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Effect of Genistein and Daidzein on the Proliferation and Differentiation of Human Preadipocyte Cell Line

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Isoflavones are known to have several biological activities, including a hypolipidemic effect. However, the mechanism of the lipid lowering effect of genistein remains to be elucidated. There is conflicting evidence on the effect of genistein for the deposition of adipocyte tissues. We examined the effect of the isoflavones on the growth and differentiation of human preadipocyte cells, AML-I. Growth arrest accompanied by the appearance of characteristics of apoptosis was observed by genistein or daidzein treatment under the adipogenic stimulation. The expressions of apoptosis-related proteins, Bad, Akt, and p-Akt, were modulated in the genistein-treated cells by Western blot analysis. On the other hand, exposure of AML-I to the isoflavones increased accumulation of cytoplasmic lipid droplets. Actually, the cytoplasmic expressions of fatty acid synthase (FAS) and peroxisome proliferator-activated receptor (PPAR)-γ were increased in the genistein-treated cells. Glycosylated forms of the isoflavones genistin and puerarin did not have such activities. These results suggested that only aglycon forms of isoflavones induced not only apoptosis but also lipogenesis in the preadipocyte cell line AML-I. The possible mechanism of these phenomena has been discussed in the text.

KEYWORDS: Isoflavone, preadipocyte, apoptosis, proliferation, differentiation

1. INTRODUCTION

The isoflavones genistein and daidzein belong to naturally occurring phenolic compounds. The main sources of dietary isoflavones are in soybeans, although they are present in many herbs and foods of botanical origins. Among the isoflavones, genistein is well-known to have a hypolipidemic effect and prevent cardiovascular diseases by regulating lipid and carbohydrate metabolism (*1*, *2*). Particularly, it appears to be one of the significant regulators of adipocyte metabolism, because supplementation of genistein in the diet decreased the adipose mass in ovariectomized or obese mice (*3*, *4*). Genistein supplementation improved hepatic steatosis and gene expression involved in the fatty acid catabolism in mice (*5*). In vitro studies demonstrated that genistein decreased insulin-induced lipogenesis in a primary culture of rat adipocytes (*6*) and preconfluent and postconfluent 3T3-L1 preadipocyte cell lines (*7*). Additionally, genistein decreased adipocyte number and lipid filling in primary bone marrow stromal cells (*8*) or it inhibited differentiation of preadipocytes from human subcutaneous adipose tissues (*9*). These studies suggest that genistein exhibits its hypolipidemic effect by inhibiting adipocyte maturation. In contrast, genistein enhanced adipogenesis and repressed osteogenesis at the concentration of 1 μM or more in the osteoprogenitor cell line KS483 (*10*). Some PPAR-γ activating isoflavones induced differentiation of 3T3L-1 preadipocytes (*11*). Furthermore, genistein increased epididymal and renal fat pads as well as adipocyte size at the doses of the isoflavone-containing human diets in male mice (*12*). Collectively, the effect of genistein on the adipocyte status remains to be elucidated.

The cellular and molecular events in the maturation of preadipocytes have been extensively studied using cell models such as a mouse preadipocyte cell line, 3T3-L1. The gene expression program leading to terminal adipocyte differentiation arises during and after the mitotic clonal expansion periods in 3T3-L1 preadipocytes (*13*, *14*), although, other data indicate that the cell proliferation process is not a necessary step in the 3T3-L1 adipocyte differentiation (*15*). At least two families of transcription factors are involved in terminal differentiation via transactivation of adipocyte-specific genes. Members of the C/EBP and PPAR-γ are key regulators in the adipogenesis process (*13*). Exposure of preadipocytes to an adipogenic cocktail containing dexamethasone, isobutylmethylxanthine, and insulin induces C/EBP, which in turn activates PPAR-γ. In the terminal phase of differentiation, adipocytes markedly increase de novo lipogenesis (*16*). Expansion of adipose tissue mass results from the increase in the adipocyte size and formation of new ones from precursor cells. The human preadipocyte cell line, AML-I, is derived from a lineage of human bone marrow stromal cells. We have shown that AML-I cells are able to differentiate into adipocytes and increase the accumulation of lipid droplets following adipogenic stimulation (*17*). Using the AML-I cell

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model, we investigated the effect of isoflavones on the proliferation and differentiation of preadipocytes for the aim of elucidating the effect of genistein on the preadipocyte status.

2. MATERIALS AND METHODS

Reagents. Daidzein (4′,7-dihydroxyisoflavone, $C_{15}H_{10}O_4$), genistein (4′,5,7-trihydroxyisoflavone, $C_{15}H_{10}O_5$), genistin (genistein 7-D-glucoside, $C_{21}H_{20}O_{10}$), and puerarin (daidzein 8-glucoside) were purchased from Sigma (St Louis, MO). Human antibodies for α-Tubulin, Bcl-2, Bad, NFκB, Akt, p-Akt, FAS, and PPAR-γ and second antibodies for horseradish-peroxidase-conjugated mouse antirabbit IgG were from Santa Cruz (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and goat antimouse IgG was from Amersham Pharmacia Biotech (Arlington Heights, IL). The chemiluminescence detection system was supplied by Amersham, Pharmacia Biotech.

Cell Culture. The human preadipocyte cell line AML-I has been maintained under standard culture condition in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air and 1% penicillin−streptomycin in our laboratory, and the medium was replaced every 4 days before leading confluency. AML-I is free from any contamination, including mycoplasma.

Culture Medium for Induction of Proliferation, Apoptosis, and Differentiation. For the experiments of proliferation, apoptosis, and differentiation, subconfluent cells were obtained on day 3 from the medium change, and the culture medium was switched to media containing 0.5 mmol/L methylisobutylxanthine (M), 0.5 μmol/L dexamethasone (D), and 0.1 mg/mL insulin (I) in dimethyl sulfoxide (DMSO) (final concentration of DMSO was 0.1%) in RPMI-1640/10% FBS (MDI medium), unless otherwise mentioned. The isoflavones were reconstituted as 100 mmol/L stock solutions in DMSO under sterile conditions and were stored at −20 °C. They were dissolved in appropriate concentrations with MDI medium immediately before the experiments. Unless otherwise noted, "vehicle" refers to 0.1% DMSO in MDI medium.

Assay for Cell Proliferation and Viability. The cells were seeded in six-well plates at a density of 2.5×10^5 cells/(mL well) in 5 mL of MDI medium on day 1. The isoflavones or vehicle (0.1% DMSO) were added to culture medium (MDI medium) at the time of plating at the concentrations of 20, 100, 200, and 400 μM. On days 3, 5, and 7 after plating, viable and dead cell numbers were measured by trypan blue dye exclusion in a Neubauer hemocytometer.

Assay for Apoptosis by Annexin V-FITC Staining. To determine whether the reduced viability was based on apoptosis or necrosis, the cells were stained using an Annexin V-FITC apoptosis detection kit (Sigma, St. Louis, MO) and analyzed qualitatively and quantitatively by fluorescent microscopy. Briefly, 1×10^6 cells were washed twice, resuspended in PBS, and stained with 5 μL of Annexin V-FITC and 2.5 μL of propidium iodide (PI) according to the manufacture's instructions with minor modification. Cytocentrifuge preparation of each sample was made, and apoptotic cells were determined by fluorescent microscopy. At least 400 cells were observed in each slide, and the percentage of Annexin V-FITC positive cells was counted in each sample. It was regarded that cells under early apoptotic process and late apoptotic process were stained with the Annexin V-FITC alone and Annexin V-FITC plus PI, respectively. Live cells showed no staining by either PI or Annexin V-FITC. Necrotic cells were stained with PI alone.

Western Blot Analysis. The expression levels of apoptosis- and differentiation-related proteins were examined by Western blot analysis. AML-I cells were plated at a density of 2.5×10^5 cells/mL in a 200 mm Petri dish in the MDI media with vehicle (0.1% DMSO) or 100 μM genistein for the indicated duration. Afterward, media were aspirated, and the cells were washed with cold PBS (pH 7.4). Cell extracts were prepared using NE-PER nuclear and cytoplasmic extraction reagents (PIERCE Biotechnology, Rockford, IL), according to the manufacturer's protocol. For Western blotting, 50 μg of protein was loaded for each lane on the compact 10% gels (Atto Corporation, Tokyo, Japan), blotted on an Immune-Blot PVDF membrane (Bio-Rad Laboratories, Hercules, CA). Blots were incubated with the indicated primary antibodies and subsequent 1:25,000 or 1:50,000 horseradish peroxidase-conjugated second antibodies. All the primary antibodies were used at the final concentration of 2 μg/mL. Blots were then visualized using an ECL detection system as described by the manufacturer (Amersham Pharmacia Biotech). For quantification analysis, the sum of the densities of bands corresponding to protein blotting with the antibodies under study was calculated and normalized to the amount of α-tubulin. After normalization with α-tubulin, changes in the expression of the proteins under study in treated samples were expressed relative to the basal levels of the proteins in untreated sample. The intensities of the bands were evaluated using a ATTO imaging analyzer system (Atto Corporation, Tokyo, Japan).

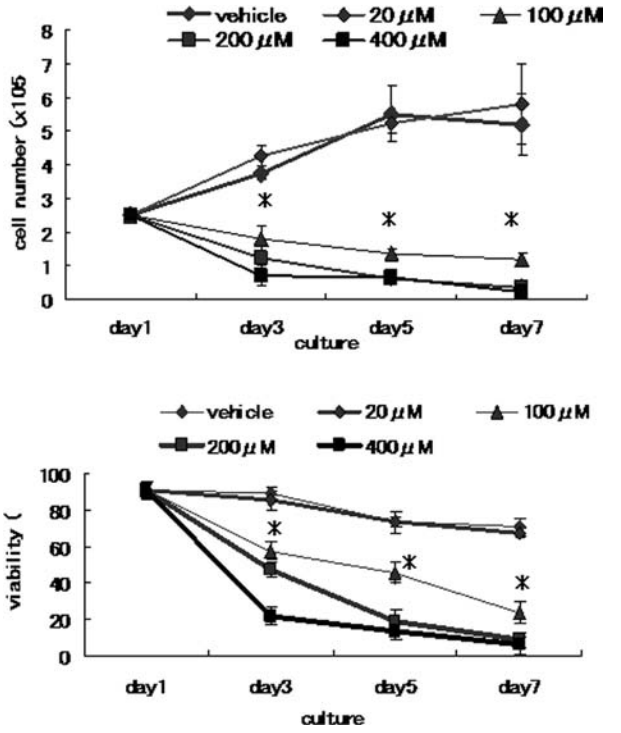

Figure 1. Effect of genistein on the growth and viability of the preadipocytes AML-I. AML-I preadipocytes were cultured in the presence of vehicle (0.1% DMSO) or genistein at the concentrations of 20, 100, 200, or 400 μM in MDI medium. On days 3, 5, and 7, cells were stained with trypan blue, and viable and dead cells were counted. Cell growth and viability are shown in upper and lower panels, respectively. Data are represented as mean ± SD of triplicate cultures. A representation of three independent experiments is shown. Significant difference ($P < 0.05$) from control cells was assessed by Student's *t* test and expressed as an asterisk (∗).

Oil Red O Staining. After culture, cytocentrifuge preparation of each sample was made and fixed with 20% formalin in phosphate buffered saline for 2 min, washed, and treated with 60% isopropyl alcohol for 1 min. Then, the cells were stained with Oil Red O dye (saturated Oil Red dye in six parts of isopropanol and four parts of water) at 60 °C for 15 min. Afterward, the cells were treated with 60% isopropyl alcohol for 1 min, washed for 2 min, and stained for nuclei with Mayer's hematoxilin solution for 8 min, washed, and covered with glycerin solution.

Assessment of Mature Adipocytes. Mature adipocytes were determined by the accumulation of cytoplasmic lipid droplets using light microscopy. The amount of fat droplets varied among the cells, but they appeared to be plenty in large adipocytes. Actually, the stage of adipocyte maturation was assessed semiquantitatively by the amounts of lipid droplets using a scoring method for evaluating leukocyte alkaline phosphatase activity in the histochemical procedure (*18*) with minor modification. Briefly, the cells were classified into three categories: stage I for the cells in which the number of cytoplasmic lipid droplets was less than 5, stage II for the cells with more than 6 scattered lipid droplets, and stage III for the cells with dense distributed lipid droplets. At least 400 cells were counted on each slide and finally expressed as a percentage (%).

Assay for Antioxidant Power. The antioxidant activity of the isoflavones was investigated using an antioxidant activity assay kit (AB-2970 CLETA-S; Atto, Tokyo, Japan) on a luminescencer-PSN AB2200 (Bioinstrument ATTO), as previously described by Shimomura et al. (*19*) Briefly, hypoxanthine−xanthine oxidase systems were used as the source

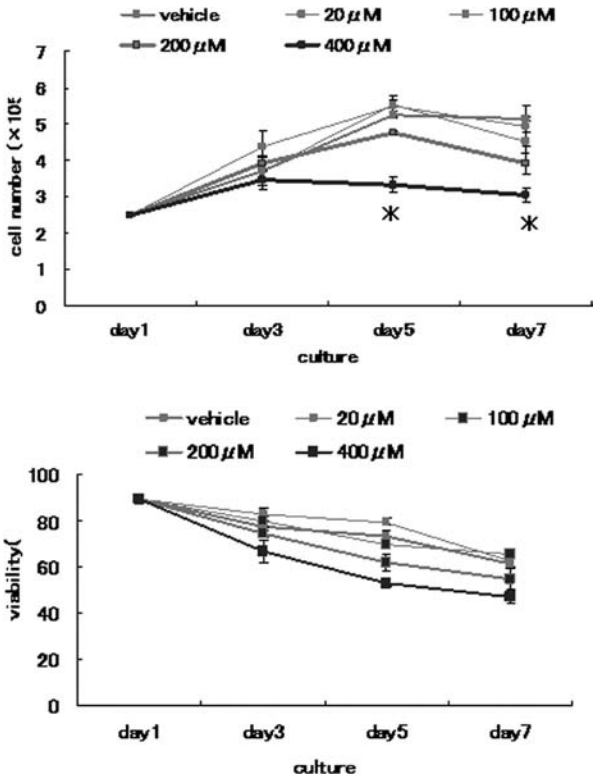

Figure 2. Effect of daidzein on the growth and viability of the preadipocytes AML-I. AML-I preadipocytes were cultured in the presence of vehicle (0.1% DMSO) or daidzein at the concentrations of 20, 100, 200, or 400 μM in MDI medium. On days 3, 5, and 7, cells were stained with trypan blue, and viable and dead cells were counted. Cell growth and viability are shown in upper and lower panels, respectively. Data are represented as mean ± SD of triplicate cultures. A representation of three independent experiments is shown. Significant difference ($P < 0.05$) from control cells was assessed by Student's *t* test and expressed as an asterisk (∗).

of superoxide anion. Chemiluminescence generation by the reaction between superoxide generator and luminous substance, MPEC, and the decay in the presence of the isoflavones was measured with a luminometer, luminescencer-PSN. The final results of antioxidant power were calculated as follows

$$\text{inhibition rate } (\%) = 1 - \frac{(\text{value in the presence of isoflavone})}{(\text{value in the presence of vehicle})} \times 100$$

Statistics. The data were expressed as mean ± SD. The Student's *t* test was used to determine significant differences. Values of $p < 0.05$ were considered significant and expressed as an asterisk (∗).

3. RESULTS

Inhibition of Cell Growth and Viability by Genistein or Daidzein but not by Genistin or Puererin Treatment. For time-course and concentration−response effects of the isoflavones on the cell growth, AML-I cells were cultured in the presence of four isoflavones, such as genistein, daidzein, genistin, or puerarin, at the concentrations of 20, 100, 200, or 400 μM, or vehicle (0.1% DMSO) in MDI culture medium. Cell proliferation and viability were assessed on days 3, 5, and 7 using a trypan blue dye exclusion test and a hemocytometer. Proliferation of AML-I cells was increased time-dependently between days 1 and 5 of the culture in the presence of vehicle in MDI medium. As shown in **Figure 1**, remarkable declines of cell proliferation and viability were observed in the presence of genistein in culture after day 3 at the concentrations of 100 μM and more. The inhibitory effect of daidzein was weak at day 3, but it was evident on day 5, as shown

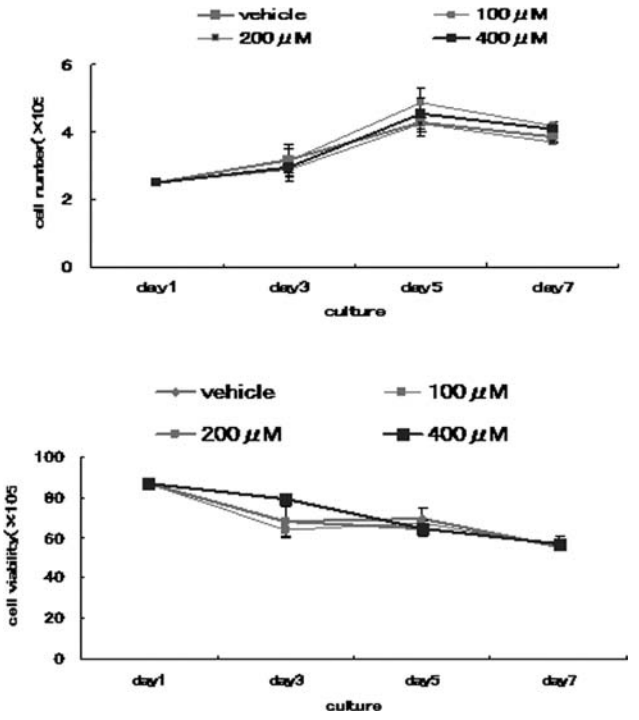

Figure 3. Effect of genistin on the growth and viability of AML-I cells. AML-I cells were cultured with vehicle or genistin (100, 200, or 400 μM) in MDI medium. On days 3, 5, and 7, cell growth and viability were detected by trypan blue dye exclusion test and hemocytometer. Data are represented as mean ± SD of triplicate cultures. A representation of three independent experiments is shown.

in **Figure 2**. In contrast, the glycosylated isoflavones, genistin or puerarin, exhibited neither growth arrest nor cytotoxicity, as shown in **Figures 3** and **4**, respectively.

Induction of Apoptosis in Genistein- or Daidzein-Treated Cells. To examine whether the reduction of genistein-treated cells was caused by apoptosis, an Annexin V-FITC apoptosis assay was performed. As shown in **Figures 5** and **6**, Annexin V-FITC positive cells were significantly increased in genistein-treated cells in a concentration-dependent manner between 100 and 400 μM on day 3. In daidzein-treated cells, the amounts of apoptotic cells were smaller than those in genistein-treated cells, which was concordant with growth inhibitory potency as shown in **Figure 6**.

Effect of Genistein on the Expression of Apoptosis-Related Proteins. We examined cytoplasmic expression of apoptosis-related proteins such as Bcl-2, NFkB, Bad, Akt, and p-Akt in vehicle- or genistein- (100 μM) treated cells after 12, 24, or 48 h of the culture by Western blot analysis. A representative one of the photographs of the bands of time course analysis was shown in **Figure 7**. The expression of Bad was significantly up-regulated in genistein-treated cells after 48 h of the culture. Akt and p-Akt were increased and decreased, respectively, after 48 h of culture in genistein-treated cells compared with vehicle-treated cells. The expression levels of Bcl-2 and NFkB were not significantly altered between genistein- and vehicle-treated cells. Taken together, genistein treatment induced the accumulation of Akt and Bad, and the reduction of pAkt proteins.

Lipid Productive Activity by Isoflavone-Treated Cells. Next, we examined the effect of isoflavones on the lipogenic activity of AML-I cells. The cells were cultured in the presence of various concentrations of isoflavones (20, 100, 200 μM) or vehicle (0.1% DMSO) in MDI medium. On day 6, the cells were harvested and the lipid droplet-containing cells were identified by Oil Red O staining under light microscopy. The maturation stages of preadipocytes

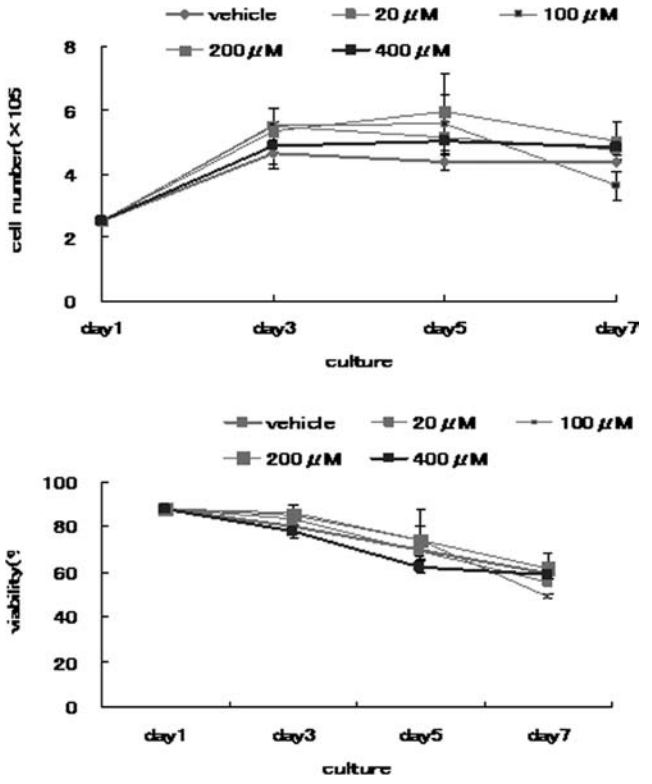

Figure 4. Effect of puerarin on the growth and viability of AML-I cells. AML-I cells were cultured with vehicle or puerarin (20, 100, 200, or 400 μM) in MDI medium. On days 3, 5, and 7, cell growth and viability were detected by trypan blue dye exclusion test and hemocytometer. Data are represented as mean $\pm$ SD of triplicate cultures. A representation of three independent experiments is shown.

were roughly assessed by the amounts of cytoplasmic lipid droplets as described in the Materials and Methods. The results showed that the cytoplasmic lipid accumulation was increased in genistein- or daidzein-treated cells in a concentration dependent manner. Especially, the cells classified as stages II and III were significantly increased under genistein or daidzein treatment at concentrations of 100 μM and higher (**Figures 8** and **9**). In contrast, cells treated with genistin or puerarin were mostly stage I, similar to the control level (data not shown). These results suggested that lipogenic activity appeared to be linked with apoptosis induction power.

Effect of Genistein on the Expression of FAS and PPAR-γ Proteins. The adipocyte specific transcription factor, PPAR-, plays a central role in the control of adipocyte gene expression and is involved in the differentiation process. FAS is a key metabolic enzyme catalyzing the synthesis of long chain fatty acids from 2-carbon precursors. The activity, proteins, and mRNA of FAS increase at the terminal stage of adipocyte differentiation (*13*). Then, we examined the expression of PPAR-γ and FAS proteins in the cells cultured in the presence of genistein or vehicle with MDI medium (adipogenic stimulation) for 48 or 96 h, by Western blot analysis. As shown in **Figure 10**, FAS was significantly increased and PPAR-γ was also increased, although not significantly, in genistein-treated cells during 96 h of culture, in comparison with the basal levels of these proteins in vehicle-treated cells.

Comparison of the Antioxidant Activity of Four Isoflavones Evaluated by the Superoxide Anion Radical Decay. It has been reported that the apoptosis induced by flavonoids is paralleled with their antioxidant acitivties in 3T3-L1 preadipocytes (*20*). We examined the antioxidant power of four isoflavones against superoxide anion radical generated in the hypoxanthine−xanthine

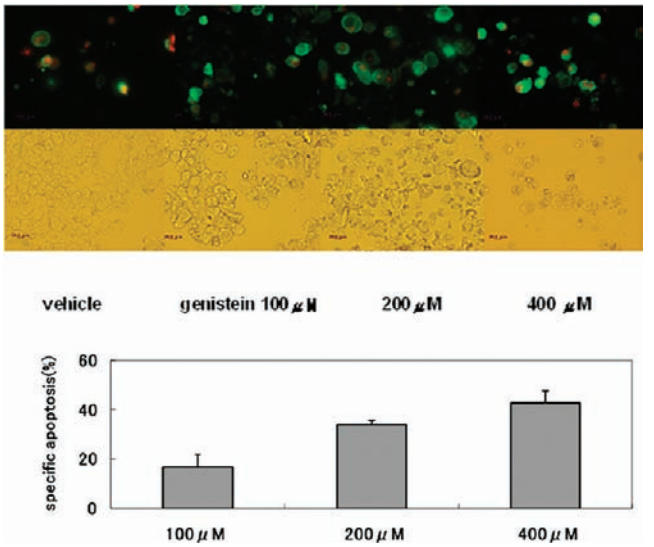

Figure 5. Isoflavone-induced apoptosis. AML-I cells were cultured in the presence of 0.1% DMSO or genistein at the concentrations of 100, 200, or 400 μM in MDI medium. On day 3, cells were stained with Annexin V-FITC and propidium iodide and were observed under fluorescence microscopy. A representative one of four independent experiments is shown. Photographs were taken on day 3 of the culture. (Original magnification $\times$400 for all photographs). Specific apoptosis (%) was calculated with the formula shown in the Materials and Methods. Experiments were repeated three times with similar results.

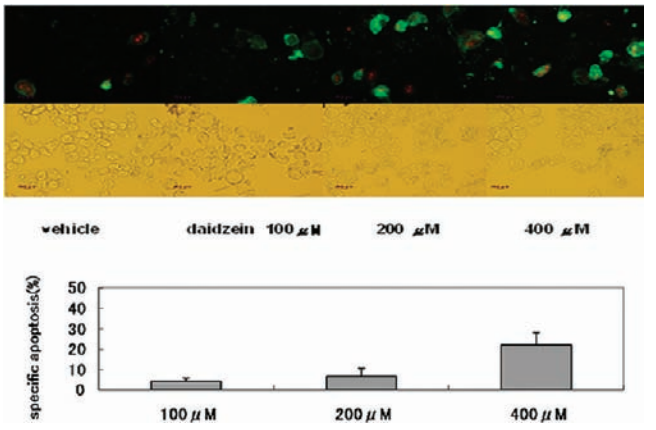

Figure 6. Isoflavone-induced apoptosis. AML-I cells were cultured in the presence of 0.1% DMSO or daidzein (Figure 6) at the concentrations of 100, 200, or 400 μM in MDI medium. On day 3, cells were stained with Annexin V-FITC and propidium iodide and were observed under fluorescence microscopy. A representative one of four independent experiments is shown. Photographs were taken on day 3 of the culture. (Original magnification $\times$400 for all photographs). Specific apoptosis (%) was calculated with the formula shown in the Materials and Methods. Experiments were repeated three times with similar results.

oxidase system. As shown in **Figure 11**, both genistein and daidzein showed strong antioxidant activities. The glycosilated forms puerarin and genistin exhibited moderate and low antioxidant activities, respectively. These results suggested that the isoflavones exhibiting strong antioxidant activity alone possess apoptosis induction capacity.

4. DISCUSSION

In the present study, we demonstrated the effect of genistein and daidzein on the growth and lipogenesis of the human

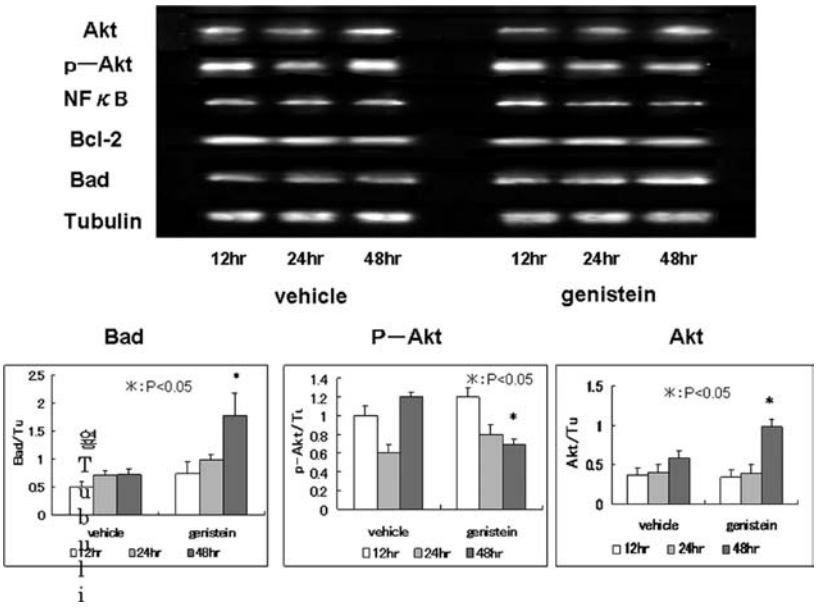

Figure 7. Effect of genistein on the expression of apoptosis-related proteins such as Akt, p-Akt, Bad, Bcl-2, and NFkB. AML-I cells were treated with vehicle (0.1% DMSO) or 100 μM genistein in MDI medium for 12, 24, or 48 h. Then, the cells were harvested and lysed for Western blot analysis as described in the Materials and Methods. Blots were incubated with mouse anti-human−tubulin α, rabbit anti-human Bcl-2, rabbit anti-human Bad, goat anti-human Akt, rabbit anti-human p-Akt, and anti-human NFkB antibodies and the second antibodies. Data shown is a representative from three independent experiments. The values of normalized densities of bands corresponding to Akt, p-Akt, and Bad are shown in each graphic chart. Significant difference ($P < 0.05$) was expressed as an asterisk (*).

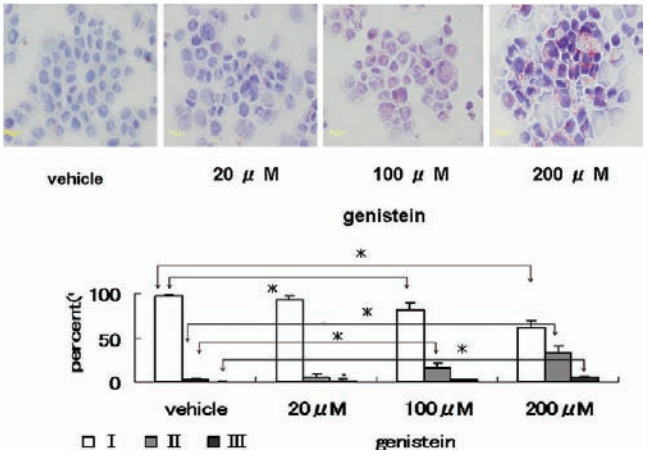

Figure 8. Effect of genistein on the lipid accumulation. AML-I cells were cultured in the presence of vehicle or genistein at the concentrations of 20, 100, or 200 μM with MDI medium. On day 6, the cells were harvested and cytoplasmic lipids were stained with Oil Red O staining and observed under light microscopy. Four hundred cells in each sample were counted and classified semiquantitatively into three categories as described in the Materials and Methods. Values are expressed as a percentage, and a representative one from three independent experiments is shown as a graph. Photographs were taken on day 6 of the culture (original magnification, $\times$400). Significant difference ($p < 0.05$) from control cells was assessed by Student's *t* test and expressed as an asterisk (*).

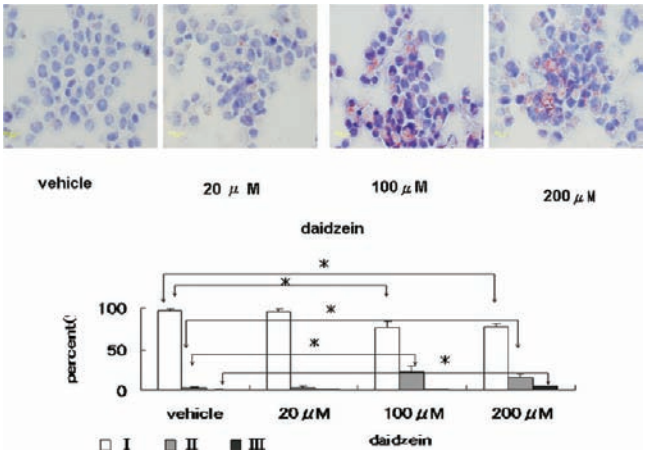

Figure 9. Effect of daidzein on the lipid accumulation. AML-I cells were cultured in the presence of vehicle or daidzein at the concentrations of 20, 100, or 200 μM with MDI medium. On day 6, the cells were harvested and cytoplasmic lipids were stained with Oil Red O staining and observed under light microscopy. Four hundred cells in each sample were counted and classified semiquantitatively into three categories as described in the Materials and Methods. Values are expressed as a percentage, and a representative one from three independent experiments is shown as a graph. Photographs were taken on day 6 of the culture (original magnification, $\times$400). Significant difference ($p < 0.05$) from control cells was assessed by Student's *t* test and expressed as an asterisk (*).

preadipocytes AML-I. An annexin V-FITC apoptosis assay verified that isoflavone-induced growth arrest was caused by apoptosis, not by necrosis. The cells cultured in the presence of genistein or daidzein increased cytoplasmic lipid droplets compared to those cultured with a vehicle. As lipid accumulation usually indicates the differentiation of preadipocyte into adipocyte, the increased lipid droplets by the isoflavones suggest that these isoflavones may accelerate adipocytic conversion of AML-I preadipocytes. Actually, PPAR-γ and FAS were increased in the cells cultured in the presence of genistein. Both PPAR-γ and FAS play the central role in *de novo* lipogenesis during the differentiation of preadipocytes into adipocytes (*13*). Previous investigators reported that genistein inhibited lipid accumulation not only in 3T3L1 mouse preadipocytes but also in primary human adipocytes (*6*−*9*). In contrast, cytoplasmic lipid droplets in AML-I cells

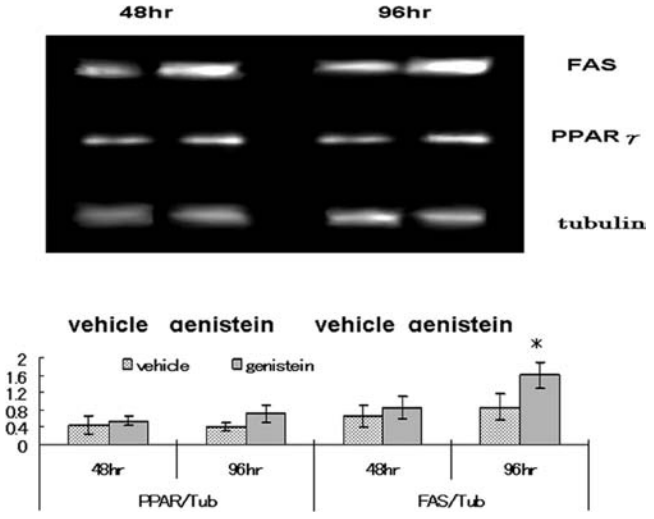

Figure 10. Expression of PPAR-γ and FAS proteins in AML-I cells by genistein treatment. Cells were treated with vehicle (0.01% DMSO) or genistein (100 μM) in the presence of MDI medium. After 48 or 96 h of culture, cells were harvested and lysed for Western blot analysis as described in the Materials and Methods. Blots were incubated with rabbit anti-FAS, mouse anti-PPAR-γ, mouse antihuman α tubulin, and the second antibodies. Experiments were repeated three times with similar results. The values of normalized densities of bands corresponding to FAS and PPAR-γ in the presence or absence of the isoflavones are shown in a graph. Significant difference ($p < 0.05$) from control cells is assessed by Student's *t* test and expressed as an asterisk (*).

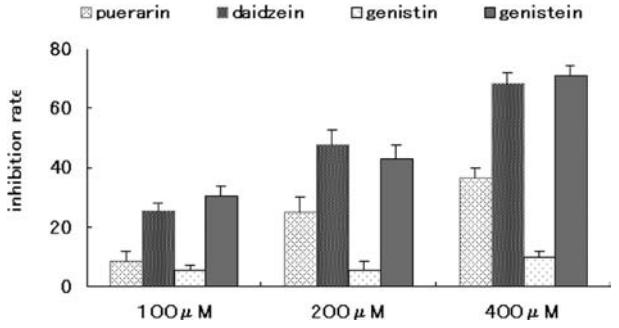

Figure 11. Antioxidant activities of four structure-related isoflavones. Antioxidant activities of four isoflavones were examined against superoxide anion radical generation, and final results of antioxidant power were calculated by the formula shown in the Materials and Methods.

were increased in the presence of genistein, as shown in this study. The primary human adipocytes originated from subcutaneous adipose tissue, while AML-I cells are established from bone marrow stromal cells. The discrepant effect of genistein between primary human adipocytes and AML-I may depend on the different origin and biology of the target cells and/or the discrete study design used.

Similar agent-induced effects have been reported in several studies. The Bcr-Abl inhibitors imatinib and PD 166326 induced apoptosis and triggered erythroid differentiation such as an increase of hemoglobin content and glycophorin A in the K562 cell line (*21*). The authors considered that apoptosis and erythroid differentiation of the K562 cell line were two independent processes which were distinguished on the basis of the caspase dependence. The PPARγ agonist thiazolidinedion promoted differentiation, inhibited proliferation, and induced apoptosis in 3T3-L1 preadipocytes (*22*). Chou described that cross-talk between the PPARγ and Wnt/β catenin signaling pathways was necessary for the induction of growth arrest, apoptosis, and differentiation in preadipocytes (*22*). Wnt/β catenin singnaling represses differentiation by inhibiting the expression of C/EBPα and PPARγ (*23*). When Akt phosphorylation is inhibited, β catenin and its downstream transciptional acitvity are downregulated (*23*). These studies suggest that inhibition of Akt phosphorylation observed in the apoptosis event led to the down-regulation of β catenin signaling and subsequent activation of PPARγ, which promotes the cell differentiation. However, another signaling pathway may be involved in the program between apoptosis and differentiation in preadipocytes. It has been reported that, in the intracellular signaling pathway, mitogen activated protein kinases (MAPKs) are able to regulate adipogenesis at each step of the process from embryonic stem cells to mature adipocytes (*24*). The MAPK inhibitor PD098059 blocks mitotic clonal expansion and accelerates adipocyte differentiation in confluent, postconfluent, and subconfluent 3T3-L1 preadipocytes (*14*). The molecular mechanisms of adipocyte differentiation induced by many compounds and their relation to the normal process of adipocyte differentiation have not been clarified. Genistein is known to activate two different types of transcriptional factors, estrogen receptor (ER) and PPARγ. When genistein acts as a ligand of PPAR, it up-regulates adipogenesis and down-regulates osteogenesis, and when genistein acts as an estrogen agonist, it stimulates osteogenesis and inhibits adipogenesis (*8*). In mice, the adipogenic and antiadipogenic action of genistein were differently regulated by low and high doses of genistein supplementation in the diet and in a gender dependent manner (*12*).

The structurally related isoflavones genistin (genistein-3-*O*-rutinoside) and puerarin (daidzein-3-*O*-rhamnoside) exhibited neither growth arrest nor apoptosis. The results suggested the possibility that attachment of a sugar moiety might attenuate the cytotoxic activity or the glycosylated forms were not taken up by the cells. Although the effect of a sugar moiety on the biological activity of flavonoids is conflicting, the glycosylated form of the flavonoid did not possess an apoptotic effect in our previous study (*25*). Others reported that aglycones were absorbed faster than glycones (*27*) and aglycons were the active form for anticancer activity (*28*).

A previous paper described that induction of apoptosis in 3T3-L1 preadipocytes by flavonoids is associated with their antioxidant activity (*20*). In our study, marked apoptosis of AML-I cells by genistein or daidzein treatment could be related to the powerful antioxidant activity of these isoflavones. However, puerarin with moderate antioxidant power showed neither apoptosis nor growth arrest, suggesting that oxidative stress was not involved in this process.

It has been reported that consumption of isoflavone brings a beneficial effect such as lowering serum cholesterol in humans. However, the absorption of isoflavone is relatively poor, and serum concentration seldom exceeds 10 μmol/L in humans (*26*). The concentration of isoflavones used in this study may be over physiological level, but still our results suggest that the hypolipidemic effect of genistein is unlikely to be due to the inhibition of preadipocyte maturation because feeding male mice with genistein at the nutritional doses increased the adipose tissue deposition in male mice (*12*). Therefore, the mechanisms of the hypolipidemic effect of genistein may be caused by the lypolytic action or modulation of lipid metabolism in the liver (*5*−*7*).

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