrMo, Ti-6Al-4V, and stainless steel (DePuy Japan, Hitachi City, Japan). In this experiment, we evaluated the antibacterial activity of the materials by the use of Staphylococcus aureus (S. aureus) FDA209P [minimum inhibitory concentration (MIC) of GM, 0.05 µg/ml], because this strain is one of the common bacteria that cause a variety of supplicative infections in humans and has been used for many research investigations thus far. A glass slide was used as a control. Antibacterial activity on S. aureus was determined by the agar diffusion test.7,8 The implant materials were coated with GM-containing HA gel, or not coated, and placed on Mueller–Hinton agar plates (Difco, Detroit, MI, USA) with S. aureus. The plates were incubated for 18 h at 37°C. Content of GM in the HA gel was 10 or 100 µg/sample. The inhibition zones around the materials were measured.

Direct contact test

We also investigated the antibacterial activity of GM-containing HA gel by the direct contact test.8 The implant materials were prepared by the same method as already mentioned. The 100 CFU S. aureus in heart infusion broth was placed on the test implant and incubated for 6 h at 37°C. Then, the implant was placed into 1 ml saline and incubated for 2, 4, or 6 h at 37°C. After the incubation, 200 µl cultured suspension was collected and put onto a heart infusion agar plate. The plate was incubated for 18 h at 37°C, and colonies that had grown on the plate were counted (n = 5).

Antibacterial assays in the mouse osteomyelitis model

Male Balb/c mice, 6–8 weeks old (Charles River Japan, Yokohama, Japan), were used. All animals received humane care, and the protocol was approved by the Toyama Medical and Pharmaceutical University Animal Research Committee. Mice were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg). The femur was surgically exposed and fractured with surgical scissors. S. aureus at 5 × 105 CFU was injected at the fracture site, and an intramedullary Kirschner wire (K-wire; diameter, 0.8 mm) coated with GM-containing HA gel (0.1 ml) was inserted from the distal end of the femur. HA gel not containing GM was used as a control.

To determine the optimal dose of GM-containing HA gel in vivo, GM concentrations of 0.1, 1, 10 and 100 mg/samples were investigated. At 7 days after the surgery, mice were euthanized by pentobarbital and infected bone marrow was collected. The collected tissues were homogenized in 1 ml phosphate buffer (pH 7.0). This homogenate was diluted 1 × 106 times with sterile saline and plated on sheep blood agar culture medium. The medium was cultured for 24 h at 35°C, and the number of colonies was counted. Five mice were used each time; in total, 20 mice were examined.

To investigate the durability of the antibacterial activity of the bioabsorbable gel, the time-course change in bacterial number after the insertion of GM-containing HA gel was studied. A mouse osteomyelitis model combined with femoral fracture was prepared as already described. GM was used at a concentration of 10 mg/sample in this study. At 0, 1, 2, 7, and 14 days after the insertion of GM-containing HA gel, the infectious bone marrow was collected to count the numbers of bacteria. Five mice were used each time; in total, 45 mice were examined.

The onset of pseudoarthrosis was also investigated because a fracture does not heal if the infection persists. A mouse osteomyelitis model combined with femoral fracture was prepared in the same manner as already described. The S. aureus (5 × 105 CFU/sample) and the HA gel containing 10 mg GM were inserted together with the K-wire into the femur. HA gel not containing GM was used as a control. Ten mice including five control mice were used for this experiment. Eight weeks after the insertion of the K-wire, the mice were killed by pentobarbital and photographed using a Softex X-ray apparatus (Softex CSM-2; Softex, Tokyo, Japan) employing HS Fuji Softex film (Fuji Film, Tokyo, Japan) at 45 cm with 30 kV and 15 mA for 20 s.

Antibacterial assays in rabbit osteomyelitis

The rabbit osteomyelitis model was examined to determine whether our antibacterial material prevents bone overgrowth in uncemented implants. Retired male New Zealand white rabbits between 3.0 and 3.8 kg in weight (Charles River Japan, Yokohama, Japan) were used in this study. A titanium (Ti-6Al-4V) intramedullary cylinder with a grid-blasted surface (mean surface roughness, 6 µm) was provided by Zimmer (Zimmer Japan, Tokyo, Japan) (Fig. 1A); this titanium cylinder was 40 mm long and 3 mm in diameter. General anesthesia was induced with ketamine (100 mg/kg), and for local anesthesia 1% lidocaine (5 ml/rabbit) was added. After the distal end of the femur was perforated using a hand reamer, S. aureus, GM-containing HA gel, and the grid-blasted titanium cylinder were inserted from the knee joint (Fig. 1B). Rabbits were divided into the following four groups: in group 1, only the titanium cylinder was inserted; in group 2, 0.5 ml HA gel not containing GM was inserted together with the titanium cylinder; in group 3, 0.1 ml HA gel containing GM was inserted; and in group 4, 0.1 ml GM was injected.