OC07

Gender specific effects of the calcium channel TRPV4 on osteoporotic fracture risk and osteoblast–osteoclast coupling

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TRPV4 is a member of the transient receptor potential (TRP) superfamily and responds to an array of stimuli, including osmolarity, pH and pressure. Recent findings showing that TRPV4 deficiency leads to reduced sensing of mechanical stimuli led us to explore the role of TRPV4 in bone.

TRPV4 mRNA was abundantly expressed in both osteoblasts and osteoclasts as assessed by qPCR. Femoral cortical and trabecular bone mass (thickness and volume) as assessed by microcomputed tomography was higher in male TRPV4 knockout mice compared to wild type mice. Despite thicker bone structures, cortical porosity was increased in the male TRPV4 knockout mice leading to reduced bone strength as assessed by 3-point bending. Osteoclast and osteoblast differentiation and function was studied, using bone marrow cultures from wildtype and TRPV4 knockout mice. Osteoclast numbers (TRAP staining) as well as the formation of resorption pits (coomassie brilliant blue staining) were significantly reduced in cultures of TRPV4 knockout mice compared to wildtype littermates. In contrast, osteoblast differentiation (alkaline phosphatase activity) and matrix mineralization (alizarin red) was significantly increased in TRPV4 knockout bone marrow cultures. None of these parameters were significantly different in bones and bone marrow cultures of female knock out mice. These data implicate a gender-specific osteoblast–osteoclast uncoupling and support the observed increase in bone mass in male TRPV4 deficient mice. To assess the possible impact of TRPV4 on osteoporotic outcome in humans, we extracted data from the genome-wide association study (Illumina 550K SNP array) within the Rotterdam Study ($\pm$6000 individuals, 55 years and older). Two single nucleotide polymorphisms (SNPs) in the *TRPV4* gene showed strong associations with osteoporotic fracture risk (Hazard Ratio 1.8; Confidence Intervals 1.2–2.7), fragility fracture risk (HR 2.6; CI 1.4–4.9) and hip fracture risk (HR 2.7; CI 1.3–5.7) in men, but not in women. This was not affected after adjusting for height, weight, age and bone mineral density (BMD).

In conclusion, TRPV4 plays an important role in male but not female bone biology. Apparently, the increased periosteal bone apposition fails to overcome the increased cortical porosity, leading to reduced bone strength in TRPV4 deficient male mice. In line with the gender-specific findings in mice, variations in the *TRPV4* gene are predicting fracture risk in men but not in women.

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OC08

Does the growth hormone-derived peptide AOD9604 have an anabolic effect on bone?

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A synthetic human Growth Hormone (hGH) 16-AA C-terminus peptide, AOD9604 (AOD, Tyr-hGH171–191), has been shown to modulate fat metabolism. As hGH is known to affect both adipocytes and osteoblasts, AOD may also affect bone. Previously, we have shown that AOD can prevent OVX-induced bone loss and fragility. This study focuses on the ability of AOD to rebuild the bone lost during ovariectomy (OVX) in a rat model of postmenopausal osteoporosis. Nine month-old female rats were ovariectomized and left without treatment for 12 weeks in order to lose bone. The OVX rats were divided into an untreated control group ($N=13$) and three groups treated with increasing doses of AOD (0.01 mg/kg/day [$N=13$], 0.03 mg/kg/day [$N=14$], 0.25 mg/kg/day [$N=14$]) administered orally by gavage for 12 weeks. A sham-OVX operated group ($N=15$) served as a negative control. The two control groups were given vehicles by gavage as per the AOD treated groups. Following sacrifice, BMD was assessed using dual energy X-ray absorptiometry (DXA) and bone quality using mechanical testing techniques (three-point bending, torsion, femoral neck fracture and vertebral compression), structural analysis of cortical and trabecular bone, mineralization (back scattered electron imaging (BSE)) and histomorphometry. DXA results showed no significant increase in femoral or vertebral BMD following AOD administration. Analysis of the femoral diaphyseal cross-section revealed that the highest dose of AOD caused a significant increase in the anterior–posterior diameter and, concomitantly, the cross-sectional area. This increase indicates that AOD caused bone deposition at the periosteal surface of the femur. Femoral torsion testing revealed a significant increase in stiffness for all three AOD-dosed groups, which, upon normalization, is preserved in shear modulus. This increase in stiffness and shear modulus is in accordance with BSE results which revealed a non-significant shift towards increased mineralization of both cortical and trabecular bone. Vertebral compression showed a dose-dependent increase in ultimate stress and elastic modulus. This restorative effect of AOD on vertebral bone strength parallels the results obtained from histomorphometric analysis which show an increase in trabecular bone volume and trabecular number and a decrease in trabecular separation versus OVX, as well as an increase in osteoid volume over the sham group. In conclusion, our results suggest that AOD has both an anabolic and anti-resorptive effect on the rat skeleton, which is more pronounced in trabecular bone.

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OC09

Neonatal bone marrow transplantation without prior conditioning rapidly reverses osteopetrosis in oc/oc mice despite only minimal donor cell engraftment

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Infantile malignant osteopetrosis (IMO) is caused by lack of functional osteoclasts leading to skeletal abnormalities, blindness due to optic nerve compression and early death. In a majority of patients TCIRG1, encoding a subunit of a proton pump essential for bone resorption, is mutated. A mouse model of the disease, the oc/oc mouse, also has a deletion in tcirg1, and die around 4 weeks of age. We have shown that oc/oc mice can be rescued by neonatal