Antibacterial Efficacy Of Ag-containing Hydroxyapatite In Vivo

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Disclosures:

Introduction: One of the serious postoperative complications associated with joint replacement is bacterial infection. To prevent the bacterial infection related to the implantation, we have developed a novel antibacterial coating with silver-containing hydroxyapatite (Ag-HA) which shows the good biocompatibility and osteoconductivity. This coating shows properties similar to HA alone, with the addition of releasing Ag ions to achieve high antibacterial activity and low cytotoxicity in vitro[1-5]. The purpose of the present study was to clarify the antibacterial efficacy of Ag-HA coating against methicillin-resistant Staphylococcus aureus (MRSA) in the rat tibia model.

Methods: Titanium rods were prepared (20 mm in length and 1 mm in diameter). Ag-HA or HA powder was sprayed onto the surface of each titanium rod using the Flame Spraying System (Sulzer Metco Japan, Tokyo, Japan), which uses an acetylene torch. The Ag-HA powder consists of 97 wt% HA mixed with 3 wt% Ag2O. HA powder consists of 100 wt% HA. Spraying was performed to achieve a uniform coating thickness of 40 ± 10 µm. The strain of bacteria used for this study was MRSA (UOEH; University of Occupational and Environmental Health Hospital, Fukuoka, Japan). Ten 10-week-old male Sprague-Dawley rats with a mean body weight of 371.5 ± 10.3 g were prepared (Kyudo, Saga, Japan). A rat tibia model was used to evaluate antibacterial activity after implantation, as a slight modification of the methods described by Lucke et al[6]. The operation was carried out under general anaesthesia induced intraperitoneally with pentobarbital. A hole was drilled with an 18-gauge needle through the tibial tuberosity of the animals. And then, 50μl of phosphate-buffered solution (PBS) containing MRSA (about 4.0 ×102CFU) were injected into the medullary cavity of the tibia. After bacterial inoculation, Ag-HA- or HA-coated rods were implanted into the medullary cavity of tibiae. The tibiae were then excised at 4 weeks postoperatively and were fixed in 10% phosphate-buffered formaldehyde, dehydrated in graded alcohol solutions and embedded in methyl methacrylate resin. Longitudinal sections in the sagittal plane were cut. Slices were stained with hematoxylin and eosin. Areas of infection around implant were evaluated under light microscopy. To assess histological finding about infection around implants, the following three regions of interest (ROIs) were determined and evaluated separately: R1, proximal epi-/metaphysis of the implant; R2, diaphysis of the implant; and R3, distal epi-/metaphysis of the implant. The following histological appearances were evaluated according to the score defined by Lucke et al.[6], with slight modification: 1) abscess formation; 2) sequestrum formation; 3) Enlargement of corticalis; 4) destruction of corticalis; 5) bone formation around implant. Parameters 1-4 were scored in each ROI as: 0, absent; 1, mild; 2, moderate; or 3, severe. Parameter 5 was scored in each ROI as: 3, absent; 2, poor; 1, mild; or 0, well. The maximum score possible was thus 45 (3 ROIs × 5 parameters × 3 points). All animal procedures were conducted with the approval of the animal research ethics committee at our institution (Approval Number, 22-003-0). SPSS version 19 software (IBM SPSS Statistic, Chicago, IL) was used for all statistical evaluations. The Mann-Whitney U-test was used to compare histological scores. A p-value < 0.05 was considered to be significant.

Results: Mean scores for bone infection were 19.2 points for the HA group and 7.4 points for the Ag-HA group. The score were significantly lower for the Ag-HA groups than for the HA groups (p=0.047). The scores of abscess formation, sequestrum formation and bone formation were significantly lower for the Ag-HA groups than for the HA groups (p=0.040, 0.034 and 0.033, respectively). Representative histological findings for the HA and Ag-HA groups are shown in Figure 1 and 2. Around HA-coated implant, severe infection was identified, some abscesses were seen close to the surface of the HA coating and bone formation was poor (Fig. 1). In contrast, Around Ag-HA-coated implant, infection appeared milder. There were a few abscesses and bone formation was uniformly good (Fig. 2).

Discussion: Ag-HA coating elutes silver ions[1]. Silver ions exhibit an effect with a broad spectrum of antimicrobial activities against bacteria. There are some reports which showed antibacterial activity of Ag-HA in vitro. However, there are few reports in vivo. The present study showed that Ag-HA coating reduced bone infection and showed good bone formation. These results indicate that Ag-HA coatings might be useful to prevent surgical-site infections associated with joint replacement.

Significance: This study shows histological antibacterial activity of silver containing hydroxyapatite coating. This coating will be helpful to prevent infection associated with joint replacement.

Acknowledgments: The authors report no external source funding for this investigation. None of the authors has received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this work.

ORS 2014 Annual Meeting
Poster No: 0296