Inhibitory effect of luteolin on titanium particle-induced inflammatory cytokine release and osteoclastogenesis

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INTRODUCTION
Periprosthetic osteolysis is the major complication following total hip arthroplasty (THA). The wear debris from orthopaedic implants induces foreign body reactions which cause formation of periprosthetic pseudomembranes composed of granulomatous tissues containing macrophages, fibroblasts, giant cells, and osteoclasts (1, 2). These cells in response to particulate debris produce inflammatory cytokines involved in osteolysis and bone loss (3, 4). Tumor necrosis factor-α (TNF-α), one of the key osteolytic cytokines, may be pivotally involved in wear debris-induced osteolysis. It has been also shown to stimulate the activation and differentiation of osteoclasts through a mechanism independent of the osteoclast differentiation factor (ODF) system (also called RANKL/Trance/OPGL).

Therefore, controlling inflammatory cytokines production, particularly TNF-α, in the periprosthetic environment may be a potential method to prevent or reduce wear particle-induced osteolysis. Recently, luteolin has been shown to inhibit osteoclast formation and function in vitro and to down-regulate osteoclast-induced bone loss. In this study, we investigated whether luteolin suppresses titanium (Ti) particle-induced inflammatory cytokines production and osteoclastogenesis. We further examined the inhibitory role of luteolin on Ti particles-induced osteolysis in mouse calvaria model.

METHODS

Osteoclast cultures: Whole bone marrow cells were isolated from ICR mice and cultured with RANKL and M-CSF in α-MEM containing 10% FBS. Osteoclasts were stained for tartrate-resistant acid phosphatase activity. To determine actin ring formation, bone marrow macrophages were cultured on bone with M-CSF and Titanium Conditioned Medium. Cells were fixed and stained with rhodamine-phalloidin.

Bone resorption assay: Osteoclasts were removed from the bone slices. After a brief rinse, the slices were incubated with 20 µg/ml peroxidase-conjugated wheat germ agglutinin for 30 min. After washing in PBS, 3,3′-diaminobenzidine was added onto the bone slices.

Enzyme-linked immunosorbent assays (ELISA): Bone marrow cells at 5×10⁸ cells/well were exposed to Titanium conditioned media with or without luteolin. The culture medium were collected at the indicated times, TNF-α levels were measured with ELISAs, according to the manufacturer's instructions (Biolegend).

Microcomputed tomography (µCT): Bone microarchitectural properties of calvaria were determined using µCT system (eXplore Locus SP, GE Healthcare).

RESULTS

The Ti particles substantially induced TNF-α and other inflammatory cytokine production in dose-and-time-dependent manner and luteolin substantially inhibited Ti particle-induced TNF-α release in mouse Bone Marrow Macrophage (Fig. 1). Luteolin effectively suppresses both the number of TRAP-positive MNCs and the activity of TRAP in Titanium particle-induced osteoclastogenesis in vitro (Fig. 2). Luteolin also inhibited the bone resorptive activity of mature osteoclasts in the presence of Titanium conditioned media. To further confirm the inhibitory effects of luteolin on osteoclast differentiation, we analyzed the expression of osteoclastogenic markers by RT-PCR. The mRNA expression levels of TRAP, NFATc1, and c-Src were significantly reduced in the presence of luteolin.

To examine the inhibitory effect of luteolin on Ti particle-induced osteolysis in mouse calvaria model, Ti particle was injected in mouse calvaria and luteolin was administered with two different doses for 10 days. Ti particles induced osteolysis compared to sham control mouse. However, administration of luteolin significantly into these mice diminished the Ti particle-induced osteolysis in a dose dependent manner.

Discussion
Pathogenesis of osteolysis and aseptic loosening after total joint arthroplasty involves activation of macrophages by particulate debris and thus release of inflammatory cytokines including TNF-α. Therefore, down-regulation of inflammatory cytokines suppresses the local inflammatory responses of macrophages in tissue adjacent to the implant and thus prevents osteolysis. Since TNF-α is the principal cytokine initiating a variety of defense mechanisms and immunological responses and has been identified as a key mediator released by particle stimulated macrophages, its synthesis may be a potential target to prevent particulate-induced osteolysis. Regulation of osteoclast formation has been considered as an effective therapeutic approach to the treatment of osteolysis. In present study, luteolin inhibits Titanium particles-induced osteoclast differentiation and function. It also diminished the Ti particle-induced osteolysis in mouse calvaria model. These results suggesting that luteolin might be useful as a therapeutic agent for the treatment of bone-related diseases.

REFERENCES

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Figure 1. Luteolin inhibits Ti induced TNF-α release in mouse Bone Marrow Macrophage

Figure 2. Luteolin inhibits Ti-particle induced osteoclast differentiation