

## 3-HMG-CoA Reductase Inhibitors and Inflammation: Underlying Mechanisms and Role in Atherosclerosis

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DOI: <http://dx.doi.org/10.13005/bbra/1181>

(Received: 16 October 2013; accepted: 04 December 2013)

**The 3-HMG-CoA reductase inhibitors, referred to as statins, are one of the most potent lipid lowering drugs. They significantly reduce incidence of cardiovascular mortality and morbidity. Data from clinical trials and experimental evidence suggest that the observed benefits can be attributed in part to their cholesterol-lowering independent effects. Statins lower the level of hs-CRP, an important inflammatory marker, thereby reducing the risk of stroke and cardiovascular events. The need for routine assay of hs-CRP is advocated in identifying patients at risk, that may benefit from statin therapy.**

**Key words:** Atherosclerosis, C-reactive Protein(CRP), Cholesterol, Inflammation and Statins.

Inflammatory cell adhesion and endothelial function are regulated by statins. Statins have been shown to decrease risk of cardiovascular disease by a novel anti-inflammatory mechanism independent of cholesterol manipulation. The reduction in lesion progression achieved in atherosclerotic plaque cannot be fully explained by cholesterol lowering alone, but could be related to thrombogenicity and anti-inflammatory effects<sup>1-3</sup>. The inflammatory response, though intended to combat the plaque, actually renders it less stable and more prone to rupture, predisposing to increased risk of cerebrovascular accident and coronary heart disease. High sensitivity C-reactive protein (hs-CRP), though a non-specific measure of inflammation, its elevation is an important predictor of heart attack; particularly in individuals without any previous incidence of heart attack. High sensitivity C-reactive protein (hs-CRP) is

an important indicator of low grade systematic inflammation.<sup>4</sup> Studies have shown that C-reactive protein levels are reduced by statins<sup>5-7</sup>. This review highlights the anti-inflammatory effects of statins and the underlying mechanisms, emphasizing the link with atherosclerosis.

### **Anti-inflammatory Effects of Statins and Underlying Mechanisms**

Statins have been shown to regulate inflammatory cell adhesion and endothelial function. Statins inhibit the expression of ICAM-1 (intracellular adhesion molecule) on human monocytes<sup>8</sup> and prevent lipopolysaccharide (LPS)-induced ICAM-1 expression in endothelial cells via inhibition of Rho activity<sup>9</sup>. Furthermore, statins decrease CD11b-dependent adhesion of monocytes stimulated or not, with monocyte chemoattractant protein-1 (MCP-1)<sup>10</sup> and reduce the leukocyte-adherence responses to platelet-activating factor and to leukotriene B4 in hypercholesterolemic rats<sup>11</sup>. Also statins block the adhesion of lymphocytes mediated by leukocyte function antigen-1 (LFA-1), a B2 integrin expressed on leukocyte surface that binds to ICAM-1<sup>12</sup>. Statins decrease the expression of chemoattractant molecules MCP-1

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and interleukin-8<sup>13</sup>. As a consequence, there is a reduction in macrophage infiltration and MMP-3 expression. The mechanism of the reduction of MCP-1 and IL-8 expression seem to be due to a decrease in the activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B). This redox-sensitive transcription factor is involved in the transmission of various signals from the cytoplasm to the nucleus of numerous cell types<sup>14</sup>. It is found in the cytosol as a trimer consisting of p50 and p65 subunits bound to its inhibitor I $\kappa$ B. The release of I $\kappa$ B from the trimer results in the migration of the p50/65 heterodimer to the nucleus and subsequent DNA binding<sup>15</sup>. This process activates genes involved in the immune, inflammatory or acute phase response, chemoattractant cytokines like MCP-1 and IL-8, pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) and procoagulant proteins as tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1)<sup>16,17</sup>. Data strongly suggest that NF- $\kappa$ B could be involved in inflammatory response<sup>18</sup> since it participates in the dysregulation of vascular smooth muscle cells (VSMC) in inflammation<sup>19</sup> and is present in the nuclei of macrophages and endothelial cells<sup>20</sup>. Cyclooxygenase-2 expression is reduced also by statin treatment. This isoform of cyclooxygenase enhances the production of the chemoattractant prostaglandin E<sub>2</sub><sup>21</sup>.

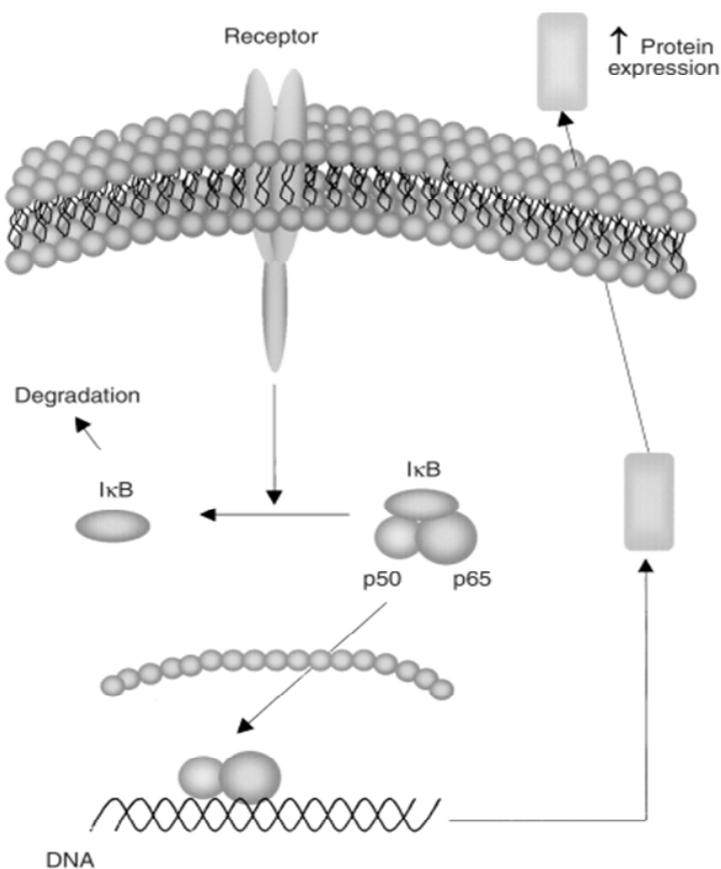
The blockade of cell recruitment into the vessel wall by statins are reinforced by further studies. Statin treatment is reported to retard enlargement of plaque size and reduces macrophage accumulation in Watanabe heritable hyperlipidemic rabbits<sup>22</sup>. Furthermore<sup>23</sup>, study demonstrates that statin treatment reduces the macrophage infiltrate and the expression of MMP-1, MMP-3, MMP-9 and tissue factor in the same model and decreases macrophage proliferation and proteolytic activity due to MMP-9 in human macrophages *in vitro*. The same group demonstrated that statins reduce MMP-3 and MMP-9 expression in the absence of modifications of the macrophage infiltrate, suggesting a reduction in their expression<sup>24</sup>. However, it has been demonstrated in an experimental design allowing the investigation of the effects of statins in human subjects; the presence of less macrophages and T cell infiltration, reduced MMP-2 expression as well as higher immunoreactivity for tissue inhibitor of

MMP-1<sup>25</sup>. Different markers of inflammation have been studied to analyze the effects of statins on inflammatory states. In particular, high-sensitivity C-reactive protein (hs-CRP) reflects low-grade systemic inflammation<sup>4</sup>. Human studies have demonstrated that statins are able to reduce CRP serum levels in hyperlipidemic patients<sup>5-6</sup>. Other inflammatory molecules are affected by statins. Serum MMP-9 levels which have been found to increase during acute coronary syndromes<sup>27</sup>, are decreased by statins in clinically healthy men, reflecting the reduction of non-symptomatic chronic arterial inflammation<sup>28</sup>. Hypercholesterolemic individuals treated with simvastatin show a reduction in the levels of the pro-inflammatory cytokines, tumour necrosis factor alpha (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\kappa$ )<sup>29,30</sup>. The activity of the transcription factor NF- $\kappa$ B in circulating leukocytes is enhanced in patients with unstable angina and in healthy volunteers after a fat-enriched meal<sup>31,32</sup>. Treatment with simvastatin reduces this activity in mononuclear circulating cells. It has been noted, in human studies, that hypercholesterolemia up-regulated the pro-inflammatory receptor CD40 and its ligand CD40L<sup>33</sup>. It has been demonstrated that oxidized LDL increases NF- $\kappa$ B activation and MMP-9 expression in cultured human monocyte-derived macrophages<sup>34</sup>. Also CD40L expression and IFN- $\gamma$  production are enhanced by lysophosphatidylcholine in human CD4<sup>+</sup> T cells<sup>35</sup>. It has been demonstrated that reducing lipid levels only by dietary modification decreases the expression of CD40 and CD40L, co-localizing with a reduction of tissue immunoreactivity<sup>36</sup>. Also, a diminution of macrophage infiltrate, MMP-1 expression and proteolytic activity are observed in the same model<sup>37</sup>.

Data accumulated over the years have suggested that some effects of statins, could be independent of their lipid-lowering ability. This is supported by clinical studies such as the WOSCOPS (West of Scotland Coronary Prevention Study Group) study, where evidence shows that patients on statins had fewer coronary events than those on placebo who had similar LDL levels<sup>38</sup>. The reduction of CRP (C-reactive protein) serum levels achieved by different statins is not related to the decrease in lipid levels<sup>7,39,40</sup>. It has been demonstrated that increment in CRP levels has a predictive value of the possibility of an acute

coronary event<sup>41</sup>. A study reported decrease in macrophage infiltration in atherosclerotic monkeys after treatment with statins and diet manipulation to avoid changes in serum lipid levels<sup>42</sup>. Simvastatin has been found to reduce inflammation in a model of carrageenan-induced foot-pad edema in mice<sup>43</sup>. Simvastatin also has been found to reduce leukocyte rolling and adherence in rats<sup>44</sup> and apolipoprotein E-deficient mice<sup>45</sup>. It has been found that the lipid-independent effects of these agents are related to the inhibition of other isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesyl pyrophosphate (FPP) and geranyl-geranyl pyrophosphate (GGPP)<sup>46</sup>. FPP and GGPP are used for the post-translational modification of several important cell proteins including nuclear lamins,

heme-a, the  $\gamma$ -subunit of heterotrimeric G proteins and small G proteins such as Ras and Ras-like proteins<sup>47</sup>. The attachment of an isoprenoid residue to those proteins is necessary for their anchorage to the cell membranes and full functionality. Statins inhibit Ras and Rho isoprenylation leading to the accumulation of their inactive forms in the cytoplasm<sup>48</sup>. These proteins are implicated in different functions in the cells such as gene expression, organization of the actin cytoskeleton, membrane trafficking, programmed cell death, proliferation and transformation<sup>49,50</sup>. Inhibition of Rho can affect intracellular transport, membrane trafficking, mRNA stability and gene transcription<sup>51</sup>. Thus, it has been seen that statins directly reduce the expression of MCP-1 and IL-8 as well as



**Fig. 1.** The redox sensitive transcriptase, nuclear factor- $\kappa$ B (NF- $\kappa$ B), is the major initiator of inflammatory cascade and activates genes involved in the mediation and perpetuation of inflammatory response. Statins inhibit the activity of NF- $\kappa$ B thereby reducing the risk of atherosclerosis. (Adapted and modified from-Barnes P.J. and Karim M. Nuclear factor- $\kappa$ B: a pivotal transcription factor in chronic inflammatory disease. *N. Engl. J. Med.* 336: 1066-1107 (1997)

NF- $\kappa$ B activation induced by angiotensin II and TNF- $\alpha$  in cultured monocytes and vascular smooth muscle cell (VSMC)<sup>52</sup>. Treatment with statins can modulate pro-inflammatory cytokines expression such as IL-1 $\beta$ , IL-6 and COX-2 by up-regulating the peroxisome proliferator activated receptor- $\alpha$  in endothelial cells<sup>53</sup>. Activator Protein-1 (AP-1) activation is decreased by statin treatment in renal epithelial tubular cells<sup>54</sup>. Simvastatin, in addition, increases the activation of the octamer transcription factor Oct-1 in mononuclear cells which represses the transcription of pro-inflammatory genes such as IL-8, CD11c/CD18, VCAM-1 and PECAM-1<sup>55</sup>. It has been shown that inhibition of VSMC proliferation is correlated with increase in the level of cyclin-dependent kinase inhibitor and is related with the inhibition of Rho A isoprenylation<sup>56</sup>.

Antiproliferative effects of statins in animal models without significant changes in serum cholesterol concentrations have also been reported<sup>57,58</sup>. Additionally, in patients treated with statins, there is decreased proliferation of VSMC *in vitro*<sup>59</sup>. It has been demonstrated that lipophilic statins induce apoptosis of VSMC in culture and this effect is related to the interference of protein isoprenylation<sup>60</sup>. Statins may alter the local fibrinolytic balance within the vessel wall toward increased fibrinolytic capacity, which is related to the inhibition of PAI-1 expression from VSMC and endothelial cells<sup>61</sup>.

### CONCLUSION

Statins possess anti-inflammatory properties independent of their cholesterol lowering effects. Significant reduction in cardiovascular events and stroke is attributable to statin therapy particularly in individuals at risk with elevated high sensitivity C-reactive proteins (hs-CRP). Experimental evidence both *in vivo* and *in vitro*, clearly suggest that anti-inflammatory effects of statins may contribute to plaque stabilization. Hence, routine assay of inflammatory markers such as hs-CRP is invaluable in identifying individuals at risk that may benefit from statin therapy.

### REFERENCES

1. Brown B.G. and Fuster V. Impact of management in stabilization of coronary disease. *Atherosclerosis* and *Coronary Artery Disease*, edited by Fuster V., Ross R., Topol E.J., Philadelphia, Lipincott-Raven, 1966; 191-205.
2. O'Driscoll G., Green D. and Taylor R.R. Simvastatin, an HMG-CoA reductase inhibitor, improves endothelial function within 1 month. *Circulation*. 1997; **95**: 1126-1131.
3. Dangas G., Badimon J.J., Smith D.A., Unger A.H., Levine D., Shao J.H., Meraj P., Fier C., Fallon J.T. and Ambrose J.A. Pravastatin therapy in hyperlipidemia: Effects on thrombus formation and the systemic hemostatic profile. *J. Am. Coll. Cardiol*. 1999; **33**: 1294-1304.
4. Baumann H. and Gauldie J. The acute phase response. *Immunol Today*. 1994; **15**: 74-80.
5. Ridker P.M., Rifai N., Clearfield M., Downs J.R., Weis S.E., Miles J.S. and Gotto AM. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N. Engl. J. Med*. 2004; **344**: 1959-1965.
6. Gomez-Gerique J.A., Ros E., Oliván J., Mostaza J.M., Vilardell M., Pinto X., Civeira F., Hernandez A., Marques da Silva P., Rodriguez-Botaro A., Zambon D., Lima J., Diaz C., Aristequi R., Sol J.M., Chaves J. and Hernandez G. Effects of atorvastatin and bezafibrate on plasma levels of C-reactive protein in combined (mixed) hyperlipidemia. *Atherosclerosis*. 2002; **162**: 245-251.
7. Ridker P.M., Rifai N. and Lowenthal S.P. Rapid reduction in C-reactive protein with cerivastatin among 785 patients with primary hypercholesterolemia. *Circulation*. 2001; **103**: 1191-1193.
8. Niwa S., Totsuka T. and Hayashi S. Inhibitory effects of fluvastatin, an HMG-CoA reductase inhibitor, on the expression of adhesion molecules on human monocyte cell line. *Int. J. Immunopharmacol*. 1996; **18**: 669-675.
9. Takeuchi S., Kawashima S., Rikitake Y., Ueyama T., Inoue N., Hirata K. and Yokoyama M. Cerivastatin suppresses lipopolysaccharide-induced ICAM-1 expression through inhibition of Rho GTPase in BAEC. *Biochem. Biophys. Res. Commun*. 2000; **269**(1): 97-102.
10. Weber C., Erl W., Weber K.S. and Weber P. HMG-CoA reductase inhibitors decrease CD11b expression and CD11b-dependent adhesion of monocytes to endothelium and reduce increased adhesiveness of monocytes isolated from patients with hypercholesterolemia. *J. Am. Coll. Cardiol*. 1997; **30**: 1212-1217.
11. Kimura M., Kurose I., Russel J. and Granger D.N. Effects of fluvastatin on leukocyte-endothelial

- cell adhesion in hypercholesterolemic rats. *Arterioscler. Thromb. Vasc. Biol.* 1997; **17**: 1521-1526.
12. Weitz Schimidt G., Welzenbach K., Brinkmann V., Kamata T., Kallen J., Bruns C., Cotton S., Takada Y. and Hommel U. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat. Med.* 2001; **7**: 687-692.
  13. Hernandez-Presa M.A., Martin-Ventura J.L., Ortego M., Gomez-Hernandez A., Hernandez-Vargas P., Blanco-Colio L.M., Mas S., Aparicio C., Ortega L., Vivanco F., Gomez-Gerique J., Diaz C., Hernandez G. and Egido J. Atorvastatin reduces the expression of cyclooxygenase-2 in a rabbit model of atherosclerosis and in cultured vascular smooth muscle cells. *Atherosclerosis.* 2002; **160**: 147-153.
  14. Grimm S. and Baeuerle P.A. The inducible transcription factor NF- $\kappa$ B: structure-function relationship of its protein subunits. *Biochem. J.* 1993; **290**: 297-308.
  15. Henkel T., Machleidt T., Alkalay I., Kronke M., Ben-Neriah Y. and Baeuerle P.A. Rapid proteolysis of I $\kappa$ B- $\alpha$  is necessary for activation of transcription factor NF- $\kappa$ B. *Nature.* 1993; **365**: 182-185.
  16. Egido J., Hernandez-Presa M.A., Tunon J., Blanco-Colio L.M., Ortego M. and Suzuki Y. Transcription factor  $\kappa$ B (NF- $\kappa$ B) and cardiovascular disease. *Cardiovasc. Risk Factors.* 1999; **9**: 159-168.
  17. Barnes P.J. and Karin M. Nuclear factor- $\kappa$ B: a pivotal transcription factor in chronic inflammatory disease. *N. Engl. J. Med.* 1997; **336**: 1066-1071.
  18. Brand K., Page S., Walli A.K., Neumeier D. and Baeuerle P.A. Role of nuclear factor-kappa B in atherogenesis. *Exp. Physiol.* 1997; **82**: 297-304.
  19. Bourcier T., Sukhova G. and Libby P. The nuclear factor kappa-B signaling pathway participates in dysregulation of vascular smooth muscle cells *in vitro* and in human atherosclerosis. *J. Biol. Chem.* 1997; **272**: 15817-15824.
  20. Brand K., Page S., Rogler G., Bartsch A., Brandt R., Knuechel R., Page M., Kaltschmidt C., Baeuerle P.A. and Neumeier D. Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. *J. Clin. Invest.* 1996; **97**: 1715-1722.
  21. Simon L.S. Role and regulation of cyclooxygenase-2 during inflammation. *Am. J. Med.* 1999; **106**: S37-S42.
  22. Shiomi M. and Ito T. Effect of cerivastatin sodium, a new inhibitor of HMG-CoA reductase, on plasma lipid levels, progression of atherosclerosis and the lesional composition in the plaques of WHHL rabbits. *Br. J. Pharmacol.* 1999; **126**: 961-968.
  23. Aikawa M., Rabkin E., Sugiyama S., Voglic S.J., Fukumoto Y., Furukawa Y., Shiomi M., Schoen F.J. and Libby P. An HMG-CoA reductase inhibitor, cerivastatin, suppresses growth of macrophages expressing matrix metalloproteinases and tissue factor *in vivo* and *in vitro*. *Circulation.* 2001; **103**: 276-283.
  24. Fukumoto Y., Libby P., Rabkin E., Hill C.C., Enomoto M., Hirouchi Y., Shiomi M. and Aikawa M. Statins alter smooth muscle cell accumulation and collagen content in established atheroma of Watanabe heritable hyperlipidemic rabbits. *Circulation.* 2001; **103**: 993-999.
  25. Crisby M., Nordin-Fredriksson G., Shah P.K., Yano J., Zhu J. and Nilsson J. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases and cell death in human carotid plaques: implications for plaque stabilization. *Circulation.* 2001; **103**: 926-933.
  26. Musial J., Undas A., Gajewski P., Jankowski M., Sydor W. and Szczekli K.A. Anti-inflammatory effects of simvastatin in subjects with hypercholesterolemia. *Int. J. Cardiol.* 2001; **77**: 247-253.
  27. Kai H., Ikeda H., Yasukawa H., Kai M., Seki Y., Kuwahara F., Ueno T., Sugi K. and Imaizumi T. Peripheral blood levels of matrix metalloproteinases-2 and 9 are elevated in patients with acute coronary syndromes. *J. Am. Coll. Cardiol.* 1998; **32**: 368-372.
  28. Kalela A., Laaksonen R., Lehtimaki T., Koivu T.A., Hoyhtya M., Janatuinen T., Pollanen P., Vesalainen R., Saikku P., Knuuti J. and Nikkari S.T. Effects of pravastatin in mildly hypercholesterolemic young men on serum matrix metalloproteinase. *Am. J. Cardiol.* 2001; **88**: 173-175.
  29. Solheim S., Seljeflot I., Arnesen H., Eritsland J. and Eikvar L. Reduced levels of TNF alpha in hypercholesterolemic individuals after treatment with pravastatin for 8 weeks. *Atherosclerosis.* 2001; **157**: 411-415.
  30. Ferro D. and Viola F. Simvastatin inhibits the monocyte expression of pro-inflammatory cytokines in patients with hypercholesterolemia. *J. Am. Coll. Cardiol.* 2000; **36**: 427-431.
  31. Ritchie M.E. Nuclear factor  $\kappa$ B is selectively and markedly activated in humans with unstable angina pectoris. *Circulation.* 1998; **98**: 1707-1713.
  32. Blanco-Colio L.M., Valderrama M., Alvarez-Sala

- L.A., Bustos C., Ortego M., Hernandez-Presa M.A., Cancelas P., Gomez-Gerique J., Millan J. and Egido J. Red wine intake prevents nuclear factor-kappa B activation in peripheral blood mononuclear cells of healthy volunteers during post prandial lipemia. *Circulation*. 2000; **102**: 1020-1026.
33. Garlichs C.D., John S., Schmeisser A., Eskafi S., Stumpf C., Karl M., Goppelt-Struebe M., Schmieder R. and Daniel W.G. Upregulation of CD40 and CD40 ligand (CD154) in patients with moderate hypercholesterolemia. *Circulation*. 2001; **104**: 2395-2400.
34. Xu X.P., Meisel S.R., Ong J.M., Kaul S., Cercek B., Rajavashisth T.B., Sharifi B. and Shah P.K. Oxidized low density lipoprotein regulates matrix metalloproteinase-9 and its tissue inhibitor in human monocyte-derived macrophages. *Circulation*. 1999; **99**: 993-998.
35. Sakata-kaneko S., Wakatsuki Y., Usui T., Matsunaga Y., Itoh T., Nishi E., Kume N. and Kita T. Lysophosphatidylcholine upregulates CD40 ligand expression in newly activated human CD4+ T cells. *FEBS Lett*. 1998; **433**: 161-165.
36. Aikawa M., Voglic S.J., Sugiyama S., Rabkin E., Taubman M.B., Fallon J.T. and Libby P. Dietary lipid lowering reduces tissue factor expression in rabbit atheroma. *Circulation*. 1999; **100**: 1215-1222.
37. Aikawa M., Rabkin E., Okada Y., Voglic J.S., Clinton S.K., Brinckerhoff C.E., Sukhova G.K. and Libby P. Lipid lowering by diet reduces matrix metalloproteinase activity and increases collagen content of rabbit atheroma. *Circulation*. 1998; **97**: 2433-2444.
38. West of Scotland Coronary Prevention Study Group. Influence of pravastatin and plasma lipids on clinical events in the West of Scotland Coronary Prevention Study (WOSCOPS). *Circulation*. 1998; **97**: 1440-1445.
39. Jialal I., Stein D., Balis D., Grundy S.M., Adams-Huet B. and Devaraj S. Effect of hydroxymethyl glutaryl co-enzyme A reductase inhibitor therapy on high sensitive C-reactive protein levels. *Circulation* 2001; **103**: 1933-1935.
40. Chang J.W., Yang W.S., Min W.K., Lee S.K., Park J.S. and Kim S.B. Effects of simvastatin on high-sensitivity C-reactive protein and serum albumin in hemodialysis patients. *Am. J. Kidney Dis*. 2002; **39**: 1213-1217.
41. Biasucci L.M., Liuzzo G., Grillo R.L., Caligiuri G., Rebuzzi G.A., Buffon A., Summaria F., Ginnetti F., Fadda G. and Maseri A. Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. *Circulation*. 1999; **99**: 855-860.
42. Willams J.K., Sukhova G.K., Herrington D.M. and Libby P. Pravastatin has cholesterol-lowering independent effects on the artery of atherosclerotic monkeys. *J. Am. Coll. Cardiol*. 1998; **31**: 684-691.
43. Sparrow C.P., Burton C.A., Hernandez M., Mundt S., Hassing H., Patel S. and Rosa R. Simvastatin has anti-inflammatory and anti-atherosclerotic activities independent of plasma cholesterol lowering. *Arterioscler. Thromb. Vasc. Biol*. 2001; **21**: 115-121.
44. Preufer D., Scalia R. and Lefer A.M. Simvastatin inhibits leukocyte-endothelial cell interactions and protects against inflammatory processes in normocholesterolemic rats. *Arterioscler. Thromb. Vasc. Biol*. 1999; **12**: 2894-2900.
45. Scalia R., Gooszen M.E., Jones S.P., Hoffmeyer M., Rimmer D.M., Trocha S.D., Huang P.L., Smith M.B., Lefer A.M. and Lefer D.J. Simvastatin exerts both anti-inflammatory and cardio-protective effects in apolipoprotein E-deficient mice. *Circulation*. 2001; **103**: 2598-2603.
46. Goldstein J.L. and Brown M.S. Regulation of the mevalonate pathway. *Nature*. 1990; **343**: 425-430.
47. Van Aelst L. and D' Souza-Schorey C. Rho GTPases and signaling networks. *Genes Dev*. 1997; **11**: 2295-2322.
48. Blanco-Colio L.M., Villa A., Ortego M., Hernandez-Presa M.A., Pascual A., Plaza J.J. and Egido J. 3-hydroxy-3-methyl glutaryl co-enzyme A reductase inhibitors atorvastatin and simvastatin induce apoptosis in vascular smooth muscle cells by downregulation of Bcl-2 expression and Rho A prenylation. *Atherosclerosis*. 2002; **161**(1): 17-26.
49. Mackay D.J. and Hall A. Rho GTPases. *J. Biol. Chem*. 1988; **273**: 20685-20688.
50. Zohn I.M., Campbell S.L., Khosravi-Far R., Rossman K.L. and Der C.J. Rho family proteins and ras transformation: The RHOad less traveled gets congested. *Oncogene*. 1998; **17**: 1415-1438.
51. Takemoto M. and Liao J. Pleiotropic effects of 3-hydroxy-3-methyl glutaryl co-enzyme A reductase inhibitors. *Artheroscler. Thromb. Vasc. Biol*. 2001; **21**: 1712-1719.
52. Ortego M., Bustos B., Hernandez-Presa M.A., Tunon J., Diaz C., Hernandez G. and Egido J. Atorvastatin reduces NF- $\kappa$ B activation and chemokine expression in vascular smooth muscle cells and mononuclear cells. *Atherosclerosis*. 1999; **147**: 253-261.
53. Inoue I., Goto S., Mizotani K., Awata T., Mastunaga T. and Kawai S. Lipophilic HMG-CoA reductase

- inhibitor has an anti-inflammatory effect: reduction of mRNA levels for interleukin-1 $\alpha$ , interleukin-6, cyclooxygenase-2, and p22phox by regulation of peroxisome proliferator-activated receptor- $\alpha$  (PPAR- $\alpha$ ) in primary endothelial cells. *Life Sci.* 2000; **67**: 863-876.
54. Vrtovsnik F., Couette S., Prie D., Lallemand D. and Friedlander G. Lovastatin-induced inhibition of renal epithelial tubular cell proliferation involves a p21 ras activated, AP-1-dependent pathway. *Kidney Int.* 1997; **52**: 1016-1027.
55. Ortego M., Gomez-Hernandez A., Bustos C., Blanco-Colio L.M. and Hernandez-Presa M.A. 3-hydroxy-3-methyl glutaryl co-enzyme A reductase inhibitors increase the binding activity and nuclear level of Oct-1 in mononuclear cells. *Eur. J. Pharmacol.* 2002; **448**: 113-121.
56. Laufs U., Marra D., Node K. and Liao J.K. 3-hydroxy-3-methyl glutaryl co-enzyme A reductase inhibitors attenuate vascular smooth muscle proliferation by preventing rho GTPase induced downregulation of p27 (Kip1). *J. Biol. Chem.* 1999; **274**: 21926-21931.
57. Corsini A., Mazzotti M., Raiteri M. and Soma M.R. Relationship between mevalonate pathway and arterial myocyte proliferation: *in vitro* studies with inhibitors of HMG-CoA reductase. *Atherosclerosis.* 1993; **101**: 117-125.
58. Soma M.R., Donetti E., Parolini C., Mazzini G., Ferrari C., Fumagalli R. and Paoletti R. HMG-CoA reductase inhibitors: *in vivo* effects on carotid intimal thickening in normocholesterolemic rabbits. *Arterioscler. Thromb. Vasc. Biol.* 1993; **13**: 571-578.
59. Corsini A., Pazzucconi F., Arnaboldi L., Pfister P., Fumagalli R., Paoletti R. and Sirtori C.R. Direct effects of statins on the vascular wall. *J. Cardiovasc. Pharmacol.* 1998; **31**: 773-778.
60. Guijarro C., Blanco-Colio L.M., Ortego M., Alonso C., Oritz A., Plaza J.J., Diaz C., Hernandez G. and Egido J. 3-hydroxy-3-methyl glutaryl co-enzyme A reductase and isoprenylation inhibitors induce apoptosis in vascular smooth muscle cells in culture. *Circ. Res.* 1998; **83**: 490-500.
61. HMG-CoA reductase inhibitors reduce plasminogen activator inhibitor-1 expression by human vascular smooth muscle and endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 2000; **20**: 556-562.