Effects of an HMG-CoA reductase inhibitor on cytokine production by human monocytes/macrophages

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Summary

It has been reported that the HMG-CoA reductase inhibitor simvastatin does not always effectively lower plasma LDL. This drug acts to monocytes/macrophages directly and inhibits cholesterol ester accumulation in these cells. However, cytokine production in macrophages when simvastatin was administered has not been described. In this study, we examined whether simvastatin affects cytokine production in human monocyte-derived macrophages. Simvastatin at doses ranging from 10^{-9} to 10^{-5} M did not affect the synthesis of proinflammatory cytokines (IL-1β, IL-6, IL-8) from human peripheral mononuclear cells. In addition, any changes in cytokine-induced cytokine production (IL-1-induced IL-8 synthesis) were not detected after the addition of simvastatin. The present results suggest that simvastatin suppresses foam cell formation in monocyte/macrophage, without affecting the immunological or inflammatory functions of these cells.

Key words: HMG-CoA reductase inhibitor, simvastatin, cytokines, macrophage, atherosclerosis

Introduction

HMG-CoA reductase (HMG-CoA-R) inhibitors inhibit the synthesis of cholesterol from mevalonic acid by suppressing the conversion of HMG-CoA, which is in the membrane of the endoplasmic reticulum. They are also known to enhance the expression of LDL receptors in the liver, increase the incorporation of LDL, and reduce serum levels of cholesterol. It has recently been reported that HMG-CoA-R inhibitors, which have closed lactone rings, act directly on macrophages and suppress the production of superoxides and LDL oxide, and that they also suppress the accumulation of cholesterol esters in human monocytic leukemia cells, THP-1. There have been no reports concerning the effects on immunity and secretion

Abbreviations: HMG-CoA; 3-hydroxy-3-methylglutaryl coenzyme A, LDL; low density lipoprotein, DMSO; dimethyl sulfoxide, PBMC; peripheral blood mononuclear cell, LPS; lipopolysaccharide, IL-1; interleukin-1, IL-6; interleukin-6, IL8; interleukin-8
of cytokines in monocytes/macrophages when HMG-CoA-R inhibitors are administered. The purpose of the present study was to determine the effects of the HMG-CoA-R inhibitor, on the cytokine synthesis in human monocytes/macrophages.

Methods

1) Preparation of HMG-CoA-R inhibitor solution: A stock solution was prepared by adding 10 mg of simvastatin (Banyu Pharmaceutical Co., Ltd.) to 1 ml of DMSO. Five microliters of this solution was transferred to 5 ml of culture medium, then diluted again to final concentrations ranging from 10^{-9} to 10^{-5} M in the culture medium.

2) Production of cytokines by human peripheral blood mononuclear cells (PBMC): Heparinized fresh blood were taken from three healthy male volunteers using Histopaque (Sigma Chemical Co., St. Louis, Mo). The mononuclear cell layer was diluted and centrifuged at 400 × g for 10 min and the cells were washed three times in PBS. The cells were adjusted to 5 × 10^6 cells/ml in ultra-filtrated (endotoxin-free) RPMI-1640 medium (Gibco Co.) containing 2% v/v human AB serum, 2 mM L-glutamine plus 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.01 M Hepes buffer, pH 7.4.

Lipopolysaccharide from E. coli serotype 055: B5 (Sigma) or recombinant human IL-1α or β (Banyu Tsukuba Research Institute) was added to PBMC suspension, to final concentrations 10 or 100 ng/ml, respectively with or without simvastatin. Simvastatin doses were adjusted from 10^{-9} to 10^{-5} M. The PBMC suspension, as above, was distributed in 0.2 ml portions into 7 mm-diameter wells of 96-well microtiter plates (Corning, Corning, NY) and they were incubated at 37°C in 5% CO₂ for 24 hours. The cells were dissolved with detergent, 0.9% w/v CHAPS (Wako Junyaku, Osaka, Japan) at 4°C for 20 minutes. Total amounts of produced cytokines (secreted and cell-associated), IL-1α, IL-1β, IL-6, and IL-8 were measured by radioimmunoassay based on the method of van der Meer et al. The detection range for the cytokines was 0.10-10.00 ng/ml, and the produced cytokines were measured after appropriate dilution.

Results

In preliminary study we recognized that when LPS was not added, cytokine production was almost the same as that with no stimulation (data not shown). Therefore, cytokine production was assayed on the cells stimulated with 10 ng/ml of LPS at various concentrations of simvastatin. Production of proinflammatory cytokines, IL-1α, IL-1β, IL-6 and IL-8 was induced in human PBMC after stimulation with 10 ng/ml LPS, whereas simvastatin at various concentrations had no effect on the production of LPS-induced cytokines (Fig. 1). Next, the effects of simvastatin on the production of a cytokine (IL-8) induced by addition of other cytokines (IL-1α or IL-1β) were studied. Both IL-1α and IL-1β markedly enhanced the production of IL-8 at 100 ng/ml, but simvastatin had no effect on the production of these cytokines at concentrations ranging from 10^{-9} to 10^{-5} M (Fig. 2).
Fig. 1 Effect of HMG-CoA reductase inhibitor (simvastatin) on cytokine productions by peripheral monocytes/macrophages stimulated with LPS. Human monocytes stimulated with LPS were incubated in various concentrations of simvastatin for 72h at 37°C. Several cytokines (IL-1α: (a), IL-1β: (b) Il-6: (c) IL-8: (d) produced from PBL were assayed by specific RIA)(PBL: peripheral blood lymphocytes)

Fig. 2 IL-8 in PBL stimulated with 100 ng/ml each of IL-1α or β. Human peripheral mononuclear cells stimulated with cytokines (IL-1α or β) were incubated with various concentration of simvastatin for 72 h at 37°C. Then IL-8 produced from PBL were assayed by specific RIA. (PBL: peripheral blood lymphocytes)

Discussion

HMG-CoA-R inhibitors significantly reduce the production of cholesterol by inhibiting the synthesis of mevalonic acid, but it has been suggested that HMG-CoA-R inhibitors also
interfere the production of the metabolites downstream in the enzyme cascade\(^4,5\), such as dolichol, ubiquinone and isopentenyl tRNA. Pravastatin, a HMG-CoA-R inhibitor has strong cell specificity and hepatocyte selectivity because it is very soluble in water\(^6\). On the other hand, lipid-soluble simvastatin has been reported to act not only on hepatocytes but also directly on other cells, especially monocytes or macrophages\(^1,2\). There have been no reports on the effects of HMG-CoA-R inhibitors on the production of cytokines in PBMC. It has been reported that 70% of simvastatin in the blood is in open acid from and the closed lactone ring form is bound to LDL. The few LDL receptors on the cell membrane of macrophages recognize simvastatin-bound LDL complexes and actively incorporate them\(^7\). When a complex is incorporated, the production of superoxide in the macrophage is suppressed. The production of oxidized LDL is also inhibited in endothelial cells cultured with the complex\(^1\). The mechanisms of these actions are considered to involve inhibition of NADPH oxidase by simvastatin-induced inhibition of the isoprenylation of GTP-bound protein in macrophages, and the resultant suppression of production of superoxides\(^8\). These findings suggest that simvastatin suppresses macrophage functions. On the other hand, production of superoxides in stimulated macrophages is known to be induced by a variety of physiological activities including immunomodulators such as cytokines. Cytokines play major roles in immunity and inflammation. Therefore, we examined the effects of simvastatin on cytokine production in human PBMC including monocytes. In this experiment, we had prior examined that the production of IL-1\(\alpha\), IL-1\(\beta\), IL-6 and IL-8 was markedly enhanced by stimulation of LPS in PBMC. Under these conditions, the cell density was adjusted to the optimum level for cytokine production and various concentrations of simvastatin were examined. However, even at the highest concentration (10\(^{-5}\) M), simvastatin had no effect on cytokine production in PBMC. It also had no effect on the induction of cytokine (IL-8) synthesis induced by other cytokines (IL-1\(\alpha\) or IL-1\(\beta\)). These findings suggest that the mevalonate pathway is not involved in the cytokine production in monocytes/macrophages stimulated with LPS or other cytokines. It has been known that LPS-stimulated production of cytokines takes place through pathways involving protein kinase C\(^9\), cyclic AMP\(^{10}\), and prostaglandins\(^{11}\). Recently, Palkama et al.\(^{12}\) found that production of IL-1 was inhibited by administration of the protein kinase inhibitor H-7, but enhanced by an inhibitor of cyclic AMP degradation (IBMX), and suggested that the production of cytokines by the macrophage expressing scavenger-receptor might be caused by a signal transduction network involving protein kinase C or cyclic AMP. In the present experiments, the production of cytokines was neither enhanced nor suppressed by simvastatin. This suggests that simvastatin has no direct effect on these signal transduction pathways. Fong et al.\(^{13}\), who studied production of cytokines in macrophages by adding modified LDL, reported that modified LDL did not alter the expression of the mRNA of IL-1\(\beta\) and TNF\(\alpha\), but that oxidized LDL inhibited the expression of both mRNAs. It has also been reported that oxidized LDL can suppress transcription of the IL-1\(\beta\) gene, but the mechanism of action is not clear\(^{14}\). From the results of the present experiments, we concluded that simvastatin does not directly affect the production of cytokines in PBMC including monocytes. Therefore simvastatin may have little effect on
immunity or inflammation related to cytokines.

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