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Effect of FGF-2 and melatonin on implant bone healing: a histomorphometric study

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Abstract Melatonin influences the release of growth hormone and cortisol in humans, and it was recently reported that it promoted bone formation. On the other hand, fibroblast growth factor-2 (FGF-2) was reported to facilitate the proliferation of osteoblasts. In the present study, we examined the effect of recombinant human FGF-2 and melatonin on the promotion of osteogenesis around titanium implants. Twenty-four 10-week-old female rats of the Wistar strain received titanium implants in both tibiae. In the experimental groups, 100 mg/kg body weight of melatonin was administered by intraperitoneal injection for 4 weeks after implantation and 10 μg of FGF-2 was locally injected around the implant sites 5 days after implantation. The control groups were administered saline only. In the control group, few newly formed bone could be seen around the implants. It was observed to be in direct contact with the implant surface, but otherwise unmineralized connective tissue was occasionally interposed. In the experimental group, newly formed bone was observed around the titanium implant. In addition, in contrast to the control group, abundant bone trabeculae were seen in the medullary canal region. Bone trabeculae were directly connected to existing cortical bone. These results strongly suggested that melatonin and FGF-2 have the potential to promote osseointegration.

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1 Introduction

Since there is an increase in the elderly population, occlusal reconstruction is of importance from the point of view of the quality of life of patients. In order to accomplish this using dental implants, osseointegration should be completed promptly, and once completed, it should be maintained for as long as possible. Also, it is very important for patients to achieve mature bone of high integrity as soon as possible after treatment. In fact, however, it often takes a long time to achieve bone which is identical to existing bone in its properties. In order to obtain functional bone as soon as possible, it is critical to enhance both the proliferation and differentiation of osteogenic cells. Basic fibroblast growth factor-2 (FGF-2) is well known to facilitate the proliferation of osteoblasts in vitro and bone repair in vivo [1–4]. On the other hand, melatonin, a hormone that is secreted by the pineal gland during the night and controls circadian rhythms, was recently reported to promote the differentiation of osteoblasts in vitro and bone formation in vivo [5–9]. From these facts, we focused on the possibility that FGF-2 and melatonin synergistically promote bone formation in vivo by enhancing both the proliferation and differentiation of osteogenic cells. The aim of the present study was to evaluate the effect of FGF-2 and melatonin on osseointegration around titanium implants.

2 Materials and methods

2.1 Animal model and implants

Twenty-four 10-week-old female rats of the Wistar strain, obtained commercially (Charles River, Japan) and fed standard pellets and water ad libitum, were used for the implantation study. The rats were anesthetized by i.p. injection of sodium pentobarbital (Nembutal®, Abbott Co., Chicago, IL). The legs were shaved and infiltration anesthesia with 0.6 ml of 2% lidocaine–epinephrine solution (Xylocaine®, Fujisawa Pharmaceutical Co., Osaka, Japan) was applied around the medial end of the tibia to arrest the bleeding from bone marrow and to control early postoperative pain. The surgery was performed under aseptic conditions. A 3-cm incision was made in the proximal-anterior part of the tibiae. The medial end of the tibia was exposed, and a bone cavity was formed with a 2-mm guide drill and twist drill under irrigation. All rats received commercially pure titanium implants (2 mm in diameter, 4 mm long, 0.438-μm average surface roughness; Kyocera, Kyoto, Japan) in both tibiae (Fig. 1). After implantation, the animals were divided into four groups. In the FGF group, 10 μg of FGF-2 (Kaken Pharmaceutial Co., Ltd, Japan) was locally injected around the implants at 5 days after implantation. The Melatonin group was intraperitoneally administered 100 mg/kg body weight of melatonin (Sigma Chemical Co., St. Louis, MO) daily for 4 weeks from the implant installation. The FGF + Melatonin group was administered both FGF-2 and melatonin. Isotonic saline was administered to the control group. This study was approved by the Animal Experimentation Committee of Tokushima University.

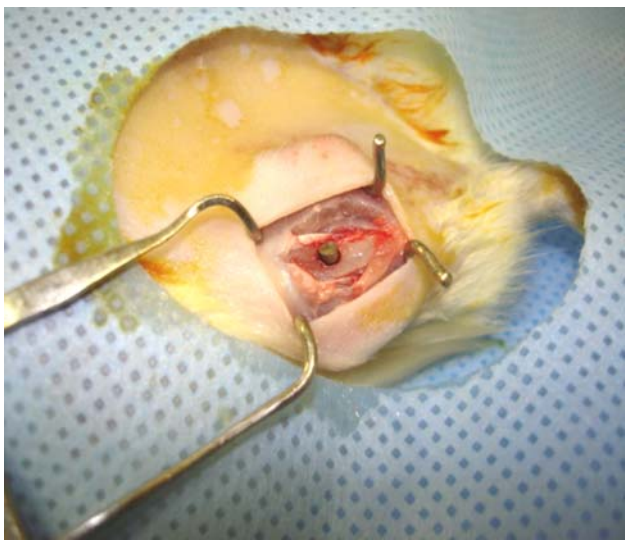

Fig. 1 Titanium implants (2 mm in diameter, 4 mm long) inserted in the cavity of tibiae

2.2 Specimen preparation

Tibiae containing the titanium implants were removed at 4 weeks after surgery. Specimen were fixed in 10% buffered formalin for 7 days, and then dehydrated in graded ethanol and embedded in polyester resin (Rigolac®; Oken Co., Tokyo, Japan). Thin sections about 200 μm thick were cut with a low-speed diamond saw. They were then hand-ground to approximately 70 μm parallel to the long axis of the implants. After staining with 5% toluidine blue, the sections were light-microscopically observed.

2.3 Histomorphometrical evaluation

The bone-implant contact (BIC) ratio and bone density (BD) were calculated for the quantitative evaluation of new bone formation. The BIC was indicated as the percentage of bone-titanium contact along the total length of the implant surface. The BD was indicated as the percentage of bone in the whole area of the bone marrow cavity. BIC and BD were calculated using NIH Image, and the data were statistically analyzed by one-way ANOVA. $P < 0.05$ was considered to be significant.

3 Results

3.1 Histological findings

In the control group, few newly formed bone was observed around the implants. Some newly formed bone was noted to contact directly with the implant surface. However, unmineralized connective tissue was also seen between the bone and implants (Fig. 2a). In contrast, in the FGF group and Melatonin group, more new bone formation was observed on the surface of titanium implants compared with the control group (Fig. 2b, c). In the FGF + Melatonin group, much more bone was formed in direct contact with the implant surface and the newly formed bone showed an immature pattern and a woven bone structure (Fig. 2d).

3.2 Histomorphometrical findings

The mean BIC in the FGF group and Melatonin group was 51.2 ± 8.5 and 57.4 ± 7.4%, respectively (Fig. 3). In the FGF + Melatonin group, the mean BIC was 87.0 ± 8.7% (Fig. 3). The mean BIC in the control group was 30.6 ± 10.2% (mean value ± SD, Fig. 4). As a result, the FGF + Melatonin group showed a significantly higher BIC than any other group.

With respect to the BD in the medullary canal, the mean BD in the control group was 41.6 ± 5.1% (Fig. 4). The

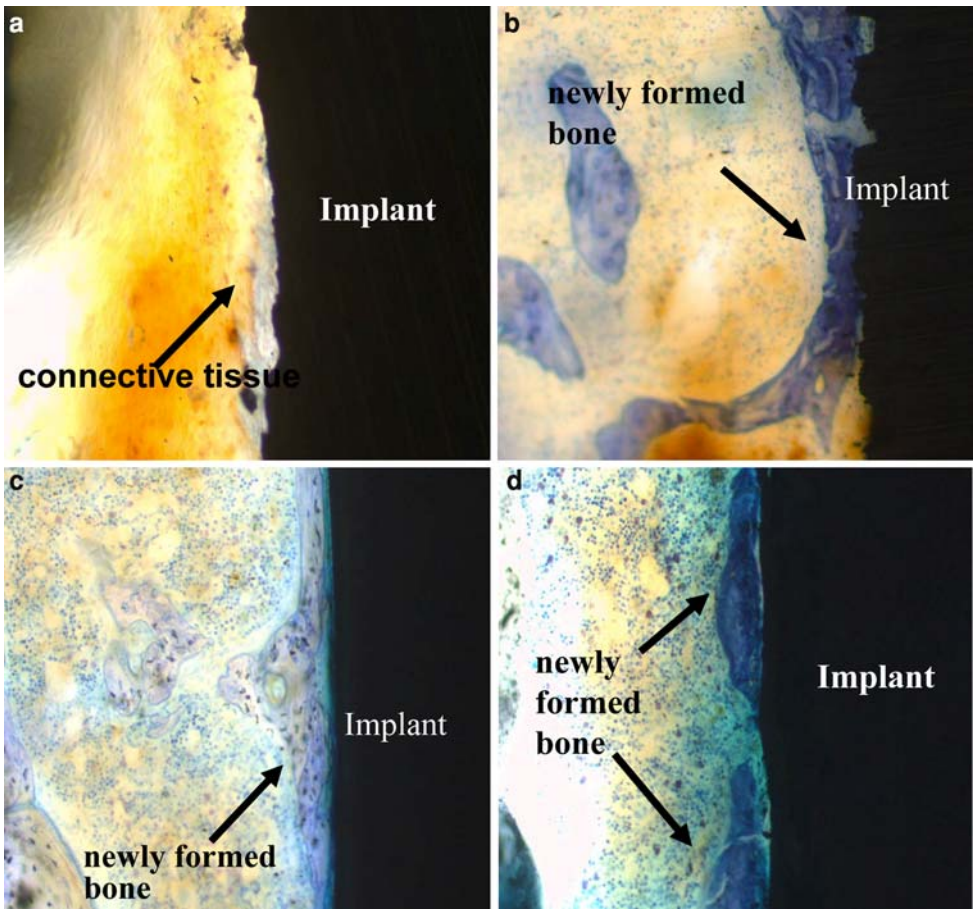

Fig. 2 Histological pictures of the implant. Ground section of the peri-implant bone showing osseointegration at 4 weeks post implantation. (**a**) Control group; (**b**) FGF group; (**c**) Melatonin group; (**d**) FGF + Melatonin group (toluidine blue stain, original magnification 200×)

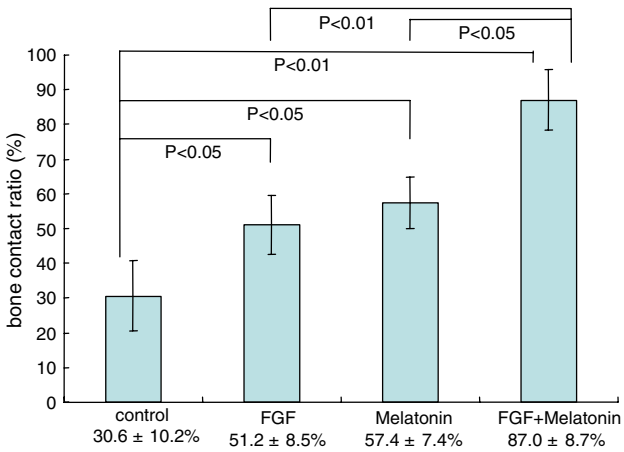

Fig. 3 Bone-implant contact ratio of the titanium implant at 4 weeks post implantation. All data are expressed as the mean ± SD (error bars). $P < 0.05$ was considered to be significant

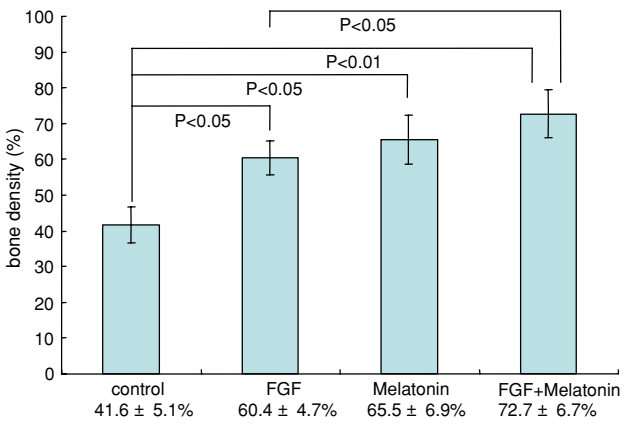

Fig. 4 Bone density of each group at 4 weeks post implantation. All data are expressed as the mean ± SD (error bars). $P < 0.05$ was considered to be significant

mean BD in the FGF group and Melatonin group was 60.4 ± 4.7 and 66.5 ± 6.9, respectively (Fig. 4). In the FGF + Melatonin group, the mean BCR was 72.7 ± 6.7% (Fig. 4). The BD results demonstrated a tendency toward a significantly higher mean value for the FGF + Melatonin group compared to any other group.

4 Discussion

Several reports have shown that FGF-2 accelerates fracture healing. The stimulation of fracture repair was demonstrated histologically and radiologically. Kawaguchi et al. [10] have reported that FGF-2 stimulates fracture repair in diabetic rats as well as in normal rats. In a study by

Nakamura et al. [11], it was demonstrated that mechanical strength during fracture healing was also enhanced by the local application of FGF-2. Also, a single local injection of FGF-2 induced the healing of segmental bony defects in rabbit tibiae [12]. Furthermore, FGF-2 delivered in a collagenous carrier was shown to improve bone healing of segmental bone defects in rabbit femurs [13]. It has also been shown to stimulate both ectopic bone formation in rats and to promote bone ingrowth in titanium chambers placed in rat tibiae [1, 14]. From these results, FGF-2 generally stimulates cellular proliferation rather than cellular differentiation [15, 16].

On the other hand, in the case of melatonin, Roth et al. [5] have reported that melatonin can similarly promote MC3T3-E1 cell differentiation and mineralization. In addition, preliminary studies have demonstrated that melatonin can stimulate the differentiation of primary osteoblasts grown in culture, confirming that the actions of melatonin are not restricted to transformed cells [17]. Furthermore, melatonin increased procollagen type I c-peptide production in normal bone cells and could modulate in vitro the expression of rat bone sialoprotein in pre-osteoblast cell lines; increased gene expression of sialoprotein and other bone marker proteins, including alkaline phosphate, osteopontin, secreted protein, and osteocalcin was reported [5, 18]. Therefore, melatonin appears to be capable of promoting osteoblast differentiation and mineralization of matrix in vitro. Also, in an in vivo study, a daily injection of young mice with 5 mg/kg or 50 mg/kg of melatonin for 4 weeks significantly increased bone mineral density and the thickness and volume of trabeculae [8]. In the present study, a daily injection of rats with 100 mg/kg of melatonin for 4 weeks significantly increased BIC and BD. Consequently, these findings provide conclusive evidence that melatonin treatment alone could lead to an increase in bone volume in vivo. Histomorphometry is an efficient method to evaluate the formation of new bone [19]. This histomorphometrical analysis showed that both FGF-2 and melatonin stimulated bone formation around the implants at almost the same level. Interestingly, the concomitant use of FGF-2 and melatonin greatly enhanced new bone formation on the surface of implants. These findings strongly suggest that these two molecules have the potential to promote osseointegration. Because of their different effects on different stages of cells, they could be synergistic in promoting bone formation. Consistent with the novel finding that FGF and melatonin act additively to promote the osseointegration of titanium implants, FGF could act synergistically with melatonin in the induction of bone formation. Although, the exact roles of these molecules during osteogenesis are not well understood, it is likely that FGF and melatonin work through related mechanisms but separately play important and different roles from each other. The stimulatory effects of these two molecules are not identical: FGF is typically thought to control osteoprogenitor cell proliferation whereas melatonin is more important in osteoblast differentiation.

In conclusion, the present study has shown that FGF-2 and melatonin, each of which has nearly equal stimulatory effects on osteogenesis, synergistically enhanced new bone formation around titanium implants.

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