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Theaflavin-3,3′-digallate represses osteoclastogenesis and prevents wear debris-induced osteolysis via suppression of ERK pathway

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ABSTRACT

Peri-implant osteolysis (PIO) and the following aseptic loosening is the leading cause of implant failure. Emerging evidence suggests that receptor activator of nuclear factor kappa-B ligand (RANKL)-induced osteoclast formation and osteoclastic bone resorption are responsible for particle-stimulated PIO. Here, we explored the effect of theaflavin-3,3′-digallate (TF3) on titanium particle-induced osteolysis *in vivo* and *in vitro*. Twenty-eight male C57BL/6 mice were randomly separated into four groups: sham control (sham), titanium particles only (titanium), titanium particles with low TF3 concentration (low-TF3, 1 mg/kg TF3), and titanium particles with high TF3 concentration (high-TF3, 10 mg/kg TF3). Two weeks later, micro-computed tomography and histological analysis were performed. Bone-marrow-derived macrophages and RAW264.7 murine macrophages were applied to examine osteoclast formation and differentiation. TF3 significantly inhibited titanium particle-induced osteolysis and prevented bone destruction compared with titanium group. Interestingly, the number of mature osteoclasts reduced after treatment with TF3 *in vivo*, suggesting osteoclast formation might be inhibited by TF3. *In vitro*, TF3 suppressed osteoclast formation, polarization and osteoclastic bone resorption by specifically targeting the RANKL-induced ERK signal pathway. Collectively, these results suggest that TF3, a natural active compound derived from black tea, is a promising candidate for the treatment of osteoclast-related osteolytic diseases, such as wear debris-induced PIO.

Statement of Significance

Total joint arthroplasty is widely accepted for the treatment of end-stage joint diseases. However, it is reported that aseptic loosening, initiated by peri-implant osteolysis, is the major reason for prosthesis failure. Although the pathophysiology of PIO remains unclear, increasing evidence indicates that osteoclasts are excessively activated at the implant site by wear debris from materials. Here, we demonstrated that theaflavin-3,3′-digallate, a natural active compound derived from black tea, inhibited osteoclast formation and osteoclastic bone resorption mainly via suppressing the ERK pathway. Moreover, the findings of this study have confirmed for the first time that theaflavin-3,3′-digallate has a protective effect on particle-induced osteolysis in a mouse calvarial model, thus preventing bone loss. These results indicate that theaflavin-3,3′-digallate may be a suitable therapeutic agent to treat wear debris-induced peri-implant osteolysis.

1. Introduction

Total joint arthroplasty (TJA) is widely accepted for the treatment of end-stage joint diseases such as severe trauma, osteoarthritis and rheumatoid arthritis [1]. However, it is reported that aseptic loosening, initiated by peri-implant osteolysis (PIO), is the major reason for prosthesis failure [2,3], resulting in more than

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one-third of patients undergoing revision surgery within two decades after TJA [4]. Although revision surgery has a positive effect on aseptic loosening, we cannot overlook the negative impacts of revision surgery, such as higher cost, shorter survival duration and poorer clinical outcomes. Although the pathophysiology of PIO remains unclear, increasing evidence indicates that osteoclasts are excessively activated at the implant site by wear debris from materials such as titanium (Ti), polymethyl methacrylate, polyethylene, and cobalt chromium [5,6]. Two essential factors are reported for osteoclastogenesis: macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL). M-CSF is an important regulator for the proliferation and survival of osteoclast precursor cells and upregulates the expression of RANK, which is vital for the differentiation of osteoclast precursor cells into mature osteoclasts [7–9]. Binding of RANKL to its receptor, RANK, promptly activates a number of signaling pathways such as mitogen-activated protein kinases (MAPKs), nuclear factor-κB (NF-κB) and phosphatidylinositol 3-kinase/AKT (PI3K/Akt) [10–13]. This in turn leads to the activation of downstream signaling factors including activated protein 1 (AP1) and nuclear factor of activated T-cells 1 (NFATc1), which are crucial for the formation of osteoclasts and expression of osteoclast-related genes [14].

Considering the critical role of osteoclasts in osteolytic diseases, inhibitors that specifically target the suppression of osteoclast formation and/or function are ideal candidates to prevent wear debris-induced PIO and bone loss. In recent years, attention has been paid to more natural compounds with the potential to inhibit osteoclastogenesis and osteoclast-related diseases. Our research group has screened a number of natural compounds to identify candidates to treat osteoclast-related osteolytic diseases, including wear debris-induced PIO.

Black tea, extracted from the leaves of *Camellia sinensis*, is one of the most popular beverages worldwide [15]. The fermentation process, which is indispensable in the process of making black tea, results in oxidation of polyphenols and subsequent formation of oligomeric flavanols containing theaflavins, thearubigin and other oligomers [16]. Compared with green tea, which is rich in catechin, black tea has a high content of tea theaflavins. Of the four main theaflavins found in black tea, theaflavin-3,3′-digallate (TF3) is formed by polyreaction of epicatechin gallate (ECG) and (−)-epigal locatechin-3-gallate (EGCG), which are the major catechins found in green tea [17]. Previous studies have reported that similar to EGCG, TF3 has many positive effects on human health, including antioxidant [18], anti-angiogenic[17] and anti-tumor properties [19]. Recently, TF3 has been reported to inhibit osteoclast formation [20] and prevent ovariectomy-induced bone loss [21]. However, whether TF3 can prevent wear debris-induced PIO remains unknown.

Because osteoclasts play a crucial role in PIO [5,6], it is logical that TF3 could be an optimal treatment candidate. We hypothesized that TF3 acts to prevent debris-induced PIO through the inhibition of osteoclast formation to prevent bone loss. The aims of this study were therefore to investigate the effect of TF3 on particle-induced PIO in a mouse calvarial model and osteoclasts formation *in vitro*, as well as the underlying mechanism of TF3 during osteoclastogenesis.

2. Materials and methods

2.1. Preparation of metal particles

Commercially pure titanium (Ti) particles (catalog #00681) were obtained from Johnson Matthey chemicals (Ward Hill, Massachusetts, USA). A Coulter counter™ (BeckmanCoulter Inc., USA) with five consecutive measurements was used to test the distribution and size of the Ti particles. Ninty precents of the Ti particles were <3.6 mm. As previously described [22,23], it was used to eliminate endotoxins that the particles were sterilized at 180 °C for 6 h and washed in 75% ethanol for 48 h. Endotoxin levels were confirmed by a quantitative Limulus Amebocyte Lysate Assay (Bio-Whittaker, Walkersville, MD, USA) and only free of endotoxin particles were used.

2.2. Experimental animals and drug treatment

All experiments were performed in strict accordance with the guidelines for Care and Use of Laboratory Animals and were approved by the Animal Care Committee of our institute. Based on the previous studies [24–26], we established a murine calvarial osteolysis model with Ti particle-stimulation. Briefly, 28 6- to 8-week-old male C57BL/6 mice were separated at random into four groups (n = 7): (1) sham group (underwent sham surgery only), (2) Ti group (received 30 mg Ti particles to induce osteolysis), (3) low-TF3 group (received 30 mg Ti particles with low TF3 concentration, Sigma-Aldrich, St. Louis, MO, USA), and (4) high-TF3 group (received 30 mg Ti particles with high TF3 concentration). According to the products instruction, TF3 was resuspended in absolute alcohol until fully dissolved and then diluted in phosphate-buffered saline (PBS). Mice in the low- and high-TF3 groups were intraperitoneally injected with TF3 (1 mg/kg and 10 mg/kg, respectively) every second day for 2 weeks, whereas the rest of groups were injected with PBS. For endotoxins elimination, Ti particles were prepared according to the method established by Geng et al. [24]. The animals were sacrificed after 2 weeks of Ti particle implantation and the calvariae were removed, fixed and prepared for further analysis.

2.3. μCT scanning

After fixed with 10% formaldehyde for 24 h, the mice calvariae were analyzed with a SkyScan1176 μCT. The calvariae (n = 4) were scanned at an equidistant resolution of 18 μm with 80 kV and 100 μA energy X-ray. To avoid metal artifacts, wear particles were removed before scanning. Images were reconstructed using the software provided by the manufacturer. As previously described [27], the combined region of interest (ROI; $3 \times 3 \times 1$ mm) with the midline suture at its center was identified for further quantitative analysis. Bone mineral density (BMD, mg/cc), number and area of pores in each sample and the ratio of body volume to tissue volume (BV/TV,%) were measured by CT Analyser software (Skyscan).

2.4. Histological and histomorphometric analysis

The calvariae(n = 3, per group) were fixed in 4% paraformaldehyde (PFA) for 48 h, followed by decalcification in 10% EDTA for 3 weeks and paraffin embedding. Sections (5 μm) were cut and prepared for hematoxylin and eosin (H&E) and tartrate-resistant acid phosphatase (TRAP) staining as previously described [26]. The TRAP staining was performed using a commercial TRAP kit (#387A, Sigma-Aldrich). Photos of the stained sections were observed in a Zeiss microscope (Zeiss, Dreseden, Germany). The eroded surface (mm^2), periosteum thickness (mm), osteoclasts number and the ratio of osteoclast to bone surface (OCs/BS,%) were measured and quantified with the help of image analysis software (Image Pro-Plus 6.0) according to the methods established by Kauther [28].