Melatonin enhances peri-implant osteogenesis in the femur of rabbits

Shin-ichiro YAMAZAKI[*], Morio OCHI[**], Yukito HIROSE[**], Yasuhiro NAKANISHI[**], and Osamu NAKADE[***]

Abstract: **Purpose:** Expeditious postoperative ingrowth of bone surrounding implants is desired for clinically successful fixation of implants. We have recently demonstrated that melatonin stimulates proliferation and type I collagen synthesis in osteoblastic cells *in vitro*. In addition, we have also shown that daily I.P. injection of melatonin could increase cancellous bone mass in young growing mice *in vivo*, indicating that melatonin may stimulate bone formation. However, the effects of melatonin on bone wound healing remains unknown. **Materials and Methods:** The present study examined the effect of melatonin on peri-implant osteogenesis *in vivo*. 20 adult male Japanese white rabbits were divided into 4 groups, vehicle control group, 5, 20 and 50 mg melatonin-treated groups. A titanium alloy POI 2-piece-type implant was placed in the distal end of both right and left femurs in each rabbit. All implants were stable at the time of placement, and the wounds were sutured. After implant placement, the rabbits were given sodium ampicillin, 20 mg/kg per day for 3 days. Daily I.P. injection of melatonin or vehicle performed between 3 p.m. and 5 p.m. was initiated on the day following implant placement. The rabbits were sacrificed 2 weeks after implantation to histologically examine the effect of melatonin on the peri-implant osteogenesis. **Results:** Daily I.P. injection of melatonin significantly increased bone ingrowth in the implants, accompanied by increased two of its indices of bone contact ratio and bone area ratio, in comparison with those of the vehicle control. **Conclusion:** These results provide new evidence that daily I.P. injection of melatonin increases peri-implant osteogenesis in the rabbit femurs, suggesting that melatonin is a useful agent to stimulate bone wound healing.

Keywords: melatonin, dental implant, osteogenesis, femur, rabbit

1. Introduction

Rapid postoperative bone-ingrowth is essential for clinically successful fixation of oral implants[(1)]. However the agents that stimulate peri-implant osteogenesis are very few.

Melatonin, a secretory product synthesized nocturnally by the pineal gland, plays a major role in the coordination of seasonal reproduction[(2)] and pubertal development in some mammals[(3)]. Melatonin is also a potent antioxidant agent and may possess anti-aging property[(4,5)]. It has been also demonstrated that melatonin influences release of growth hormone in humans[(6,7)], serum levels of corticosterone in rats[(8)] and the circadian rhythm of osteoblast metabolism in rats[(9)], suggesting that melatonin may affect bone and mineral metabolism. In addition, we have recently demonstrated that melatonin at μM concentrations stimulates proliferation and type I collagen synthesis in human bone cells *in vitro*[(10)] and daily I.P. injection of melatonin could increase cancellous bone mass in young growing mice *in vivo*[(11)], indicating that melatonin may stimulate bone formation *in vitro* and *in vivo*. However, little is known about the effects of melatonin on healing process of bone wound such as bone fracture, tooth extraction and placement of the implant.

Received 19 Aug 2008, Accepted 16 Nov 2008.
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The present study was performed to test whether melatonin stimulates peri-implant osteogenesis. In this report, we provide new evidence that melatonin accelerates peri-implant osteogenesis in the rabbits.

2. Materials and Methods

Animal Model and Surgical Procedure

The protocols for animal experimentation described in this paper were previously approved by the Animal Ethics and Research Committee of Health Sciences University of Hokkaido, Japan; all of the animal experiments adhered to the Guidelines for the Care and Use of Laboratory Animals of the University.

20 adult male Japanese white rabbits weighing approximately 2.5 kg were divided into 4 groups consisted of a vehicle control group and three of melatonin-treated groups (5, 20, and 50 mg / kg body weight per day). Under general anesthesia with venous administration of 10 mg / kg pentobarbital (Nembutal, Abbott, North Chicago, IL, USA), a hole, 3.6 mm in diameter and 10.0 mm in depth, was drilled at the distal end of both right and left femurs in each rabbit. After cleaning the surgical area with sterile saline several times, a titanium alloy (Ti-6Al-4V) POI 2-piece-type implant (3.2 mm in diameter and 10.0 mm in length) whose surface had been treated with anodic oxidation (JMM Co., Kyoto, Japan), was placed there in the same position and direction being adopted wherever possible as previously described (Fig. 1) [12]. All implants were stable at the time of placement, and the wounds were sutured. After implant placement, the rabbits were given sodium ampicillin, 20 mg/kg per day for 3 days.

Daily I.P. injection of melatonin (5, 20, or 50 mg / kg per day) or vehicle performed between 3 p.m. and 5 p.m was initiated on the day following implant placement.

At the end of the 2 weeks of experiment period, the rabbits were anesthetized and sacrificed by exsanguinations to histologically examine the effect of melatonin on the peri-implant osteogenesis, using the undecalcified ground sections. The rationale for the observation-period of 2 weeks was based on our previous study[12].

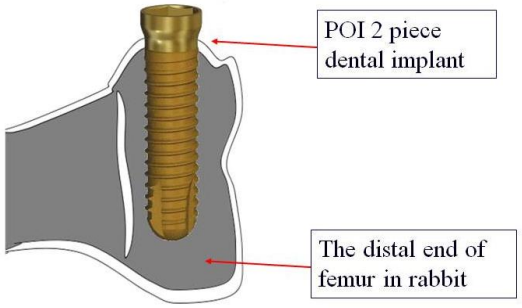

Fig. 1 Schematic drawing of the implant placed at the distal end of right femur in rabbit.

Histological examination

After sacrifice, the rabbits were immediately fixed by perfusion with 10 % neutral formalin via the abdominal aorta. Excised samples from both side of femurs (n = 10 for each group) fixed 3 days by immersion in 10 % neutral formalin. Samples consisting of the implant and surrounding bone tissue were then embedded in polyester resin (Ohken Co., Tokyo, Japan). Following polymerization, each sample was sliced perpendicular to the major axis of the implant into 200-μm sections using the Exact cutting-grinding system (Exact; Apparatebau, Norderstedt, Germany). Each section was polished to a thickness of 100-μm using a series of polishing discs (800 to 2400 grit) on the Exact micro grinding system. Six sections per implant were made, the section for each implant was randomly chosen for histological and histomorphometrical evaluation[12]. Contact microradiography (CMR) using Sofron BSTI 1505 CX ® (Soken, Tokyo, Japan) was performed on each section, and the CMR images were used for histomorphometric measurement of the calcified bone. Each section was further polished to a thickness of 50-μm for histological observation after double staining with basic fuchsin - methylene blue as previously described [12,13].

Histomorphometric Measurements

On each image, new bone at the edge and inside the drilled hole was traced. The bone contact ratio was defined as the length of bone surface border that is in direct contact with the implant / perimeter of the implant [× 100 (%)] (Fig. 2). The bone area ratio was defined as the area of new bone inside the edge of the drilled hole / area of drilled hole [× 100 (%)] (Fig. 3). The resultant data were then subjected to analysis

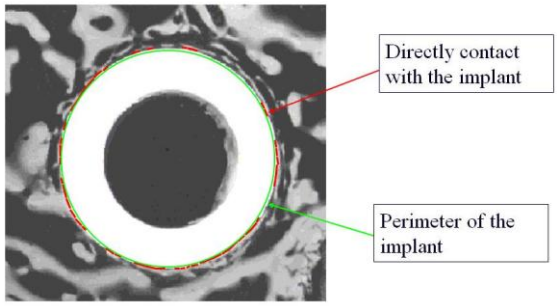

Fig. 2 Dental implant surrounded by new bone, showing the calculation of the bone contact ratio. The bone contact ratio = length of bone surface border that is in direct contact with the implant / perimeter of the implant [× 100 (%)]

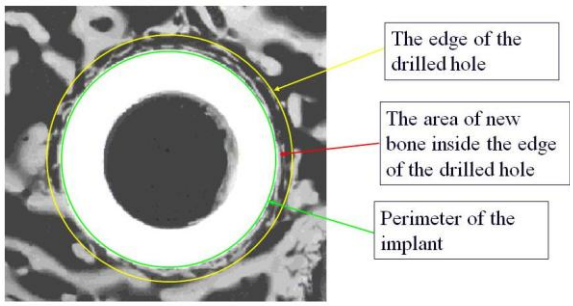

Fig. 3 Dental implant surrounded by new bone and pre-existing bone, showing the calculation of the bone area ratio. The bone area ratio = area of new bone inside the edge of the drilled hole / area of drilled hole [× 100 (%)].

of variance (ANOVA). Comparison of group means was performed using Scheffe's multiple comparison test. Differences between group means were considered to be statistically significant at a level of $p < 0.05$.

3. Results

There were no postoperative complications following placement of the implants, and the healing process was uneventful in all of the rabbits. There was no clear difference in the mean body weight during experiment among the groups (n = 5 for each group, data not shown).

The effect of melatonin on peri-implant osteogenesis was observed on the undecalcified grind sections stained with basic fuchsin-methylene blue stain, and the CMR images (n = 10 from both sides of femurs for each group).

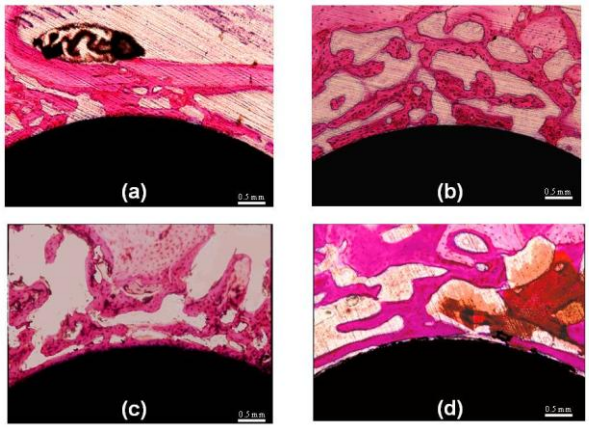

Fig. 4 Histological findings of bone ingrowth into the implant. A representative section of a femur with implant of the indicated group is shown. The sections were stained with basic fuchsin-methylene blue. The original bone, which stained light pink, was surrounded by new bone which stained reddish-violet.
(a) Control group (×50,bar = 0.5 mm)
(b) Melatonin-treated group (5 mg; ×50,bar = 0.5 mm)
(c) Melatonin-treated group (20 mg; ×50,bar = 0.5 mm)
(d) Melatonin-treated group (50 mg; ×50,bar = 0.5 mm)

As shown in Fig. 4, newly formed immature bone which did not contain lamella structures, was stained reddish-violet by basic fuchsin-methylene blue stain, while the mature bone which did contain a lamella structure, stained light pink. In the CMR images, newly formed immature bone was recognized as a thin trabecular bone without the structure of a lamella, while mature bone was recognized as a thick lamella bone (Fig. 5). In comparison with untreated control group, melatonin treatment clearly enhanced peri-implant osteogenesis accompanied by the ingrowth of immature bone within the drilled hole (Control; Fig. 4(a) and 5(a), melatonin-treated; Fig. 4(b-d) and 5(b-d)).

Quantitative data

The bone contact ratio and bone area ratio of the melatonin-treated and control groups are summarized in Figs. 6 and 7. As shown in Fig. 6, melatonin treatment also significantly increased the bone contact ratio by 56% with maximal effect at 20 mg/kg/day. Melatonin significantly increased the bone area ratio compared to that of control group with maximal effect between 20 to 50 mg/kg/day (Fig. 7). These results indicate that melatonin treatment accelerated bone repair in the space of the drilled hole between the pre-existing bone and implant.

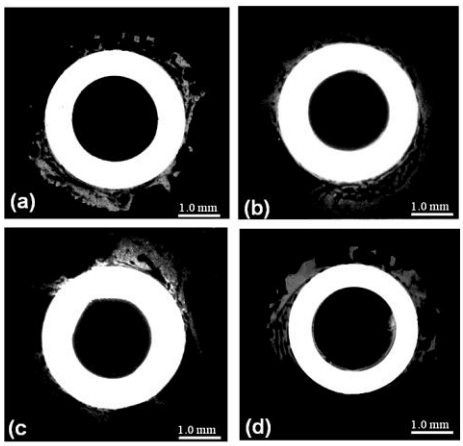

Fig. 5 CMR images of bone ingrowth into the implant.
A representative CMR image of a femur with implant of the indicated group is shown.
(a) Control group (× 15,bar = 1.0 mm)
(b) Melatonin-treated group (5 mg; × 15,bar = 1.0 mm)
(c) Melatonin-treated group (20 mg; × 15,bar = 1.0 mm)
(d) Melatonin-treated group (50 mg; × 15,bar = 1.0 mm)

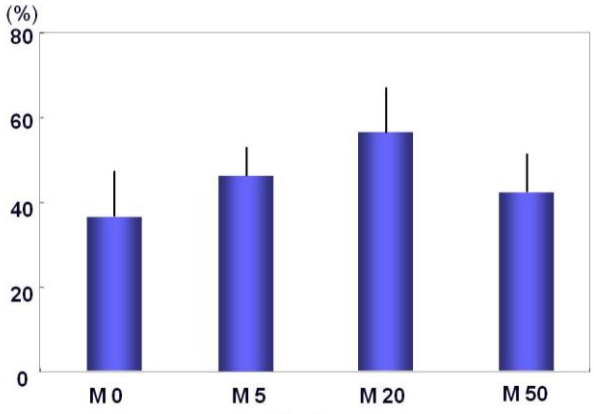

Fig. 6 Effect of Melatonin on the bone contact ratio of the oral implant inserted in the femur of rabbits. Each column represents the mean of the bone contact ratio of 10 implants (the vertical line are one standard deviation). Groups connected by a horizontal line are not significantly different. $p > 0.05$ compared with control by Scheffe's multiple comparison test.
M0: Control group
M5: Melatonin-treated group (5 mg/kg)
M20: Melatonin-treated group (20 mg/kg)
M50: Melatonin-treated group (50 mg/kg)

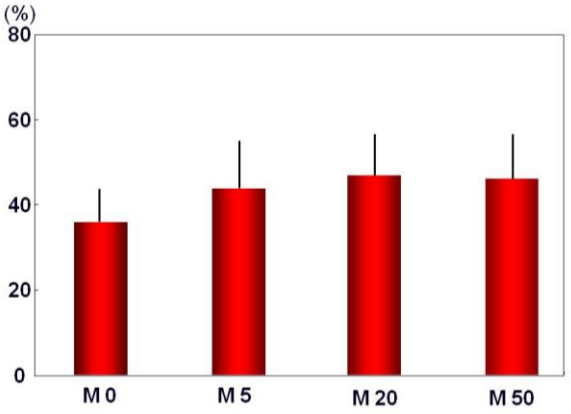

Fig. 7 Effect of Melatonin on the bone area ratio of the oral implant inserted in the femur of rabbits. Each column represents the mean of the bone area ratio of 10 implants (the vertical line are one standard deviation). Groups connected by a horizontal line are not significantly different. $p > 0.05$ compared with the control by Scheffe's multiple comparison test.
M0: Control group
M5: Melatonin-treated group (5 mg/kg)
M20: Melatonin-treated group (20 mg/kg)
M50: Melatonin-treated group (50 mg/kg)

4. Discussion

Considerable indirect evidence links melatonin to osteoporosis[14]. For example, melatonin secretion decreases sharply during menopause[15], declines with immobility[16,17], while osteoporosis increases after menopause and immobility. Osteoporosis and pineal calcification are uncommon among black population[18].

Based on these reports, we postulated that melatonin supplementation may have beneficial effects on bone. At first, we have recently shown that melatonin, a secretory product of pineal gland, at μM concentrations stimulates proliferation and type I collagen synthesis in normal human bone cells *in vitro*. In addition, we have recently demonstrated that daily I.P. injection of melatonin (5 and 50 mg/kg/day) could increase bone mineral density and cancellous bone mass in the tibia in young growing mice *in vivo*. However, in this study, bone histomorphology indicated that while there was no difference in bone formation parameters such as bone formation rate and osteoblast surface between melatonin-treated and control mice, bone resorption parameters such as osteoclast surface and the number of osteoclasts in each melatonin-treated group were reduced ($p<0.05$ for each), suggesting that melatonin increases bone mass *in vivo* through suppression of bone resorption. However, the effects of melatonin on bone wound healing have not been elucidated. Therefore, we first tested whether melatonin affects peri-implant osteogenesis, because the agents that stimulate peri-implant osteogenesis are very few, despite the fact that rapid postoperative bone-ingrowth is essential for

clinically successful fixation of oral implants. Accordingly, we provide here new evidence that melatonin clearly enhanced the ingrowth of bone within the drilled hole without significant side effects. The mechanisms thereby melatonin enhances bone repair around the implants are under investigation and further studies are needed in the future studies, however, our results indicate that melatonin has beneficial effects on oral implantology.

5. Conclusion

The present study offers new evidence that melatonin increases the peri-implant osteogenesis in vivo, indicating that melatonin has a beneficial effect on clinical implantology where accelerated bone healing around the implants is desired.

Acknowledgment

The authors wish to thank Prof. T. Kaku at the Health Sciences University of Hokkaido for helpful discussions. This study was supported, in part, by a grant-in-aid from Hokkaido Foundation for the Promotion of Scientific and Industrial Technology.

References

(1) Adell R, Lekholm U, Rockler B, Brånemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. ***Int J Oral Surg*** 10: 387−416, 1981.

(2) Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interaction. ***Endocr Rev*** 12: 151−180, 1991.

(3) Silman RE, Leone RM, Hooper RJL, Preece MA. Melatonin, the pineal gland and human puberty. ***Nature*** 282:301−303, 1979.

(4) Sandyk R. Possible role of pineal melatonin in the mechanisms of aging. ***Int J Neuroscience*** 52:85−92, 1990.

(5) Armstrong SM, Redman JR. Melatonin: a chronobiotic with anti-aging properties? ***Med Hypotheses*** 34: 300−309, 1991.

(6) Smithe GA, Lazarus L. Growth hormone responses to melatonin. ***Science*** 184: 1373−1374, 1974.

(7) Valcavi R, Zini M, Maestroni GJ, Conti AI. Portioli. Melatonin stimulates growth hormone secretion through pathways other than the growth hormone-releasing hormone. ***Clin Endocrinol*** 39:193−199, 1993.

(8) Oxenkrug GF, MeIntyre IM, Gershon S. Effects of pinealectomy and aging on the serum corticosterone circadian rhythm in rats. ***J Pineal Res*** 1:181−185, 1984.

(9) Cane V, Farneti D, Marotti G, Criscuolo M, Trentini G P, Marotti F. The effect of melatonin on bone growth in rats kept under constant light. ***Calcif Tiss Int*** A:47, 1991.

(10) Nakade O, Koyama H, Ariji H, Yajima A, Kaku T. Melatonin stimulates proliferation and type I collagen synthesis in human bone cells in vitro. ***J Pineal Res*** 27: 106−110, 1999.

(11) Nakade O, Koyama H, Ochi M, Ariji H, Kaku T, Takada J, Nishi M, Matsuyama T. Melatonin increases tibial bone mass in young growing mice. ***Bone*** 23 (suppl):S585, 1998.

(12) Matsumoto H, Ochi M, Abiko Y, Hirose Y, Kaku T, Sakaguchi K. Pulsed electromagnetic fields promote bone formation around dental implants inserted into femur of rabbits. ***Clin Oral Implants Res*** 11:354−360, 2000.

(13) Shigino T, Ochi M, Kagami H, Sakaguchi K, Nakade O. Application of capacitively coupled electric field enhances periimplant osteogenesis in the dog mandible. ***Int J Proshodont*** 13:365−372, 2000.

(14) Sandyk R, Anastasiadis P G, Anninos PA, Tsagas N. Is postmenopausal osteoporosis related to pienal gland functions? ***Int J Neurosci*** 62:215−225, 1992.

(15) Sack RL, Lewy AJ, Erb DL, Vollmer WM, Singer CM. Human melatonin production decreases with age. ***J Pineal Res*** 3:379−388, 1986.

(16) Yocca FD, Freidman E. Effect of immobilization stress on rat pineal beta-adrenergic receptor-mediated function. ***J Neurochem*** 42:1417−1432, 1984.

(17) Vauhan GM, McDonald SD, Jordan RM, Allen JP, Bohmfalk GL, AbouSamra M, Story JL. Melatonin concentration in human blood and cerebrospinal fluid: relationship to stress. ***J Clin Endocrinol Metab*** 47: 220−223, 1978.

(18) Adeloye A, Felson B. Incidence of normal pineal gland calcification in skull roentgenograms of black and white Americans. ***Am J Radiol*** 122:503-507, 1974.