ALENDRONATE ENHANCE OSTEOGENIC DIFFERENTIATION OF MOUSE MESENCHYMAL STEM CELLS

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Introduction: One of the most potent anti-osteoporotic agents is alendronate, which strongly inhibits bone resorption (1-4). Consequently, alendronate interferes with the stability of the ruffled border and stimulates osteoclast apoptosis, which reduces bone resorption, lowers bone turnover, and promotes a positive bone balance (5). Moreover, studies indicate that bisphosphonates also influence osteoblasts and increase bone formation, and more recently, others have reported that bisphosphonates enhance osteoblast proliferation and maturation (6-8), and inhibit osteoblast apoptosis (9). In this study, we examined the effect of alendronate 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 for 7 days, then treated with alendronate, 2 days later the cells were used for the test. 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 spectrometric analysis (EDX). Osteogenic gene expression was analyzed using RT-PCR. The change of CD 44 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 treated with 0.1, 1 and 10 ug/ml of alendronate. In the measurement of the density of staining, which was measured by densitometer program (Multi Gauge V3.0, Fujifilm, Japan), it was increased with the control CDG media culture (Fig. 1A). The cell cytotoxicity of alendronate was not detected (Fig. 1B). The activity of ALP, a marker of early osteoblasts differentiation, was increased after alendronate treatment, as 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 with 0.1, 1, and 10 ug/ml alendronate, osteocalcin gene expression was increased compared to those of 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 peaks in the undifferentiated D1 cells, but revealed calcium peak in CDG alone and alendronate plus CDG group (Fig. 2). In the immunofluorescence staining, which were carried out with antibodies against CD 44 (an antigen detected on osteoblasts) and CD 45 (the leukocyte common antigen) showed that alendronate treated cells expressed the osteoblast-specific marker, CD 44, however, the expression of the leukocyte marker, CD 45, was not detected (Fig. 3).

Discussion: In this study, we could demonstrate that mouse MSCs (D1 cells) were converted toward osteoblasts by CDG media culture and alendronate treatment. Alendronate treatment in combination with CDG media enhanced osteogenic differentiation and was found to be dependent on the dose of alendronate. ALP activity and osteocalcin expression were considered to be the markers of osteoblasts differentiation (9, 10). The stimulation of ALP activity may reflect the stimulation of both differentiation and proliferation of cells at the CDG culture resulting in a significant increase in the number of differentiated cells that express differentiation markers. In the present experiment, ALP activity was significantly stimulated by alendronate (Fig. 1B). This phenomenon was supported by the fact that the gene expression of osteocalcin was increased by alendronate (Fig. 1C). In the present study, the presence of calcium peaks in the EDX analysis (Fig. 2) indicated that the main mineral component of calcified bone matrix, or its precursors were formed (11). Adhesion molecule CD44 is a cell surface transmembrane glycoprotein encoded by single gene. As a receptor for hyaluronic acid (HA), CD44 is involved in lymphocyte activation, recirculation and homing, adhesion of extracellular matrix, angiogenesis, cell proliferation, cell differentiation and migration (12). The CD44 modulation may play an 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 CD 44 is an important marker for osteoblast differentiation. In conclusion, the combined CDG/alendronate treatment biostimulates the differentiation of MSCs into osteoblasts during culture.

These data suggests that alendronate enhances proliferation and differentiation of the mouse D1 cells.

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Figure 1. Alizarin red S staining (A), MTT and ALP activity (B), and osteocalcin and collagen type I gene expression (C)

Figure 2. EDX analysis

Figure 3. Fluorescence microscopy (A), Confocal microscopy (B), and FACS (C) analysis