NF-κB Decoy Oligodeoxynucleotide Increases Bone Mineral Density in the Murine Femur during Continuous Infusion of Polyethylene Particles

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Statement of Purpose: Total joint replacement is a very effective surgical procedure in patients with end-stage arthritis. However, wear particle-induced tissue responses may cause chronic inflammation and local bone loss (periprosthetic osteolysis). We have previously shown that modulation of NF-κB activity via decoy NF-κB oligo-deoxynucleotide (ODN) could efficiently suppress multiple pro-inflammatory cytokine expression in macrophages exposed to polyethylene particles. In addition, decoy ODN can protect cell viability and osteogenic differentiation ability of mesenchymal stem cells exposed to the particles. In the current study, we examine the effects of decoy ODN on particle induced bone loss in our established continuous murine particle infusion model.

Methods: Male athymic nude mice at 10-12 weeks of age were used for the experiments. Decoy NF-κB (ODN) was used to modulate NF-κB activity. Ultra-high molecular weight polyethylene (UHMWPE) particles (0.48±0.10μm) were obtained from joint stimulation test samples provided by Dr. Tim Wright at the Hospital of Special Surgery. Alzet mini-osmotic pumps (Model 2006) with a mean pumping rate of 0.15 μl/hour were connected to silicon tubing and a hollow titanium rod place in the distal femur. The pump was filled with various combinations of UHMWPE particles (15mg/ml), decoy ODN (50μM), lipopolysaccharide (1 μg/ml) and connected to the rod implanted into left distal femur (Group 1, Saline; Group 2, UHMWPE; Group 3, UHMWPE + Decoy ODN; Group 4, UHMWPE + scrambled ODN; Group 5, LPS + UHMWPE; Group 6, LPS + UHMWPE + Decoy ODN; and Group 7, LPS+ UHMWPE + scrambled ODN). The mice underwent μCT scans at Day 0 (before surgery) and Day 28 (after sacrifice and removal of the titanium rod) using a GEHC μCT scanner with 49 μm resolution. A 3D region of interest (ROI, 4mm × 3mm ×3mm) was created within the distal part of the femur, and began 3 mm from the distal end of the femur and proceeded proximally. The threshold bone mineral density (TBMD) was quantified by GEMS MicroView (threshold: 700 HU). Each group included 4-5 mice. Statistical analysis was performed using Graph-Pad Prism. The animal protocol was approved by the Stanford University Animal Care Committee.

Results: Infusion of UHMWPE particles significantly reduced BMD in the ROI from 15.79±9.47 mg/ml to -40.22±3.03 mg/ml, while the values for treatment with decoy ODN and scrambled ODN in particle-infused mice were -34.36±4.46 and -52.46±10.30 mg/ml, respectively. Infusion of UHMWPE particles plus LPS significantly increased BMD to 19.76±11.3 mg/ml when compared with the particle alone group. Combined treatment with particles, LPS and decoy ODN showed a trend for increasing the BMD to 54.32±13.95 mg/ml on the operative side (p=0.10). Furthermore, combined treatment also showed similar effects on BMD on the non-operated site, which is consistent with our previous reports. No significant difference in bone volume fraction was observed in all the treatment groups.

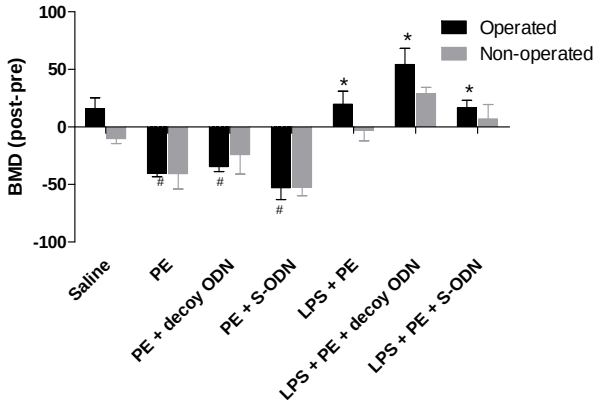

Figure. 1 Bone marrow density (BMD) in the operative and non-operative femur. BMD values in the region of interest were normalized by comparing the scanning data before surgery (post-pre). PE: UHMWPE particles; S-ODN: scrambled ODN. #significant difference compare with saline control, *significant difference compare with PE group.

Conclusions: This preliminary study suggests that targeting NF-κB activity via decoy ODN may have the potential to mitigate wear particle-induced bone loss. Surprisingly, addition of LPS to the UHMWPE infusion increased BMD. Investigation of the underlying mechanisms using histology and immunostaining of osteoblast/osteoclasts as well as infiltrated immune cells markers are currently ongoing.

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