Local Delivery Of Mutant Mcp-1 Protein Reduced Osteolysis Induced By Wear Particles Orthopaedic Implant Wear Particles In Vivo

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Introduction: Total joint replacement (TJR) has been widely used for treating end-stage arthritis with great success. However, undesirable wear particle production is inevitable for all TJRs, which begins during the initial “bedding in” phase and continues during use of the TJR. Wear particles activate foreign body giant cells and macrophages and secrete pro-inflammatory chemokines and cytokines, including MCP-1 TNF-α, and IL-1β (Fig.1). This in turn leads to delayed osseointegration, periprosthetic bone loss (osteolysis) and eventual TJR failure. Macrophage Chemo-attractant Protein-1 (MCP-1) is one of the most potent chemokines regulating systemic and local macrophage recruitment in chronic inflammation. Previous studies suggest that mutant MCP-1 proteins such as 7ND may be used as a decoy agent to block CCR2 receptor and reduce inflammatory cell migration (1-2). We hypothesize that local administration of 7ND will decrease wear particle induced inflammation and osteolysis (Fig.1). The purpose of this investigation is to evaluate the efficacy of local 7ND delivery on modulating orthopaedic implant wear-particle induced osteolysis in vivo using a mouse calvarial model.

Methods: Conventional ultra-high molecular weight polyethylene (PE) particles (a gift from Dr. Timothy Wright, Hospital for Special Surgery, New York, NY) were obtained from knee joint stimulator tests. 7ND protein was provided by Dr. Kensuke Egashira in Kyushu University (Japan). The efficacy of 7ND delivery on modulating wear particle-induced osteolysis in vivo was examined using C57BL/6 male mice (8-9 weeks old, Jackson Laboratory). To mimic wear particle induced osteolysis, PE wear particles (7 mg of PE in 50 μL of PBS) were injected at day 1 into the subcutaneous space above the calvarial bone and periosteeum. Mice were treated with either 7ND (1 μg in 50 μL of PBS) or PBS alone every other day via local injection above the mouse cranium. At day 14, micro-CT imaging was performed on all animals to measure changes in bone volume fraction (BVF) and bone mineral density (BMD). Mice were then sacrificed for histology. Histomorphometric analysis was used to evaluate the bone volume differences, and tartrate resistant acid phosphatase (TRAP) staining was used to assess osteoclast formation. Macrophages were identified by immunohistochemical staining with FITC-labeled mouse anti-CD11b antibodies.

Results: Fourteen days after injection, PE particles led to diffuse and extensive osteolysis across the entire calvarial bone compared to treatment with PBS alone, as shown by micro-CT imaging (Fig. 2A). In contrast, local injection of 7ND reduced the level of osteolysis, which was also restricted to the central cranium (Fig. 2B). Compared with the PE treated group, 7ND treatment significantly increased both bone volume fraction (Fig. 2D) and bone mineral density (Fig. 2E). H&E staining of calvarial bones showed similar trends (Fig. 3A-D). The PE treated group exhibited loss of cranial bone integrity, and lost bone
was replaced with fibrous tissue. The 7ND treated group showed a more preserved cranial bone with a healthy bone marrow cavity. To quantify the degree of osteolysis, we calculated the ratio of bone thickness (BT) over total tissue thickness (TTT). The BT/TTT ratio in the 7ND group (0.84 ± 0.11) was statistically higher than that of PE group (0.53 ± 0.09) (p < 0.001), suggesting 7ND reduced osteolysis. TRAP staining of osteoclasts also showed 7ND significant decreased number of osteoclasts in the calvarial bone section (Fig. 3F-H). To evaluate the degree of inflammation, we stained CD11b, a marker for macrophages. PE group (Fig. 3I-K) showed scattering of CD11b+ cells across the calvarial bone. In contrast, CD11b+ cells was contained within bone marrow cavity in the 7ND treated group (Fig.3L-N).

**Discussion:** Our results show that local delivery of 7ND significantly reduced PE wear particle-induced osteolysis and inflammation using the mouse calvarial model. Modulation of macrophage polarization via local 7ND delivery may offer an effective strategy to reduce undesirale wear particle-induced osteolysis, thus decreasing the failure rate of TJRs.

**Significance:** Mitigating wear particle-induced inflammation and osteolysis would prolong the lifetime of orthopaedic implants, reduce the number of revision surgeries, and substantially reduce the associated medical costs.

**Fig. 1.** Biological processes involved in orthopaedic implant wear particle-induced inflammation and osteolysis, which involves recruitment and activation of systemic macrophages in response to wear particles.
**Fig. 2.** Representative μ-CT images of mouse calvarial bone at day 14 treated with PE (A) or PE + 7ND (B) by local injection. The region of interest (ROI) indicated by the yellow shaded region (7.7 × 6.6 × 2.3 mm, 1 × d × h) box (C) was used to quantify the BVF and BMD. Graphical representation of μ-CT quantifying the BVF (D) and BMD (E) after 7 treatments.

***p<0.001, (n=5).

**Fig. 3.** Histological staining showed 7ND reduced wear particle-induced osteolysis in a mouse calvarial model. H&E staining of calvarial bone section treated with PE+PBS (A, C) or PE+7ND (B, D). Yellow arrows: total tissue thickness (TTT); green arrows: bone thickness (BT). (E) Calculated ratio of BT/TTT in PE+PBS vs. PE+7ND. ***p < 0.001. TRAP staining showed higher number of osteoclasts in PE+PBS group (F) than 7ND treated group (G). Yellow arrow: multinucleated osteoclasts. (H) Quantifying the number of osteoclasts. (I-N) Immunostaining with FITC-labeled mouse anti-CD11b antibodies. White arrow indicates bone marrow cavity.