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Melatonin attenuates titanium particle-induced osteolysis via activation of Wnt/β-catenin signaling pathway

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ABSTRACT

Wear debris-induced inhibition of bone regeneration and extensive bone resorption were common features in peri-prosthetic osteolysis (PPO). Here, we investigated the effect of melatonin on titanium particle-stimulated osteolysis in a murine calvariae model and mouse-mesenchymal-stem cells (mMSCs) culture system. Melatonin inhibited titanium particle-induced osteolysis and increased bone formation at osteolytic sites, confirmed by radiological and histomorphometric data. Furthermore, osteoclast numbers decreased dramatically in the low- and high-melatonin administration mice, as respectively, compared with the untreated animals. Melatonin alleviated titanium particle-induced depression of osteoblastic differentiation and mineralization in mMSCs. Mechanistically, melatonin was found to reduce the degradation of β-catenin, levels of which were decreased in presence of titanium particles both *in vivo* and *in vitro*. To further ensure whether the protective effect of melatonin was mediated by the Wnt/β-catenin signaling pathway, ICG-001, a selective β-catenin inhibitor, was added to the melatonin-treated groups and was found to attenuate the effect of melatonin on mMSC mineralization. We also demonstrated that melatonin modulated the balance between receptor activator of nuclear factor kappa-B ligand and osteoprotegerin via activation of Wnt/β-catenin signaling pathway. These findings strongly suggest that melatonin represents a promising candidate in the treatment of PPO.

Statement of Significance

Peri-prosthetic osteolysis, initiated by wear debris-induced inhibition of bone regeneration and extensive bone resorption, is the leading cause for implant failure and reason for revision surgery. In the current study, we demonstrated for the first time that melatonin can induce bone regeneration and reduce bone resorption at osteolytic sites caused by titanium-particle stimulation. These effects might be mediated by activating Wnt/β-catenin signaling pathway and enhancing osteogenic differentiation. Meanwhile, the ability of melatonin to modulate the balance between receptor activator of nuclear factor kappa-B ligand and osteoprotegerin mediated by Wnt/β-catenin signaling pathway, thereby suppressing osteoclastogenesis, may be implicated in the protective effects of melatonin on titanium-particle-induced bone resorption. These results suggested that melatonin can be considered as a promising therapeutic agent for the prevention and treatment of peri-prosthetic osteolysis.

1. Introduction

Peri-prosthetic osteolysis (PPO) and ensuing aseptic loosening remains the principal reason of implant failure and revision surgery [1]. Accumulation of wear particles at the interface of the bone-implant is important for the initiation of PPO [2]. Although

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the precise mechanism has not been fully elucidated, we and several other groups have demonstrated that the interaction of receptor activator of nuclear factor kappa-B ligand (RANKL) with osteoprotegerin (OPG) are fundamental in the process of wear debris-provoked PPO [3–5].

Bone homeostasis is acquired through dynamic cycle of osteoclastic bone resorption as well as osteoblastic bone regeneration, and imbalances of bone remodeling result in metabolic bone disorders such as PPO. Osteoclasts have been considered the primary factor involved in the process of particle-irritated osteolysis, and for many years anti-bone resorption agents such as bisphosphonates have been studied for the treatment of PPO and implant loosening [6–8]. However, even when osteoclasts are controlled, bone integrity cannot be restored, and lytic bones fail to heal, suggesting that bone formation is substantially compromised by wear debris in osteolytic diseases. Indeed, loss of osteogenic cell viability or differentiation capacity due to stimulation of wear particles is prominent in osteolytic disease. Wear debris is found to directly impair the survival of osteoblasts, determined by decreased alkaline phosphatase (ALP) activity [9]. Moreover, wear debris can stimulate osteoblasts to produce local osteoclastogenic mediators [10]. Although the precise mechanism involved in the inhibition of bone formation is poorly elaborated, it is obvious that modulation of bone regeneration is predominant in destructive bone diseases and is a novel approach in the treatment of wear debris-irritated osteolytic disease.

Melatonin (*N*-acetyl-5-methoxytryptamine), a bioactive indolamine secreted mainly by the pineal gland, has been verified to be involved in numerous physiological conditions, including antioxidant, regulation of sleep, anti-inflammation and anti-tumor [11–14]. Recently, a melatonin bone-protective effect has been suggested [15–22]. Melatonin stimulates the proliferation of osteoblasts and enhances osteoprogenitor differentiation towards the osteoblast lineage [15]. In addition, melatonin has also been found to promote bone formation [16], reduced age-related bone loss [17] and elevate bone mineral density (BMD) in postmenopausal women with osteopenia [18,19] and accelerate osteointegration in a calvaria-defect model [20]. Meanwhile, several authors have demonstrated that local administration of melatonin increased new bone formation around dental implants and reduced the risk of crestal bone loss [21,22]. Researches of the molecular mechanisms whereby melatonin exhibits promotive bone regeneration indicate its involvement in increased synthesis of Runt-related transcription factor 2 (Runx2) and Osterix, thereby accelerating transduction of Wnt/β-catenin signaling pathway and making melatonin a potential agent in intervention of osteolytic disease [23]. Moreover, melatonin has been confirmed to restrain osteoclast formation and increase bone regeneration by disturbing the balance between RANKL and OPG [24,25]. Collectively, these data indicate that melatonin is beneficial for bone remodeling. However, whether melatonin can reverse wear debris-induced osteolysis is yet to be determined.

In the current study, we speculated that melatonin administration could attenuate wear debris-irritated lytic bone destruction by accelerating bone regeneration and inhibiting bone resorption. We assessed this hypothesis *in vivo* and *in vitro* using the particle-induced osteolysis murine calvaria model and cultured mouse-mesenchymal-stem cells (mMSCs), respectively.

2. Materials and methods

2.1. Ti particles

Ti particles were obtained from Johnson Matthey Chemicals (Ward Hill, MA, USA). The specification was 3.25 ± 2.18 μm in diameter, of which 95% were less than 4 μm [4]. Ti particles were immersed in 75% ethanol for 48 h and rinsed for 4 times with sterile ultra-pure water at room temperature followed by firing at 180 °C for 6 h [10]. The Limulus assay was performed to ensure the particles without endotoxin (<0.25 EU/ml). Subsequently, the particles were mingled with sterile phosphate-buffered saline (PBS) with appropriate proportion [26] thereafter tightly sealed and deposited at 4 °C until use.

2.2. Surgery procedure

All the experiments were approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. The Ti particle-irritated calvarial model was established as previously described [27]. In brief, forty 2-month-old male C57BL/6J mice were randomized into four groups: PBS-only (sham group), PBS with Ti particles (vehicle group), Ti particles with low- (5 mg/kg) or high- (50 mg/kg) melatonin concentration (low- and high-melatonin groups, respectively). The dosage adopted in the current study has been demonstrated to exert bone protective effects *in vivo* [24,28]. Melatonin (Sigma, St. Louis, MO, USA) was dissolved in 100% ethanol at a dilution of 50 mg/ml according to the protocol of manufacture, and then diluted in sterile PBS. Mice were anesthetized with chloral hydrate intraperitoneally (500 mg/kg). Then a 10-mm midline sagittal incision over the parietal bone was cut. Mice in the sham group were sutured without any intervention, while animals in other groups received 40 μl Ti suspension. This concentration of Ti particles administrated to mice was suitable to mimic the clinical scenario of peri-implant osteolysis as determined by Schwarz et al. [26]. Mice in the drug treatment groups underwent intraperitoneally administration of melatonin during 3:00 pm–5:00 pm for consecutive 14 days [24]. Mice in un-treatment groups were given PBS only. During the experiment, dark/light was set as 12 h/12 h, and water and food were given *ad libitum*. Peripheral blood was collected prior to sacrifice on day 14 postoperatively for serological assessment, and the calvariae were harvested for radiological and histological analyses.

2.3. Radiological analyses

Fixed specimens (n = 5 per group) were analyzed with high resolution μCT (SkyScan1176; SkyScan, Knotich, Belgium). The calvaria were scanned with 9 μm per layer. The X-ray parameters were set at voltage of 50 kV with a current of 500 μA together with 0.7° rotational step. Then a round region of interest (ROI), 3 mm in diameter through the midline suture, was chosen to perform related analysis including the number of pores, bone volume (BV), bone volume/tissue volume (BV/TV) and bone mineral density (BMD) [29]. All analyses were performed blindly to the treatment as reported previously [30].

2.4. Histological and immunohistochemical analysis

After fixed in 10% formalin for 2 days, calvaria (n = 5 per group) were decalcified in 10% Ethylene Diamine Tetraacetic Acid (EDTA, Sigma) for 21 days and then embedded. Calvaria were cut in 5 μm thick to perform hematoxylin and eosin (H&E) staining and tartrate-resistant acid phosphatase (TRAP) staining. Section images (4 consecutive sections per calvaria) were captured microscopically with a scale bar of 100 μm. The ROI was set around the center of the middle suture as previously described [30]. The eroded bone surface area (mm^2) and bone thickness (BT, mm) were quantified and determined with the protocols introduced by Parfitt et al. [31,32]. Dark-purple stained granules intensely distributed at the osteolytic sites were deemed as osteoclasts. Additionally, the per-