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EFFECTS OF CALCIMIMETICS ON OSTEOCLAST-DEPENDENT BONE MINERALIZATION

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Objectives:

Patients with chronic kidney disease (CKD) have a high risk of osteoporosis and bone fracture owing to the mineral and bone disorder associated with CKD. Since calcium receptor plays an important role in differentiation and apoptosis of osteoclasts, we believe that calcimimetic agents might have a definitive role to influence osteoclast bone resorption, and subsequent osteoblast bone formation locally, by inducing different bone formation-stimulating osteoclast-derived factors (clastokines). Through studies of cinacalcet-induced clastokine Wnt10b secretion, we evaluate its physiological role in bone mineralization.

Methods:

Bone marrow hematopoietic mononuclear cells were isolated from rat femur and tibia. They were induced into osteoclasts by M-CSF and RANKL treatments. Wnt10b concentration in supernatant collected from the cinacalcet-treated osteoclasts was analysed by ELISA. Western blot analysis was used for measurement of intracellular Wnt10b in the cinacalcet-treated osteoclasts. Alizarin red staining was used to evaluate the mineralization effect of culture osteoblasts, which was isolated from neonatal rat calvarias and cultured with cinacalcet-treated osteoclasts supernatant.

Results:

In osteoclasts, cinacalcet induced a decrease of TRAP stain-positive reaction. However, ELISA analysis showed increases of Wnt10b expression in supernatant collected from the cinacalcet-treated osteoclasts. Western blot analysis also showed an increase in intracellular Wnt10b in the cinacalcet-treated osteoclasts. Culture of osteoblasts with the cinacalcet-treated osteoclasts supernatant showed an increase of mineralization as indicated by alizarin red staining.

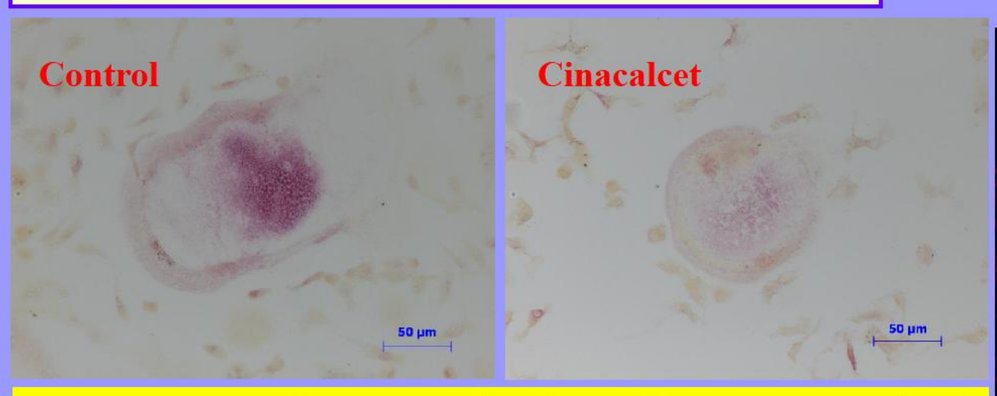

Fig 1. TRAP stain analysis in osteoclast derived from RANKL-stimulated monocytes. (A) TRAP stain (red) of osteoclasts, control. (B) TRAP stain (red) of osteoclasts, treated with 400 nM cinacalcet 6 hr. Bar = 50 µm.

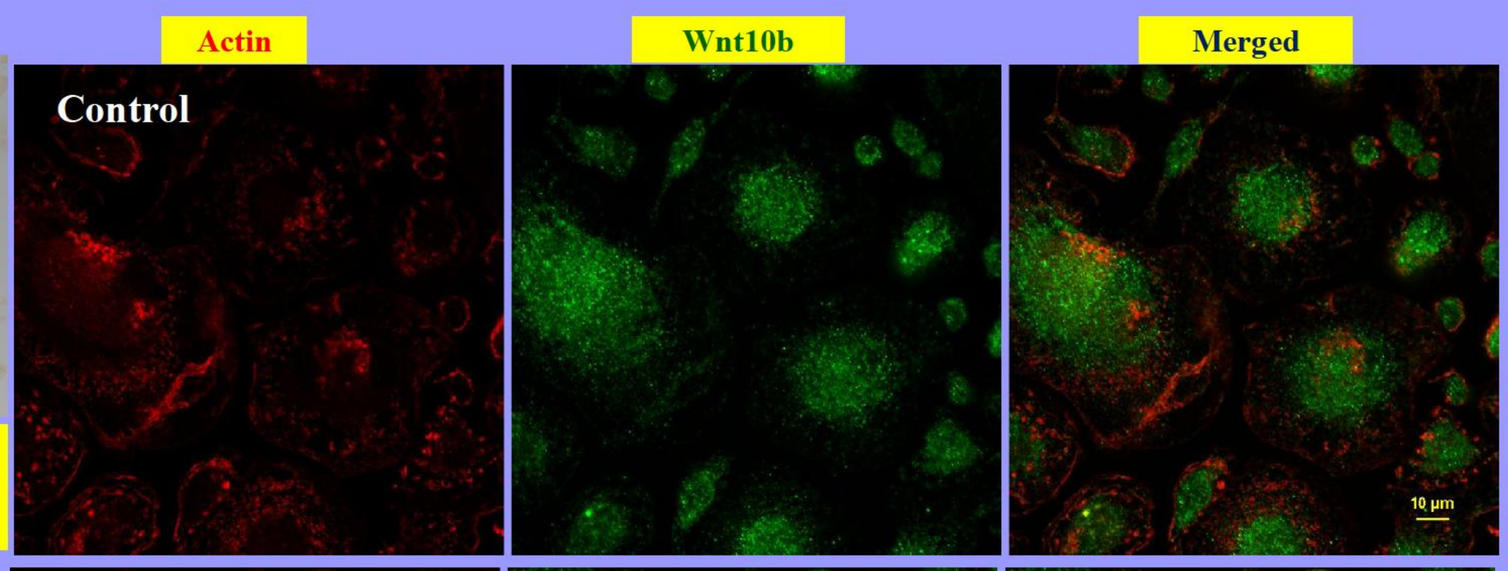

Fig 3. Confocal analysis of im munofluorescent labeling of Wnt10b (green) in cinacalcet-treated osteoclasts. Actin was labeled with Cy3 (red). Bar = 10 µm.

Wnt 10b of conditional medium

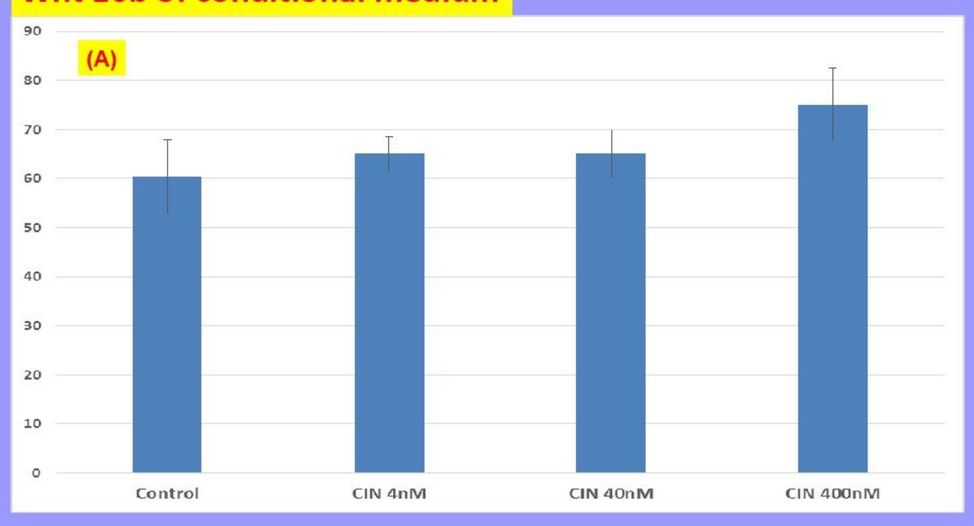

Fig 2 A & B. Wnt10b expression in osteoclasts. Osteoclasts were treated with culture medium plus RANKL (Control), 4nM, 40nM and 400nM cinacalcet. Increased expression of Wnt10b was found to be increased and dose dependent in (A) osteoclasts was analyzed by Western blot and (B) osteoclasts analyzed by ELISA.

Alizarin red stain

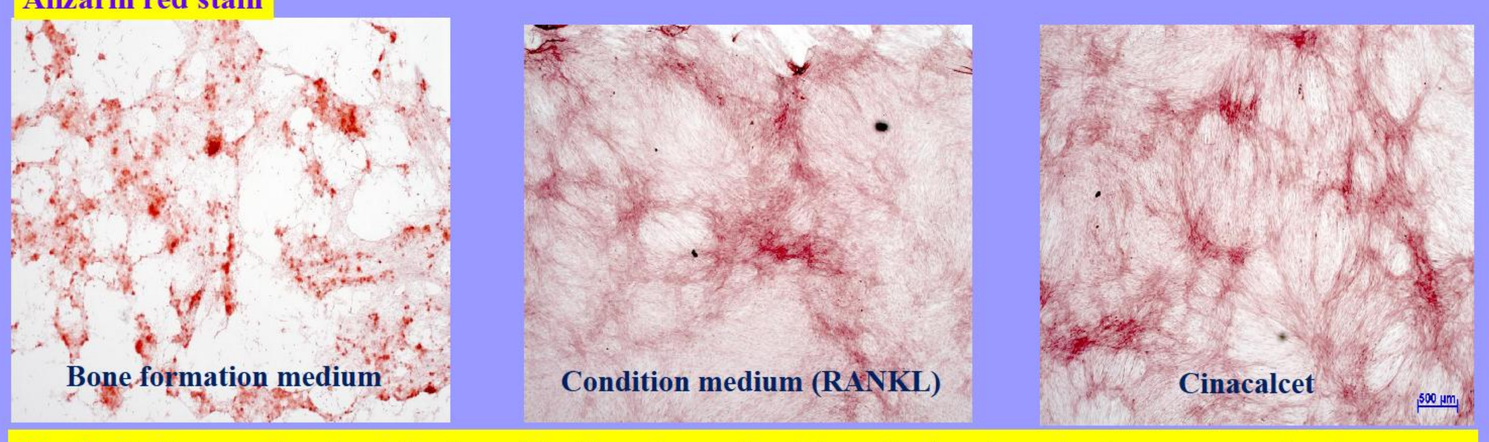

Fig 4. Culture of osteoblasts with the cinacalcet-treated osteoclasts supernatant showed an increase of mineralization as indicated by alizarin red staining.

Conclusions:

Cinacalcet decrease osteoclasts activity, but increase Wnt10b secretion which may work as osteoblast anabolic factors contributing to the coupling activity at the previous resorption sites.

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