Fullerol antagonizes dexamethasone-induced oxidative stress and adipogenesis while enhancing expression of osteogenetic markers in a cloned bone marrow mesenchymal stem cell

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INTRODUCTION
Adipogenesis may play a significant role in the development of steroid-induced osteonecrosis (ON). Previous findings implicate that oxidative stress is involved in the pathogenesis of ON. A bone marrow derived mesenchymal cell line D1 isolated from Balb/c mice in our laboratory can differentiate into either osteoblasts or adipocytes depending on culture conditions. This study was designed to answer two questions. First, is oxidative stress involved in adipogenic differentiation by D1 cells? Second, can fullerol, a powerful antioxidant with unique chemical structure, inhibit adipogenesis and promote osteogenesis?

METHODS
The D1 cells in a 12 or 24-well plate were divided into 5 groups and treated for up to 21 days: (A) D1 cells; (B) D1 cells treated with 10 µM dexamethasone (DEX); (C) D1 cells with 10 µM DEX and 10 µM glutathione (GSH); (D) D1 cells with 10 µM DEX and 0.1µM fullerol (formula as C₆₀(OH)₂₂₋₂₄(ONa)₆₋₈); (E) D1 cells with 10 µM DEX and 1.0 µM fullerol. For fullerol preparation, a stock solution of 1 mM was made in pH 10.2 buffer. Diluted solutions were made in culture medium and sterilized with a 0.22 µm membrane. Adipogenic differentiation was assessed by staining with Oil Red O at day 21. Cellular reactive oxygen species (ROS) was detected by using a fluorescent dye CM-H₂DCFDA and measuring the fluorescence intensity under a flow cytometer. Cellular mRNA levels of target genes were determined by real-time RT-PCR, using 18s as internal control. Data are expressed as mean ± SD. Statistically significant differences between two groups were determined using two-tailed Student t-tests.

RESULTS
The results showed that fullerol could inhibit adipogenesis in D1 cells (Fig. 1). There were significantly increased levels of aP2 and PPARγ mRNAs after DEX treatment (p<0.01) (Fig. 2). Fullerol inhibited intracellular ROS Level (Fig. 3) and promoted gene expression of antioxidative enzymes superoxide dismutase (SOD) and catalase (Fig. 4). Furthermore, fullerol enhanced gene expression of osteogenic markers Runx-2 and osteocalcin (Fig. 5).

DISCUSSION
In this study, we provided evidence showing that DEX induces adipogenesis and suppresses osteogenesis in D1 cells and that treatment of fullerol can inhibit this activity of DEX. A limitation of the present study is that we did not perform an in vivo analysis of fullerol for its protective activity against steroid-induced bone loss. For this purpose, use of animal models of steroid induced ON will be a good choice.

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