Activation of Bile Acid Receptor (FXR) Attenuates Osteoclast Differentiation, Survival and Function

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Introduction: Farnesoid X Receptor (FXR) is a nuclear receptor that plays a role in regulating bile acids homeostasis. It has been known that FXR is expressed in liver, adipose tissue, small intestine, and kidneys, and most recently was shown that FXR is expressed in calvaria and bone marrow; thus suggesting a possible role for FXR in regulating bone homeostasis. Recent studies demonstrated that the activation of FXR in osteoblasts increased the expression of osteoblast related-genes such as bone sialoprotein (BSP), osteocalcin (OC), osteopontin (OPN) and alkaline phosphatase (ALP). Other studies also showed the in vivo FXR deletion (FXR-/-) in male mice significantly decreased bone mass and increased numbers of multi-nucleated osteoclasts in FXR-/- when compared to wild type (WT) mice. In this study, we examine the effects of FXR activation on osteoclast formation and activity. Thus, our hypothesis is that activation of FXR inhibits osteoclast differentiation and function.

Methods: Bone marrow-derived hematopoietic stem cells (HSCs) from 4-8 weeks old C57Blk6 male mice were cultured in a 96-well plate at a density of 100,000 cell/well in α-MEM culture media. For the evaluation of the effects of FXR activation on HSCs toxicity and proliferation, cells were treated with different concentrations of GW4064 and evaluated for proliferation using CyQuant DNA fluorescent labeling, and survival using MTT assays. For osteoclast differentiation, HSCs were primed with 20ng/ml MCSF. On day 3 and day 5, media was replaced with α-MEM containing 20ng/ml MCSF and 20ng/ml RANKL to initiate osteoclast differentiation. Cultures were terminated on day 7 for the measurements of TRAP activity and staining. For osteoclast survival assay, multinucleated osteoclasts were cultured for additional two days in the presence of either α-MEM only, RANKL, GW4064 or GW4064 and RANKL together. For the control condition, cells were treated with MCSF and RANKL only. For the GW treatment groups, cells were treated with 1 μM GW4064 (Sigma) either on: day 0, day 3, and day 5, or on day 3 and 5 of culture.

Osteoclasts function was determined using Corning disc Osteoassay and resorption area was quantitated using binary pixel classification using the Nikon NIS Elements Imaging software. Statistical analysis was carried out by unpaired two-tailed t-test. Asterisk (*) indicates the significant differences where p-value < 0.05.

Results: GW4064 has no significant effects on HSC proliferation or survival. In contrast, GW4064 inhibits HSCs differentiation into multinucleated osteoclasts, where osteoclast TRAP activity and number are significantly decreased in cells treated with GW4064 and RANKL compared to RANKL alone (Figure. 1). Interestingly, GW4064 significantly inhibits survival of multinucleated osteoclasts when added to RANKL compared to RANKL alone. For osteoclast function, following differentiation of HSCs on corning discs, our results show that GW4064 significantly inhibited osteoclast activity in disc resorption compared to RANKL alone (Figure. 2). Gene expression analysis of osteoclast-related markers showed that mRNA for TRAP, cathepsin K, RANK receptors, and calcitonin receptors is significantly lower in osteoclasts differentiated with GW4064 and RANKL compared to RANKL alone.

Discussion: As a result of this study, our data demonstrated that FXR activation using the FXR agonist; GW4064 negatively regulates osteoclast differentiation by decreasing TRAP activity, number and size. Osteoclast markers were also significantly decreased in treated cells as a result of cell differentiation suppression. From the proliferation and survival Assays of HSCs, GW4064 has no toxic effect on HSCs; while the effect of GW4064 on survival of fully differentiated osteoclasts is significantly suppressing. This study also demonstrated that activation of FXR causes inhibition of osteoclast function. Taken together, these data suggest that FXR plays a role in osteoclast differentiation and function.

Significance: In clinical practice, the control of osteoclast function is the key for treating osteoporosis and osteoarthritis that could possibly resulted from the invasion of osteoclasts from the subchondral bone to the articular cartilage. GW4064 might be a therapeutic treatment for limiting osteoclasts function and treat osteoporosis and osteoarthritis.

Acknowledgments:

Id Boufker, H., et al., Role of farnesoid X receptor (FXR) in the process of differentiation of bone marrow stromal cells into osteoblasts. Bone, 2011. 49(6): p. 1219-
Figure 1: GW inhibits differentiation of bone marrow-derived hematopoietic stem cells (HSC) into osteoclasts. HSC from BMM6 were differentiated with RANKL with and without GW4064 then cultures were fixed for TRAP staining and activity. (A) Microscopic pictures of TRAP stained osteoclasts showing smaller osteoclasts in GW treated conditions. (B) TRAP activity is measured in RANKL with and without GW. Data in panel (A) and (B) represent Mean ± SEM in n-wells per experiment. Experiment was repeated 3 times and showed similar pattern. *** (p<0.01).

Figure 2: GW4064 inhibits osteoclasts function. HSC from BMM6 were differentiated with RANKL with and without GW4064 then cultures were terminated using 10% bleach. (A) Microscopic pictures of cortical bone disks showing less resorbed area from osteoclasts in GW treated conditions. (B) Resorbed area quantification was measured using the NIS Elements imaging software. Data in panel (A) and (B) represent Mean ± SEM in n-wells per experiment. Experiment was repeated 3 times and showed similar pattern. ** (p<0.05).