Antioxidative Actions of Statins: Potential Mechanisms for Antiatherosclerotic Effects

Takanori Watanabe, Kenichi Yasunari* and Munehoro Nakamura

Department of Medicine and Cardiology, Osaka City University Graduate School of Medicine, Osaka 545-8585, Japan

Abstract: Inhibitors of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase (statins) are widely used for the prevention of atherosclerotic diseases. The effects of statins on the generation of reactive oxygen species (ROS) by *in vitro* and *in vivo* were studied. Administration of statins significantly decreased ROS generation *in vitro* and *in vivo*.

Keywords: Statins, oxidative stress, atherosclerosis, carotid artery, leukocytes, flow cytemetory.

INTRODUCTION

Clinical and experimental studies have shown that reduction of plasma cholesterol, particularly low-density lipoprotein (LDL) cholesterol, reduces the risk of cardiovascular events in both primary and secondary prevention [1, 2]. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), which are cholesterol-reducing drugs, can achieve a relatively large reduction of plasma cholesterol [3]. The beneficial effects of HMG-CoA reductase inhibitors are usually assumed to be a result from their ability to reduce cholesterol synthesis [4]. However, a variety of experimental findings suggest that statins can interfere with major events involved in the formation of atherosclerotic lesions, independent of their hypocholesterolemic properties [4, 5, 6]. Among the pleotrophic effect of statins, anti-inflammatory effects of statins have extensively studied [7, 8]. We have recently demonstrated that oxidative stress of monocytes is related to C-reactive protein, a marker of intravascular inflammation [9]. Therefore, if the anti-inflammatory effects of statins are suggested, antioxidative effects of statins are also suggested.

In this review, we discuss the possibility that chemical structure of statins themselves or their metabolites may have antioxidative effects, and that HMG-CoA inhibition may have antioxidative effects. We describe *in vivo* evidence of antioxidative effects of statins in experimental animals and humans. Finally, we review the effects of statins on regression of carotid atherosclerosis in humans.

CHEMICAL STRUCTURE

Among the several statins, the only compound that the molecule itself has antioxidative action is fluvastatin. Fluvastatin, one of the HMG-CoA reductase inhibitors, and its metabolites M2 and M3 (Fig. **1**) have been reported to have the ability to scavenge hydroxyl radical [10]. *In vitro* superoxide anion scavenging activities of fluvastatin and its metabolites have been also reported [11]. Fluvastatin showed dose-dependent superoxide anion scavenging activity in the cell-free NADH/phenazine methosulphate (PMS)/nitroblue tetrazolium (NBT) system, and the effect was as potent as the reference antioxidant, trolox, which is a water-soluble α-tocopherol derivative [12]. The superoxide anion scavenging activities of the major metabolites of fluvastatin (M2, M3) were also determined in this system. M2, M3 which possess a phenolic hydroxyl group at the 5 or 6-position of the in dole moiety, respectively, showed 3 times stronger activities than that of fluvastatin. The effects of fluvastatin, M2 and M3 on phorbol myristate acetate (PMA)-induced superoxide anion generation in human peripheral blood polymorphonuclear leukocytes (PMN) have been reported [11]. The compounds tested also showed a depressing effect on the amount of superoxide anion in this system. It seems that fluvastatin and its metabolites have a potential to protect cells or lipids from oxidative modification mediated by superoxide anion [13, 14].

Fluvastatin has two chiral carbons in its chemical structure and it is clinically used as a racemic mixture (3RS, 5SR). The hypolipidemic action of fluvastatin *in vivo* is thought to be predominantly caused by 3R, 5S-enantiomer but not by 3S, 5R-enantiomer, because the former has 30-fold stronger inhibitory activity on HMG-CoA reductase than its optical counterpart [15]. Concerning the inhibitory activity on the oxidation of lipids however, the 3S, 5R-enantiomer seems to have equal antioxidative ability as its optical counterpart.

IN VITRO STUDY

Although superoxide anion scavenging properties of fluvastatin and its metabolites have been reported [10], fluvastatin and its hydroxylated metabolites, M2 and M3, decreased the increase in SMC oxidative stress induced by lysophosphatidylcholine (lyso-PC) through the suppression of PLD and PKC [16]. Since PLD and PKC inhibitors completely blocked the increased oxidative stress induced by lyso-PC, the predominant mechanism of antioxidative effect of statins may be through PLD and PKC suppression rather than direct scavenging properties. Increased oxidative stress by Lyso-PC may be mediated by the activation of NADPH oxidase. Since critical processes in the activation of NADPH oxidase are the prenylation of Rac1 at its C-terminal domain, which determines the translocation to the membrane and the exchange of GDP for GTP at its regulatory domain [17], statins, in addition to inhibiting cholesterol synthesis, may downregulate Rac1-GTPase activity by reducing prenylation

*Address correspondence to this author at the Department of Medicine and Cardiology, Osaka City University Graduate School of Medicine, Osaka 545-8585, Japan; Tel: 81-6-6645-3803; Fax: 81-6-6646-6808; E-mail: yasunari@med.osaka-cu.ac.jp

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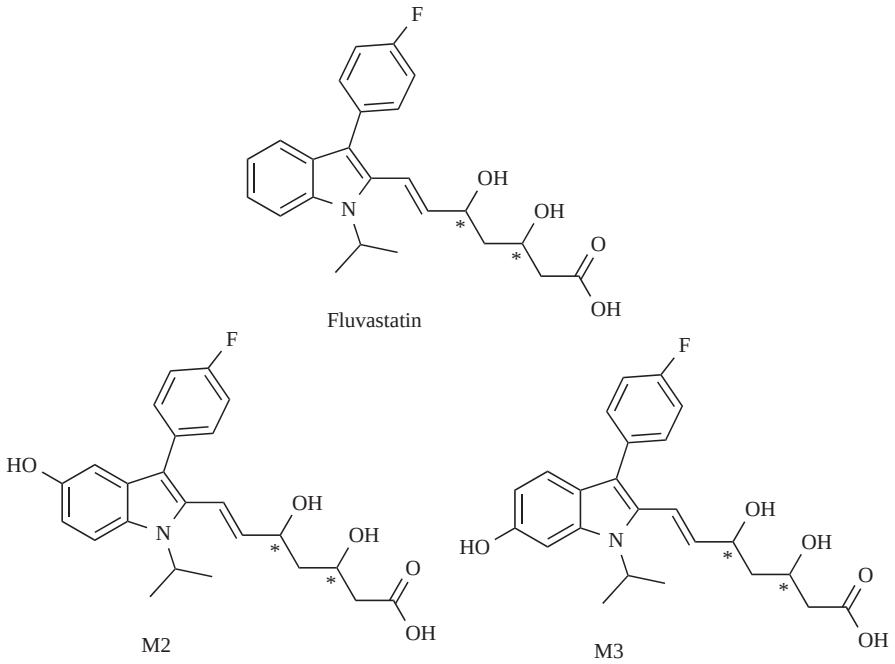

Fig. (1). Chemical structures of Fluvastatin and its major metabolites. * indicates chiral carbon.

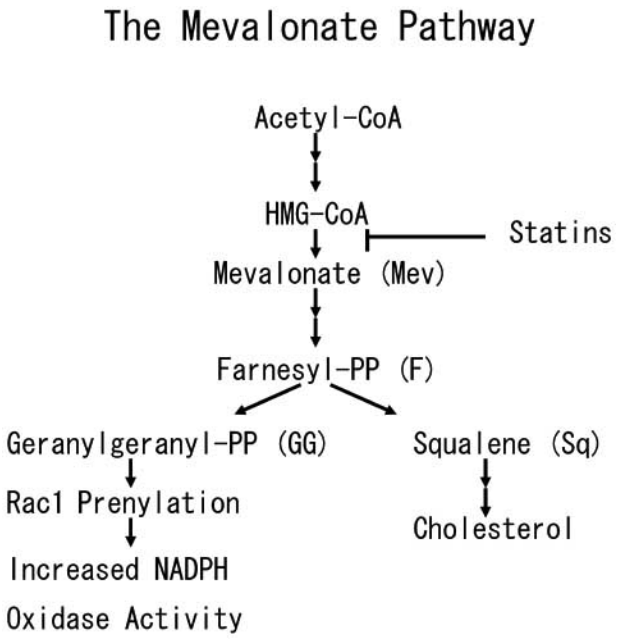

Fig. (2). Mevalonate pathway and oxidative stress.

and translocation of rac1 to the cell membrane [18, 19, 20] (Fig. **2**). Inhibition of Rac1 by statins decreases NADPH oxidase-related ROS production in vascular smooth muscle cells and cardiac myocytes. However, the fact that fluvastatin and metabolites, which have less HMG CoA reductase inhibitory action than simvastatin, has an equivalent effect of antioxidation to simvastatin [11]. This suggests that two different mechanisms (suppression of mevalonate pathway-mediated process and direct scavenging caused by two metabolically linked compounds) may exist in the action of fluvastatin (Fig. **3**).

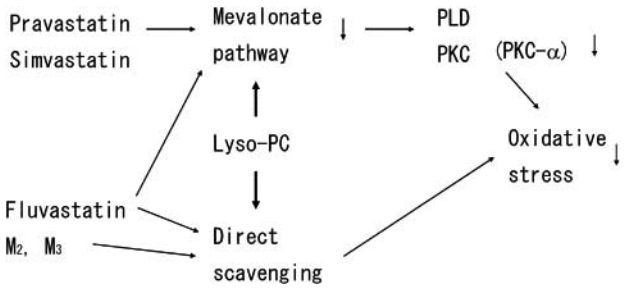

Fig. (3). *In vitro* effects of dual actions of antioxidative effect.

IN VIVO STUDY

In cynomolgus monkeys, pravaststin improved plaque morphology and endothelial function independent of cholesterol lowering effect [21]. A possible explanation is the redution of circulating mevalonate and isoprenoids by hepatic inhibition of HMG-CoA reductase, resulting in decreased prenylation of GTP proteins in peripheral tissue.

To obtain further insight into the mechanism of action of statins, we studied the effect of pitavastatin on the generation of reactive oxygen species (ROS) by peritoneal PMN obtained from control and hyperlipidemic guinea pigs [22]. Flow cytometric analysis revealed that the amount of ROS generated by PMN from the hyperlipidemic animals that had been administered a laurate-containing diet (LD) for 4 weeks was larger than that from the normal diet (ND) group. Administration of pitavastatin to the LD group significantly decreased plasma levels of total cholesterol (TC) and low-density lipoprotein (LDL) with a reduction in ROS generation by PMN (Fig. **4**). Western blotting analysis

revealed that the expression of protein kinase C α (PKC α) was higher in PMN from the LD group than in PMN from the ND group. Expression of NADPH oxidase gp91phox in PMN from the LD group was higher than that in PMN from the ND group. By administration of pitavastatin to the LD group, the expression of PKC α, and gp91phox was suppressed compared with the control LD. These results indicate that PMN from hyperlipidemic animals is associated with an accelerated respiratory burst of ROS by increasing the expression of PKC α, and gp91phox, and pitavastatin inhibits this by suppressing the expression of those proteins.

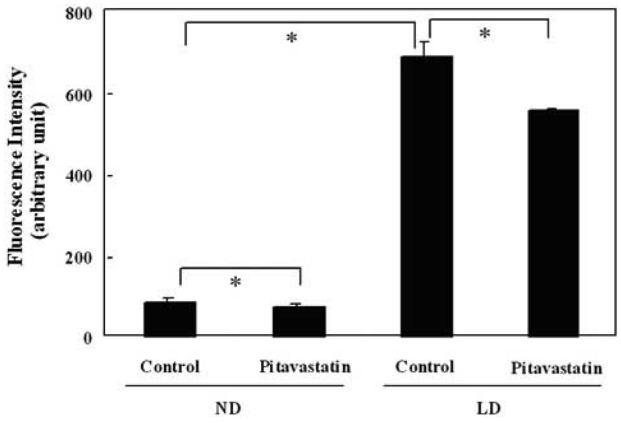

Fig. (4). Effect of pitavastatin in ROS generation by PMN from ND and LD.

Fluorescence intensities of peritoneal PMN from ND and LD were analyzed using flow cytometry in the absence or presence of 1 mg/kg pitavastatin for 2 weeks. Values (arbitrary units) are means±SD for 6 animals, P<0.01 (Reference 16).

CLINICAL STUDY-REGRESSION OF CAROTID ATHEROMA BY STATINS

Inconsistent effects of antioxidant on clinically coronary endpoints, in sharp contrast with the studies with statins has been reported [23], although antioxidative actions of statins were observed *in vitro* [24, 25, 26]. Therefore, it is important from the clinical point of view to confirm that statins actually reduces atherosclerosis in humans.

It has been reported that atrovastatin therapy was effective in atherosclerosis regression in the 2-year Atrovastatin versus Simvastatin on Atherosclerosis Progression (ASAP) study [27, 28]. The effect of statin intervention on the atherosclerosis process was monitored in this trial by measuring carotid intima-media thickness (IMT). Atorovastatin provides superior efficacy to pravastatin for carotid atherosclerosis (IMT) regression for 1 year [29]. The effects were related to the reduction of LDL, which is the marker of HMG-CoA reductase inhibition.

We have performed a randomized, control study with pitavastatin 2mg, which efficacy is comparable to atrovastatin 10mg in HMG-CoA reductase inhibition. Patients are randomly assigned pitavastatin 2mg (n=12) or placebo (n=13) and have been treated for six months. Carotid intima-media thickness (IMT) were measured by echocardiography before and after treatment. Statistical analysis was performed by the paired t-test for tntragroup comparisons and post hoc test for intergroup comparisons preceded by analysis of variance (ANOVA). There was no significant difference in IMT between both pitavastatin treated group and placebo treated group Pitavastatin 2mg showed the significant carotid IMT regression at 6 months compared with control in patients with and essential hypertension (Fig. **5**). Since IMT is a validated surrogate marker of the cardiovascular endpoint [30, 31, 32, 33],

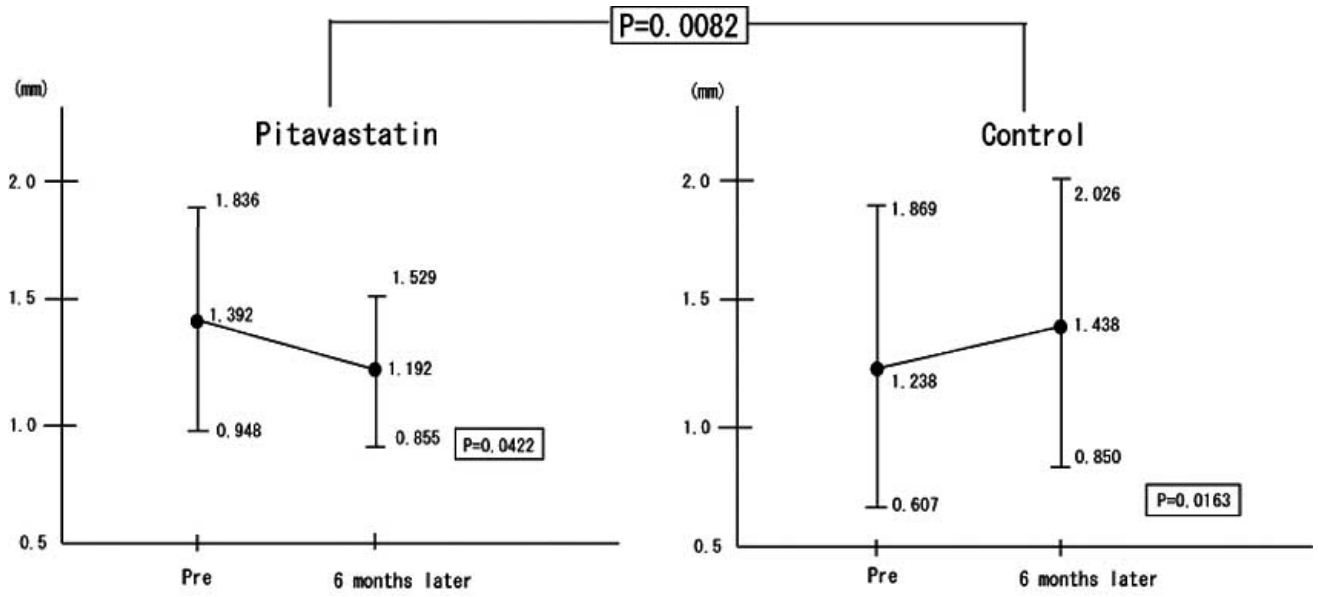

Fig. (5). Effect of pitavastatin on carotid IMT in patients with essential hypertension.

Patients are randomly assigned pitavastatin 2mg or placebo and have been treated for six months. Carotid intima-media thickness (IMT) were measured by echocardiography before and after treatment. P<0.05 is considered to be significant different.

marked HMG-CoA reductase inhibition by atrovastatin or pitavastatin, which are related to marked decrease in oxidative stress, may provide enhanced reduction in clinical coronary event rate.

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