Mini Review

Anti-inflammatory Effects of Simvastatin on Human Oral Cells

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Periodontitis is a chronic inflammatory disease associated with degradation of periodontal tissues and is highly prevalent in late middle age.

Statins, such as simvastatin, are pharmacologic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that inhibit cholesterol synthesis, which is important in development of arteriosclerosis. Many cardiovascular studies have suggested that statins also have anti-inflammatory effects which are independent of cholesterol lowering. As a chronic inflammatory disease, periodontitis shares some mechanisms with atherosclerosis. Since oral epithelial cells are implicated in periodontal inflammation, we measured simvastatin effects on cytokine [interleukin (IL)-1 β , IL-6, IL-8, IL-10, IL-12p70, and tumor necrosis factor α (TNF α)] production by cultured human epithelioid cell line KB cells in response to IL-1 α . Simvastatin decreased production, an effect reversed by adding mevalonate or geranylgeranyl pyrophosphate (GGPP), but not farnesyl pyrophosphate (FPP). Simvastatin was found to reduce NF- κ B and AP-1 promoter activity in KB cells. Our results support an anti-inflammatory effect of simvastatin on human oral epithelial cells.

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Introduction

3-Hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, constitute the most powerful class of lipid-lowering drugs. Clinical trials have demonstrated a marked reduction in cardiovascular mortality in patients taking statins. The benefits observed with statin therapy appear to be related to their pleiotropic effects, independent to cholesterol-lowering¹⁾. Extensive research carried out, mainly in the last decade, suggests that the clinical benefits of statins could be related to anti-inflammatory properties²⁾, an improvement in endothelial dysfunction³⁾, a reduction in blood thrombogenicity⁴⁾, and immunomodulatory actions²⁾. Many of these effects are related to the inhibition of isoprenoid synthesis, which serves as a lipid attachment for small G proteins implicated in intracellular signaling. In fact, small G proteins, whose proper membrane localization and function are dependent on isoprenylation, play an important role in the pleiotropic effects of statins.

Periodontal disease is one of the most common diseases, affecting about 75% of the adult population in Japan. Periodontitis is a chronic inflammatory degradation of the tissue and bone supporting the teeth, which is composed of gingiva, cementum, periodontal ligament, and alveolar bone. Periodontitis is considered to result from an imbalance between destruction and repair of periodontal tissues, triggered by oral bacteria. A number of bacterial products stimulate local host responses that enhance the production of prostaglandins and inflammatory cytokines (such as IL-1 α , IL-1 β , IL-6, and IL-8), the recruitment of inflammatory cells, the elaboration of lytic enzymes, and subsequent damage of periodontal tissue.

It is known that oral pathogens and inflammatory mediators (such as IL-1 and TNF α), from periodontal lesions, intermittently reach the bloodstream inducing systemic inflammatory reactants, such as acute-phase proteins, and immune effectors including systemic antibodies to periodontal bacteria^{5,6)}. Furthermore, periodontitis can be accompanied with severe systemic complications⁷⁾. Epidemiological studies have also indicated that inflammatory and immunological reactions induced by periodontal infections contribute to the pathogenesis of atheroma formation, which leads to cardiovascular disease (CVD)^{8,9)}.

At present the tissue regenerative therapy is applied to treatments of periodontal disease, in addition to therapies focusing on eradication of the cause of the disease. For these therapies to be successful, control of inflammatory conditions is indispensable. Furthermore, since many patients in the current clinical dentistry are suffering from various systemic diseases and taking a number of drugs in combination, it is extremely important to find

effective therapies adequate for respective patient.

Here, we focused on the anti-inflammatory effect of statins, since there is a similar progress of the disease state between CVD and periodontitis. In this mini review, we describe the anti-inflammatory effect of simvastatin, which could modulate atherogenesis, on periodontal cells.

Effects of Simvastatin on IL-1 α -induced Inflammatory Cytokines in KB cells

First, we provide evidence that simvastatin reduces expression of IL-1 α -induced inflammatory cytokines, such as IL-6 and IL-8, in human gingival fibroblasts (HGFs) and human epithelial cell line (KB cells), which has been extensively used as a model for the study of gingival epithelial cells. Comparison of 0 M with 10^{-8} – 10^{-6} M simvastatin showed dose-dependent down-regulation of IL-1 α -induced IL-6 and IL-8 production by KB cells (Fig. 1A). Moreover, 10^{-6} M simvastatin decreases IL-6 and IL-8 production in HGFs (Fig. 1B). Similar results were obtained by the use of the LPS deriving from *Porphyromonas gingivalis*, a pathogenic bacterium causing periodontal disease. Conceivably this is because the signals of both IL-1 and LPS might have almost the same transmitting pathway in common.

The physiological plasma concentration of simvastatin in CVD patients ranges from 10^{-9} to 10^{-7} M¹⁰⁾. Generally, the concentration of medications in gingival crevicular fluid tends to be 10 or 100 times higher than those in plasma; accordingly, clinical doses would be expected to exert anti-inflammatory effects upon oral tissues.

Because statins are inhibitors of HMG-CoA reductase, incubation of cells with these compounds results in depletion of mevalonate. To test whether simvastatin-mediated inhibition of IL-6 and IL-8 production was specific and dependent on mevalonate depletion, KB cells were incubated with simvastatin in the presence or absence of mevalonate. The effect of simvastatin was due to specific inhibition of HMG-CoA reductase, since it was reversed by the addition of the product of the HMG-CoA reductase enzyme, mevalonate (Fig. 2A). This indicates that the mevalonate pathway is involved in regulation of inflammatory cytokine expression.

Moreover, we observed that the reduction of IL-6 and IL-8 production by simvastatin was completely reversed by addition of geranylgeranyl pyrophosphate (GGPP), but not by farnesyl pyrophosphate (FPP) (Fig. 2A). This is a similar observation to the pleiotropic effects of simvastatin in other type of cells, including endothelial cells, cardiac myocytes, and macrophages¹¹⁾. The small GTP-binding protein Ras and Ras-like proteins, such as

Fig.1 Reduction of IL-6 and IL-8 production by simvastatin in cell cultures

A: Simvastatin has a dose-dependent effect (0, 10⁻⁸-10⁻⁶ M) in KB cells. KB cells were stimulated with 1.0 ng/mL of IL-1α in the presence or absence of the specified concentration of simvastatin for 5 h.

B: Simvastatin (10⁻⁶ M) decreased IL-6 and IL-8 production in HGFs. Data are expressed as means ± SD (n = 4). **p* < 0.01 and ***p* < 0.001 versus control (treated with IL-1α)

Fig.2 The effect of simvastatin (10⁻⁶ M) alone, or in combination with mevalonate (10⁻⁴ M), GGPP (5×10⁻⁶ M), or FPP (5×10⁻⁶ M) on KB cells

A: The inhibitory effect of simvastatin on KB cells was reversed by co-treatment with immediate down-stream metabolites of HMG-CoA reductase. The inhibitory effect of simvastatin on KB cells was reversed by co-treatment with mevalonate or GGPP, but not with FPP. Data are expressed as means ± SD (n = 4). **p* < 0.05 versus control (treated with IL-1α).

B: Cross-talk between the statin and mevalonate pathway. Statins inhibit conversion of HMG-CoA to mevalonate by competitive inhibition of the rate-limiting enzyme HMG-CoA reductase. Statins not only inhibit the cellular production of cho-

lesterol but also the biosynthesis of several intermediates of the mevalonate pathway, such as FPP (farnesyl pyrophosphate) and GGPP (geranylgeranyl pyrophosphate). These proteins are essential for the posttranslational modification of several proteins involved in important intracellular signaling pathways, such as the small GTP-binding proteins Ras and Rho.

Fig.3 Simvastatin (10^{-6} M) suppresses NF- κ B and AP-1 activity in IL-1 α -stimulated KB cells

KB cells were transiently cotransfected with a mammalian NF- κ B-responsive luciferase reporter vector (pNF- κ B-luc) or a mammalian AP-1-responsive luciferase reporter vector (pAP-1-luc), together with β -galactosidase expression vector (pCMV- β gal). The cells were analyzed 48 h later, and after a 5h stimulation with IL-1 α (1 ng/mL). All results were normalized for transfection efficiency using the expression of β -galactosidase. Data are expressed as means $\pm$ SD (n = 4). * p < 0.05 versus control (treated with IL-1 α)

Rho, Rab, Rac, and Rap are known to play crucial roles in varied cellular events, including cell proliferation, cell migration, and cellular responses to extra cellular stimuli^{12,13}. FPP and GGPP mediate the post-translational modification (prenylation) of Ras and Rho respectively. Therefore this observation indicates the isoprenylation of Rho is involved in the IL-1 α -stimulating inflammation in KB cells.

Second, since NF- κ B and AP-1 are essential for IL-1 α -stimulated IL-6 and IL-8 expression, we examined whether simvastatin is pharmacologically useful for down-regulation of NF- κ B and AP-1 activity in KB cells. The participation of Rho protein, such as RhoA, Rac1 and Cdc42, in signal transduction cascades from extra cellular stimuli to cell nucleus is clarified in previous reports, with varied interaction of the Rho family and phosphorylation pathways depending on type of cell and stimulation. A number of groups have reported that the interaction of Rac with the ser/thr kinase, p65^{PAK}, might lead to activation of the JNK and p38 pathways and in some cell types, overexpression of p65^{PAK} leads to JNK activation¹⁴⁻¹⁶. Furthermore, there have been reports that Rac can activate NF- κ B when transfected into cells¹⁷⁻²⁰. Our results demonstrated that simvastatin suppresses NF- κ B and AP-1 activity in IL-1 α -stimulated KB cells (Fig. 3). Since NF- κ B and AP-1 coordinate the expression of a wide variety of genes that control immune responses, and are involved in many inflammatory diseases, statins may also have beneficial effects in periodontal diseases in addition to atherosclerosis.

Periodontitis and Statins

Periodontitis is widely accepted as a risk factor for CVD due to elevated inflammatory mediators (such as IL-1 and TNF α) in periodontal lesions and consequently increased serum levels⁸. It is conceivable that administration of statins to cardiovascular patients may have additional benefits on atherosclerotic suppression through inhibition of inflammation in oral tissue. Since simvastatin is well established and frequently used, and we also verified the anti-inflammatory effects of simvastatin in HGFs and KB cells²¹, our findings of inhibition of IL-1 α -induced IL-6 and IL-8 expression, *in vitro*, may have clinical impact.

Cunha-Cruz et al. reported the association of statin use with a decreased tooth loss rate in chronic periodontitis patients²². Moreover, Mundy et al. revealed that statins enhance new bone formation *in vitro* and in rodents²³. This effect was associated with increased expression of the bone morphogenetic protein-2 (BMP-2) gene in bone cells. These findings indicate that statins might have beneficial effects in periodontal disease as well as in CVD.

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