Effects of a Dynamin Inhibitor on Osteocytes in Ovariectomized Mice.

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Abstract

Osteocytes perform a significant role in the maintenance and integrity of bone. Osteocytes reside in mineralized matrix within specialized structures called lacunae. They are involved in cell-to-cell networking with each other as well as with osteoblasts and osteoclasts via long extensions called dendrites. Changes in the viability of osteocytes and disruption of the dendritic network can lead to changes in bone remodeling which leads to changes in bone quality and quantity. Inhibition of the dynamin GTPase affects the bone resorbing activity of osteoclasts and promotes the bone-forming activity of osteoblasts.

In this study, we examined the role of dynamin on osteocytes in vivo. Female mice were subject to ovariectomy (OVX) to deplete estrogen and promote bone loss and then treated without/with the dynamin inhibitor Dynasore (DS) for 4 weeks (10 mg/kg, 3 days/week). Three groups were examined: sham controls, OVX, and OVX+DS. After sacrifice, midshaft coronal sections of tibia were stained with H&E. Total lacunae density per cortical area, and the number of lacunae containing osteocytes (full lacunae) versus empty lacunae (likely due to osteocyte death) were counted using Bioquant software. The lacunae density per cortical area was increased in OVX but not OVX+DS bones. OVX also had the lowest percentage of full lacunae and, correspondingly, the highest percentage of empty lacunae, while OVX+DS bones were intermediate between OVX and sham (OVX=OVX+DS>sham) (p<0.05). Although more studies are needed, the increase in lacunae density with OVX may be caused by a decrease in osteocyte survival (decreased % full lacunae) and loss of matrix-mineral making lacunae more visible by histology. Dynasore may partially protect cortical bone from the negative effects of OVX by potentially increasing osteocyte survival (increased % full lacunae) and reducing the loss of matrix-mineral. These findings have implications for dynamin-targeted strategies to improve bone mass and integrity.

Introduction

- Osteoporosis is a disease that causes low bone density and bone fracture more easily.
- Bone loss is also associated with oral health problems such as periodontal disease and temporomandibular joint (TMJ) disorder.
- Osteocytes are matrix-embedded dendritic bone cells responsible for bone maintenance and integrity.
- Aging affects osteocyte viability and leads to dendrites that are shorter with lesser connectivity compared to the young human bone.
- Estrogen plays a pivotal role in bone diseases such as osteoporosis because it causes an increase in the ratio of osteoclasts to osteoblasts.
- Estrogen loss after menopause or after ovariectomy (OVX) in mice, leads to an increase in osteocyte apoptosis.
- Dynasore, a specific chemical inhibitor of dynamin GTPase, is vital for actin remodeling and migration in osteoclasts. Dynasore regulates differentiation and migration of osteoclasts, however, effects of dynamin on osteocyte dendrite remodeling is unknown.

Methods and Materials

Cell Culture. 1.2x10⁶ MLO-Y4 cells (murine long bone osteocyte Y4) were cultured on collagen-coated 6-well plates with 5% serum-osteocyte culturing medium (α-MEM supplemented with 2.5% (v/v) FBS, 2.5% (v/v) FCS and 100 U/ml penicillin/100 µg/ml streptomycin) in an incubator at 37°C with 5% CO₂. Three groups of 4 mice each were examined: sham controls, OVX and OVX+DS. A sham ovariectomy surgery (OVX) was done on the control group. Removal of ovaries were done on another two group of mice. One OVX group of mice received intraperitoneal injection of dynasore (DS 10mg/kg for 3 days a week for 4 weeks). After sacrifice, midshaft coronal sections of tibia were stained with H&E (Hematoxylin and eosin stain).

Measuring Lacunae Density. To determine if there were any changes as a result of the treatment with OVX and Dynasore, total lacunae density per cortical area, and the number of lacunae containing osteocytes (occupied lacunae) versus empty lacunae (likely due to osteocyte death) were counted using Bioquant software, were measured.

Results

- **Effect of Dynasore on Lacunae Density**
  - Total lacunae density/area
  - sham vs. OVX vs. OVX+Dynasore
  - Figure 1.1: Total Lacunae density/area. The total lacunae density was increased in OVX mice. There was no difference between OVX and OVX+DS. The increase in lacunae density with OVX may be caused by a decrease in osteocyte survival (decreased % full lacunae) and/or loss of matrix-mineral. (*p<0.05)

- **Effect of Dynasore on Osteocytes in Lacunae**
  - In Cortical Bone Coronal Sections
  - Figure 2. Effect of Dynasore on Osteocytes in Lacunae. OVX had the lowest percentage of full lacunae and, correspondingly, the highest percentage of empty lacunae. (*p<0.05)

Summary & Conclusion

- Female mice were subject to ovariectomy (OVX) show evidence of depleted estrogen and resulting bone loss.
- The increase in lacunae density with OVX was unexpected and may be caused by a increase in lacunae size or visibility of osteocytes.
- Dynasore may partially protect cortical bone from the negative effects of OVX by potentially increasing osteocyte survival (increased % full lacunae) and reducing the loss of matrix-mineral.
- Our data suggest that the connection between dynamin GTPase activity in controlling activity of osteocytes may be valuable in understanding bone homeostasis. These findings have implications for dynamin-targeted strategies to improve bone mass and integrity.
- Future work will need to be done to discover more about the function of Dynamin in osteocytes, mainly in the signaling pathway.

References


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