Effects of genistein, daidzein and glycitein on the osteogenic and adipogenic differentiation of bone marrow stromal cells and on the adipogenic trans-differentiation of osteoblasts*

LI Xianghui[1], ZHANG Jinchao[2,3], SUI Senfang[1]** and YANG Mengsu[3]**

(1. State Key Laboratory of Biomembrane, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, China; 2. Department of Chemistry, College of Chemistry & Environmental Science, Hebei University, Baoding 071002, China; 3. Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, China)

Received February 24, 2005; revised April 18, 2005

Abstract The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), alkaline phosphatase (ALP) activity and oil red O assays were used to examine the effects of genistein, daidzein and glycitein on the osteogenic and adipogenic differentiation of primary mouse bone marrow stromal cells (MSCs) and the adipogenic trans-differentiation of primary mouse osteoblasts. The results indicated that daidzein, genistein and glycitein at concentrations from 1×10^{-8} mol/L to 1×10^{-5} mol/L promoted the proliferation of MSCs and osteoblasts; genistein, daidzein and glycitein promoted osteogenic differentiation and inhibited adipogenic differentiation of MSCs, and inhibited adipocytic transdifferentiation of osteoblasts at appropriate concentrations as 17β-estradiol. It suggests that genistein, daidzein and glycitein regulate a dual differentiational process of MSCs into the osteogenic and adipogenic lineages, and trans-differentiational process of primary osteoblasts into the adipocyte lineages, causing a lineage shift toward osteoblast. Protective effects of them on bone may be mediated by a reversal of adipogenesis which may promote the proliferation, differentiation and mineralization of osteoblasts, and make adipocytes secrete less cytokines which may promote osteoclast formation and activation. In addition, the results also indicated that genistein, daidzein and glycitein may be helpful in preventing the development of steroid induced osteonecrosis.

Keywords: genistein, daidzein, glycitein, bone marrow stromal cells, osteoblasts, osteogenic differentiation, adipogenic differentiation, adipogenic trans-differentiation.

Bone contains two distinct cell types, the osteoblasts which are also called bone-forming cells and the osteoclasts which are also called bone-resorbing cells. The osteoblasts are of mesenchymal origin, while osteoclasts are multinucleated cells formed by the fusion of mononuclear progenitors of the hematopoietic monocyte/macrophage family. The imbalance between the osteoblasts and osteoclasts leads to the osteoporosis which is characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures. Osteoporosis is a major health problem, especially in elderly women. Estrogen has been used in hormone replacement therapy (HRT) to prevent osteoporosis in postmenopausal women, however such therapy may have serious side-effects including increased risk of breast and ovarian cancer. So many postmenopausal women are looking for alternatives to hormone therapy, of particular interest is phytoestrogens such as soy isoflavones, which are plant compounds with estrogen-like biological activity but tend to have weaker side-effects than most estrogen[1].

Now more and more evidence on the action of isoflavones on osteoblasts and osteoclasts has been reported. Genistein and daidzein have a stimulatory effect on the proliferation and differentiation of osteoblastic MC3T3-E1 cells. The genistein (10^{-6} and 10^{-5} mol/L) or daidzein (10^{-6} and 10^{-5} mol/L) caused a significant elevation of protein content, ALP and DNA content in the osteoblastic MC3T3-E1 cells, and the elevation of protein content, ALP and DNA content in the cells were clearly inhibited in the presence of tamaxifen (10^{-6} mol/L), suggesting that the effect of them is partly mediated through esteogen action[2]. Genistein has been shown to bind to estrogen receptor β in the osteoblastic cells although it has not been reported whether daidzein can bind to estrogen receptors[3]. Genistein (10^{-7}—10^{-5} mol/L) inhibited osteoclast-like cell formation, its inhibitory effect was equal to that of other anti-bone-resorbing agents (calcitonin and 17β-estrodiol)[4]. The inhibitory effect of genistein on osteoclast-like formation in

* Supported by the National 863 Project (Grant No. 2003AA2Z2052)
** To whom correspondence should be addressed. E-mail: bhmyang@cityu.edu.hk; suisf@mail.tsinghua.edu.cn

?1994-2018 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

mouse marrow culture seemed greater than that of daidzein. In addition, genistein may partly induce apoptosis of osteoclasts by inhibiting protein tryosine kinases in the cells.

So far these studies have mainly focused on the effect of daidzein and genistein on osteoblasts and osteoclasts. MSCs are pluripotent cells which have the capacity to differentiate into osteoblasts, adipocytes, chondrocytes, myoblasts or fibroblasts[5]. Thus, lineage determination between osteoblasts and adipocytes may be a critical component in the regulatory pathways of osteoblastogenesis. Consistently, an increased lipid accumulation in bone marrow has been reported in association with age-related bone loss implying an inverse relationship between osteoblastogenesis and adipogenesis. Indeed, it is now hypothesized that an increase in the number of adipocytes occurs at the expense of osteoblasts in osteopenic disorders. Furthermore, there is more and more evidence that suggests a large degree of plasticity exists between osteoblasts and adipocytes and this trans-differentiation is reciprocal[6]. Nuttall et al. pointed out that there was a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis[7]. Therefore it is possible that inhibition of marrow adipogenesis with a concomitant increase in osteoblastogenesis could provide a therapeutic target with which to either prevent further increases in adipocyte formation or divert existing adipocytes to become more osteoblastic with a resulting increase in functional bone cells. Moreover, recent studies *in vitro* have demonstrated that 17β-estradiol suppresses expression of lipoprotein lipase (LPL), a marker of adipocyte differentiation in an extramedullary preadipocytic cell line, 3T3L1[8]. Zhou et al. reported that 17β-estradiol may enhance bone formation by activating bone morphogenetic protein-2 (BMP-2) gene transcription in mouse MSCs[9]. Okazaki et al. reported that 17β-estradiol directly modulates differentiation of bipotential stromal cells into the osteoblast and adipocyte lineages, promoting osteoblast differentiation and inhibiting adipocyte differentiation, causing a lineage shift toward the osteoblast[10]. Such effects would lead to direct stimulation of bone formation and thereby contribute to the protective effects of 17β-estradiol on bone. Genistein, daidzein and glycitein, belonging to the phytoestrogen, are currently heralded as offering potential alternative therapies for a range of hormone-dependent conditions, including cancer, menopausal symptoms, cardiovascular disease, osteoporosis and osteonecrosis. The aim of the present investigation is to address the question of whether genistein, daidzein and glycitein affect osteogenic and adipogenic differentiation of primary mouse MSCs and the adipogenic trans-differentiation of primary mouse osteoblasts, and the comparison with 17β-estradiol's effect is also made.

1 Materials and methods

1.1 Materials

KM mice were obtained from the Guangming Weiwu Biological Products Factory. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Gibco. 17β-estradiol, Benzylpenicillin, streptomycin, MTT, β-glycerophosphate, dexamethasone, ascorbic acid, insulin and oil red O were obtained from Sigma. Genistein, daidzein and glycitein were from Shanghai Tongtian Company. ALP activity kit was obtained from Nanjing Jiancheng Biological Engineering Institute, microprotein assay kit was from Beyotime Biotechnology.

1.2 Methods

1.2.1 Isolation and culture of primary bone MSCs The mouse bone MSCs were obtained from adult KM mice (4 to 6 weeks old) using a modification of the method previously reported[11]. Briefly, mice were sacrificed by decapitation. Femora and tibiae were aseptically harvested, and the whole bone marrow was flushed using DMEM in a 1 mL syringe and a 25-gauge needle. The cells were collected and cultured in a culture flask. After incubated in a 37 ℃, 5% CO_2 humidified incubator for 3 days, the nonadherent cells were removed from the cultures by gentle aspiration and the medium replaced with fresh DMEM. Then the medium was changed every 3 days in all the experiments.

1.2.2 Isolation and culture of primary marrow osteoblasts The mouse osteoblasts were isolated mechanically from newborn mice skull using a modification of the method previously reported[12]. Briefly, skull was dissected from KM mice, endosteum and periosteum were stripped off, and the bone was cut into approximately 1—2 mm^2 pieces and digested with trypsin (2.5 g/L) for 30 min then with collagenase A (2.0 g/L) twice with each time for 1 h, and the cells were collected and cultured in a culture flask. After a 24 h incubation in a 5% CO_2 humidified incubator,

?1994-2018 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

the DMEM was removed. Then the medium was changed every 3 days in all the experiments.

1.2.3 Assay for bone MSCs and osteoblasts proliferation The protocol described by Mosmann was followed with some modifications[13]. Briefly, MSCs (3×10^6 cells per well) and osteoblast (10^4 cells per well) were plated in 96-well culture plates and cultured overnight at 37 ℃ in a 5% CO_2 humidified incubator. Genistein, daidzein, glycitein and 17β-estradiol were then added separately to the wells. Control wells were prepared by addition of DMEM. Wells containing DMEM without cells were used as blanks. The plates were incubated at 37 ℃ in a 5% CO_2 incubator for 48 h. Upon completion of the incubation, stock MTT dye solution (20 μL, 5 mg/mL) was added to each well. After 4 h incubation, the supernatant was removed and DMSO (100 μL/well) was added to solubilize the MTT. The optical density of each well was measured on a microplate spectrophotometer (BioRad Model 3550, USA) at a wavelength of 570 nm. The proliferation rate was calculated according to the formula: $(OD_{treated}/OD_{control}-1)\times 100\%$.

1.2.4 Effect of genistein, daidzein and glycitein on the osteogenic differentiation of bone MSCs The bone MSCs were isolated as above. MSCs (3×10^6 cells per well) were plated in 48-well culture plates, after being induced by osteogenic supplement (10^{-8} mol/L dexamethasone, 5.0 mmol/L β-glycerophosphate, 50 μg/mL ascorbic acid) and treated with genistein, daidzein, glycitein and 17β-estradiol at final concentrations of 1×10^{-8}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6}, 1×10^{-5} mol/L for 7 days. The plates were washed thrice with ice-cold PBS and lysed by two cycles of freezing and thaw. Aliquots of supernatants were subjected to ALP activity and protein content measurements using an ALP kit and a microbradford assay kit respectively. The osteogenesis promotion rate was calculated according to the formula: $(\text{ALP activity}_{treated}/\text{ALP activity}_{control}-1)\times 100\%$.

1.2.5 Effect of genistein, daidzein and glycitein on the adipogenic differentiation of bone MSCs The bone MSCs were isolated as above. MSCs (3×10^6 cells per well) were plated in 48-well culture plates, after being induced by adipogenic supplement (10 μg/mL insulin, 10^{-7} mol/L dexthamethone) they were treated with genistein, daidzein, glycitein and 17-estradiol at final concentrations of 1×10^{-8}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6}, 1×10^{-5} mol/L for 14 days. Fat droplets within differentiated adipocytes from MSCs were observed using the oil red O staining method described by Sekiya et al. with some modification[14]. Briefly, Cell monolayers were fixed in 4% formaldehyde, washed in water and stained with a 0.6% oil red O solution (60% isopropanol, 40% water) for 15 min at room temperature. For quantification of adipogenesis, cell monolayers were then washed extensively with water to remove unbound dye, then 1 mL of isopropyl alcohol was added to the stained culture dish. After 5 minutes, the absorbance of the extract was assayed by a spectrophotometer at 510 nm. The adipogenic inhibition rate was calculated according to the formula: $(1-OD_{treated}/OD_{control})\times 100\%$.

1.2.6 Effect of genistein, daidzein and glycitein on the adipogenic trans-differentiation of osteoblasts The mouse osteoblasts were isolated as above. Osteoblasts (10^4 cells per well) were plated in 48-well culture plates, after being induced by adipogenic supplement (10 ug/mL Insulin, 10^{-7} mol/L dexthamethone) they were treated with genistein, daidzein, glycitein and 17β-estradiol at final concentrations of 1×10^{-8}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6} mol/L for 9 days. The adipogenic trans-differentiation of osteoblasts was quantificated as above. The adipogenic trans-differentiation inhibition rate was calculated according to the formula: $(1-OD_{treated}/OD_{control})\times 100\%$.

1.2.7 Statistical analyses Data were expressed as means ± SD of at least three separate experiments. The statistical differences were analyzed using SPSS' *t*-test. *p* values less than 0.05 were considered to be statistical significance.

2 Results

2.1 Effect of genistein, daidzein and glycitein on the MSCs proliferation

The effect of genistein, daidzein and glycitein on the MSCs proliferation is shown in Fig. 1. Daidzein has no effect on the MSCs proliferation at concentrations of 1×10^{-8}, 1×10^{-6}, 5×10^{-6} and 1×10^{-5} mol/L ($P>0.05$), but it promotes MSCs proliferation at concentration of 5×10^{-7} mol/L ($P<0.05$). Genistein and glycitein (1×10^{-8}—1×10^{-5} mol/L) promote MSCs proliferation ($P<0.01$). Moreover, the effect of 17-estradiol on the MSCs proliferation is greater than that of genistein, daidzein and glycitein.

?1994-2018 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

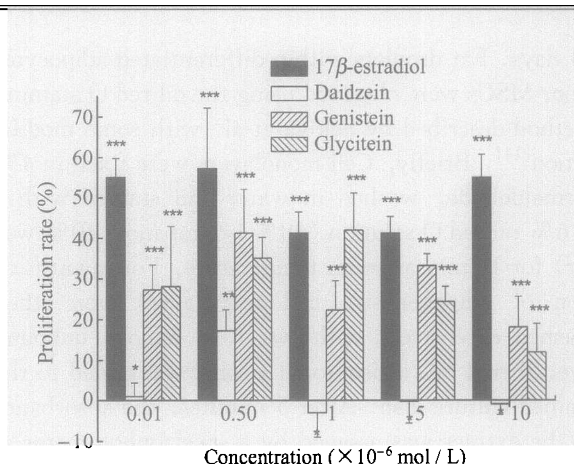


Fig. 1. Effect of genistein, daidzein and glycitein on the MSCs proliferation ($^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control group, $n=4$).

2.2 Effect of genistein, daidzein and glycitein on the osteoblasts proliferation

The effect of genistein, daidzein and glycitein on the osteoblasts proliferation is shown in Fig. 2. Glycitein had no effect on the osteoblasts proliferation at concentration of 5×10^{-6} mol/L ($P>0.05$), but it promoted osteoblasts proliferation at concentrations of 1×10^{-8}, 5×10^{-7}, 1×10^{-6} mol/L ($P<0.01$). Genistein and daidzein (1×10^{-8}—5×10^{-6} mol/L) promoted osteoblasts proliferation ($P<0.05$, $P<0.01$). In addition, the proliferation rate reached the maximal value at concentration of 1×10^{-8} mol/L, the proliferation rate is 36.66%, 47.26% and 41.95% for genistein, glycitein and daidzein respectively. Moreover the effect of them (1×10^{-8}—1×10^{-6} mol/L) on osteoblasts proliferation increases in the sequence of glycitein > daidzein > genistein > 17-estradiol.

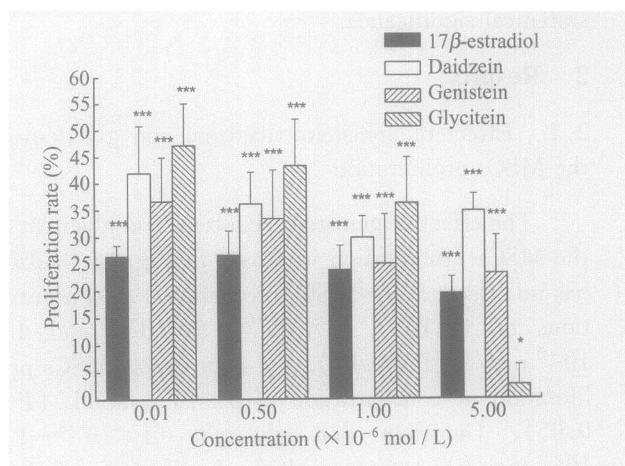


Fig. 2. Effect of genistein, daidzein and glycitein on the osteoblasts proliferation ($^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control group, $n=4$).

2.3 Effect of genistein, daidzein and glycitein on the osteogenic differentiation of MSCs

Effect of genistein, daidzein and glycitein on the osteogenic differentiation of MSCs is shown in Fig.3. The results indicate that daidzein at the concentrations of 1×10^{-8}, 1×10^{-6}, 5×10^{-6} and 1×10^{-5} mol/L has no effect on the osteogenic differentiation of MSCs ($P>0.05$), whereas it promotes osteogenic differentiation of MSCs at concentration of 5×10^{-7} mol/L ($P<0.05$), and the promotion rate is 42.44%. For genistein, it promotes osteogenic differentiation of MSCs at concentrations of 1×10^{-6} and 5×10^{-6} mol/L ($P<0.05$), and the promotion rate is 22.01% and 31.41% respectively, but has no effect on the osteogenic differentiation of MSCs at other concentrations ($P>0.05$). Glycitein has no effect on the osteogenic differentiation of MSCs at concentrations of 1×10^{-8} and 1×10^{-5} mol/L ($P>0.05$), but promotes osteogenic differentiation of MSCs in a dose-dependent manner at concentrations of 5×10^{-7}, 1×10^{-6} and 5×10^{-6} mol/L ($P<0.05$), and the promotion rate is 37.70%, 49.54% and 67.93% respectively. For 17-estradiol, it (5×10^{-7}—1×10^{-5} mol/L) also promots osteogenic differentiation of MSCs ($P<0.05$), and promotion rate reaches the maximal value at concentration of 5×10^{-6} mol/L.

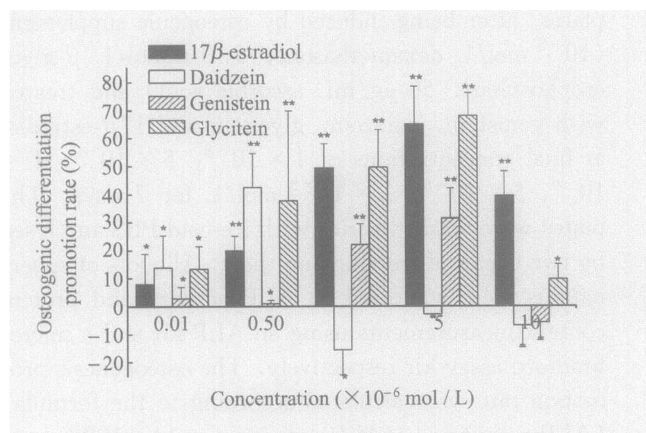


Fig. 3. Effect of genistein, daidzein and glycitein on the osteogenic differentiation of MSCs for 7 days ($^*P>0.05$, $^{**}P<0.05$ *vs* control group, $n=4$).

2.4 Effect of genistein, daidzein and glycitein on the adipogenic differentiation of MSCs

Effect of genistein, daidzein and glycitein on the adipogenic differentiation of MSCs is illustrated in Fig.4. The results indicate that both daidzein and

?1994-2018 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

17β-estradiol (1×10^{-8}—1×10^{-5} mol/L) inhibit adipogenic differentiation of MSCs ($P<0.05$, $P<0.01$), and inhibition rate reaches the maximal value at concentration of 5×10^{-7} and 1×10^{-6} mol/L respectively. For genistein and glycitein, they do not affact the adipogenic differentiation of MSCs at concentration of 5×10^{-6} mol/L ($P>0.05$), but inhibit adipogenic differentiation of MSCs at other concentrations ($P<0.05$, $P<0.01$), the inhibition rate reaches the maximal value at concentration of 1×10^{-6} and 1×10^{-8} mol/L, and inhibition rate is 42.86% and 26.27% respectively. Moreover the experimental results are in accordance with morphological observations (Fig. 5).

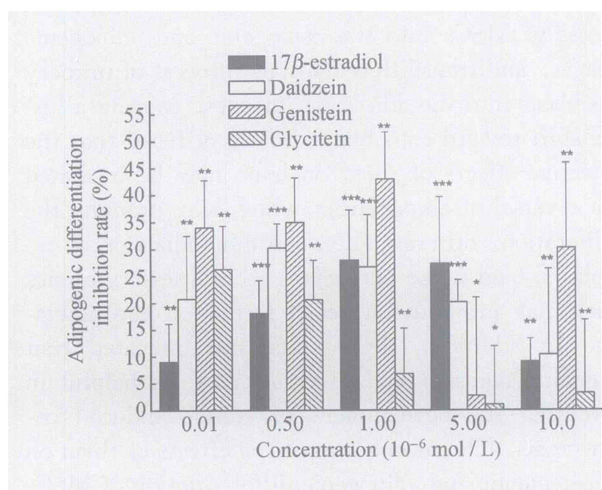


Fig. 4. Effect of genistein, daidzein and glycitein on the adipogenic differentiation of MSCs for 14 days ($^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control group, $n=4$).

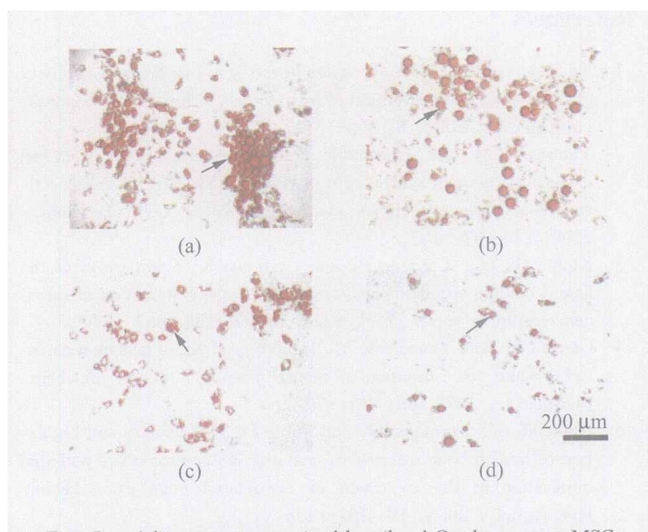


Fig. 5. Adipocytes were stained by oil red O when mouse MSCs were cultured in the presence of 10 μg/mL insulin and 1×10^{-7} mol/L dexthamethone for 14 days. (a) Control group; (b) 1×10^{-6} mol/L glycitein; (c) 1×10^{-6} mol/L daidzein; (d) 1×10^{-6} mol/L genistein.

2.5 Effect of genistein, daidzein and glycitein on adipogenic trans-differentiation of osteoblasts

Effect of genistein, daidzein and glycitein on adipogenic trans-differentiation of osteoblasts is indicated in Fig. 6. The results indicate that daidzein, genistein and 17β-estradiol (1×10^{-8}—1×10^{-5} mol/L) can inhibit adipogenic trans-differentiation of osteoblasts ($P<0.05$, $P<0.01$), with the maximal inhibition rate of 18.75%, 17.87% and 16.73% respectively at concentration of 1×10^{-6} mol/L. For glycitein, it has no effect on adipogenic trans-differentiation of osteoblasts at concentration of 1×10^{-8} mol/L ($P>0.05$), but it inhibits adipogenic trans-differentiation of osteoblasts at concentrations of 5×10^{-7}, 1×10^{-6} and 5×10^{-6} mol/L ($P<0.05$, $P<0.01$), the inhibition rate is 3.45%, 10.34% and 9.48% respectively. Moreover the experimental results are accordance with morphological observations (Fig. 7).

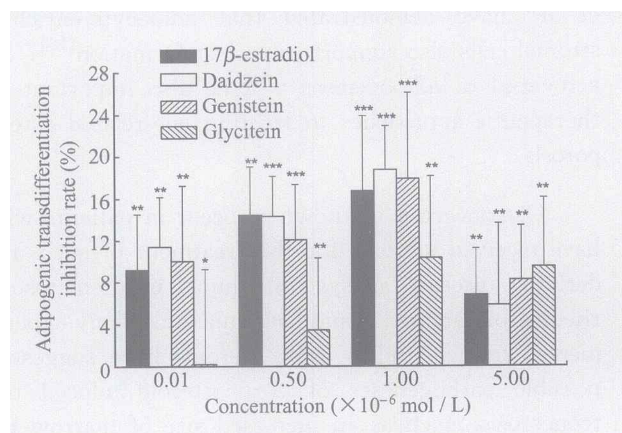
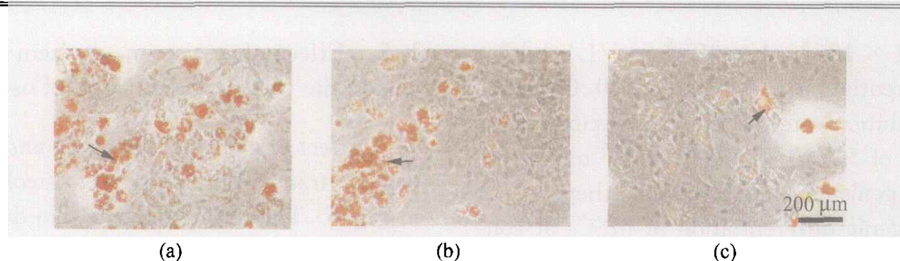


Fig. 6. Effect of genistein, daidzein and glycitein on the adipogenic trans-differentiation of osteoblasts for 9 days ($^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$, *vs* control group, $n=4$).

3 Discussion

Adipocytic and osteogenic cells are believed to derive from multipotential stromal cells in the marrow, and *in vitro* studies have shown an inverse relationship between the differentiation of adipocytic and osteogenic cells[6]. It was reported that preadipocytes isolated from mouse marrow may regulate the activity and final differentiation of marrow precursors of osteoblasts. The condition medium harvested from mouse stromal preadipocytes had a decreased ALP activity in a mouse stromal osteoblastic cell line. Besides having a passive role of space-filling in bone marrow cavity, recent data suggest that medullary adipocytes are secretory cells that may influence hematopoiesis and osteogenesis. A variety of peptide

?1994-2018 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net



(a) (b) (c)

Fig. 7. Adipocytes were stained by oil red O when mouse primary osteoblasts were cultured in the presence of 10 μg/mL insulin and 10^{-7} mol/L dexthamethone for 9 days. (a) Control group; (b) 1×10^{-6} mol/L glycitein; (c) 1×10^{-6} mol/L genistein.

and nonpeptide compounds are synthesized by and released from adipocytes. Adipocytes also secrete cytokines such as tumor necrosis factor-α (TNF-α) and interleukin 6(IL-6), and the main effect of these cytokines is a stimulation of bone resorption[16]. In addition, Benayahu et al. reported that preadipocytes have the potential to stimulate osteoclast differentiation[17]. In the relationship between adipogenesis and the ability to support osteoclast formation, Sakaguchi et al. have demonstrated that adipocyte-enriched stromal cells also support osteoclast formation[18]. So a reversal of adipogenesis will be also important as therapeutic approaches to treating age-related osteoporosis.

Osteonecrosis is known to occur in patients who have received steroids for the treatment of such underlying diseases as systemic lupus erythematosus, rheumatoid arthritis, and leukemia etc. Many experimental animal studies using steroids have suggested possible pathogeneses of corticosteroid-induced osteonecrosis, such as an increased size of marrow fat cells, a high intraaosseous pressure, an accumulation of lipid within the osteocytes, and fat emboli[19]. Cui et al. reported that steroid-induced adipogenesis by bone progenitor cells in marrow may influence the development of osteonecrosis[20]. Lipid clearing agents, such as lovastatin which promotes osteogenic differentiation and inhibits adipogenic differentiation of MSCs, may be helpful in preventing the development of steroid-induced osteonecrosis[21].

In this study we have examined the effects of genistein, daidzein and glycitein on osteogenic and adipogenic differentiation of MSCs and on the adipogenic transdifferentiation of osteoblasts in an *in vitro* assay employing isolated mouse primary MSCs and osteoblasts. The results indicated that genistein, daidzein and glycitein promoted osteogenic differentiation and inhibited adipogenic differentiation of MSCs, and inhibited adipocytic transdifferentiation of osteoblasts at appropriate concentrations as 17β-estradiol in a dose-independent manner. These results were further supported by the facts that genistein, daidzein and glycitein did not inhibite proliferation of the MSCs and osteoblasts. It suggests that genistein, daidzein and glycitein regulate a dual differentiational process of MSCs into the osteogenic and adipogenic lineages, and transdifferentiational process of primary osteoblasts into the adipocyte lineages, causing a lineage shift toward osteoblast. So we deduced that the protective effects of them on bone may be mediated by a reversal of adipogenesis which may promote the proliferation, differentiation and mineralization of osteoblasts, and make adipocytes secrete less cytokines which may promote osteoclast formation and activation. In addition, the results also indicated that genistein, daidzein and glycitein may be helpful in preventing the development of steroid induced osteonecrosis. The mechanism of the effects of them on the osteogenic and adipogenic differentiation of MSCs and the adipogenic trans-differentiation of osteoblasts remains to be further studied.

References

1 Na X.L. and Cui H.B. Progress in estrogen-like biological activity of isoflavones and prevention of osteoporosis. Chin. J. Osteoporos. (in Chinese), 2002, 8: 370—371.

2 Yamaguchi M. and Sugimoto E. Stimulatory effect of genistein and daidzein on protein synthesis in osteoblastic MC3T3-E1 cells: activation of aminoacyl-tRNA synthetase. Mol. Cell. Biochem., 2000, 214: 97—102.

3 Kuiper G. G., Carlsson B. and Grandien K. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptor α and β. Endocrinol., 1997, 138: 863—870.

4 Gao Y. H. and Yamaguchi M. Inhibitory effect of genistein on osteoclast-like cell formation in mouse marrow cultures. Biochem. Pharmacol., 1999, 58: 767—772.

5 Ahdjoudj S., Fromigué O. and Marie P. J. Plasticity and regulation of human bone marrow stromal osteoprogenitor cells: potential implication in the treatment of age-related bone loss. Histol. Histopathol., 2004, 19: 151—158.

6 Song C. L. and Dang G. T. Adipocytes in bone marrow space and osteoporosis. Chin. J. Osteoporos. (in Chinese), 2002, 8: 266—269.

7 Nuttall M. E. and Gimble J. M. Is there a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis? Bone, 2000, 27: 177—184.

?1994-2018 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

8 Homma H., Kurachi H., Nishio Y. et al. Estrogen suppresses transcription of lipoprotein lipase gene. Existence of a unique estrogen response element on the lipoprotein lipase promoter. J. Biol. Chem., 2000, 275: 11404—11411.

9 Zhou S., Turgeman G., Harris S. E. et al. Estrogens activate bone morphogenetic protein-2 gene transcription in mouse mesenchymal stem cells. Mol. Endocrinol., 2003, 17: 56—66.

10 Okazaki R., Inoue D., Shibata M. et al. Estrogen promotes early osteoblast differentiation and inhibits adipocyte differentiation in mouse bone marrow stromal cell lines that express estrogen receptor (ER) α or β. Endocrinol., 2002, 143: 2349—2356.

11 Kelly K. A. and Gimble J. M. 1, 25-dihydroxy vitamin D3 inhibits adipocyte differentiation and gene expression in murine bone marrow stromal cell clones and primary cultures. Endocrinol., 1998, 139: 2622—2628.

12 Carpenter T. O., Moltz K. C., Ellis B. et al. Osteocalcin production in primary osteoblast cultures derived from normal and *Hyp* mice. Endocrinol., 1998, 139: 35—43.

13 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods, 1983, 65: 55—63.

14 Sekiya I., Larson B. L., Smith J. R. et al. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. Stem Cells, 2002, 20: 530—541.

15 Benayahu D., Zipori D. and Wientroub S. Marrow adipocytes regulate growth and differentiation of osteoblasts. Biochem. Biophys. Res. Comm., 1993, 197: 1245—1252.

16 Mundy G. R., Boyce B. F., Yoneda T. et al. Cytokines and bone remodeling. In: Osteoporosis. San Diego: Academic Press, 1996, 301—313.

17 Benayahu D., Peled A. and Zipori D. Myeloblastic cell line expresses osteoclastic properties following coculture with marrow stromal adipocytes. J. Cell Biochem., 1994, 56: 374—384.

18 Sakaguchi K., Morita I. and Murota S. Relationship between the ability to support differentiation of osteoclast-like cells and adipogenesis in murine stromal cells derived from bone marrow. Prostaglandins Leukotrienes and Essential Fatty Acids, 2000, 62: 319—327.

19 Miyanishi K., Yamamoto T., Irisa T. et al. Bone marrow fat cell enlargement and a rise in intraosseous pressure in steroid-treated rabbits with osteonecrosis. Bone, 2002, 30: 185—190.

20 Cui Q., Wang G. J. and Balian G. Steroid-induced adipogenesis in a pluripotential cell line from bone marrow. J. Bone Joint Surg. Am., 1997, 79: 1054—1063.

21 Cui Q., Wang G. J., Su C. C. et al. Lovastatin prevents steroid induced adipogenesis and osteonecrosis. Clin. Orthop., 1997, (344): 8—19.

?1994-2018 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net