Estrogen Deficiency is Protective Against Particle-Induced Osteolysis

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Introduction
Patient-related factors may cause variability in the host response to wear particles. Postmenopausal osteoporosis is a common disorder that results from increased osteoclastic activity caused by estrogen deficiency. Whether it can alter bone response to periprosthetic particulate debris is unknown. Our purpose was to evaluate particle-induced osteolysis in an ovariectomized (OVX) murine model.

Materials and Methods
Polyethylene (PE) particles were implanted onto the calvaria of normal controls, OVX mice, OVX mice supplemented with estrogen (OVX+E2), and sham-OVX mice (twelve mice per group). Sham-implanted mice served as internal controls. After 14 days, 7 skulls per group were harvested and analyzed with a high-resolution micro-CT. Then, calvariae were prepared for undecalcified histology. Sections were stained with Stevenel Blue and picrofuchsine, and serial sections were stained for tartrate-specific acid phosphatase-positive (TRAP) osteoclasts. Histomorphometric parameters included sagittal suture area (SSA), bone thickness, total tissue thickness, and the number of osteoclasts in the region of interest.

Five calvariae per group were cultured for 24 hours. Culture media were then collected for the assay of IL-1β, IL-6, TNF-α and RANKL secretion using quantitative ELISA. IL-6 concentrations were measured in blood samples obtained at the time of sacrifice. The expression of RANKL and OPG mRNA was evaluated using Real-time PCR.

Results
Micro-CT evaluation of osteolysis
In particle-implanted animals, the bone/tissue volume ratio significantly decreased as compared to sham-implanted animals in normal control group (p=0.0002, t test), in OVX group (p=0.002), in OVX+E2 group (p=0.004), and in sham-OVX group (p=0.001). As compared to internal controls, particles induced a significant decrease in bone thickness in normal controls (-13.6%, p=0.01) and OVX+E2 mice (-11.6%, p=0.005), while bone thickness remained stable in OVX mice (+3.8%, p=0.08).

Histology
Histological sections showed a consistent erosion of the superficial side of the calvarial bone after particles implantation in all groups (Fig.1). However, the tissue response to particles appeared limited in OVX mice without E2 supplementation. Two-way ANOVA revealed a significant effect of PE particles on SSA (p=0.0001). In particle-implanted calvariae, the number of osteoclasts was 2.2±0.7 in normal controls, and 2.1±0.8 in OVX+E2 mice as compared to 0.7±0.5 in OVX mice (p<0.0001). Mean bone loss was 12±5% and 17±5% in controls and OVX+E2 mice respectively, as compared to 6.7±4.9% in OVX mice (p<0.001).

Discussion and Conclusion
This is the first in depth characterization of consequences of estrogen privation on a murine model of osteolysis. In this study, the combination of two well-known bone resorative mechanisms ultimately attenuated osteolytic response, suggesting that estrogen deficiency had a protective effect on particle-induced osteolysis. These observations were associated with a downregulation of pro-resorptive cytokines TNF-α and RANKL. This phenomenon appears paradoxical as extensive clinical trials and experimental studies have demonstrated the protective effects of estrogen against inflammatory processes, such as rheumatoid arthritis. However, the relationship between estrogen and inflammation remains controversial. Additionally, recent studies delineated a direct role for T cells not only in pathogen clearance but also in regulating adaptive and innate immunity. Regulatory T cells were found to suppress innate-immunity-driven inflammation. Similarly, it is hypothesized that a potential mechanism controlled excessive inflammatory response in our model, illustrated by the absence of increase of serum IL-6 in OVX mice after PE implantation. In this group, we speculate that osteoclastogenesis was controlled by the RANK/RANKL/OPG pathway, synergistically with TNF-α, which both appeared downregulated.