Epimedium-derived Flavonoids Promote Osteoblastogenesis of Bone Marrow Mesenchymal Stem Cells during Exerting Anabolic Effect on Osteoporotic Bone

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INTRODUCTION: Epimedium-derived Flavonoids (EFs) have been reported to exert beneficial effects on treatment of osteoporosis in late postmenopausal women without detectable hyperplasia effect on the endometrium1, and the anabolic effects on the trabecular bone was also found in EFs-treated rats during ovariectomy-induced osteoporosis development2. However, there are two open questions: one is that the anabolic effect of EFs on bone during progress of osteoporosis remains to be evidenced histomorphometrically; the other one is that the underlying mechanism remains to be explored if the anabolic effect could be validated. Accordingly, this study evaluated the effect of EFs on osteoporotic bone histomorphometrically, and the effect of EFs on osteoblastogenesis of bone marrow mesenchymal stem cells (MSCs) at cellular and molecular level was also explored since it is of the known important cellular events during bone anabolism.

MATERIALS AND METHODS: Eleven-month-old female Wistar rats were divided into five groups with ten animals per group: (1) sham operated (SHAM); (2) ovariectomized (OVX); (3) SHAM+ solvent vehicle (SHAM-SV); (4) OVX+ sv (OVX-SV); (5) OVX + EF 10 mg/kg body weight/day (OVX-EFs). Groups 1 and 2 were sacrificed after three months to establish that bone loss in proximal tibia had occurred due to OVX. The remaining groups began four months of treatment, at the end of which the animals were also sacrificed. At sacrifice, serum was collected for analysis of C-terminal telopeptides of type I collagen (ICTP) and tartrat-resistant acid phosphatase 5b (TRACP 5b). Trabecular bone mineral density (BMD) in proximal tibia was analyzed by both microCT for static histomorphometric parameters and un-decalcified histology examination for dynamic histomorphometric parameters. The effect of the conditioned serum on proliferation / differentiation of rat-derived bone marrow MSCs were assessed for colony formation assay (CFU-F, CFU-Osteo and CFU-Adipo). Osteogenic and adipogenic related genes expression was analyzed by real-time PCR. MAPK and Wnt signaling pathways were examined by Western Blotting in MSCs cultures.

RESULTS: A significantly low trabecular BMD in proximal tibia was found by MicroCT at three-month post ovariectomy in OVX Group compared to SHAM Group (p<0.01). EFs treatment significantly increased serum bone formation marker ICTP and decreased bone resorption marker TRACP 5b compared to OVX-SV rats (p<0.05 for both). Bone volume (BV/TV) and Trabecular Thickness (Tb. Th) were significantly increased in OVX-EFs Group compared to OVX-SV Group (p<0.05 for both) by MicroCT analysis (Figure 1). Un-decalcified histological examination demonstrated that EFs significantly increased trabecular bone mineralizing surface (MS/BS, p<0.05), bone formation rate (BFR/BS, p<0.01) and mineral apposition rate (MAR, p<0.05) compared to OVX-SV Group. CFU-F assay demonstrated that there is no significant difference for the proliferation rate of MSCs in OVX-EFs Group and OVX-SV Group (p>0.05). EFs significantly increased osteogenesis and decreased adipogenesis of MSCs as evidenced by the colony formation examination and real-time PCR analysis compared to OVX-SV Group (Figure 2). Western Blotting indicated that EFs can significantly enhance the phosphorylation level of ERK and p38 MAPK signaling and accelerated the degradation of phosphorylated β-catenin (Figure 3).

DISCUSSION: Our findings indicated that EFs could exert the anabolic effect on osteoporotic bone at the micro- and tissue level. The underlying mechanism was partially explained by increased osteogenic differentiation of bone marrow MSCs, which required MAPK and Wnt signaling pathway.


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Fig. 1. Representative 3-D image of trabecular bone core in different groups.

Fig. 2. Effect of conditioned serum from OVX rats treated with SV or EFs on colony formation of BMSCs cultured in 24-well plate.

Fig. 3. Detection of phosphorylation of MAPK and Wnt signaling molecules during the treatment of BMSCs with conditioned serum from SV or EFs treated rats.