PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ ACTIVATION BY TROGLITAZONES INDUCES ADIPOGENESIS IN HUMAN MESENCHYMAL STEM CELLS

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Introduction
Troglitazones are a recently identified class of antidiabetic agents that act by improving insulin sensitivity in various animal models of obesity and diabetes as well as in humans. However, the clinical application of troglitazones has been limited by their potential effects on blood cell production and cardiac hypertrophy. In vitro, troglitazones have been found to induce adipocyte differentiation in preadipocyte cell lines derived from murine fetal tissue (3T3-L1) or ob/ob mice (Ob17). Recently, the troglitazones have been identified as ligands for peroxisome proliferator-activated receptor-γ (PPARγ), an adipogenic transcription factor. The effects of troglitazone in human mesenchymal stem cell (MSC) remain unknown. It has been demonstrated that MSCs are multipotential cells, which are present in adult marrow, that can replicate as undifferentiated cells, and that have the potential to differentiate to lineages of mesenchymal tissues, including bone, cartilage, fat and muscle. There were several well-known recipes for adipogenic differentiation from human MSCs. Through these protocols, uniform adipogenesis cannot be obtained and it takes relatively long time for adipogenesis. To overcome these disadvantages, we have tried to make a new protocol to induce adipogenesis. In this study, we determined that the troglitazones could induce uniform adipogenesis of human MSCs within a short time in a dose- and time-dependent manner.

Materials and Methods
Cell culture: Human MSCs were isolated from bone marrow and cultured in basal medium (Dulbecco’s modified Eagle’s medium (DMEM)-low glucose supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution). Confluence was achieved within 7 days. Cells were cultured in basal medium or adipogenic medium (DMEM-low glucose supplemented with 1% antibiotic-antimycotic solution plus 0.5 mM isobutyl-methylxanthin, 1µM dexamethasone, 10 µM insulin, 200 µM indomethacin) in the presence of troglitazone (5 µM to 50 µM) for 5 days.

Flowcytometry: Analysis of cell surface molecules was performed on human MSCs using flowcytometry. Cell layers were detached by incubation with a solution of 5 mM EDTA for 10 min at 37°C. Nonspecific fluorescence was determined using equal aliquots of an anti-biotin antibody (PharMingen). Data were analyzed by a FACSort (Becton Dickinson, San Jose, CA).

Semi-quantitative reverse-transcription polymerase chain reactions (RT-PCR): RT-PCR was performed to detect RNA expression of lipoprotein lipase (LPL), fatty acids binding protein (aP2), and PPARγ. Total RNA was isolated from cell pellets by the RNeasy kit (Qiagen). Integrated density values for the genes were then normalized to GAPDH values.

Results
Treatment of confluent human MSC cultures in basal medium or adipogenic medium for 5 days with troglitazone (5 µM to 50 µM) increased the number of adipocytes comparing to control. This was accompanied by increased mRNA levels for the adipocyte gene markers LPL, aP2 and PPARγ. Based on quantification relative to the GAPDH, PPARγ mRNA induction was achieved maximum at concentration of 25 µM troglitazone in adipogenic medium. LPL and aP2 mRNA expression did not change. In FACS assay, troglitazone induced adipocyte differentiation in a dose-dependent manner. At concentration of 25 µM troglitazone in adipogenic medium, over 50% of the cells differentiated into adipocytes. But human MSCs died at concentration of 50 µM troglitazone in adipogenic medium.

Discussion
The current study has examined the effects of the troglitazones on human MSCs from bone marrow. MSCs underwent adipogenic differentiation in a time- and dose-dependent manner following troglitazone treatment. The troglitazone increased mRNA levels of adipocyte-specific genes, especially PPARγ. Through the addition of troglitazone as a PPARγ agonist, we could get the uniform adipogenic differentiation within a short time. Thus, troglitazone directly regulates human MSCs differentiation into adipocytes; induced PPARγ expression may play a key regulatory role in this process.

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Troglitazone showed positive Oil-red-O staining in dose-dependent manner (data not shown).

![Fig 1. The total RNA harvested from human MSCs cultures after 5 days in the presence of different mediums including troglitazone (5 µM to 50 µM) was examined through semi-quantitative RT-PCR.](image)

![Fig 2. FACS analysis of human MSC adipogenesis in response to troglitazone concentrations for 5 days.](image)