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Effect of combined local treatment with zoledronic acid and basic fibroblast growth factor on implant fixation in ovariectomized rats

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ABSTRACT

Osteoporosis is a skeletal disorder characterized by low bone mass and deterioration of bone microarchitecture resulting in bone fragility, which impairs fixation of the implants. Zoledronic acid (ZOL) is a potential inhibitor of osteoclast-mediated bone resorption and basic fibroblast growth factor (bFGF) is a growth factor that stimulates osteoblast-mediated bone formation, and these drugs could enhance fixation of implants under osteoporotic conditions. In this study, 40 ovariectomized (OVX) rats were randomly divided into 4 groups ($n=10$ for each group) and underwent bilateral tibiae implantation using hydroxyapatite (HA)-coated titanium implant: Control group (distilled water immersing before implantation), ZOL group (1 mg/ml of ZOL immersing), bFGF group (20 μg/ml of bFGF immersing), and ZOL+bFGF group (1 mg/ml of ZOL and 20 μg/ml of bFGF immersing). At 3 months after implantation, all animal were sacrificed and the tibiae were harvested for histology, micro-CT examinations and biomechanical testing. Bone area and contact, determined by histomorphometric analysis, were 2.7-fold and 1.8-fold in the ZOL-treated implants, 1.9-fold and 1.8-fold in the bFGF-treated implants, 3.6-fold and 2.3-fold in the both-treated implants compared with controls ($p<0.01$). Such significant effects were further confirmed by microstructure parameters, the bone volume ratio and the percentage osteointegration were significantly increased by ZOL treatment (3.0-fold and 1.8-fold), bFGF treatment (1.2-fold and 1.9-fold) and ZOL+bFGF treatment (3.3-fold and 2.7-fold) ($p<0.001$). In addition, push-out test showed that the maximum force and the corresponding interfacial shear strength of the implants treated by ZOL, bFGF and ZOL+bFGF was 8.4-fold and 8.6-fold, 3.8-fold and 3.7-fold, 10.8-fold and 10.7-fold of the control levels, respectively ($p<0.05$). The combined treatment was better than either treatment alone for force, but was not different from ZOL alone for interfacial strength. The significant correlation between biomechanical and micro-CT parameters demonstrates the role of microstructure assessments in predicting mechanical fixation of implants ($p<0.01$). Our study suggests that locally applied ZOL or bFGF may improve implant fixation in the ovariectomized rats, and that combined treatment has more beneficial effects on osseointegration, peri-implant bone formation and maximum force than either intervention alone.

Introduction

Postmenopausal osteoporosis is a common debilitating systemic disease, characterized by gradually reduced bone mineral density and compromised bone strength due to the bone microarchitectural deterioration induced by estrogen deficiency [1]. This skeletal disorder is primarily caused by increased bone fragility in older women, and is also a potential risk factor for endosseous implants in orthopedics and dental surgery. Without proper intervention, the compromised bone in osteoporosis could further increase implants loosening [2,3].

Previous studies have shown the promising potency of the bisphosphonates (BPs) in reducing osteolysis around implants due to its high affinity for bone mineral and powerful inhibitory effect on osteoclast-mediated bone resorption through suppressing osteoclast function, inhibiting osteoclast differentiation, and promoting osteoclast apoptosis [4-7]. While basic fibroblast growth factor (bFGF), a beneficial molecule on cell proliferation and differentiation, has an anabolic effect in the process of bone tissue regeneration due to its ability to induce osteoblasts development, bone formation and vascularization [8,9]. Some investigations also demonstrated that locally used bFGF can promote bone formation and bone ingrowth into porous hydroxyapatite (HA) and HA-coated implants [10–12].

Although these studies had reported the positive effects of BPs and bFGF on implant osseointegration, there is no experimental study that described the effects of locally applied BPs or bFGF on fixation of the implants following osteoporosis. Considering the abnormal peri-implant bone metabolism in osteoporosis patients [2], aim of locally administered interventions should target two aspects: to increase bone formation or reduce bone resorption around implants.

We hypothesized that the locally administered BPs and bFGF may promote peri-implant bone regeneration and lead to stronger

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mechanical stability of implants in osteoporotic bone. Zoledronic acid (ZOL), as a new parenteral bisphosphonate, has a high affinity for hydroxyapatite and binds directly to mineralized bone [13]. Based on an ovariectomized animal model, our study was designed to observe the effects of individual or combined use of ZOL and bFGF on implant fixation following osteoporosis. These effects were evaluated separately by histology, micro-CT and push-out test.

Materials and methods

Animals

Forty female Sprague–Dawley rats weighing 190–210 g were bred individually in cages. All received a standard diet, and were raised in relative steady temperature and humidity in an air-conditioned environment, with lighting controlled in a cycle of light 12 h/dark 12 h. The in vivo study was approved by the Animal Care Committee of Sichuan University.

Treatment

After 7 days of acclimatization, all rats underwent bilateral ovariectomization (OVX) and the surgical techniques were similar to the previous reports [3,8]. At 3 months after OVX, the animals were randomly assigned into following 4 groups (10 rats per group) for bilateral tibiae implantation using hydroxyapatite (HA)-coated titanium implant: Control group, ZOL group, bFGF group and ZOL+ bFGF group, and each animal received two implants with the same local treatment. The implants used in this study were supplied by Dr. Liu (Engineering Research Center in Biomaterials, Sichuan University). These custom-made titanium cylinders, measuring 1 mm in diameter and 12 mm in length, were plasma sprayed with HA using a METCO MN Plasma System and an AR-2000 Thermal Spray Robot (Metco, USA). The average thickness of the HA coating was 50 μm, and crystallinity was 80%. Fig. 1 shows the porous surface morphology of the HA sample.

Before implantation, these implants were sterilized and immersed into 4 special solutions for 24 h, including distilled water, a solution containing ZOL (1 mg/ml) (Novartis Pharma AG, Switzerland) [14–16], a solution containing bFGF (20 μg/ml) (Cytolab, USA) [17–19], and a solution containing both ZOL (1 mg/ml) and bFGF (20 μg/ml), Then, these implants were lyophilized under aseptic conditions and stored at 4 °C for in vitro release testing and the following implantation [20].

All OVX rats were anesthetized by intraperitoneal injections of 10% chloral hydrate (3.3 ml/kg). An incision to expose the knee in both hind limbs was made longitudinally, a pilot hole was drilled through the intercondylar eminence and 1 mm-diameter titanium wire was gradually twisted to make a channel from the proximal tibia metaphysis into the medullary canal. The implants were inserted into the channel and positioned as far as possible beneath the growth plate (Fig. 1). The incision of skin and soft tissue was closed. Prophylactic i.m. antibiotic was administered at the time of surgery and for 3 postoperative days.

At 3 months after implantation, all rats were sacrificed and the tibiae were harvested for structural and functional analysis. For each rat, one tibia was used for histological examination, and the contralateral tibia was used for micro-CT and biomechanical testing.

In vitro release testing

The release of ZOL was tested by reverse phase liquid chromatography at 220 nm [21], using a Waters 1525 binary HPLC pump (Waters Co., USA), a Rheodyne (Cotati, CA, USA) model 7725i injection valve fitted with a 20 μl loop, and a Waters 2487 dual λ absorbance detector (Waters Co., USA). ZOL in the samples was separated on a Phenomenex C_{18} column (USA) at 25 °C using a mixture of acetonitrile and n-amylamine, and the flow-rate was 1 ml/min. The release of bFGF was determined using an immuno-ligand-assay (Molecular Devices Cooperation, Menlo Park, USA) as previously described [20]. Briefly, the implants treated with bFGF and ZOL, alone or in combination, were rinsed in 1 ml of buffer or eluting agent (adjusted to pH 7.0) for 24 h, then the solutions were collected, and the concentrations of bFGF and ZOL were measured (n=5/group). The procedures of implant elution, solution collection and quantitative assay were performed once a day over 21 days.

Histological examination

For hard tissue histology, proximal tibiae with implants (n=10/group) were fixed in 10% neutral buffered formalin for 3 days, dehydrated with increasing concentrations of alcohol, then embedded in methylmethacrylate without decalcification. Horizontal cutting of 100 μm thick sections were performed using a model SP1600 microtome (Leica Microsystems, Wetzlar, Germany). The proximal tibiae were continuously cut until the section at approximate 2 mm below the growth plate was selected. The sections of interest were stained in 1% toluidine blue.

Histomorphometric analysis of the percentages of bone contact and bone area were performed with a semi-automated digitizing image analyzer system, consisting of a Nikon ECLIPSE E600 stereomicroscope,

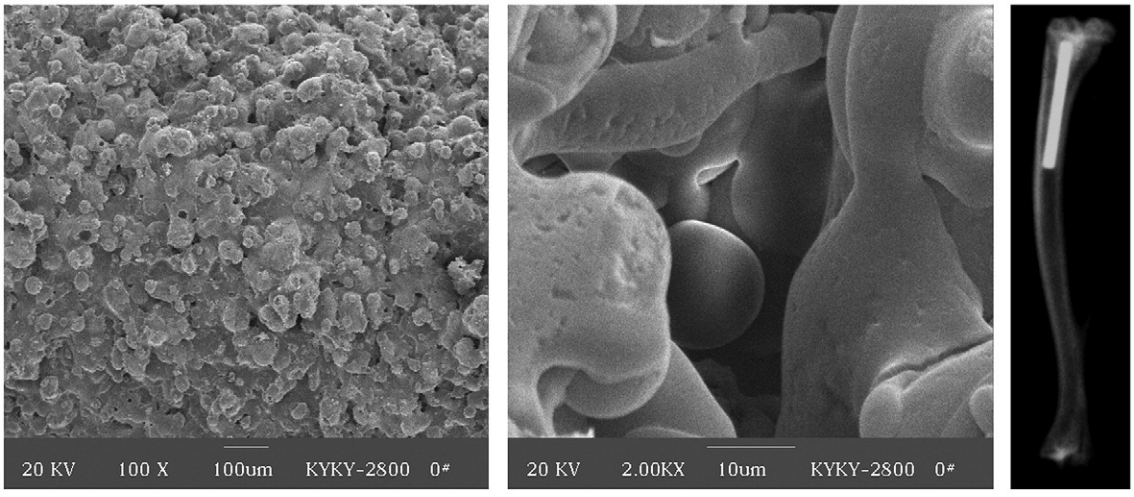

Fig. 1. SEM micrographs of HA coating on the surface of titanium implant at various magnifications and a radiograph of the rat tibia with implant.