Note

Glycitein Effect on Suppressing the Proliferation and Stimulating the Differentiation of Osteoblastic MC3T3-E1 Cells

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Received September 18, 2000; Accepted December 18, 2000

Glycitein, as one of the three major isoflavones in soybeans, directly but significantly (about 5%) suppressed the proliferation of MC3T3-E1 and stimulated bone-related protein (alkaline phosphatase (ALP) and osteocalcin (OC)) expression. These results indicate that glycitein suppresses the proliferation of osteoblasts and promotes differentiation from its progenitor.

Key words: glycitein; isoflavone; alkaline phosphatase; osteocalcin

The post-menopausal reduction in the circulating level of estrogen (E2) results in bone loss. This loss of bone has been attributed to uncoupling between the functions of osteoblasts and osteoclasts and is characterized by an increase in bone formation and a further increase in bone resorption. Genistein, daidzein, and glycitein (Fig. 1; 4', 7-dehydroxyisoflavone), which are known as isoflavonoids, have estrogenic activity because of the similarity of their chemical structures to estrogen. Soybean contains a much smaller amount of glycitein than the amounts of genistein and daidzein, and the characteristics of glycitein and its effects on bone metabolism have not been widely investigated. We show in this study that glycitein directly and significantly suppressed osteoblastic proliferation. We also confirm that glycitein increased ALP activity and the production of OC, and we prove that glycitein promoted the differentiation of osteoblasts. ALP is produced in osteoblasts and is thought to participate in bone formation,10 while OC is secreted from osteoblasts and are taken exclusively in bones and teeth. The results of the present study are the first to demonstrate that glycitein directly suppressed cell proliferation and promoted differentiation of osteoblasts.

The proliferation of osteoblastic cells was determined by first plating the cells (1–2 × 10^5/ml) in a 24-well culture plate (100 μl/well) and allowing them to attach for 24 hours in an alpha-minimum essential medium (α-MEM; Gibco BRL, Gaithersburg, MD, U.S.A.) supplemented with 10% fetal bovine serum (FBS; ICN Biochemicals, Aurora, OH, U.S.A.) After 24 hours, the culture medium was changed to α-MEM containing only 0.1% bovine serum albumin (BSA; Wako Pure Chemical Industries, Osaka, Japan) for 24 hours. This medium was then removed and replaced by α-MEM plus 0.1% BSA containing graded concentrations of glycitein (0, 10^-4, 10^-3, 10^-2 M; Fujico, Kobe, Japan). After 48 hours, the cell growth was estimated by using the Cell Titer 96 non-radioactive cell proliferation assay kit (Promega, Madison, WI, U.S.A.) which is based on the cellular conversion of a tetrazolium salt to a formazan product that can be detected spectrophotometrically. All data were normalized to 100% cell proliferation by the control medium. As shown in Fig. 2, 10^-7 mol/l of glycitein significantly suppressed the proliferation of MC3T3-E1 cells by 95.1 ± 5.2% when compared to the vehicle control value.

ALP activity was determined by first plating cells (1–2 × 10^5/ml) in a 24-well culture plate (1ml/well) and allowing them to attach for 24 hours in α-MEM supplemented with 10% FBS. After 24 hours, the culture medium was changed to α-MEM containing only 0.1% BSA for 24 hours. The cells were then incubated in α-MEM plus 10% FBS containing glycitein at similar concentrations to those just mentioned for 48 hours. The cells were harvested in 0.1 ml of a 0.05 % trypsin solution, before 0.4 ml of Ca-Mg-free phosphate-buffered saline was added. A 0.1 ml aliquot of cell suspension was counted by a particle analyzer CDA-500 (Sysmex, Kobe, Japan). The remaining cell suspension was centrifuged, the supernatant being removed and replaced by 0.2 ml of 0.2% Nondit P-40 and further disrupted by sonication. After centrifugation for 5 min, the ALP activity in the supernatant was measured by the method of Lowry et al.11 As shown in Fig. 3, 10^-7 mol/l of glycitein significantly increased ALP activity of MC3T3-E1 cells by 159 ± 12.7% in comparison with the vehicle control value.

OC production was determined by plating cells
(8 × 10⁷/ml) in a 6-well culture plate (2 ml/well) and allowing them to attach for 24 hours in α-MEM supplemented with 10% FBS. After 24 hours, the culture medium was changed to α-MEM containing only 0.1% BSA for 24 hours. They were then incubated with glycitein at concentrations of 0, 10⁻⁵, 10⁻⁴, 10⁻³ M for 48 hours. OC released in the culture medium was determined by a radioimmunoassay according to the manufacturer's instructions (Biomedical Technologies, Stoughton, MA, U.S.A.), using a mouse OC antiserum with mouse OC as the standard. As shown in Fig. 4, glycitein concentrations as low as 10⁻⁵ mol/l began to induce significantly increased OC secretion (p<0.05) and elicited a maximal response (148 ± 8.6% compared to the vehicle control) with 10⁻³ mol/l of glycitein.

Glycitein in this study significantly suppressed cell proliferation with some scattering of the results. Glycitein increased the ALP activity and promoted the production of OC, although their results were scattered. In MC3T3-E1 cells, the expression patterns of bone-related proteins are regulated in a temporal manner during successive developmental stages of proliferation, bone matrix formation/maturation, and mineralization. The ALP activity has been found to increase during cell differentiation, and OC was expressed during the late stage of differentiation and mineralization. It is therefore possible that glycitein accelerated to the late stage of the differentiation of osteoblasts.

We have speculated that glycitein suppressed the proliferation of osteoblasts, but promoted the differentiation of osteoblasts from its progenitor. Previous studies have shown that genistein and daidzein have a direct stimulating effect on bone formation in a tissue culture system in vitro. We also confirmed here that genistein and daidzein increased ALP activity and the production of OC, while significantly suppressing proliferation in the same manner as glycitein (data are not shown). These results are consistent with the report that, in a mouse bone culture, E₂ increased the ALP and OC mRNA levels, although the stimulation of proliferation by E₂ could not be seen except in the early phase of culture by Q. Qu et al. These effects of glycitein may therefore be partly attributable to their estrogenic activity.

Regarding the effects of E₂ on bone formation, the discovery of the E₂ receptor (ER) in osteoblasts has implicated osteoblasts as a direct target for E₂. More recently, evidence from experimental studies has suggested that E₂ may directly promote bone formation in vivo, although some studies have reported an E₂-mediated decrease in bone formation.

The results of present study show that glycitein significantly suppressed the proliferation of osteoblastic cells and promoted cell differentiation. Glycitein, therefore, appears to have promoted bone formation. Further investigations about glycitein can promote bone formation in vivo are needed.

![Structure of Glycitein](image)

![Effect of Glycitein on Proliferation of MC3T3-E1 Cells](image)

![Effect of Glycitein on ALP Activity of MC3T3-E1 Cells](image)
Glycitein Regulation of Osteoblast Proliferation and Differentiation

Fig. 4. Effect of Glycitein on the Production of Osteocalcin in MC3T3-E1 Cells.
Cells were treated with α-MEM plus 0.1% BSA containing the vehicle or various concentrations of glycitein for 48 hours. Each column and bar represent the mean±SE of the results from 5 cultures, expressed as a percentage of the vehicle control value. The mean value for 100% control was 236 µg of OC/10^6 cells. *, p<0.05 (compared to the vehicle control by one-way ANOVA)

References


