INHIBITORY EFFECT OF (-)-EPIGALLOCATECHIN GALLATE ON TITANIUM PARTICLE-INDUCED TNF-α RELEASE THROUGH INHIBITION OF MAP KINASE AND AP-1/NF-κB PATHWAYS IN MACROPHAGES

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Introduction: Periprosthetic osteolysis and subsequent aseptic loosening are the most common causes of failure of 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 loosening (3, 4).

Tumor necrosis factor-α (TNF-α), one of the key osteolytic cytokines, augments osteoclast differentiation and function directly or indirectly. Therefore, controlling TNF-α synthesis in the periprosthetic environment may be a potential target to prevent or reduce wear particle-induced osteolysis. Recently, EGCG has been shown to induce the apoptotic cell death of osteoclast, and inhibit osteoclast formation and lipopolysaccharide (LPS)-induced TNF-α release in macrophages. In this study, we investigated whether EGCG suppresses titanium (Ti) particle-induced TNF-α release and underlying molecular mechanism in vitro. We further examined the inhibitory role of EGCG on Ti particles-induced osteolysis in mouse calvaria model.

Materials and Methods: 1) Preparation of Ti particles: Commercially pure Ti particles (1-3 μm) were purchased from Cerac (Milwaukee, WI). The Ti particles were sterilized as previously described (5).

2) Production of TNF-α was analyzed by Enzyme-linked immunosorbent assays (ELISA)

3) Activation of NF-κB and AP-1 was measured by Electrophoretic mobility shift assay (EMSA)

4) Mouse calvarial osteolysis model and surgical procedure: Ti particles were injected under the periosteum of mouse calvaria. EGCG were fed or left for 7 days. The calvaria were sectioned and stained with hematoxylin-eosin (HE).

Results: 1) TNF-α release by Ti particles and inhibitory effect of EGCG on Ti induced TNF-α release

Ti particles substantially induced TNF-α release in dose-and time-dependent manner and EGCG substantially inhibited particle-induced TNF-α release in dose-dependent manner in Raw264.7 and J774 (Fig.1A and B).

2) Effect of EGCG on Ti particle-induced activation of JNK/AP-1 and NF-κB pathways

To define the molecular mechanism(s) by which EGCG inhibits Ti-induced TNF-α release, we examined activities of MAP kinases and the protein level of IkBα. Ti-particles induced phosphorylation of p38MAPK, ERK and JNK and degradation of IkBα from Ti particles stimulation. Treatment of EGCG dramatically inhibited activation of MAP kinases and degradation of IkBα in a dose-dependent manner, suggesting that EGCG exerts its inhibitory function on the signaling pathways leading to activation of MAP kinases and NF-κB. In fact, activation of AP-1 and NF-κB was detected 15 or 30 min after Ti stimulation, and AP-1 and NF-κB activation were inhibited by EGCG. Taken together, these data suggest that the inhibitory effect of EGCG on Ti-induced TNF-α release is in part mediated by down regulation of the activities of JNK/AP-1 as well as NF-κB pathways.

3) Inhibitory effect of EGCG on Ti particle-induced osteolysis in mouse calvaria model in vivo

Ti particles induced osteolysis compared to sham control mouse (Fig. 2A and B). However, administration of EGCG significantly into these mice diminished the Ti particle-induced osteolysis in a dose dependent manner (Fig. 2C and D).

Discussion: This is the first report demonstrating that Ti particles-induced TNF-α release is mediated through JNK/AP-1 and NF-κB pathways, and EGCG has inhibitory effect on Ti particle-induced TNF-α release in vitro. We also show that EGCG inhibits Ti particles-induced osteolysis in mouse calvaria model.

These results suggest that EGCG may be a potential candidate natural compound for the prevention and/or treatment of osteolysis and loosening after THA.

References:

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Figure 1. EGCG inhibits Ti induced TNF-α release in a concentration dependent manner in Raw264.7 cells (A) and J774 cells (B).

Fig.2. Representative photographs of calvarial histology stained with HE staining. Mouse calvaria were left (A) or injected with Ti particle (B). Some mice injected with Ti particles were fed with 1mg/kg/day (C) or 2mg/kg/day (D) of EGCG for 7 days. Seven days after operation, calvaria were sectioned and stained with HE.