Inhibition of geranylgeranylation reduces MCP-1 production in IL-1β-stimulated human synoviocytes

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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 (MMPs) plays important roles in initiating or amplifying cartilage destruction in osteoarthritis (OA). 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 (1). We previously demonstrated that intra-articular administration of Mevastatin can reduce inflammatory cell infiltration and matrix-degrading enzyme expression, thus limiting cartilage degradation in rabbit experimental osteoarthritis (2). It was considered that these beneficial effects of statin reflect the ability of statins to block the synthesis of isoprenoid intermediates, such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), which are required for the posttranslational modification of the small GTP-binding proteins such as Rho.

The purpose of this study was to investigate whether Simvastatin are able to prevent production of Monocyte chemotactic protein-1 (MCP-1), the predominant chemokine for monocyte/macrophages, in IL-1β-stimulated human synoviocytes. We subsequently evaluated the reverse effect of isoprenoids on the inhibition of MCP-1 by Simvastatin and the effects of two different isoprenyl-transferase inhibitors and ROCK inhibitor on MCP-1 production.

Materials and Methods:
Cell isolation and culture: Human synoviocytes were purchased from Cell Applications Inc. Cells were cultured in 6 well microplates at a density of 1.0 × 10^5 cells/well and overlaid with 2 ml of Dulbecco’s Modified Eagle Medium containing 10 % fetal bovine serum, at 37˚C in a humidified atmosphere of 95 % air and 5 % CO2.

Cell Applications Inc. Cells were cultured in 6 well microplates at a density of 1.0 × 10^5 cells/well and overlaid with 2 ml of Dulbecco’s Modified Eagle Medium containing 10 % fetal bovine serum, at 37˚C in a humidified atmosphere of 95 % air and 5 % CO2. RT-PCR: Human synoviocytes were pre-treated for 18 h in culture medium with or without Simvastatin (10 µM) 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 genes (GAPDH, MCP-1). Enzyme-linked immunosorbent assay (ELISA): Human synoviocytes were pre-treated for 18 h with or without Simvastatin (10 µM) and then stimulated with 1 ng/ml of IL-1β for 18 h. The level of MCP-1 produced by synoviocytes was measured using ELISA kits for MCP-1. 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 synoviocytes. Subsequently, IL-1β-stimulated human synoviocytes were treated with the isoprenyl-transferase inhibitors, FTI-276 and GTI-2133, (sigma) (1, 10, 50, and 100 µM), and MCP-1 production was measured by ELISA. Finally, synoviocytes were treated with the two RhoA kinase inhibitor Y27632, (sigma) (1, 10, 50, and 100 µM), and MCP-1 production was measured. Statistical analysis: Statistical significance was evaluated by unpaired t-test. P values less than 0.05 were considered significant.

Results:
Inhibitory effects of Simvastatin on IL-1β-induced MCP-1 production in human synoviocytes: Incubation with IL-1β enhanced the production of MCP-1 by synoviocytes in the absence of Simvastatin (Figure 1). Simvastatin at 50 µM significantly inhibited IL-1β stimulation of MCP-1 production.

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 1). In contrast, no reverse effect was observed when the culture was co-incubated with 10 µM FPP.

Inhibitory effects of GTI-2133 and Y27632 on IL-1β-induced MCP-1 production in synoviocytes: Farnesyl-transferase inhibitor did not show inhibitory effect at any concentration (Figure 2). In contrast, geranylgeranyl-transferase inhibitor demonstrated inhibitory effect on MCP-1 production dose-dependently. Treatment with Y27632 also reduced MCP-1 production from synoviocytes (Figure 3).

Discussion:
Our results show that Simvastatin are able to inhibit MCP-1 production from IL-1β-stimulated human synoviocytes by interfering protein geranylgeranylation. Geranylgeranyl-transferase inhibitor, but not farnesyl-transferase inhibitor, demonstrated inhibitory effect on MCP-1 production dose-dependently. This result was similar to the inhibitory effect of statin by the depletion of GGPP but not FPP. Moreover, Rho kinase inhibitor also reduced IL-1β-stimulation of synoviocytes. Rho is one of the major geranylgeranylated proteins, therefore, the inhibition of RhoA/Rho kinase pathway can be related to the process of inhibitory effect of statins on MCP-1 production.

References

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Our findings suggest that statins have potential as novel therapeutic agents for OA, through inhibition of protein geranylgeranylation.