Differential effects of biologic vs bisphosphonate inhibition of wear debris-induced osteolysis assessed by longitudinal micro-CT

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Introduction: While bisphosphonates and RANK antagonists have been developed to prevent osteolysis, there is no clinical evidence supporting their effectiveness in patients with aseptic loosening. Thus, pre-clinical studies to determine superiority are warranted. Here we aimed to develop an in vivo micro-CT outcome measure of wear debris-induced osteolysis for the mouse calvaria model, and utilize this approach to quantify differences between zoledronic acid (ZA) and osteoprotegerin (OPG).

Materials and Methods: The in vivo mouse calvaria experiments were performed as previously described (1). No particles (sham surgery), or 5mg ultra high molecular weight polyethylene (PE) particles were used. A single intraperitoneal injection of PBS (placebo), OPG (5 mg/kg), or ZA (0.1 mg/kg), was administered to the mice 3 days after surgery. In vivo micro CT imaging and volumetric osteolysis analysis. High resolution in vivo micro-CT was performed on day 0 and day 10 after surgery. Using Amira3.1 software, quantification of the osteolytic volume was performed by subtracting a day 10 image from its baseline counterpart. Next, a region of interest defined by the operator as the largest osteolytic volume that fits in a 125x125x125 voxels template was manually isolated using the Crop Editor. Within this sectioned volume, the Segmentation Editor was used to "label" all voxels above a threshold value of 5,000 as calcified bone (Figure 1). Histological evaluation. Ten days after surgery the mice were sacrificed for histology as previously described (2). Osteoclast number were quantified from three contiguous sections 500 μm apart, stained for tartrate-resistant acid phosphatase (TRAP).

Statistical analysis. Statistical significance was determined using a Student’s t-test with Bonferroni correction for six tests of the four treatment groups.

Results: Pre-clinical and clinical testing has determined that OPG and ZA are the most potent drugs in their respective class. We developed in vivo micro-CT methods for the murine calvaria model and compared these drugs head-to-head study. Figure 2 showed the representative volumetric quantification of osteolysis from an 89.6 mm3 region of interest in each treatment group.

Discussion: The major obstacle towards a therapeutic intervention for aseptic loosening is the absence of an effective drug. Although bisphosphonates have proven efficacy for metabolic bone diseases such as osteoporosis, Paget’s disease and, bone cancers, their consistent failure in treating focal erosions in rheumatoid arthritis, has cast doubt as to whether these drugs prevent inflammatory bone loss. On the other hand, RANK antagonists inhibit all forms of bone resorption. Our data supports the theory that inflammatory bone loss is refractory to bisphosphonate therapy, while OPG can completely inhibit PE-induced osteolysis, while the ability of ZA to do so is more variable, and failed to reach statistical significance (p = 0.18) (Figure 3). Traditional histomorphometry from PE treated mice confirmed similar result with micro-CT (not shown). The difference in OPG vs. ZA effects on osteolysis were largely explained by the significant difference in osteoclast numbers observed between these groups in both Ti (0.11 +/- 0.21 vs. 5.53 +/- 3.20; p<0.001) (not shown) and PE (0.25 +/- 0.32 vs. 12.61 +/- 7.09; p<0.001) treated calvaria (Figure 3).

Fig 1. Subtraction of the day 10-bone volume from the day 0-bone volume of a defined region of interest to quantify the volume of osteolysis

Fig 2. Representative reconstructed images of the calvaria and osteolytic volume from each group are shown

Fig 3. The osteolytic volumes quantified using microCT are presented (A). Calvaria were stained for TRAP to quantify osteoclast numbers (B).

References:

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