Statins prevent production of monocyte chemoattractant protein-1 and matrix-metalloproteinase-3 in IL-1β stimulated synoviocytes through inhibition of protein isoprenylation

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Introduction
Recently, there are increasing evidences that synovial inflammation such as the migration of inflammatory cells and the overproduction of chemokines and matrix-metalloproteinases plays an important role in initiating or amplifying cartilage destruction in osteoarthritis (OA). Monocyte chemoattractant protein-1 (MCP-1) is the predominant chemoattractant for monocyte/macrophages that is produced in inflamed synovial tissue. MMPs, particularly MMP-3, play a significant role in the development of cartilage degradation in joint disease. To block these catabolic factors is one of the major current therapeutic strategies for OA.

Statins, competitive inhibitors of hydroxymethylglutaryl (HMG)-CoA reductase, are effective lipid-lowering agents and used worldwide in medical practice. Moreover, recent experimental and clinical evidences confirmed that statins have a wide range of effects on cells and tissues involved in inflammation, such as the inhibition of production of MCP-1 and MMPs (1). We previously demonstrated that intra-articular administration of statin can reduce inflammatory cell infiltration and matrix-degrading enzyme expression, thus limiting cartilage degradation in rabbit experimental osteoarthritis (2). It is considered that these beneficial effects of statins reflect the ability of statins to block the synthesis of isoprenoid intermediates, such as farnesyl pyrophosphate (FPP) and geranylgeranylated pyrophosphate (GGPP), which are required for the posttranslational modification of the small GTP-binding proteins such as Rho. The purpose of the present study was to investigate whether statins are able to prevent production of MCP-1 and MMP-3 in IL-1β stimulated synoviocytes and to evaluate the mechanism underlying the inhibitory effects of statins.

Materials and Methods:
Cell isolation and culture: Synovial tissues were obtained from Japanese white rabbit knees and human osteoarthritis knee undergoing total knee arthroplasty. Synovium fragments were digested by collagenase. Cells obtained after digestion were cultured at a density of 1.0×10^5 cells/well.

RT-PCR: Rabbit synoviocytes were pre-treated for 18 h in culture medium with or without mevastatin (sigma) at 1, 10, and 50 µM and then stimulated with 1 ng/ml of IL-1β for 3 h. PCR was performed by amplification of target gene: (GAPDH, MCP-1 and MMP-3). Enzyme-linked immunosorbent assay (ELISA): Rabbit synoviocytes were pre-treated for 18 h with or without mevastatin (1, 10, and 50 µM) and then stimulated with 1 ng/ml of IL-1β for 18 h. The levels of MCP-1 and MMP-3 produced by synoviocytes were measured using ELISA kits for MCP-1 and MMP-3. The effect of the addition of exogenous FPP (10 µM) and GGPP (10 µM) (sigma) was evaluated in the 50 µM simvastatin (sigma) with IL-1β in human OA synoviocytes. Finally, human OA synoviocytes were treated with the RhoA kinase inhibitor Y27632 (sigma) (1, 10, 50, and 100 µM), and MCP-1 production was measured by ELISA. Cell viability assay: The cell viability assay was carried out using CellTiter-Glo Luminescent Cell Viability Assay kit (Promega) in order to assess the adverse effect of statins on cell viability. Statistical analysis: Statistical significance is evaluated by unpaired t-test. P values less than 0.05 were considered significant.

Results
Inhibitory effects of statin on IL-1β-induced MCP-1 and MMP-3 gene expressions in synoviocytes: Incubation with 50 µM of mevastatin inhibited IL-1β stimulation of MCP-1 and MMP-3 gene expression in synoviocytes (Figure 1). Inhibitory effects of statin on IL-1β-induced MCP-1 and MMP-3 productions in synoviocytes: Incubation with IL-1β enhanced the productions of MCP-1 and MMP-3 by synoviocytes in the absence of mevastatin (Figure 2). Mevastatin at 50 µM significantly inhibited IL-1β-stimulation of MCP-1 and MMP-3 productions without adverse effect on cell viability (Figure 2). Reverse effect of GGPP on the inhibition of MCP-1 production by statin: The inhibiting effect of simvastatin was reversed by the addition of 10 µM GGPP (Figure 3). In contrast, no reverse effect was observed when the culture was co-incubated with 10 µM FPP. Effect of Rho kinase inhibitor on MCP-1 production: Treatment with Y27632 reduced MCP-1 production from synoviocytes dose-dependently (Figure 4).

Discussion:
Our results show that statins are able to inhibit MCP-1 and MMP-3 productions from IL-1β-stimulated OA synoviocytes by blocking HMG-CoA reductase and interfering in the prenylation processes, particularly through the inhibition of protein geranylgeranylation. Moreover, Rho kinase inhibitor also reduced IL-1β-stimulation of synoviocytes. Rho is one of the geranylgeranylated proteins, therefore, the inhibition of Rho/Rho kinase pathway can be related to the process of inhibitory effect of statins on MCP-1 and MMP-3 productions. These findings suggest that statins have potential as novel therapeutic agents for OA.

References