**Statins and angiotensin II-induced vascular injury**

Ralf Dechend, Dominik Müller, Jeun Koon Park, Anette Fiebeler, Hermann Haller and Friedrich C. Luft

HELIOS Klinikum Berlin, Franz Volhard Clinic and Max Delbrück Center for Molecular Medicine, Medical Faculty of the Charité, Humboldt University of Berlin, Germany and the Department of Nephrology, Hannover Medical School, Hannover, Germany

**Effects of statins unrelated to lipid lowering**

Statins may have pleiotropic properties that complement their cholesterol-lowering effects. These properties include nitric oxide-mediated improvement of endothelial dysfunction and attenuation of endothelin-1 expression, antioxidant effects, anti-inflammatory properties, inhibition of cell proliferation with anti-carcinogenic actions in animals, stabilization of atherosclerotic plaques, anti-coagulant effects and inhibition of graft rejection after heart and kidney transplantation [1]. In a remarkable short-term human study, Tsunekawa et al. [2] showed that cerivastatin improved endothelial dysfunction in elderly diabetic men within 3 days, independent of lipid lowering. The effect may have been partly due to upregulation of nitric oxide (NO) production.

**Angiotensin II-induced vascular injury—a model to study non-clinical effects of statins**

We have been interested in angiotensin II (Ang II)-induced vascular injury and were curious to see if a statin would ameliorate the effects. We investigated this issue in a double transgenic rat model (dTGR) that produces copious amounts of Ang II locally [3,4]. We gave the animals cerivastatin, 0.5 mg/kg, daily for 3 weeks by gavage. Both NF-κB and AP-1 transcription factor activation in the kidney, as shown by electrophoretic mobility shift assays, were significantly reduced with statin treatment (Figures 1A and 2). Immunostaining for the p65 NF-κB component was similarly reduced (Figure 1B) as was c-fos mRNA expression (Figure 2).

In the heart, the effects were no less dramatic. Cerivastatin treatment reduced cardiac hypertrophy. Cerivastatin treatment also reduced extracellular matrix deposition. Fibronectin and laminin staining were decidedly less in the hearts of statin-treated dTGR compared with SD rats. NF-κB and AP-1 activation were reduced to a similar degree as in the kidneys. Untreated dTGR show markedly increased expression of the interleukin (IL)-6 and basic fibroblast growth factor (bFGF) in the media of coronary vessels, which was reduced by cerivastatin treatment. bFGF and IL-6 were also present in the perivascular space and between the myofibrils. With cerivastatin treatment, the staining for both substances was reduced, as was macrophage infiltration (Figure 3).

**Statins block the response to Ang II**

We did not do a detailed analysis of signal transduction in these studies. However, we were able to show that activation of the extracellularly regulated kinase (ERK) was highly activated in dTGR and that this activation was reduced by statin treatment. We also tested this response in cultured vascular smooth muscle cells and showed that exposing the cells to Ang II resulted in ERK phosphorylation. This response was abolished by 30 min pre-treatment of the cells with cerivastatin. When mevalonate was given to circumvent the statin effect, the ERK phosphorylation was no longer inhibited, indicating that the action does indeed involve 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase inhibition.

The rationale for employing statins to ameliorate Ang II-induced vascular injury comes from various sources [5]. In cell culture experiments that are clearly independent of any low-density lipoprotein cholesterol-dependent (LDL) effects, HMG-CoA reductase inhibition was effective in blocking platelet-derived growth factor and Ang II-mediated induction of c-jun and c-fos, components of AP-1 [6]. Vascular smooth muscle cells were also exposed to phorbol ester in the presence of the HMG-CoA reductase inhibitor lovastatin. Phorbol ester-induction of AP-1 activation was inhibited, indicating that the action does indeed involve 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase inhibition.

The protection was blocked by the concomitant addition of mevalonate, farnesylpyrophosphate, and geranylgeranyl pyrophosphate suggesting that the mechanisms indeed involved inhibition of mevalonate synthesis by lovastatin. In a rat study of...
aortic banding, simvastatin was successful in reducing left ventricular hypertrophy almost to the same degree as an ACE inhibitor [7]. Furthermore, hydroxyproline deposition, tissue ACE activity, and vascular Ang II content were reduced. Clinical data also suggest that statins may modulate the renin–angiotensin–aldosterone system. Nickenig et al. [8] recently showed that hypercholesterolaemic men have greater hypertensive responses to infused Ang II and high AT-1 receptor expression compared with normocholesterolaemic men. Statin treatment rapidly reversed the exaggerated response to Ang II infusion and led to a down-regulation of AT-1 receptors.

Our findings, that Ang II-induced NF-κB activation can be reduced by statins fits well with those of Ortego et al. [9] who found that atorvastatin inhibited NF-κB activation induced by TNF-α. As a result, surface adhesion molecule expression, inflammatory infiltration, tissue factor production, matrix protein production, and cellular proliferation were all attenuated. The same group had reported similar findings with red wine earlier [10]. Possibly additive effects could be achieved. However, this hypothesis has not yet been tested.

The role of prenylation of Ras superfamily proteins

The mechanisms may involve G proteins involved in receptor-coupled signal transduction, particularly Rho. The role of RhoA/Rho-kinase in vascular biology has recently been reviewed [11]. The Rho proteins belong to the Ras superfamily. The Ras proteins alternate between an inactivated GDP-bound form and activated GTP-bound form, allowing them to act as molecular switches for growth and differentiation signals. Prenylation is a process involving the binding of hydrophobic isoprenoid groups consisting of farnesyl or geranylgeranyl residues to the C-terminal region of Ras protein superfamily. Farnesyl pyrophosphate and geranylgeranyl pyrophosphate are metabolic products of mevalonate that are able to...
supply prenyl groups. The prenylation is conducted by prenyl transferases. The hydrophobic prenyl groups are necessary to anchor the Ras superfamily proteins to intracellular membranes so that they can be translocated to the plasma membrane [12]. The final cell-membrane fixation is necessary for Ras proteins to participate in their specific interactions. Statins decrease the production of mevalonate, geranyl pyrophosphate, and farnesyl pyrophosphate, and subsequent products on the way to construction of the cholesterol molecule. Thus, statins could act, independently of circulating LDL, by intracellularly interfering with Ras superfamily protein function. An abbreviated schema is provided (Figure 4) [13]. Ikeda et al. [14] recently showed that statins decrease matrix metalloproteinase-1 expression through inhibition of Rho. Laufs et al. [15] showed that suppression of endothelial nitric oxide production after withdrawal of statin treatment is mediated by negative feedback regulation of rho GTPase gene transcription.

Fig. 2. Electrophoretic mobility shift assay for AP-1 DNA binding from whole kidney. c-fos and c-jun mRNA expression was determined separately by RT–PCR and quantitated. c-fos expression was reduced.

Fig. 3. Immunohistochemical staining for bFGF, IL-6, and macrophage infiltration. Inserts show confirmatory studies and quantification.
Effects of statins on endothelial cell function

Other novel statin-related effects, independent of extracellular cholesterol, have been described recently. Feron et al. [16] observed that atorvastatin reduced caveolin-1 abundance in endothelial cells, irrespective of whether or not LDL cholesterol was present in the medium. Atorvastatin also restored the agonist-induced eNOS activity in the cells. These findings may explain how endothelial dysfunction is restored by statins. The atorvastatin-related effects were completely restored by the addition of mevalonate. The caveolin-1 gene contains sterol response elements in its promoter, consistent with these findings. Scalia et al. [17] reported that simvastatin exerts anti-inflammatory effects in apolipoprotein (Apo) E-deficient mice, without lowering their serum cholesterol concentrations. Similar to our findings, leukocyte–endothelial cell relationships were restored. Furthermore, NO production was increased in the Apo E-deficient mice.

Potential human relevance?

Whether or not the animal findings we report, or the in vivo and in vitro findings observed by others, have clinical importance is unknown. Clinically, the vasculo-protective effects of lipophilic and lipophobic statins appear similar. The salubrious protective effects of a statin that is confined to acting in hepatocytes, has been well documented in clinical studies [18]. Clinical studies are now in progress to resolve these issues.

References

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3. Park JK, Muller DN, Mervaala EM et al. Cerivastatin prevents angiotensin II-induced renal injury independent of blood


