—Short Communication—

Effects of an HMG-CoA reductase inhibitor on
cholesterol esterification in THP-1 cells

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Foam cells in atherosclerotic lesions contain large amounts of cholesterol esters. It is well known that these foam cells originate from monocyte-derived macrophages. Cultured macrophages are able to accumulate cholesterol esters from various forms of modified low density lipoprotein (LDL) through a so called scavenger receptor. 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (HMG-CoA-R) inhibitors inhibit the synthesis of cholesterol by suppressing the conversion of HMG-CoA to mevalonic acid. These inhibitors are also known to enhance the expression of LDL receptors in the liver, increase the incorporation of LDL and reduce serum levels of cholesterol. Pravastatin, one of the HMG-CoA reductase inhibitors, has strong cell specificity and hepatocyte selectivity because it is water soluble¹⁾, while simvastatin has been reported to act not only directly to hepatocyte but also on other cells, especially monocytes and macrophages, because it is lipid soluble^{2,3)}. Furthermore, 70% of simvastatin in the blood circulates in the form of an open acid and the closed lactone ring fraction is bound to LDL. It has been reported that the few LDL receptors present in macrophages recognize the complex of LDL-simvastatin and are actively incorporated in this complex⁴⁾. Therefore, simvastatin has cellular affinity to monocytes and macrophages and can be expected to inhibit the foam cell formation which causes the initial change in atherosclerosis. The present study sought to verify the direct effect of this drug on cholesterol ester accumulation in cultured monocyte-leukemia derived cells, THP-1 cells.

1) Regulation of the HMG-CoA-R inhibitor: A stock solution was prepared by adding 10 mg of simvastatin (Banyu Pharmaceutical Co., Ltd.) to 1 ml of DMSO, and 5 μ l of this solution was added to 5 ml of medium which was then diluted to various concentrations and used in the experiments. 2) Cells: Monocytic-leukemia-derived THP-1 cells were purchased from ATCC. The cells were adjusted to $8 \times 10^6/4$ ml with RPM-1640 medium (Gibco Lab.) containing 10% FCS in 10-ml Petri dishes. After the addition of 15 μ l (5 nM) of phorbol myristate acetate (PMA: Sigma Chemical Co.) and incubation for 24 hours at 37°C in 5% CO₂, simvastatin, prepared as indicated above, was added at various concentrations. These reaction mixtures were incubated for another 24 hours. 3) After 200 μ g/ml of LDL oxide prepared by the method of Basu et al⁵⁾ was added and incubated for 4 days, the cells were removed and recovered using a rubber policeman. Intracellular lipids of the developed cells were extracted with a mixture of chloroform: methanol (1:2) after homogenization and the analysis of lipids was carried out by ordinary enzymatic

Fig. 1 Changes in lipid accumulation in THP-1 cells treated by simvastatin

methods.

The changes of intracellular lipids distribution are shown in **Fig. 1**. Intracellular phospholipids showed no significant changes, but triglyceride and free cholesterol tended to increase on the concentration of simvastatin. In contrast, a decrease in the ratio of cholesterol ester/total cholesterol was observed with an increase in simvastatin and this decrease was statistically significant ($p < 0.05$). Monocytic-leukemia-derived THP-1 cells can be expected to incorporate the denatured LDL as well as monocytes and macrophages. The increase in the triglyceride contents of THP-1 cells due to the increase of simvastatin indicates that simvastatin does not inhibit the incorporation of denatured LDL. The contents of phospholipid and total cholesterol did not change significantly, regardless of the concentration of simvastatin. These results also support that there is no potential in simvastatin to inhibit the incorporation of denatured LDL which may supply cholesterol and fatty acid. Concerning the formation of cholesterol esters in macrophages, Kempen et al reported that simvastatin inhibited the accumulation of ¹⁴C-oleate more than pravastatin³⁾. They stated that the incorporation of denatured LDL via the scavenger-receptor is not inhibited by simvastatin and suggested the possibility that simvastatin directly inhibits the synthesis of cholesterol esters in macrophages. However, it has recently been reported²⁾ that, when simvastatin bound LDL is incorporated, the production of superoxide in macrophages is

inhibited, and then the production of LDL oxide is also inhibited owing to the addition of simvastatin. Therefore *in vivo*, simvastatin may suppress the incorporation of LDL in monocytes and macrophages. One of the mechanisms is considered to involve the inhibition of NADPH oxidase by the simvastatin induced inhibition of the isoprenylation of GTP bound protein of macrophages and the resultant inhibition of producing superoxides⁶⁾. In monocytic leukemia derived THP-1 cells, it was confirmed that simvastatin decreased the amount of cholesterol ester in spite of a large supply of LDL oxide which may become the large supply of cholesterol and fatty acid. In contrast, the contents of free cholesterol show a tendency to increase depending on the concentration of simvastatin, in spite of simvastatin being an inhibitor of HMG-CoA R. Therefore, it is suggested that simvastatin in this cells might be much stronger inhibitor of cholesterol ester synthesis than that in HMG-CoA-R. Cholesterol esterification is carried out by acyl CoA transferase (ACAT) in the liver, intestine, and macrophages. In the intestines, cholesterol becomes free cholesterol and after the esterification owing to ACAT, cholesterol ester is incorporated in chylomicrone. In macrophages, the supplied free cholesterol from LDL oxide is esterified by ACAT, and this may become one of the signals to induce foamed cells. Therefore, the inhibition of ACAT in macrophages and the intestine is expected to suppress or defend foam cell formation in the artery. In an experiment with simvastatin administrated to cholesterol-loaded rabbits, it was shown that the absorption of cholesterol in the small bowel was inhibited⁷⁾. Other reports also indicated that the mechanism involves the inhibition of ACAT by microsomes in the small bowels⁸⁾. Simvastatin which inhibits cholesterol synthesis, and also suppresses the incorporation of LDL in macrophages *in vivo*, inhibited cholesterol esterification in THP-1 cells. This study suggests that simvastatin may have enough potential to defend or suppress foamed cell-formation and atherosclerosis.

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