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Enhancement of bone-bonding ability of bioactive titanium by prostaglandin E2 receptor selective agonist

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Abstract

Systemic administration of prostaglandin E2 receptor (EP4) selective agonist increases both bone formation and resorption, and consequently leads to an increase in bone mass. Although previous studies have reported that EP4 agonist enhanced bone remodeling and fracture healing, it was not known if EP4 agonist activates the bone–biomaterial interface. Bioactive titanium prepared by chemical and thermal treatment can bond to living bone and is suitable for use in clinical applications in cementless fixation devices. Therefore, we examined whether the administration of EP4 agonist enhances the bonding strength between bone and bioactive titanium. Bioactive titanium plates were inserted into the tibia bone of rabbits and examined histologically and biomechanically at 4, 8, and 16 weeks. EP4 agonist was administrated systemically every 2 weeks after surgery. A non-administrated control group, a low-dose group (10 μg/kg body weight (BW)), and a high-dose group (100 μg/kg BW) were compared. The bonding strength of bioactive titanium in the EP4 agonist groups was significantly higher than that in the control group at both 4 and 8 weeks, and enhanced bone remodeling and direct bonding around the bioactive titanium plates was observed only in the EP4 agonist groups at 4 weeks. EP4 agonist enhanced bone formation around the bioactive titanium plate, and achieved early direct bone bonding.

Keywords: Prostaglandin; Titanium; Biomaterial; Animal experiment

1. Introduction

Cementless fixation of metal implants is widely used clinically as joint arthroplasty for osteoarthrosis or rheumatoid arthritis [1]. Several types of manipulation of the surface morphology or surface functionalization have been used to enhance bone bonding. Conventional porous coatings using plasma spraying or beads coatings provide a stable interface through mechanical interlocking [2]. On the other hand, bioactive ceramic coatings, such as hydroxyapatite and glass-ceramic A-W, provide chemical bonding, which is related to early and stable fixation [3]. However, there are concerns about hydroxyapatite plasma spray-coated implants with regard to resorption or delamination of the coating layer [4,5]. We have developed bioactive titanium using surface chemical and thermal treatments [6–9]. Through this surface treatment, titanium and its alloys form bioactive materials, which bond to living bone via a spontaneously formed apatite layer. These bioactive titanium implants are designed for use as cementless fixation devices and their effectiveness has already been confirmed in clinical trials. Early bonding between implant and bone will accelerate the onset of activity of patients soon after an operation, with subsequent good long-term results. Two types of approach must be considered to accelerate early bone bonding. The acceleration of the bioactive capability of an implant will provide early bonding. As previously reported, the bioactivity of bioactive titanium was accelerated using an additional treatment, such as hot water and diluted HCl to remove the sodium ions in the treated layer [8,9]. On the

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other hand, enhancement of the bone formation around an implant will provide early and stable bone–implant bonding.

Prostaglandin E2 (PGE2) has the potential to stimulate bone formation and prevent bone loss when given locally or systemically [10–12]. Fracture healing failed in rats that received a selective cyclooxygenase-2 (cox-2) inhibitor, which reduced endogenous PGE2 production [13]. PGE2 exerts its influence through an interaction with specific cell surface receptors. Four subtypes of PGE receptor have been identified: EP1, EP2, EP3, and EP4 (prostaglandin E2 receptor). These belong to the G-protein-coupled receptor family and activate different second messenger systems such as adenylate cyclase or phospholipase C(PLC). Of these four receptors, EP4 and EP2 activate adenylate cyclase, EP1 activates PLC and EP3 either lowers intracellular cAMP levels or activates PLC, depending on the alternatively spliced isoform [14,15]. Expression of EP4 and its regulation by PGE has been observed in osteoblastic cell lines and bone marrow osteogenic cells [16]. Osteoblastic recruitment from bone marrow stromal cells was the major mechanism for the anabolic effect of PGE2 [17]. The EP4 receptor is considered to be the most potent receptor among well-known several prostanoid receptors [18]. Yoshida et al. [19] reported a lack of PGE2-induced bone formation in EP4-deficient mice and PGE2-induced differentiation of osteoblasts through the activation of the EP4 receptor. They also showed that cultured cells from EP4-deficient mice lacked mineralized nodules. They reported that a selective EP4 agonist increased the parameters of bone formation significantly in ovariectomized or immobilized rats. The intermittent systemic administration of an EP4 agonist was shown to have an anabolic effect on ectopic ossicles induced by recombinant human bone morphogenetic protein 2 (rhBMP2) [20], and accelerated the healing of cortical bone defects after drill-hole injury by increasing the local turnover of the regenerating bone [21]. In addition, Machwate et al. [18] reported that EP4, but not EP2, was detected in adult bone tissue. Recent observations showed that PGE2 stimulates the osteoblastic commitment of bone marrow stromal cells via activation of the EP4 receptor [17]. From these findings, it is suggested that activation of EP4 induces osteoblasts and stimulates new bone formation.

In this study, the effect of the systemic administration of EP4 agonist on new bone formation around bioactive titanium was examined histologically, and the bonding strength between bone and bioactive titanium was analyzed using animal models.

2. Materials and methods

2.1. EP4 agonist

EP4 agonist (ONO-AE2-724, Ono Pharmaceutical Co., Osaka, Japan) was subcutaneously injected into each subject's back every 2 weeks after surgery. The first injection was performed the following day after the operation. The effective dose in the rabbit models was estimated from previous results on rat models. In the fracture models, the minimum effective dose of EP4 agonist was 100–300 μg/kg body weight (BW) in rats. In this study, the experimental animals were divided into three groups: a control group, a 10 μg/kg BW (low-dose group), and a 100 μg/kg BW (high-dose group).

2.2. Implant preparation

Pure titanium (cp-Ti) plates (Japan Medical Material Co., Osaka, Japan) $15 \times 10 \times 2\,mm^3$ in size, were polished with No. 400 diamond paste, washed with distilled water, and dried at room temperature. For bioactivation, the pure titanium plates were soaked in a 5.0 M NaOH aqueous solution at 60 °C for a period of 24 h, gently washed with distilled water, and dried at 40 °C for a period of 24 h at room temperature, as reported in a previous publication [8]. The plates were subsequently heated to 600 °C in electric furnace using a heating rate of 5 °C/min, kept at 600 °C for a period of 1 h, and then allowed to cool to room temperature.

2.3. Experimental design

The implants were conventionally sterilized using ethylene oxide gas, and implanted into the metaphyses of the tibiae bone of mature male Japanese white rabbits weighing 2.8–3.5 kg. The surgical methods used have been described previously [7,8,22]. Briefly, the rabbits were anesthetized with an intravenous injection of pentobarbital sodium (0.5 ml/kg), an intramuscular injection of ketamine hydrochloride (10 mg/kg), and a local administration of a solution of 1% lidocaine. A 3 cm longitudinal skin incision was made on the medial side of the knee, and the fascia and periosteum were incised and retracted to expose the tibial cortex. Using a dental burr, a $16 \times 2\,mm^2$ hole was made from the medial to the lateral cortex running parallel to the longitudinal axis of the tibial metaphyses, as shown in Fig. 1. After irrigating the hole with saline, titanium plates were implanted in the frontal direction, perforating the tibia and protruding from the medial to lateral cortex. The fascia and skin were closed in layers. The same surgical procedures were performed bilaterally. One group was a baseline control without EP4 administration. The remaining two groups were divided into two dosing groups. These were treated with a subcutaneous injection of the EP4-selective agonist in the back every 2 weeks using a dose of 10 μg/kg BW (low-dose group) and 100 μg/kg BW (high-dose group), until sacrifice. The first injection was performed the following day after the operation.

The animals were housed individually in standard rabbit cages and fed standard rabbit food and water ad libitum. Four rabbits from each group were sacrificed using an overdose of intravenous pentobarbital sodium at 4, 8, and 16 weeks after implantation, with a total 36 rabbits being used. The Kyoto University guidelines for animal experiments were observed in this study.

2.4. Measurement of detaching failure load

Following euthanasia, the segments of the proximal tibial metaphyses containing the implanted plates were harvested and prepared for the detaching tests [22]. All the samples were kept moist after harvesting. The bone tissue surrounding the plates was carefully removed on both sides and at the ends using a dental burr to remove periosteal bone growth. Traction was applied vertically to the implant surface using a crosshead speed of 35 mm/min employing an Instron-type autograph (Model 1011, Aikoh Engineering Co., Ltd., Nagoya, Japan). Specially designed hooks held the bone–plate–bone construct. The detaching failure load was measured when the plate was detached from the bone. If the plate was detached before the test, then the failure load was defined as zero newton. Data was recorded as the mean ± the standard deviation (SD) and assessed using a one-way factorial ANOVA and Fisher's PLSD method as a post hoc test. Differences of $p < 0.05$ were considered to be statistically significant.