INTRODUCTION: 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, have been described as the most effective class of drugs to reduce serum cholesterol levels. It has recently been reported that statins induced up-regulation of bone-morphogenetic protein-2 (BMP-2) expression in osteoblasts and in animal bone defect models. However, the effects of statins on mesenchymal stem cells (MSCs), which are the progenitor cells of osteoblasts, were not reported. Therefore, the purpose of this study is to investigate the effects of fluvastatin (Lescol®), a commonly prescribed lipid-lowering agent, on the proliferation and differentiation of MSCs. We hypothesized that fluvastatin increases proliferation and osteogenic differentiation of bone marrow-derived mesenchymal stem cells.

METHODS: Human bone marrow MSCs were harvested using the methods previously reported in the literature with slight modification. Briefly, bone marrow was collected during orthopedic surgeries with informed consent. Mononuclear fraction was obtained using Ficoll (Sigma, St Louis, MO, USA). Cells were then transferred to T-75 flasks at the density of 5x10^5 cells/cm^2. Iscove’s Modified Dulbecco Medium plus 10% fetal calf serum (HyClone, Logan, UT, USA) was used as growth medium. Unattached cells were removed after 24 hours and medium change was carried out twice a week. Fibroblast-like, colony-forming cells were observed 10-14 days after seeding. Cells were then collected and limiting dilution was performed to get single cell derived clones. The final density of limiting dilution was 0.3 cell/well (30 cells for each 96-well plate). Cell pellets in the wells were obtained and the most rapidly proliferating clone was selected. Surface phenotyping was performed using flow cytometry. After obtaining enough number of cells for experiments, MSCs were seeded into six-well plates at the density of 4x10^5 cells/cm^2. Fluvastatin (Novartis International AG, Basel, Switzerland) was added into the MSC cultures in seven different concentrations (0.0064 µg/ml, 0.032 µg/ml, 0.16 µg/ml, 0.8 µg/ml, 4 µg/ml, 20 µg/ml, 100 µg/ml) for 7 days. Six replicates were performed for each concentration. MTT assay was used to evaluate the rate of cell proliferation. Reverse transcription polymerase chain reaction (RT-PCR) was performed to evaluate the expression of osteoblast-related genes such as type I collagen, osteopontin, osteonectin, osteocalcin and BMP-2.

RESULTS: Clonally-expanded MSCs from human bone marrow were obtained and the morphology was shown in Figure 1. The phenotype of the MSCs was CD115^+, CD73^+, CD29^+, CD44^+, CD90^+, CD166^+, SH2^+* HLA-ABC^+, but CD45^-, CD14^-, CD34^-, CD38^- and HLA-DR^- (Figure 2). Surprisingly, change of morphology of the MSCs into neuroglial cell-like was observed in all seven experimental groups 24 hours after adding fluvastatin (Figure 3). Immunohistochemical stains showed positive findings after 7 days in all groups (Figures 4-7), indicating that MSCs were undergoing neural differentiation after being treated with fluvastatin. RT-PCR for MAP2 and GFAP was also performed and expression of MAP2 and GFAP mRNA was noted in all experimental groups (data not shown). MTT results were shown in Figure 8. After being treated with fluvastatin for seven days, proliferation rate was decreased and this effect was dose-dependent. RT-PCR tests for type I collagen, osteopontin, osteonectin, osteocalcin and BMP-2 showed negative results in all groups.

DISCUSSION: It has been demonstrated in this study that fluvastatin did not induce osteogenic differentiation of MSCs and BMP-2 expression was not up-regulated. However, fluvastatin did induce neural differentiation of MSCs. Furthermore, fluvastatin inhibited the proliferation of MSCs in a dose-dependent manner. More work is needed to prove the functionality of the neuroglial-differentiated MSCs.


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