

The role of HMG-CoA reductase inhibition in endothelial dysfunction and inflammation

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Abstract: Statin-induced inhibition of HMG-CoA reductase reduces cholesterol production and prevents the formation of many non-steroidal isoprenoid compounds, such as farnesylpyrophosphate and geranylgeranylpyrophosphate, that act as lipid attachments for the post-translational modification of various proteins, including the G-proteins and transcription factors involved in a number of cell processes. However, the blockade of isoprenylation elicited by statin treatment also has biological effects on cell function that go beyond the decrease in cholesterol synthesis: these are the so-called “pleiotropic” effects that mainly relate to vascular function. Endothelial dysfunction is an independent predictor of cardiovascular events that correlates with inflammation markers/mediators and robust predictors of cardiovascular diseases such as increased high-sensitivity C-reactive protein levels. The results of in vivo and in vitro studies indicate that the statins have beneficial effects unrelated to cholesterol lowering, such as improving endothelial function, increasing myocardial perfusion, and enhancing the availability of nitric oxide. This review describes the pleiotropic effects of statins that may be involved in modulating/preventing endothelial dysfunction and inflammatory processes, as well as the cellular and molecular mechanisms through which they improve endothelial function.

Keywords: statins; inflammation; endothelial dysfunction; nitric oxide; HMG-CoA reductase

Introduction

The endothelium is a monocellular layer lining the inside of vessels that normally provides a non-adhesive, non-thrombogenic surface for blood constituents, and acts as a dynamic interface regulating blood vessel functions (Behrendt and Ganz 2002). It influences responses to environmental and endogenous factors by generating paracrine and autocrine mediators that control the biology of the entire vessel wall. The endothelium plays a pivotal role in regulating vascular tone, but also controls other physiological process such as inflammation, coagulation and thrombosis.

Persistent hemodynamic or inflammatory factors activate the endothelium and lead to it becoming dysfunctional. In particular, endothelial cell activation by cytokines or other inflammatory mediators increases the expression of a variety of cell surface adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), procoagulants and anticoagulants, and substances regulating vasomotor tone. One hallmark of endothelium dysfunction is an altered response to endothelium-dependent and endothelium-independent stimuli, such as acetylcholine and bradykinin (Harrison 1997). These effects are due to a reduced bioavailability of nitric oxide (NO), which may be caused by a decrease in the synthesis, release and/or activity of endothelial-derived NO.

It is now known that abnormal endothelium function can be detected before the establishment of obvious intimal lesions in patients with risk factors for atherosclerosis (Celermajer et al 1992), and endothelial dysfunction of the coronary or peripheral

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arteries is an independent predictor of cardiovascular events, even after adjusting for traditional factors (Ganz and Vita 2003). Endothelial dysfunction correlates with inflammation markers/mediators and robust predictors of cardiovascular diseases, such as increased high-sensitivity C-reactive protein (hs-CRP) levels in subjects with coronary artery disease (Fichtlscherer et al 2000). This observation is of particular interest because CRP stimulates the expression of VCAM-1, thus highlighting once again the link between inflammation and endothelial dysfunction (Pasceri et al 2000). Furthermore, it has been suggested that enhanced endothelial function may contribute to improved clinical status (Anderson et al 1995; Treasure et al 1995).

Experimental and clinical studies have shown that hypercholesterolemia, a major risk factor for vascular diseases, impairs endothelium function (Creager et al 1990; Egashira et al 1993), and LDL apheresis alone rapidly exerts beneficial effects on endothelial vasodilator function within a few hours (Tamai et al 1997).

It has been shown that, in addition to reducing atherosclerosis and cardiovascular events, lipid-lowering therapies and particularly 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors (commonly known as statins) improve endothelium function, and numerous clinical studies have demonstrated that this is not necessarily related to a detectable decrease in serum cholesterol levels.

The first evidence that statins may inhibit cardiovascular events regardless of their effects on blood cholesterol levels came from the WOSCOPS study, which found that the incidence of cardiovascular events in a subgroup of patients treated with placebo or statin with the same LDL-cholesterol level was markedly lower in the statin group (WOSCOPS Study Group 1998). The beneficial effects of statins may occur relatively soon after the start of therapy and are different from those observed after the reduction of plasma cholesterol levels (Buchwald et al 1995). The cholesterol-independent, anti-thrombotic, anti-oxidative and anti-inflammatory vascular effects of statins are known as pleiotropic effects (Davignon 2004; Halcox and Deanfield 2004). The most important underlying mechanism is mediated by a reduction in the synthesis of mevalonate, which is not only a precursor of cholesterol, but also of a variety of non-steroidal isoprenoid compounds that are essential for normal cell activity (Corsini et al 1999; Wolfrum et al 2003). Isoprenoids, such as farnesylpyrophosphate and geranylpyrophosphate, are essential for the cell membrane attachment of important regulatory proteins,

particularly small GTPase: by inhibiting its synthesis, statins deplete cells of these lipids and thus elicit the retention of small GTPase in the cytosol, where they cannot exert their biological actions.

Statins and endothelial NO synthase (eNOS) expression

The initial studies of endothelial function concentrated on vasomotion, which became the major parameter of endothelial health. Endothelium-dependent vasodilatation primarily occurs via the release of a humoral mediator (identified as NO), rather than prostacyclin and an endothelium-derived hyperpolarisation factor (Kansui et al 2004).

Well documented data from experimental and clinical studies show that statins increase eNOS expression and activation, which may be the principal mechanism by which statins improve endothelial dysfunction in addition to reduce cholesterol levels. Interestingly, statins can modulate NO bioavailability by increasing mRNA expression or increasing eNOS activity: the first is a late effect and involves inhibiting the isoprenylation of Rho, small GTPase proteins, whereas the second is much more rapid and requires a lower statin concentration. It must also be remembered that statins maintain NO availability by preventing its degradation by free radical molecules (Koh 2000).

NO bioavailability is mediated by Rho inhibition

Rho proteins are small GTPases that regulate cytoskeleton organization and cell adhesion, thus contributing to cell migration and endothelial permeability (Nobes and Hall 1999; Ridley 1995). Their function is strictly regulated by their membrane localization, which is favored by prenylation, a post-translational modification that helps anchor them to membranes.

Statins modulate the stability and activity of Rho GTPases by acting on their sub-cellular localization and inhibiting the synthesis of farnesyl- and geranylgeranyl pyrophosphate, the isoprenoids required for the prenylation of Rho proteins. They also alter Rho expression at transcriptional level, although the underlying mechanism is still unclear.

However, the inhibition of Rho (and particularly RhoA) is a determinant factor in stabilizing the mRNA of endothelial NO synthase (eNOS) and improving endothelium-dependent relaxation (Figure 1).

It has been demonstrated that RhoA affects vasomotion by activating Rho-kinases (ROCK), which inactivates

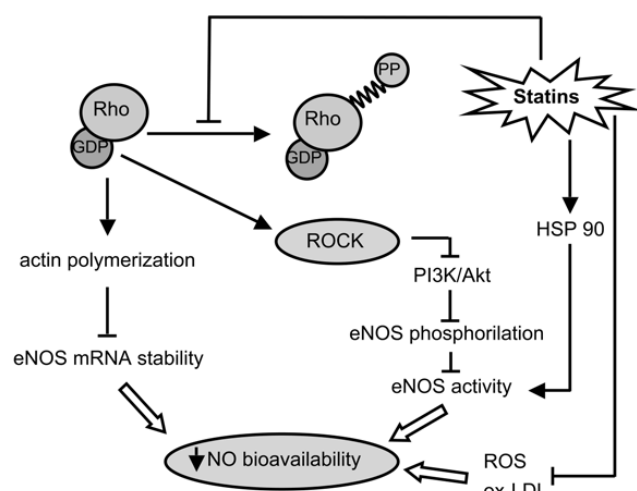


Figure 1 Statins inhibit the activation of Rho signaling, which negatively influences eNOS mRNA stability and activity, leading to an increased NO bioavailability.

myosin light chain phosphatase (MLCP) and reduces the expression of eNOS (Somlyo 2002). Direct inhibition of ROCK by specific inhibitors such as hydroxyfasudil and Y27632 increases the half-life and expression of eNOS mRNA (Rikitake et al 2005).

Furthermore, ROCK inhibits the serin/threonine kinase Akt phosphorylation and activity, and thus has negative effects on eNOS activity. Akt can phosphorylate eNOS on Ser 1179, and that phosphorylation enhances the enzyme's ability to generate NO (Fulton et al 1999). Interestingly, the membrane compartmentalization of both proteins (inside the Golgi region and plasma membrane of endothelial cells) is required for Akt's functional interaction with eNOS. It is not fully understood how the phosphorylation of eNOS enhances NO release, but it seems to be mediated by the introduction of a negative charge that 'opens' the structure and thus permits activated calmodulin binding at lower calcium concentrations (Salerno et al 1997). In vitro data suggest that enzyme activity is enhanced in a Ca^{2+} -independent manner, but is due to greater Ca^{2+} -sensitization (Dimmeler et al 1999).

The bioavailability of NO may also be influenced by RhoA in a ROCK-independent manner. Non-filamentous actin (G-actin) interacts with eNOS mRNA, and changes in actin polymerization affect eNOS mRNA stability and down-regulate eNOS expression (Searles et al 2004). Recent studies have demonstrated that cytoplasmic filaments and microtubules are necessary to transport mRNA within the cytoplasm and anchor them at specific sub-cellular locations (Bassell and Singer 1997; Nasmyth and Jansen 1997). The cytoskeleton anchoring of mRNAs, and their co-localisation

with ribosomes and RNA-binding protein complexes are necessary for their translational expression and stability. The Rho-controlled reorganisation of the actin cytoskeleton may therefore play a key role in the movement and compartmentalisation of specific mRNAs.

Endothelial dysfunction and vasoactive agonists

The partial reversion of endothelial dysfunction induced by statin treatment is not totally due to improved NO bioavailability, but also to the better regulated expression of vasoactive factors. The regulation of vascular tone is a complex process that involves the concerted action of many factors; in particular, endothelin-1 (ET-1) and angiotensin II (Ang II) elicit contractile and proliferative activities in the vascular smooth muscle layer. An imbalance between Ang II and NO is often caused by a loss of NO due to endothelial dysfunction and oxidative stress and/or the enhancement of Ang II local tissue activity (Dzau 2001).

Ang II, which is the primary effector of the renin-angiotensin system (RAS), is a multifunctional hormone that plays important autocrine and paracrine roles in vascular function (Campbell and Habener 1986). Albeit indirectly, Ang II and NO interact with each other in the vasculature to influence vascular tone, cell growth, apoptosis and inflammation (Fig. 2). Via AT_1 receptor-coupled G protein, Ang II activates phospholipase C (PLC) which in turn produces inositol 1,4,5-triphosphate (IP_3), and stimulates Ca^{2+} mobilisation. The Ca^{2+} -mediated activation of myosin light-chain kinase (MLCK) leads to the phosphorylation of MLCK and smooth muscle contraction. Ang II also induces Ca^{2+} sensitization of the contractile apparatus by activating RhoA/Rho kinase, which in turn inactivates myosin light-chain phosphatases (MLCP). A recent *in vivo* study has shown that Ang II infusion decreases NO production and uncouples eNOS in rat aortas, thus causing superoxide rather than NO production (Mollnau et al 2002). Interestingly, the long-term infusion of Ang II causes endothelial dysfunction associated with decreases in guanylyl cyclase (GC) expression and cGMP-dependent protein kinase (PKG) activity in rat aorta (Mollnau et al 2002).

On the contrary, by activating soluble guanylyl cyclase (sGC), NO stimulates PKG and reduces Ca^{2+} levels by down-regulating IP_3 production and decreasing Ca^{2+} mobilization. PKG inactivates the RhoA/Rho kinase signaling pathway to inhibit RhoA-induced Ca^{2+} sensitization. The results of a number of experimental studies suggest that NO may directly inhibit ACE activity reducing plasma Ang II concentrations

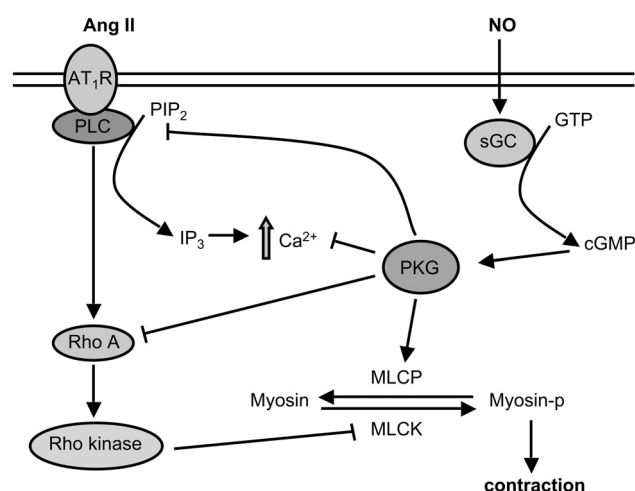


Figure 2 Ang II and NO functions interplay to influence vascular tone, through different effects on RhoA pathway.

and decrease AT₁ receptor mRNA expression at transcriptional level (Kumar and Das 1997; Ackermann et al 1998; Wu et al 2000).

Recent studies have demonstrated that statins may prevent harmful Ang II-induced events, such as the production of reactive oxygen species in vascular smooth muscle cells (VSMCs), cardiac hypertrophy and end-organ damage (Wassmann et al 2001; Park et al 2000). In particular, it has been found in an *in vivo* model of arterial neointimal thickening that fluvastatin inhibits Ang II-mediated ERK phosphorylation, and the tyrosine- and serine-induced phosphorylations of STAT1 and STAT3 that are known to be activated by many extracellular signaling proteins, including cytokines, growth factors, and Ang II via the AT₁ receptor. Furthermore, *in vitro* and *in vivo* studies have shown that the effects of statins on AT₁ receptor-mediated actions affecting VSMCs may also be mediated by decreasing AT₁ receptor expression (Ichiki et al 2001; Wassmann et al 2001). Various findings indicate that patients with high levels of LDL-cholesterol may also increase vascular responses to Ang II, and it is known that hypercholesterolemia is closely associated with AT₁ receptor upregulation (Nickenig et al 1997, 1999). Statin treatment improves vascular responsiveness to Ang II (although not in a dose-dependent manner), and seems to be closely related to serum cholesterol levels.

In vascular endothelial cells, statins also affect the expression of pre-pro-endothelin-1, a precursor of endothelin-1 (ET-1), which elicits potent contractile and proliferative action in VSMCs (Hirata 1996). Statins-mediated inhibition of the activity of Rho proteins downregulates

pre-pro-endothelin-1 gene expression, an effect that is independent of their action on NO.

Statins and caveolae

The endothelial cell plasma membrane consists of liquid-ordered microdomains (lipid rafts), that are assembled from lipid constituents and have distinct biophysical characteristics and limited random movement (Brown and London 2000; Simons and Ehehalt 2002). These regions are involved in the local sequestration of proteins that mediate signal transduction in a variety of cell types, including endothelial and vascular smooth muscle cells. In certain pathological situations, such as hyperlipidemia, the composition of some membrane microdomains are altered and thus contribute to the mechanisms of atherogenesis in vascular cells.

Caveolae are the most widely studied lipid rafts. Their principal component is the protein caveolin, a scaffolding element that efficiently binds cholesterol and interacts with various signalling macromolecules, including G proteins (Smart et al 1999; Gargalovic and Dory 2003). Caveolin also inhibits eNOS by blocking its access to cofactors, and regulates the production of NO in the endothelium (Ju et al 1997; Feron et al 1998).

The high caveolae levels under condition of hypercholesterolemia are associated with reduced endothelial NO synthesis and increased superoxide levels, SMC proliferation and leukocyte adhesion (Vergnani et al 2000). The therapeutic benefit of statins is mainly due to their restoration of normal endothelial NO levels by means of various mechanisms, including the upregulation of eNOS expression (Laufs et al 1998). They also stimulate endothelial NO production by greatly decreasing plasma membrane caveolin levels: a recent study on endothelial cells (ECs) found that atorvastatin reduced the abundance of caveolin-1 in the absence or presence of LDL-cholesterol, and promoted NO production regardless of the level of extracellular LDL-cholesterol (Feron et al 2001). These results highlight the central role of inhibiting the mevalonate pathway in peripheral cells by reducing the synthesis of isoprenoid intermediates regardless of cholesterol synthesis. Moreover, the beneficial effect of atorvastatin on eNOS activity was greater in the cells expressing high levels of caveolin. Finally, the statin promoted the agonist-induced association of eNOS and chaperone Hsp90, thus leading to increased activity (Feron et al 2001).

In addition to modulating the physical and chemical properties of membrane lipids, hypercholesterolemia has also been associated with the disruption of active L-arginine transport, which affects the capacity of endothelial cells to

generate NO: ie, L-arginine deficit leads eNOS to overproduce superoxide from oxygen instead of NO. By improving L-arginine uptake through amino acid transport, statins may also enhance NO production and interfere with superoxide formation.

Effects of statins on endothelial dysfunction

Many studies have demonstrated the beneficial clinical effects of statins on endothelial dysfunction, but the underlying mechanisms remain largely unknown. Nevertheless, all researchers believe that, in addition to the reduction in cholesterol, a NO-dependent process is also involved. *In vitro* and *in vivo* studies have confirmed that statins enhance the expression of eNOS by means of post-transcriptional/translational mechanisms (Laufs et al 1998, 2000). In particular, the use of different animal models has been useful in improving our understanding of the role of individual risk factors, such as hypertension, hypertriglyceridemia, hyperinsulinemia, hyperglycemia and insulin resistance in endothelial dysfunction, and correlating the improvement in endothelial function due to statins treatment with changes in these factors.

Animal studies

Chronic treatment with simvastatin improves endothelium-dependent acetylcholine relaxations of aorta from hypertensive rats (SHR) by means of a mechanism that is independent of the cyclo-oxygenase pathway (de Sotomayor et al 1999). The improved endothelial function in the treated animals can be attributed to the normalization of deranged NOS activity, partly mediated by the promotion of superoxide dismutase (SOD) (Carneado et al 2002).

The same authors have also shown that simvastatin improves endothelium-dependent acetylcholine relaxations in vessels from aged Wistar rats. The mechanisms involved enhanced endothelial NO vasodilatation due to increased eNOS expression, decreased participation of TXA₂ associated with the decreased expression of the COX-2 isoform, and enhanced vessel antioxidant properties (de Sotomayor et al 2005).

Statins improve endothelial health in many situations, but have failed in the well-known model of cardiovascular disease offered by DOCA-salt rats that develop hypertension, cardiovascular hypertrophy, inflammation and endothelial dysfunction. At a dose that decreased plasma cholesterol levels, rosuvastatin attenuated aortic media thickness and vascular hypertrophy, but did not affect the developing hypertension. It surprisingly increased aortic responses to

acetylcholine in male Wistar rats, but had no effect on the reduced responses to noradrenaline, sodium nitroprusside and acetylcholine of DOCA-salt rats. These results may be attributed to species-related differences and variations in the capacity of statins to penetrate vascular cell membranes, but it is also conceivable that lowering blood pressure is necessary to improve endothelial dysfunction in DOCA-salt rats (Loch et al 2006).

A recent study investigated *in vivo* a possible molecular mechanism of vascular dysfunction and the effects of fluvastatin in obese Zucker rats, a model of diabetes mellitus (Nishimatsu et al 2005). Fluvastatin partially (but significantly) reduced Ang II-induced vasoconstriction and improved endothelium-dependent vasorelaxation via the phosphatidylinositol 3-kinase/protein kinase Akt (PI3K/Akt)-dependent and NO/cGMP-dependent pathways in rat aorta. This had previously been observed in an *in vitro* study of endothelial cells in which statins seemed to stimulate the membrane translocation of Akt and its activating phosphorylation by PI3-kinase (Skaletz-Rorowski et al 2003): statin stimulation promoted the association of tyrosine phosphorylated protein with the p85 subunit of PI3-kinase, and Akt translocation was inhibited by mevalonate and wortmannin, a PI3-kinase inhibitor, thus leading to the inactivation of the enzyme. It has been reported that the Akt-dependent phosphorylation of eNOS is necessary for the full activation of eNOS and endothelium-dependent vasorelaxation, and so impaired PI3K/Akt activation may have been involved in the reduced endothelium-dependent vasorelaxation (Dimmeler et al 1999; Fulton et al 1999).

Furthermore, Akt signaling is subject to regulation by a rapidly exchanging pool of cholesterol within cells. Some authors suggest that this endothelial cholesterol pool is more sensitive to the statin-mediated inhibition of endogenous cholesterol synthesis than it is to changes in exogenous cholesterol delivery from the serum by LDLs. In this regard, it may be relevant that PI3-kinase activity in fibroblasts is negatively regulated by the recruitment of caveolin-1, an intracellular cholesterol transport protein, to PI3-kinase-associated receptor complexes within lipid rafts (Zundel et al 2000). It has also been shown that the statin-induced inhibition of cholesterol synthesis in endothelial cells can improve the inhibitory action of caveolin-1 on eNOS (Feron et al 2001), the activity of which is controlled by Akt-mediated phosphorylation (Fulton et al 1999; Dimmeler et al 1999).

Cerivastatin treatment improves endothelial dysfunction in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a useful model of obese type 2 diabetes. The restoration of

endothelial function was related to an increased in the aortic expression of CD36, a gene encoding a fatty acid transporter, and PPAR- γ . This study is an interesting example of an inter-relationship between cholesterol and fatty acid metabolism which may lead to marked beneficial effects on endothelial function in patients with diabetic hyperlipidemia and insulin-resistance syndromes (Nakamura et al 2004).

Human studies

Strong evidence that lowering LDL alone is not enough to improve endothelial dysfunction has been provided by the inability of ezetimibe, in comparison with atorvastatin alone or in combination with ezetimibe, to improve endothelial vasodilator function in the forearm circulation of patients with coronary artery disease (CAD), despite its LDL-cholesterol lowering effect (Fichtlscherer et al 2006). These findings suggest that the lipid-lowering capacity of atorvastatin is not the primary mechanism underlying the beneficial effects of short-term atorvastatin therapy in patients with CAD.

In subjects with moderately high total serum cholesterol levels, the vasodilator response to acetylcholine and baseline blood flow significantly increased after four weeks' treatment with simvastatin, which simultaneously increased the vasoconstrictor response to L-NMMA, whereas the response to the endothelium-independent vasodilator sodium nitroprusside remained unchanged. None of these effects were related to the decrease in cholesterol levels (O'Driscoll et al 1997). Statin therapy also improves endothelial function in normocholesteremic patients with chronic heart failure (CHF) and, if the treatment is long, stabilizes neurohormonal imbalances and provides measurable clinical benefits. Furthermore, these beneficial effects on the endothelium are dose dependent (van der Harst et al 2005).

Some new aspects have been highlighted by a recent study of atorvastatin in hyperlipidemic patients. The improvement in endothelial function preferentially occurred in patients with pre-existing endothelial dysfunction and completely disappeared within the 36 hours following the withdrawal of the statin (Taneva et al 2006). This last observation corresponds to results from cell cultures and animal experiments in which the lowering of eNOS and/or NO levels occurred after the discontinuation of statins (Laufs et al 2000; Gertz et al 2003; Xing et al 2005). One possible molecular mechanism of this may be related to the increase in membrane Rho expression after statin withdrawal that has been found both in vitro and in vivo (Laufs et al 2000).

However, as the findings conflict with the results obtained in non-diabetic subjects, it is intriguing to note

that statins do not seem to improve endothelial function consistently in patients with type 2 diabetes. Only a few published clinical studies have assessed the effect of statins on the microcirculation in subjects with type 2 diabetes, and their results were varied. Some found that statin therapy failed to improve endothelial dysfunction, and the authors suggested that lowering LDL alone may not be sufficient to improve endothelial function in the absence of glycemic control (Mansourati et al 2001; van Etten et al 2002; van Venrooij et al 2002; Fegan et al 2005). Another recently suggested explanation for the failure of statins to improve endothelial function in such cases is the diabetes-specific accumulation of advanced glycosylation end-product (AGE) products (Sowers 2002) that leads to vascular thickening, loss of elasticity, and the cross-linking of subendothelial structural proteins.

However, two studies have demonstrated improved endothelial function with statins in diabetes and, although CRP levels did not decrease significantly, the change correlated with the change in endothelium-dependent vasodilatation (Tsunekawa et al 2001; Tan et al 2002).

Anti-inflammatory properties of statins

Inflammation plays a pivotal role in all stages of atherosclerosis, from the nascent lesion to acute coronary syndromes (Libby et al 2002).

A number of in vitro studies have described the beneficial effects of statins in decreasing the levels of CD 11b adhesion molecules (Weber et al 1997), leukocyte function antigen-1 (LFA-1) (Weitz-Schmidt et al 2001), and ICAM-1 and VCAM-1 (Bernot et al 2003; Zapolska-Downar et al 2004; Landsberger et al 2006), and other studies have shown that they reduce the secretion of pro-inflammatory cytokines (such as interleukin IL-6, IL-1 β and TNF- α) and chemokines, such as IL-8 and MCP-1 (Romano et al 2000; Wang et al 2005). Interestingly, statins inhibit the production of TNF- α in endothelial cells as well as the CRP-stimulated activation of NF- κ B, a strong biomarker of systemic inflammation in cardiovascular diseases. It has been shown that CPR induces plasminogen activator inhibitor (PAI-1) expression and complement activation, and decreases eNOS expression, thus leading to a propensity for thrombosis, inflammation and endothelial dysfunction. Recent clinical studies have found that various statins reduce CRP levels, and that this is at least partially independent of their lipid-lowering properties (Ridker 2003; Nawawi et al 2003; Sugiyama et al 2005).

Numerous *in vitro* and *in vivo* experiments have shown that the anti-inflammatory effects of statins are partially mediated by vascular endothelial NO (Scalia et al 2001; Stalker et al 2001). This cholesterol-independent effect of statins is absent in eNOS-deficient mice, thus suggesting that eNOS mediates the protective vascular effects of statins (Stalker et al 2001).

NO mediates the beneficial effects of statins on vascular health

Recent studies have shown that endothelial dysfunction plays an important role as an independent risk factor (Widlansky et al 2003) and, together with inflammation, triggers cardiovascular diseases (Drexler et al 1992; Tousoulis et al 2005). Furthermore, it has been recognized that endothelium-derived NO is an anti-inflammatory and anti-arteriosclerotic molecule as it protects nuclear transcription factor (NF- κ B) from activation by oxidized LDL or cytokines, and thus prevents or attenuates the transcription and expression of adhesion molecules (Marui et al 1993). It has also been shown that the inhibition of NO synthesis in cultured endothelial cells increases the expression of the gene coding for MCP-1, and that MCP-1 expression is associated with the activation of NF- κ B (Zeiber et al 1995). Therapies that increase NO bioactivity may reduce the synthesis of pro-inflammatory proteins on the endothelial cell surface, which may reduce inflammation.

The capacity of statins to improve endothelial dysfunction and reduce inflammation has been demonstrated in numerous experimental studies. Long-term treatment with simvastatin normalizes acetylcholine-induced relaxation in rats treated with L-NAME without affecting response to the nitrovasodilator itself (Perez-Guerrero et al 2003). The inhibition of NO synthesis by N-nitro-arginine methyl ester induces early inflammation characterized by increased monocyte infiltration coronary vessels and increased MCP-1 expression (Takemoto et al 1997; Tomita et al 1998). In the same animal model, pravastatin and cerivastatin inhibited vascular inflammation by increasing eNOS expression and restoring NO-generating capacity by inhibiting Rho activity (Ni et al 2001). A recent study has suggested a novel molecular mechanism by which statins regulate vascular inflammation by finding that simvastatin increased NO production in human aortic endothelial cells (HAECs), and this covalently modified N-ethylmaleimide sensitive factor (NSF), a key regulator of endothelial exocytosis. The nitrosylation of NSF blocked the externalization of P-selectin to the endothelial surface, which otherwise activates leukocyte rolling, the first

step in leukocyte inflammation (Yamakuchi et al 2005). The statin also modified the second step in leukocyte trafficking by blocking the interaction of LFA-1 with intercellular adhesion molecule-1 (VCAM-1) but, interestingly, not in eNOS knockout mice.

In patients with heart failure, atorvastatin treatment significantly improves forearm vasodilatory response to reactive hyperemia and reduced serum levels of IL-6, TNF- α and soluble VCAM-1, but has no effects on MCP-1 (Tousoulis et al 2005). Statins also improve arterial stiffness and decrease the plasma levels of hsCRP, a sensitive marker of the chronic inflammation of arteriosclerotic lesions in patients with hypercholesterolemia (Matsuo et al 2005).

In hypercholesterolemic patients with angiographically-documented coronary artery disease, simvastatin significantly improved the percent flow-mediated dilator response to hyperemia, whereas the response to nitroglycerin was not significantly modified (Koh et al 2003). In the same patients, it significantly lowered the plasma levels of TNF- α , CRP, fibrinogen and ICAM-1, but had no effect on E-selectin and VCAM-1; furthermore, the greatest reduction in plasma TNF- α and CRP levels occurred in the patients with the highest baseline levels. It is interesting to note that there was a significant inverse correlation between the percentage of flow-mediated dilatation and plasma TNF- α levels, and a positive correlation between the latter and changes in plasma nitrate levels.

High-dose atorvastatin acutely increased endothelium-dependent forearm blood flow (FBF) in subjects with normal vascular function, and rapidly decreased the levels of the inflammation marker hs-CRP (Laufs et al 2001). Furthermore, its withdrawal has been found to induce a rapid deterioration in endothelial function, a rebound-like decrease in NO bioavailability, and increased inflammation in clinical and experimental studies (Thomas and Mann 1998; Laufs and Liao 2000; Laufs et al 2000). These data are in line with the recent finding that the discontinuation of statin treatment induces vascular complications in patients with acute coronary syndromes (Heeschen et al 2002; Li et al 2006).

Statin modulates thrombosis and coagulation

Nitric oxide is the major mediator synthesized by the endothelium. It regulates vascular homeostasis and blood flow, and a decrease in its bioavailability is related to vasoconstriction, vascular smooth muscle proliferation, platelet aggregation and endothelial-leukocyte adhesion

(Palmer et al 1987; Radomski et al 1992; Gauthier et al 1995). These pathological conditions (together known as endothelial dysfunction) is considered to be an early marker of the atherothrombosis that underlies cardiovascular, and particularly coronary heart disease. Numerous experimental and clinical studies have shown that statins have beneficial effects on atherothrombosis by reducing the progression of the atheroma and the incidence of acute thrombosis-related vascular events. The mechanisms by which statins inhibit thrombosis have not been totally clarified, although several pathways seem to be involved. In particular, they increase the stability of the plaque whose rupture leads to thrombosis by exposing blood to the highly thrombogenic contents of its lipid core (Liao 2002). Rather than reducing lipid levels (which reduces plaque size and modifies the physiochemical compositions of the lipid core) (Koh 2000; Takemoto et al 2001), statins exert their beneficial effects by decreasing the infiltration and activity of macrophages and T-lymphocytes within the plaque, and inhibiting proteolytic enzymes such as matrix metalloproteinases (MMP), which are thought to be responsible for the plaque rupture induced by the thinning, ulceration and fissuring of the fibrous cap (Bellosta et al 1998; Aikawa et al 2001).

Platelet hyperactivity is an important factor contributing to the enhanced risk of thrombotic complications in hypercholesterolemic patients (Oppen et al 1995). It is linked to increases in the biosynthesis of thromboxane A_2 (TXA₂), platelet α_2 -adrenergic density, and cytosolic calcium (Baldassarre et al 1997; Notarbartolo et al 1995). Studies of platelets taken from statin-treated hypercholesterolemic patients have led to contrasting results: platelet aggregation has been found to be reduced, unchanged or increased by lovastatin treatment (Colli et al 2004); some studies have found that fluvastatin reduces platelet aggregation as well as soluble P-selectin (a marker of α -granule platelet secretion or endothelial cell dysfunction) and ICAM-1 levels (Osamah et al 1997; Romano et al 2000); and it has also been found that simvastatin inhibits the production of TXA₂, and urinary excretion of its metabolite 11-dehydrothromboxane B₂ (Notarbartolo et al 1995). These discrepant results may be explained by the different experimental conditions used to assess platelet function.

Statins also inhibit the expression of tissue factor (TF), a transmembrane glycoprotein whose binding with coagulation factor VII initiates blood coagulation by activating proteolytically factor IX and X (Colli et al 1997). Various studies have demonstrated that statins affect this pathway by inhibiting Rho/Rho-kinase and the activation of Akt (Eto et al 2002).

The (ATROCAP) study provides definite in vivo evidence that statins affect TF expression and activity, and macrophage infiltration in human vessels (Cortellaro et al 2002). These data strongly indicate that statins attenuate atherosclerotic plaque thrombogenicity by reducing cell-mediated thrombin generation. Studies of the effects of statins on plasma fibrinogen and factor VII levels have led to very contrasting results (Colli et al 2004).

Additional features of endothelial cell dysfunction include atheroma fibrinolytic imbalance. In advanced lesions, a state of hypofibrinolysis prevails because of the high levels of plasminogen activator inhibitor-1 (PAI-1) released by activated cells within the atheroma and the platelets incorporated in mural thrombi (Robbie et al 1996). Although, in vitro and ex vivo studies have shown that different statins induce tissue-type plasminogen activators (t-PA) and reduce PAI-1, the results of in vivo studies are conflicting (Colli et al 2004). These results may be explained by differences in metabolic profiles and genetic backgrounds, which are known to have a considerable effect on PAI-1 levels.

Conclusion

Many of the beneficial pleiotropic effects of statins occur as a result of modulated endothelial function and reduced inflammatory processes. Attempting to understand these properties of statins is an exciting field of research that will also improve our understanding of vascular biology in health and disease, and thus enable the better use of this drug class in clinical practice.

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