Toxicity In Vivo Of Thermal-sprayed Silver Containing Hydroxyapatite Coating In The Rat Tibia

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Introduction: Silver has a broad antibacterial spectrum, strong antibacterial activity and low toxicity. Silver metal-coated megaprostheses have already been used in European countries and good clinical results have been reported. However, such silver metal coatings have not been applied to the surface of the stem, in the region of bone contact, because of potential toxicity. To resolve this problem, we used HA as a support material for silver ions, because it offers good biocompatibility and osteoconductivity. We previously developed a novel coating technology composed of HA containing silver (Ag) to reduce the incidence of implant-associated infections, and have reported that this coating has the properties of HA [1], induces the release of silver ions, and shows high antibacterial activity with inhibition of bacterial adhesion and low cytotoxicity in vitro [2]. In addition, using rat subcutaneous [3] and tibial [4] implantations, we demonstrated that the Ag-HA coating possesses antibacterial activity against methicillin-resistant Staphylococcus aureus in vivo. Furthermore, the Ag-HA coating has been shown to provide good osteoconductivity, and the effect of silver toxicity on in vivo osteoconductivity depends on the dose [5]. However, no reports have described the in vivo toxicity of Ag-HA. The aim of this study was to clarify local and systemic adverse effects of Ag-HA-coated implants in rat tibiae.

Methods: The implants used were Ti wire 20 mm in length and 1 mm in diameter. Ag₂O powder was mixed with HA powder in concentrations of 2% and 50%. The mix was then sprayed onto the surface of the Ti wire using the Frame Sprayed System, which uses an acetylene torch. We prepared three types of implant, as follows: i) HA, ii) 2% Ag-HA and iii) 50% Ag-HA coating on Ti. We used 10-week-old male Sprague-Dawley rats. One transverse 10-mm incision was made on both knees. To access the medullary cavity, a hole was drilled with an 18-gauge needle through tibial tuberosity, and the same implants were inserted into the medullary cavity of both tibiae in each rat. Rats were divided into three groups according to the implant inserted. Three rats from each group were euthanized at acute phase (2-4 days after treatment). Six rats from each group were euthanized at subacute phase (4-12 weeks after treatment). The abdomen was then opened under general anesthesia and 10 ml of blood was collected from the right common iliac vein. The liver, kidneys, spleen, and brain were obtained after euthanasia. Postmortem examinations included macroscopic inspection of the skin after shaving. The inspection focused on the presence of ash-colored skin, which can occur in argyria (systemic silver intoxication). The concentration of silver in serum, brain, liver, kidneys and spleen were determined by inductively coupled plasma-mass spectrometric analysis. In addition, the following parameters were determined: glutamic-oxalo-acetic transaminase (GOT); glutamic-pyruvic transaminase (GPT); lactate dehydrogenase (LDH); blood urea nitrogen (BUN); and creatinine. Histological examinations of brain, liver, kidneys and spleen were also performed.

Results: The HA, 2% Ag-HA, and 50% Ag-HA coating groups showed mean serum silver concentrations of 0.60 ± 0.17 ppb, 1.75 ± 1.08 ppb, and 13.8 ±3.95 ppb at 2 days, respectively (Fig. 1). The silver concentration of serum decreased gradually over the experimental period. Mean concentration of silver in the 50% Ag-HA coating group differed significantly from that in the other two groups at acute and sub-acute phase (p < 0.01 for all comparisons at all periods, Tukey’s HSD test). Mean concentration of silver in the 2% Ag-HA coating group showed no significant difference compared with the HA coating group in any experimental periods. Mean GOT, GPT, LDH, creatinine, and BUN of the HA, 2% Ag-HA, and 50% Ag-HA coating groups were not significantly higher than in controls at 2, 3, and 4 days. At 4, 8 and 12 weeks, no significant differences were seen between the controls, HA, 2% Ag-HA and 50% Ag-HA coating groups for any laboratory parameters. Figure 2-a, 2-b, 2-c and 2-d shows that mean silver concentration in the brain, liver, kidney and spleen, respectively. During the experimental periods mean silver concentrations in all organs examined of the 2% Ag-HA coating groups showed no significant differences compared with those of the HA coating groups. The silver concentrations of 50% Ag-HA were significantly elevated in all organs examined at 4 and 8 weeks. At 12 weeks, no significant differences were found between the three groups. Growth of fibrous tissue, chronic inflammatory infiltrates, foreign body granulomas, and fatty degeneration were not detected in the brain, liver, kidneys, or spleen of the HA, 2% Ag-HA, and 50% Ag-HA coating groups at any time during the experimental period. Histological examination of organs revealed no abnormal pathological findings.
Discussion: A concentration of silver in the blood of more than 300 ppb has been reported to cause argyria, argyrosis and liver and kidney damage. In our study, the highest concentrations of serum silver resulting from the 2% Ag-HA and 50% Ag-HA coatings were 1.75 ± 1.08 ppb at 2 days and 16.2 ± 5.85 ppb at 3 days, respectively. These levels were low enough to avoid harmful effects. Mean silver concentrations in all analyzed organs of the 2% Ag-HA coating group showed no significant differences compared with those in the HA coating group at all experimental periods. The silver concentration of 50% Ag-HA was significantly elevated in all analyzed organs. The highest silver concentrations were found in the brain, liver, kidneys, and spleen of the 50% Ag-HA coating group at 4 weeks, with mean concentrations of 0.05 µg/g, 0.05 µg/g, 0.04 µg/g, and 0.21 µg/g, respectively. The concentration of silver decreased gradually over the experimental period, and no significant differences were found between the three groups at 12 weeks, with neither pathological changes in laboratory parameters nor histological changes in tissues. Ag-HA coating on an implant may offer a biologically safe antibacterial biomaterial and may be of value in reducing SSI related to implantation. It will be necessary to implant Ag-HA-coated megaprostheses in humans to assess possible toxicity and to determine infection rates with joint replacement.
**Significance:** Ag-HA coatings on implants may represent biologically safe antibacterial biomaterials and may be of value for reducing surgical-site infections related to implantation.

**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.

**References:**

*ORS 2014 Annual Meeting*

*Poster No: 0284*