Icariin inhibits osteoclast differentiation and bone resorption by suppression of MAPKs/NF-κB regulated HIF-1α and PGE2 synthesis

INTRODUCTION
The human bone is a highly dynamic organ that maintains its homeostasis through a delicate balance between the bone-forming osteoblasts (bone formation) and the bone-eroding osteoclasts (bone resorption). The dynamic balance between these two cell types results in bone remodeling. Increased osteoclast activity induces erosion of trabecular bone and fragile bones. Conversely, increased osteoblast activity increases bone density, which is associated with bone deformity and osteoporosis (Boyle et al., 2003). Many plant-derived natural products have been used in traditional medicine for the treatment of various diseases. Herba Epimedii is a traditional Chinese herbal medicine, which has been commonly used as tonic, aphrodisiac and anti-rheumatic in China for thousands of years. Its physical and functional characteristics have been thoroughly documented in the Chinese pharmacopoeia 2005. Icariin (C33H40O15; molecular weight: 676.67), the main active flavonoid glucoside isolated from Epimedium pubescens, has been found to have a therapeutic effect on osteoporosis by ovariectomy rat models and postmenopausal women (Zhang et al., 2007). Further studies demonstrated that icariin also suppressed mouse osteoclast differentiation (Lu et al., 2007), but more detailed molecular mechanisms underlying these effects remain unclear. Recently, we found that icariin might exert its osteogenic effects through induction of bone morphogenic protein-2 (BMP-2) and NO synthesis, subsequently regulate core binding factor A1/runt-related transcription factor 2 (Cbfal/Runx2), and RANKL genes expression (Hsieh et al. 2009). In the present study, we further examined the detailed molecular mechanisms of the effect of icariin on LPS-induced osteocytosis by primary co-culture models obtained from adult female mice. Our hypothesis is that icariin can inhibit osteoclast differentiation and bone resorption by suppressing MAPKs/NF-κB regulated HIF-1α and PGE2 synthesis.

MATERIALS AND METHODS
Cells were treated with different concentration of Icariin in 90% MEM with 1% FBS, 26gM ascobic acid 2-phosphate, 10M α2,5G02VY D3, and 1μg/ml LPS with or without icariin. After 6 days incubation, cells were fixed in 4% formaldehyde in PBS for 20 min, stained for TRAP activity using a commercialized kit (Sigma Chemical, St. Louis, MO, USA) according to the manufacturer’s instruction. After incubation at room temperature for one hour, cells were washed with distilled water for three times. Red color-TRAP-positive cells were observed and photographed for further analysis. The co-culture cells were also seeded on BD Biocult™ Osteologics™ Multitest Slides to analyze their in-vitro bone resorption ability. Their gene expression and signal pathways were analyzed by semi-quantitative real-time PCR The cytokines, including IL-6, TNF-α and prostaglandin E2 (PGE2) were analyzed by enzyme-linked immunosorbent assay (ELISA) and western blotting analysis. The results were expressed as mean ± standard deviation of these experiments and statistically analyzed by Two-way ANOVA. Statistical significance by Dunnett’s test was set at p < 0.05 between the means of the control and test groups. Each experiment was performed more than three times, and results from one representative experiment were shown.

RESULTS AND DISCUSSION
In this study, we examined the effects of icariin on the activity and differentiation of adult female mice osteoclasts. We found that icariin acted on osteoclasts to suppress osteoclast cell differentiation and ACP and TRAP activities. In the model of LPS-induced osteoclastogenesis, we observed that icariin reduced LPS-induced ACP and TRAP activities, inhibited bone resorption, while with no effect on osteoclasts viability. This suggested that the osteoclastogenesis suppression by icariin was not due to toxic effect on osteoclasts. The level of RANKL and OPG are critical in the regulation of the osteoclast formation and differentiation (Chakravarti et al., 2009). Our study showed that icariin can significantly down-regulate LPS-mediated RANKL expression and up-regulate LPS-suppressed OPG expression. Many cytokines are involved in LPS-mediated osteoclasts formation. LPS stimulates the target cells to produce pro-inflammatory cytokines such as IL-6 and TNF-α, which have been shown to support the survival of osteoclasts (Itoh et al., 2003). Our results showed that icariin diminished LPS-induced IL-6 and TNF-α mRNA and protein expression on osteoclasts; we also found that icariin decreased LPS-mediated PGE2 production by inhibiting COX-2 on both cell types. In previous study, LPS has been shown to promote osteoclastogenesis by activating various intracellular signaling pathways including NF-κB, JNK, ERK1/2, and p38. Binding of LPS to toll-like receptor 4 activates NF-κB and MAPKs, which induces production of pro-inflammatory cytokines such as IL-6 and TNF-α (Liu et al., 2009). In this study, we found that icariin suppresses LPS-mediated activation of the p38 and JNK on osteoclasts, but not on osteoblasts, supporting the idea that icariin inhibited LPS-induced osteoclastogenesis program by suppressing activation of the p38 and JNK pathway. HIF-1α plays an important role in the regulation of osteoclasts cell size, and has recently emerged as a central regulator of bone biology (Bozec et al., 2008). Activation of HIF-1α enhances osteoclastogenesis on osteoblasts, and also increases osteoclasts differentiation and bone resorption (Knowles & Athanasou, 2009). In this study, we found that LPS can induce HIF-1α expression on osteoclasts, but not osteoblasts. This data suggested that icariin reduced bone resorption through suppression of LPS-regulated HIF-1α expression of osteoclasts under normoxia condition. In summary, our results indicate that icariin has an in vitro inhibitory effects on osteoclasts differentiation that can prevent inflammatory bone loss. Icariin inhibits LPS-induced osteoclastogenesis program by suppressing activation of the p38 and JNK pathway. However, the in vivo molecular mechanisms about the icarin function on the LPS-induced osteoclastogenesis need to be further investigated.

REFERENCES

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