Introduction: Osteoporosis is well-known as the number and size of marrow adipocytes increases. Histomorphometric observations suggested that adipose replacement of the marrow functional cell population was a cause.

Glucocorticoids affect the reciprocal conversion between osteoblastogenesis and adipogenesis by binding to hormone response elements on gene promoters and modulating the transcription of growth/differentiation regulatory genes via ligand-activated nuclear receptors.

Pioglitazone, a new therapeutic for diabetes, acts via the peroxisomal proliferator-activated receptor-γ (PPARγ) pathway. In vivo studies indicate that pioglitazone can enhance adipogenesis and bone resorption and result in bone loss. Although the mechanism by which pioglitazone induces bone loss is not clear, it is considered to relate to PPARγ.

Materials and Methods: Pluripotent mesenchymal cells, D1, cloned from Balb/c mouse bone marrow cells, were maintained in DMEM. Adipogenesis was evaluated by Oil-Red O staining. Alkaline phosphatase (ALP) activity was assayed by chemiluminescent activity. Promotor activity was evaluated by luciferase assay after plasmid construction.

Results: Pioglitazone induced adipogenesis and reduced ALP activity

After 3 days of treatment, lipid droplets stained with Oil-red O stain appeared with 10 and 1 μM concentrations but not with 0.25 μM concentrations. After 6 days of treatment, lipid droplets appeared at all concentrations. With 9 days of treatment, further increases in Oil-Red O stain were found at all concentrations (Fig 1A). The adipogenic effect showed a dose-dependent and time-dependent pattern. After 2 days treatment, ALP activities after treatment with different concentrations of pioglitazone (0.5, 1, and 10 μM), decreased by 81%, 89%, and 91%, respectively.

PPARγ antagonist has no effect on pioglitazone-induced adipogenesis.

BADGE (100 μM) was unable to inhibit the adipogenic effect of pioglitazone (1 and 10 μM) and DEX (Fig. 2A). Moreover, BADGE alone did not induce adipogenesis.

PPARγ not involving in the pPPRE-SV-40-SEAP2 reporter gene activity.

Pioglitazone has no agonist activity for PPARγ. This suggested that a PPARγ-mediated pathway might not exist in D1 cells, further confirming that pioglitazone induces adipogenesis through another mechanism not involving PPARγ.

The adipogenic effect of DEX does not act through GR

D1 cells were incubated with or without 0.1μM of DEX in the presence or absence of 10μM of mifepristone, an antagonist of GR, for 8 days. Results demonstrated that mifepristone can not repress DEX-induced adipogenesis. We performed a GR-mediated reporter gene assay using the pMMTV-SEAP2 system. Neither DEX nor mifepristone showed agonist activity for GR. Thus, we concluded that D1 cells do not contain GR and the adipogenic effect of DEX is not through the GR pathway.

Discussion: We demonstrated that pioglitazone and DEX can convert a stromal cell line, D1, from an osteoblastic phenotype that reversibly expresses adipocyte characteristics to terminally differentiated adipocytes. The antagonist of both nuclear receptors cannot inhibit the adipogenic effect of pioglitazone and DEX. The adipogenic effect of both agents in D1 may not be mediated through ligand-nuclear receptor pathway. Reporter gene assays confirmed pioglitazone and DEX induced D1 adipose differentiation via a PPARγ- and GR-independent pathway.

Pioglitazone alone or DEX alone could robustly induce preosteoblastic D1 cells toward adipogenesis under conditions with neither an adipogenesis medium nor PPARγ transfection. Pioglitazone is now a popular medication for type II diabetes mellitus. A report showed that short-term therapy with rosiglitazone involves bone loss in healthy menopausal women. Pioglitazone can also cause bone loss in rats and mice, but the cellular mechanism underlying this skeletal response has not been determined. Our studies suggest that the adipocyte conversion of bone marrow stromal cells may be the mechanism of bone loss caused by pioglitazone. Considering its widespread clinical use, the detrimental skeletal effects of pioglitazone should be closely monitored.

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