Lovastatin Helps Re-differentiation of Human Nucleus Pulposus Cells during Monolayer Expansion

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INTRODUCTION

Regeneration of nucleus pulposus (NP) tissues in the early stages of degeneration can theoretically retard or even reverse the degenerative process and possibly restore a healthy intervertebral disc (IVD). The approval of autologous disc cell transplantation (ADCT) in Germany demonstrated the potential for cell-based therapies of IVD degeneration. The most clinically applicable source of cells is NP tissue from surgeries to treat lumbar disc herniation (LDH) and degenerative disc disease. In order to achieve adequate number of cells for transplantation, monolayer culture system is usually utilized. When cultured on monolayer, chondrocytes and intervertebral disc (IVD) cells “dedifferentiate” characterized by decrease in synthesis of aggrecan and collagen II and increase in synthesis of collagen I. These changes can be partially reversed by re-encapsulating the cells in a three-dimensional matrix.

Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and are highly effective cholesterol-lowering drugs. Statins has been observed to increase BMP-2 expression and stimulate chondrogenic phenotype of rat chondrocytes and IVD cells. This study aimed to investigate the effect of lovastatin on human IVD cells during monolayer expansion.

METHODS

Isolation of human NP cells: Under the regulation of Research Ethical Committee, human nucleus pulposus (NP) tissues were aseptically from six patients (age 23-29) who underwent surgery for LDH. NP cells were isolated and then expanded on monolayer.

Lovastatin stimulation: When NP cells reached 80% of confluence in 25 cm² flasks, lovastatin was added to the medium to reach the final concentration at 0.1-10 µM. After 72 hours, RNA in NP cells was extracted for determining expressions of GAPDH, collagens I (Col1), II (Col2), aggrecan (AGC), SOX9, and cytokines including TGF-β1 and BMP-2,7. Expression of each target gene was normalized to GAPDH.

Measurement of cellular proliferation and LDH cytotoxicity test: 3x10³ cells were suspended in lovastatin-containing medium and then seeded onto a 96-well plate. After 72 hours, culture medium was removed for LDH cytotoxicity test. Cell number in each well was then determined using Cell Counting Kit-8 with some modifications.

Statistical analysis: RNA expression, cellular proliferation, and OD value of LDH test with or without lovastatin stimulation were analyzed by using Wilcoxon matched-pairs signed ranks test.

RESULTS

Lovastatin significantly enhanced expression of collagen II and SOX9 as the concentration increased. (Fig. 1) The maximal stimulatory effect was found at concentration of 5 µM (16.7-fold increase of collagen II expression and 2.6-fold increase of SOX9 expression). Expression of collagen I was suppressed by lovastatin and the extent of suppression was most prominent at concentration of 5 and 10 µM (0.52-fold and 0.48-fold respectively). Aggrecan expression was not affected by lovastatin at every concentration.

When the concentrations of lovastatin in the culture medium was 1 µM or higher, expressions of BMP-2 and 7 were enhanced more than 30-fold and 7-fold respectively. (Fig. 2) The expression levels were not further increased when the concentration exceeded 1 µM. Expression of TGF-β1 was not influenced by lovastatin.

DISCUSSION

Monolayer culture is an effective system for increase of cell numbers. Altered phenotypes of monolayer-expanded cells would compromise the properties of regenerative tissues after cell transplantation. In order to optimize the properties of regenerative tissues, several studies investigated on chemical agents as well as physical stimuli that would help re-differentiation of monolayer-expanded cells.

Ideal manipulation for IVD cells should induce increased synthesis of collagen II and decreased synthesis of collagen I simultaneously. Although TGF-β1 is commonly used for promoting chondrogenic phenotypes, its effect is not optimal as the synthesis of collagen I is also enhanced. Studies regarding several other stimuli that showed enhancement of collagen II synthesis did not reveal suppression of collagen I expression. In this study, lovastatin showed both increased synthesis of collagen II and decreased synthesis of collagen I. The stimulatory effect of lovastatin was partially related to overexpression of BMP-2 and 7.

Although lovastatin showed its potential on promoting chondrogenic phenotype of IVD cells, lovastatin also slowed down the proliferation rate of cells in monolayer culture system. The phenomenon of lower proliferation rate was not resulted from cytotoxicity of lovastatin. By adding lovastatin to culture medium, IVD cells on monolayer could manifest similar behaviors as in three-dimensional system. The optimal concentration of lovastatin would be around 1-5 µM according to expressions of collagens, SOX9, and BMP-2,7 as well as cellular proliferation. Further studies are needed to determine the potentials of statins for regeneration and repair of degenerative IVDs.

REFERENCES