Modulating Angiotensin II-Induced Inflammation by HMG Co-A Reductase Inhibition

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Angiotensin (Ang) II is capable of producing inflammatory changes by signals through its AT1 receptor. Reactive oxygen species production, adhesion molecule expression, chemokines, and other mediators are involved. Nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) are two of the transcription factors activating the responsible genes. We have studied Ang II-independent modulating effects in a double transgenic rat model harboring the human renin and angiotensinogen genes. We have recently focused on the protective effects of HMG-CoA reductase inhibition and review these data here. We found that cerivastatin decreased mortality, lowered blood pressure, preserved renal function, decreased cardiac hypertrophy, and inhibited the entire chain of inflammatory events. Furthermore, NF-κB and AP-1 activation was sharply attenuated. We also observed that cerivastatin blocked ERK1/2 phosphorylation in vivo and in vitro. Cerivastatin also inhibited phorbol ester-transmitted events in vascular smooth muscle cells. Because Rho, a member of the Ras protein superfamily is important to Ang II-dependent and -independent vascular smooth muscle signaling events, we suggest that cerivastatin may act by inhibiting the prenylation, membrane anchoring, and subsequent activation of Ras proteins. These data may in part explain cholesterol-independent, HMG-CoA reductase-related, protective effects. Am J Hypertens 2001;14:55S–61S © 2001 American Journal of Hypertension, Ltd.

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the calcineurin pathway.\textsuperscript{10,11} An alternative and highly attractive approach would be to reduce the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase.

**HMG-CoA Reductase Inhibitors (Statins) and Ang II-Related Effects**

The rationale for using HMG-CoA reductase inhibition comes from various sources. In cell culture experiments that are clearly independent of any low-density lipoprotein (LDL) cholesterol-dependent effects, HMG-CoA reductase inhibition was effective in blocking platelet derived growth factor (PDGF) and Ang II-mediated induction of c-jun and c-fos, components of AP-1.\textsuperscript{12} Vascular smooth muscle cells were also exposed to phorbol ester in the presence of the HMG-CoA reductase inhibitor lovastatin in these studies. Phorbol ester induction of AP-1 activation was inhibited, suggesting that protein kinase-C (PKC) signaling is also influenced by HMG-CoA reductase inhibition. The protection was blocked by the concomitant addition of mevalonate, farnesyl pyrophosphate, 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 angiotensin converting enzyme (ACE) inhibitor.\textsuperscript{13} Furthermore, hydroxyproline deposition, tissue ACE activity, and vascular Ang II content were reduced. Clinical data also suggest that HMG-CoA reductase inhibitors (statins) may modulate the renin-angiotensin-aldosterone system. Nickenig et al\textsuperscript{14} showed that hypercholesterolemic men have greater hypertensive responses to infused Ang II and high AT1 receptor expression. Statin treatment rapidly reversed the exaggerated response to Ang II infusion and led to a downregulation of AT1 receptors. Findings such as these prompted us to test whether or not we could ameliorate Ang II-induced end-organ damage in our model.

**Cerivastatin In dTGR**

We gave 4-week-old male dTGR the HMG-CoA reductase inhibitor cerivastatin (0.5 mg/kg) by gavage for 3 weeks. Control dTGR and nontransgenic Sprague-Dawley rats received vehicle as described elsewhere.\textsuperscript{4–11,15} Blood pressure was measured by tail-cuff. We performed immunohistochemistry, immunocytochemistry, and conventional paraffin-fixed histologic sections, as described.\textsuperscript{15} For transcription factor expression, we relied on electrophoretic gel mobility shift assays (EMSA) and Western blotting.\textsuperscript{15} Statistically significant differences in mean values were tested by ANOVA and Scheffé test. In our earlier studies, we showed that dTGR develop a severe vasculopathy with fibrinoid necrosis and infiltration of inflammatory cells.\textsuperscript{4–11} This reaction was also present here. Details of the cerivastatin experiment have been published elsewhere.\textsuperscript{15}

The dTGR developed hypertension, severe renal damage, and cardiac hypertrophy with focal necrosis. Nine of 20 untreated dTGR (45%) died before the end of the study at 7 weeks. Cerivastatin showed a markedly reduced mortality (3 of 15; 20%), whereas none of the nontransgenic Sprague-Dawley rats died before the end of the study \((P < .05)\). Blood pressure was reduced by 50 mm Hg, albuminuria was reduced by 70%, and urea and creatinine values of surviving animals were reduced in cerivastatin-treated compared to untreated control rats \((all \ P < .05)\). Cerivastatin reduced perivascular leukocyte infiltration and adhesion molecule expression for ICAM-1 and VCAM-1 in the kidneys and hearts in treated dTGR, compared to controls. The same was true for mediators of inflammation. Interleukin 6 (IL-6) expression was reduced in the hearts and kidneys of cerivastatin-treated rats, as was inducible nitric oxide synthase (iNOS).

We studied transcription factor activation in our model. Fig. 1 shows immunohistochemistry staining for the p65 component (upper) of NF-κB in a coronary artery branch from cerivastatin-treated and untreated dTGR. Interleukin-6 expression (middle) and basic fibroblast growth factor (b-FGF) expression (lower) are also shown. The expression of all three parameters was markedly \((P < .05)\) reduced by cerivastatin treatment. Similar staining was performed for the AP-1 components c-fos and c-jun with similar results (data not shown). To convincingly show that transcription factor activation was reduced by cerivastatin treatment, we also performed EMSA. In Fig. 2 we show that cerivastatin treatment significantly reduces NF-κB (upper) and AP-1 (lower) DNA binding in the heart. Supershifts for subcomponents of these transcription factors were performed, as well as other controls, which corroborated the specificity of these findings.

We then analyzed possible intracellular mechanisms of the observed protective effects of cerivastatin. We first analyzed the activation of mitogen-activated protein (MAP) kinase by using specific antibodies that only detect the phosphorylated, active form of extracellular signal-regulated kinase (p-ERK). In untreated transgenic rat kidneys, we observed an increase in phosphorylated ERK in the vessel wall, glomeruli, and the peritubular space (not shown). An even more pronounced increase was also present in the medulla. Treatment with cerivastatin decreased, but did not abolish this staining pattern in the cortex and in the medulla.

To verify with certainty that cerivastatin interferes with Ang II-induced ERK phosphorylation, we performed in vitro experiments. We analyzed Ang II-induced ERK phosphorylation, with and without previous vascular smooth muscle cell incubation with cerivastatin. Vascular smooth muscle cells were incubated with cerivastatin (5 μmol/L) or buffer for 20 min. Cells were then exposed to Ang II \((10^{-7}\) mol/L) and ERK phosphorylation was assessed after 1 min using confocal microscopy. The results of these experi-
ments are shown in Fig. 3. Ang II induced a rapid increase in p-ERK immunoreactivity within 1 min. This response was almost abolished by the prior incubation with cerivastatin ($P < 0.01$). The inhibitory effect was also observed after longer cerivastatin incubation times (6, 12, and 24 h). The inhibitory effect afforded by cerivastatin could be circumvented by the addition of farnesyl (not shown).

**Modulatory Mechanisms of Statin Protection**

We found that HMG-CoA reductase inhibition with cerivastatin improved survival, decreased blood pressure, reduced proteinuria and improved renal function, reduced cardiac hypertrophy, and reduced myocardial fibrosis in an Ang II-dependent model of end-organ damage. To obtain insight into cellular mechanisms, we observed that the activation of NF-$\kappa$B and AP-1, transcription factors important to innate immunity and inflammation and cellular proliferation, respectively, was attenuated. As a result, surface adhesion molecule expression, inflammatory infiltration, tissue factor production, matrix protein production, and cellular proliferation were all attenuated. We briefly studied some possible signal transduction pathways that might be important to the process. We have evidence that cerivastatin interfered with the ERK and MAP kinase signaling pathway, as well as PKC signaling. Both pathways could have a bearing on NF-$\kappa$B- and AP-1-related effects.

Cerivastatin treatment reduced blood pressure in our model; however, we do not believe that blood pressure reduction was responsible for the effects we observed, although statins lower blood pressure effectively in hypertensive patients. We do not believe that the protective effects in our model were related to LDL reduction. We measured total cholesterol in our rats and the levels were not reduced despite the fairly high cerivastatin dose we administered. This dose, on a weight basis, is much higher than the human dose, although the pharmacokinetic area under the curve is similar in both species. Statins do not effectively lower total serum cholesterol in rats because the rat transports much cholesterol with high-density lipoproteins and because of compensatory increases in hepatic enzyme production, although the enzyme is effectively inhibited in the liver and elsewhere.

**FIG. 1.** Localization of b-FGF (b-FGF), interleukin-6 (IL-6), and the nuclear factor-$\kappa$B component p65. dTGR vessel (pink stain) is visible in all three panels, but not in cerivastatin-treated or Sprague-Dawley vessels.
The molecular mechanisms whereby cerivastatin influenced Ang II-induced cell activation are not clear. Cerivastatin could influence cell activation through inhibition of NF-κB. Previous work has shown that lovastatin inhibits lipopolysaccharide-induced NF-κB activation in human mesangial cells in vitro. Ang II has been shown to stimulate NF-κB in mesangial cells in vitro and to activate NF-κB in the renal cortex in vivo. In a model of immune-complex nephritis, NF-κB activation was reduced by ACE inhibition. Another possibility is the activation of NF-κB through oxygen-free radicals. Reactive oxygen species represent an important signal transduction pathway inside the cell and also participate in the expression of adhesion molecules on the cell surface. Our results indicate that statins interfere with the MAP kinase activation proximal to NF-κB activation. Because MAP kinase phosphorylation and activation occur through the Ras signaling pathway, statins may interfere with Ras signaling. Such a mechanism has been proposed by several investigators. This inhibition can be overcome by the simultaneous addition of either mevalonate or farnesol, but not by exogenous LDL cholesterol.

The Ras superfamily of proteins is important to cell differentiation, proliferation, apoptosis, and regulation of gene transcription. Within the superfamily are the Ras proteins themselves and the Rho family. The Ras proteins alternate between an inactivated GDP-bound form and activated GTP-bound form, allowing them to act as mo-
lecular switch growth and differentiation signals. Prenylation is a process involving the binding of hydrophobic isoprenoid groups consisting of farnesyl or geranylgeranyl residues to the carboxy-terminal region of Ras protein superfamily. Farnesyl pyrophosphate and geranyl pyrophosphate are metabolic products of mevalonate that are able to supply prenyl groups. The prenylation is conducted by prenyltransferases. 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. The final cell-membrane fixation is necessary for Ras proteins to participate in their specific interactions. HMG-CoA reductase inhibitors, as shown in Fig. 4, 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.

The Rho proteins have a key role in maintaining cytoskeletal structure and regulating cell adhesion as well as cell cycle progression. Rho-associated kinase (ROCK) is an effector of small GTPase Rho, and regulates vascular tone through a calcium sensitization mechanism. Thus, GTPase Rho has as its target ROCK, which plays a key role in the pathogenesis of hypertension. Selective inhibitors of ROCK have been shown to lower blood pressure and decrease vascular smooth muscle cell proliferation. However, members of the Rho family are prenylated by the addition of a geranyl group. Specific pre-

![FIG. 3. Effects of cerivastatin on the angiotensin (Ang) II-induced phosphorylation of extracellular signal regulated kinase (p-ERK) in vascular smooth muscle cell (VSMC). Confocal micrographs demonstrate an increase in p-ERK immunoreactivity after exposure of Ang II (10^{-7} mol/L) for 1 min. Prior exposure of the vascular smooth muscle cells by cerivastatin (10^{-5} mol/L) almost abolished the Ang II-induced p-ERK. For details, see Reference 15.](image)

![FIG. 4. Schema showing that the inhibition of HMG Co-A reductase by statins leads not only to decreased cholesterol production and thus increased LDL receptor expression, but also to decreased production of geranyl and farnesyl, leading to decreased prenylation of small proteins. The original statins were derived from the mold Penicillium brevicipactum and thus could be considered "penicillins."](image)
nylation inhibitors directed at geranylgeranyl-transfase-1 can be used to interfere with this process.\(^{32}\)

Several G(i)- and G(q)-coupled receptors, including the AT1 receptor, activate Rho and Rho-associated kinase in Swiss 3T3 cells and cardiac myocytes. Yamakawa et al.\(^ {13}\) recently presented data indicating that ROCK is involved in Ang II-induced hypertrophy of vascular smooth muscle cells. They used a specific kinase inhibitor and suggested that the Rho, ROCK, and c-fos pathways play a role in Ang II-induced vascular effects. Thus, the role of the Rho protein family in Ang II-related signaling is established. Hypertension itself may also cause vascular injury by activation of Rho. Numaguchi et al.\(^ {34}\) have shown that mecanotransduction (stretch) in vascular smooth muscle cells is dependent on intact actin filaments, that Rho is activated by stretch, and that Rho/p160ROCK mediates stretch-induced ERK activation and vascular hyperplasia. Finally, Muniyappa et al.\(^ {35}\) recently showed that inhibition of Rho protein with mevastatin stimulated IL-1β-induced iNOS expression in rat vascular smooth muscle cells. Interleukin-1β activates two different signaling pathways regulating iNOS: one increases, whereas the other decreases iNOS expression. We have observed that statin treatment decreases iNOS expression in glomeruli of dTGR.\(^ {15}\)

In summary, dTGR for human renin-angiotensin-aldosterone system genes develop severe cardiac and renal failure related primarily to the effects of Ang II through the AT1 receptor. The process is complex and involves several transcription factors including NF-κB and AP-1. The involvement of Rho proteins in Ang II-related signaling through the AT1 receptor is established as is subsequent ERK and MAP kinase activation. Blood pressure itself, by mecanotransduction, could also exert a Rho-related effect. We have shown that HMG-CoA reductase inhibition with cerivastatin has an effect on Ang II-induced vascular injury and improves survival in the dTGR model. We have shown that HMG-CoA reductase inhibition ameliorates angiotensin II-induced inflammatory damage in rats. Hypertension 2000;35:193–201.


