Effect of luteolin on bone resorption, bone loss and microarchitecture in ovariectomized mice

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INTRODUCTION
Osteoporosis is a common, systemic skeletal disease characterized by low bone mass, low bone strength and microstructural deterioration of bone tissue with a consequent increase in bone fragility. It is most common in postmenopausal women, because estrogen deficiency causes an imbalance of osteoclastic bone resorption and osteoblastic bone formation, resulting in deterioration of bone tissue and low bone mass. Current therapy for osteoporosis include agents that either inhibit bone resorption or stimulate bone formation thereby leading to a more efficient recovery of bone mass in osteoporosis. Recently, attention has been focused on phytoestrogens as possible alternatives, or at least adjuncts, to HRT. Phytoestrogens such as genistein and daidzein were found to prevent bone loss in ovariectomized rats. However, the impact of luteolin on experimental models of bone and osteoclastic cells is still unclear. Therefore, we examined the effects of luteolin on osteoclast differentiation and ovariectomized (OVX) animal model.

Materials and Methods:

In vitro
For the osteoclastogenesis experiments, bone marrow mononuclear (BMM) cells were plated in a 96-well culture plate at a density of 4X10⁵ cells/well and cultured in α-MEM containing 10% FBS in the presence of 20 ng/ml RANKL and 10 ng/ml M-CSF, with or without 1, 2, 5 and 10 µM of luteolin. In Vivo
Total of 32 female C57BL/6 mice were used at 8 weeks of age. One week later, Bilateral ovariectomy was performed on the ovariectomized (OVX) animals via a dorsal midline incision, while the SHAM mice underwent a sham procedure. Administration of luteolin started 7 days after the surgical procedure. The mice were assigned to the following treatments: SHAM and OVX groups, OVX+luteolin 5 mg/kg BW/day and OVX+luteolin 20 mg/kg BW/day administrated orally for 30 days. Effect of luteolin on the OVX mice was evaluated with µCT bone morphometric analysis, bone metabolic marker assay (alkaline phosphatase, osteocalcin, CTX) and three-point bend testing. Data were analyzed by One-Way ANOVA. A p value of less than 0.05 was considered significant.

RESULTS
In vitro, luteolin effectively suppresses both the number of TRAP-positive MNCs and the activity of TRAP, suggesting that luteolin exerts antiresorptive actions by directly inhibiting osteoclast differentiation.
In vivo, luteolin had increased bone mineral density (BMD), bone mineral content (BMC), tissue mineral density (TMD), bone volume fraction, trabecular number, cortical BMD and cortical BMC relative to control OVX mice. Luteolin treatment resulted in enhanced serum concentration, as shown by effects on alkaline phosphatase, osteocalcin and CTX. Luteolin group (20mg/kg) similarly showed significantly higher serum ALP levels as compared to the OVX groups. In osteocalcin and CTX, the luteolin group (5mg/kg, 20mg/kg) was significantly lower than the OVX groups. Also effect of luteolin on cortical bone was further supported by the biomechanical testing of femurs. A three-point bending test showed the maximal load and stiffness were higher in femurs from luteolin treatment relative to untreated OVX mice.

CONCLUSION
These results suggested that effect of luteolin is mediated by its inhibitory effects on RANKL-induced osteoclast formation and prevent bone loss in OVX mice. Luteolin may provide more effective therapeutic implication by progressive bone loss including osteoporosis.

REFERENCES

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Figure 1. Histomorphometric analysis of distal femurs in mice treated with luteolin at doses 5 and 20 mg/kg for 30 days after OVX. Luteolin treatment increased the Bone Mineral Density (BMD), Bone mineral Content (BMC), Bone volume Fraction (BVF), Tissue Mineral Density (TMD), Cortical Bone Mineral Density (Cr.BMD), Cortical Bone Mineral Content (Cr.BMC). *, P<0.05 vs. sham control; #, P<0.05 vs. OVX.