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Effect of safflower seeds supplementation on stimulation of the proliferation, differentiation and mineralization of osteoblastic MC3T3-E1 cells

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Abstract

Anti-bone resorption properties of the Korean herbal formulation, Gami-Honghwain (HJ), which comprises *Carthamus tinctorius* L. seed and hominis placenta, were investigated. We demonstrate that the production of PGE2 is inhibited by 20–100 μg/ml HJ in nontransformed osteoblastic cells (MC3T3-E1 cells), indicating that HJ inhibits PGE2 production. The effect of HJ on the proliferation and osteoblastic differentiation in MC3T3-E1 was also studied. HJ dose-dependently increased DNA synthesis (significant at 20–100 μg/ml), and increased alkaline phosphatase (ALP) and prolyl hydroxylase activities of MC3T3-E1 cells (20–100 μg/ml), while anti-estrogen tamoxifen eliminated the stimulation of proliferation and ALP activity of MC3T3-E1 which was induced by HJ. These results indicate that HJ directly stimulates cell proliferation and differentiation of osteoblasts. Also, when we assessed the effects of HJ on osteoblastic differentiation in MC3T3-E1, HJ enhanced ALP activity and mineralization in a dose- and time-dependent fashion. This stimulatory effect of the HJ was observed at relatively low doses (significant at 20–100 μg/ml and maximal at 100 μg/ml). Northern blot analysis showed that the HJ (60 μg/ml) increased in bone morphogenetic protein-2 as well as ALP mRNA concentrations in MC3T3-E1 cells. HJ (100 μg/ml) slightly increased in type I collagen mRNA abundance throughout the culture period, whereas it markedly inhibited the gene expression of collagenase-1 between days 15 and 20 of culture. These results indicate that HJ has anabolic effect on bone through the promotion of osteoblastic differentiation, suggesting that it could be used for the treatment of common metabolic bone diseases.

Keywords: Honghwain-Jahage; Osteoblast; Mineralization; Bone morphogenetic protein-2; Alkaline phosphatase; Collagenase-1; Prostaglandin E2; Proliferation; Differentiation

1. Introduction

Korean traditional medicine, Gami-Honghwain (HJ), which comprises *Carthamus tinctorius* L. seed and hominis placenta, is known to be effective for the treatment of inflammation, hyperlipemia, arteriosclerosis and gynecological diseases, such as osteoporosis and bone resorption, according to the ancient traditional herbal literature (Shi, 1983). The HJ is also regarded as an effective biological response modifier for augmenting host homeostasis of body circulation (Shi, 1983). The pharmacological action of HJ has been limitedly studied in regard to gynecological diseases. This herbal medicine has been shown to express diverse activities such as immunomodulating, anti-infarction, anti-allergic and anti-inflammatory effects (Hong, 1998). It is a hemostatic agent, promoting blood coagulation (D.I. Kim et al., 1999). Recently, Yoo et al. (2006) isolated the lignan glycoside tracheloside from seeds of *Carthamus tinctorius* as an anti-estrogenic principle against cultured Ishikawa cells by employing a bioassay-linked method. Tracheloside

Abbreviations: HJ, Gami-Honghwain; ALP, alkaline phosphatase; BMP, bone morphogenetic protein; MMP-1, matrix metalloproteinase-1; PGE2, prostaglandin E2; IL-1, interleukin-1; FBS, fetal bovine serum; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified eagle's medium.

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significantly decreased the activity of alkaline phosphatase, an estrogen-inducible marker enzyme. With regard to anti-oxidative activity, ethanol–ethyl acetate extract of *Carthamus tinctorius* seeds inhibited low-density lipoprotein oxidation in vitro. Two serotonin derivatives [*N*-(*p*-coumaroyl)serotonin and *N*-feruloylserotonin] and their glucosides were then identified as the major phenolic constituents of the extract. Orally administered *Carthamus tinctorius* seeds suppressed lipid peroxidation and decreased anti-oxidized low-density lipoprotein autoantibody titers (Koyama et al., 2006). Thus, it still occupies an important place in traditional Oriental medicine. There is also a resurgence of interest in herbal medicines in western countries as an alternative source of drugs often for intractable diseases such as bone loss (Phillipson and Anderson, 1989). The need for safer and effective anti-inflammatory drug and the lack of enough scientific data to support the claims made in ancient literature prompted the present study.

Attempts have been made to use a combination of antiresorptive agents, such as estrogen, and bone formation-stimulating agents, such as growth hormone, to treat bone loss (Tunner, 1991). A sharp decrease in ovarian estrogen production is the predominant cause of the rapid hormone-related bone loss during the first decade after menopause (Gruber et al., 1984). Therefore, traditional therapeutic agents for post-menopausal osteoporosis have been estrogen, calcitonin and bisphosphonate, which inhibit bone resorption. For example, if bone resorption exceeds bone formation, diseases of bone loss occur (Riggs and Melton, 1992). Bone forming osteoblasts arise from mesenchymal stem cell precursors and undergo differentiation in response to a number of factors including the bone morphogenetic proteins (BMPs), transforming growth factor-β, and glucocorticoids (Spelsberg et al., 1999). In addition, several different molecules are associated with the development and maintenance of mineralized skeletal elements. Once matrix synthesis begins in osteoblast culture models such as primary osteoblast cultures and osteoblast-like cell (MC3T3-E1), the cells differentiate in accordance with the gene activation of osteoblast markers such as alkaline phosphatase (ALP), type I collagen and matrix metalloproteinase-1 (MMP-1) or collagenase-1. Finally, osteoblasts become embedded in the extracellular matrix, mainly collagen fibrils, and then matrix mineralization involves mineral growth on and within collagen fibrils (Lian et al., 1999; Franceschi and Iyer, 1992). It is generalized that inflammation induces bone resorption and osteophoresis. Therefore, anti-bone resorption activity may be assessed by the effect on osteoblastic cells.

Prostaglandins are complex regulators of bone metabolism, being simultaneously potent stimulators of osteoclastic bone resorption and of osteoblastic bone formation (Kawaguchi et al., 1995; Robinson et al., 1975). Prostaglandin E2 (PGE2) is the most abundant eicosanoid present in bone, and excess level of PGE2 have been implicated in a number of pathological states associated with bone loss, such as hypercalcemia of malignancy, periodontal disease, and rheumatoid arthritis (Dekel et al., 1981). PGE2 is a mediator in a net anabolic effect on bone and bone matrix destruction in a variety of inflammatory processes, including fracture callus remodeling (Dekel and Francis, 1981), rheumatoid arthritis (Galasko and Bennett, 1976), osteomyelitis (Dekel and Francis, 1981), metastatic disease (Galasko and Bennett, 1976) and periprosthetic osteolysis (Goldberg et al., 1983). To date, many of the cellular and intracellular events involved in these matrix-remodeling states remain unclear.

Thus, it would be most helpful to discover a natural dietary substance that minimizes degenerative bone loss. As a possibility, the phytoestrogen-containing herbs are potentially important in the prevention of post-menopausal osteoporosis caused by estrogen deficiency. As HJ contains phenolic compounds and possesses estrogenic activity, we have assayed the inhibitory activities of HJ on the bone resorption and collagenolysis in the mouse calvarial bone cells. In our previous paper (Hong et al., 2002), HJ inhibited the interleukin-1 (IL-1)-stimulated bone resorption in the fetal mouse bone culture system. Results of in vitro cytotoxicities showed that HJ has no cytotoxicity on the cultured osteoblast cells derived from mouse calvarial bone explants. The HJ was shown to have inhibitory effect against the synthesis of PGE2. Pretreatment of the HJ reduced the synthesis of PGE2 and showed protective effects against plasminogen-dependent fibrinolysis induced by IL-1β. Although the effectiveness of HJ for inflammatory diseases has been widely demonstrated by clinical administration, the scientific and acting mechanisms for those are not understood and elucidated.

In this study, we examined the effect of HJ on the proliferation and differentiation of osteoblastic MC3T3-E1 cells (Varghese and Canalis, 1997) in vitro. We also investigated whether HJ regulates the differentiation and function of osteoblasts using nontransformed osteoblasts (MC3T3-E1). The results suggest that HJ enhances bone formation through the induction of BMP-2 and ALP and by the accumulation of bone matrix proteins such as type I collagen. The present paper reports the effect of HJ on ALP activity and mineralization in MC3T3-E1 cells. From the results, it was concluded that the HJ is applicable to clinical uses in osteoporosis.

2. Materials and methods

2.1. Materials

HJ is consisted of *Carthamus tinctorius* L. seed and hominis placenta. Dried human placenta was purchased from Dongduk Pharmaceutical Co. Ltd., (Seoul, Korea). One hundred grams of the medicinal mixed materials (50 g plus 50 g in each material) were extracted with distilled water (250 ml) for 2 or 1 h at 100 °C, and then centrifuged at 3,0000 rpm for 15 min. The supernatant was stored at 4 °C and used as the aqueous extract. The extract was pre-warmed to room temperature before use. Alternatively, the aqueous extract of HJ is massproduced as for clinical use and was kindly supplied by the Cardiovascular Medical Research Center, Dongguk University (Kyungju, Korea).

2.2. Cell culture of MC3T3-E1 cells

MC3T3-E1 cells were grown in plastic dishes containing αMEM (ICN Pharmaceuticals, Inc., Aurora, OH) with 10% fetal