**Introduction:** One of the most potent anti-osteoporotic agents is alendronate, which strongly inhibits bone resorption (1). In addition to gains in bone mass due to prolonged secondary mineralization, both the vertebral and nonvertebral fracture rates were significantly lower in those treated with this drug (2, 3). Recent results regarding 10 years use of alendronate show that the therapeutic effects of the drug were well-sustained and were acceptable in many aspects (4). Furthermore, even though the approved daily dosage of alendronate from the USA Food and Drug Administration is 10 mg, some reports revealed that a 5 mg daily dose of alendronate in Asian osteoporotic women was quite effective, almost as effective as a 10 mg daily dose in Caucasian women (5, 6). In this study, we examined the effect of alendronates on osteogenesis of the mouse mesenchymal stem cells (D1 cells).

**Materials and Methods:** The mouse mesenchymal stem cells were cultured in the presence of osteogenic medium (DMEM with 10% FBS, antibiotics, 50 μg/ml sodium ascorbate, 100 nM dexamethasone, 10 mM b-glycerophosphate; CDG) for 7 days, then alendronates were treated, 2 days later the cells were used for the tests. The cell proliferation was analyzed using MTT assay. Alizarin red staining was done for mineralization. Alkaline phosphatase activity was measured using a commercial ELISA kit. Calcification was analyzed using energy dispersive X-ray spectrophotometric analysis (EDX). Osteogenic gene expression was analyzed using RT-PCR. The change of CD44 expression was observed using confocal microscopy and FACS analysis.

**Results:** The D1 cells in the culture of CDG media, differentiated into osteoblasts. The stain by Alizarin red S revealed much higher intensity in CDG media cultures with concentrations of 0.1, 1 and 10 μg/ml of alendronate. In the measurement of the density which was measured by Digital Image system, it was increased with the control CDG media culture (Fig. 1A). The cell cytotoxicity of alendronate was not detecting (Fig. 1B). The activity of ALP, a marker of early osteoblasts differentiation, was increased after alendronate treatment which is depicted in Fig 1B. The mRNA expressions of osteocalcin of the alendronate plus CDG treated-cells were compared with the CDG alone cells. After treatment concentrations of 0.1, 1, and 10 μg/ml alendronate, osteocalcin gene expression was increased in comparisons to those CDG alone cells (Fig. 1C). EDX analysis provided evidence for the mineralization via the appearance of a calcium peak. EDX did not show any calcium peak in the undifferentiated D1 cells, but revealed calcium peaks in CDG alone and alendronate plus CDG group (Table 1). The immuno fluorescence staining, which were carried out with antibodies against CD 44 (an antigen detected on osteoblasts) and CD 45 (the leukocyte common antigen). As can be seen in Fig. 2, alendronate treated cells expressed this osteoblasts-specific marker, CD 44. Expression of the leukocyte marker, CD 45 was not detected control and irradiated group.

**Discussion:** In this study, we demonstrate that mouse MSCs (D1 cells) were converted toward osteoblasts by CDG media culture and alendronate treatment. ALP activity and osteocalcin expression were considered to be the markers of osteoblasts differentiation (7, 8). It was reported that early progenitor cells do not express osteoblast markers such as ALP and osteocalcin and differentiate through a defined number of cell divisions to express ultimately a mature osteoblast phenotype that is a postmitotic cell expressing such markers and capable of bone formation (9). In the present experiment, ALP activity was significantly stimulated on days 5 after treatment of 1 μg/ml concentration of alendronate (Fig. 1B). These phenomena are supported by the fact that the gene expression of osteocalcin was increased by alendronate (Fig. 1C). It is likely that alendronate treatment stimulated cellular differentiation, alendronate treatment might have stimulated cellular proliferation of osteocalcin and ALP gene expression cells. In the present study, the presence of calcium peaks in the EDX analysis (Table 1) indicated that the main mineral component of calcified bone matrix, or its precursors were being formed (10). Our findings are in agreement with recent works suggesting that local calcium concentration may regulate osteoblast activity and most likely acting through the calcium-sensing receptor (CaR), is a key regulator of osteoblast cell fate (11, 12). Adhesion molecule CD44 is involved in lymphocyte activation, recirculation and homing, adhesion of extracellular matrix, angiogenesis, cell proliferation, cell differentiation and migration (13). The CD44 modulation maybe play important role in differentiation of MSCs. In the present experiment, the expression of CD 44 in Fluorescence microscopy, Confocal microscopy and FACS analysis indicated that the CD 44 is important marker for osteoblast differentiation. These data suggest that alendronate enhances proliferation and differentiation of the mouse D1 cells.

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**References:**


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