Statin-Sensitive Dysregulated AT1 Receptor Function and Density in Hypercholesterolemic Men

Georg Nickenig*, MD; Anselm T. Bäumer*, MD; Yavuz Temur, MS; Daniela Kebben, MS; Friedrich Jockenhövel, MD; Michael Böhm, MD

Background—Hypercholesterolemia causes an upregulation of vascular angiotensin II type 1 (AT1) receptor expression in cell culture and animal models. The presented studies were undertaken to examine AT1 receptor overexpression in hypercholesterolemic men and therapeutic interventions thereof by HMG CoA reductase inhibitors (statins).

Methods and Results—Effects of AT1 receptor activation were measured by assessing the blood pressure increase after infusion of angiotensin II in normo- (cholesterol 181±11 mg/dL) and hypercholesterolemic (cholesterol 294±10 mg/dL) men (n=19 and 20, respectively). AT1 receptor expression was assessed on isolated platelets. Some patients were investigated before and after cholesterol-lowering therapy with statins. Hypercholesterolemia led to a significant increase of angiotensin II-induced blood pressure elevation. AT1 receptor expression was significantly enhanced in hypercholesterolemic individuals (Bmax=5.2±1.2 fmol/mg protein) compared with normocholesterolemic men (Bmax=2.1±0.2 fmol/mg protein). Cholesterol-lowering treatment with statins reversed the elevated blood pressure response to angiotensin II infusion (P<0.05) and downregulated AT1 receptor density (P<0.05).

Conclusions—Hypercholesterolemia induces AT1 receptor overexpression and enhances biological effects of angiotensin II in men. These findings provide novel insights into the pathogenesis of hypertension and atherosclerosis and may initiate rational and new therapeutic concepts. (Circulation. 1999;100:2131-2134.)

Key Words: lipids ■ hypertension ■ angiotensin ■ receptors ■ atherosclerosis

Hypercholesterolemia is a major risk factor for coronary heart disease.1 Several cholesterol-lowering interventions have reduced cardiovascular events in secondary and primary prevention trials.2,3 Hypercholesterolemia is frequently associated with hypertension, another potent cardiovascular risk factor.4 It is thought that interactions of lipoproteins with other neurohumoral systems may play an important role.5 The renin-angiotensin system and especially the angiotensin II type 1 (AT1) receptor have been implicated in cardiovascular pathophysiology.6 Previous studies have shown that low-density lipoprotein (LDL) induces AT1 receptor upregulation in isolated vascular smooth muscle cells (VSMC) and that hypercholesterolemic rabbits display an enhanced vascular expression of AT1 receptors.7,8 These interactions could explain the association of hypercholesterolemia with hypertension and atherosclerosis, because AT1 receptor overexpression may account for enhanced release of free radicals and increased vasoconstriction and cell proliferation. The present study was designed to evaluate whether hypercholesterolemia causes enhanced AT1 receptor density and function in men and whether these potential alterations could be modulated by treatment with statins.

Methods

Patients

Male hypercholesterolemic individuals were compared with normocholesterolemic volunteers. The exclusion criteria included any cardiovascular disease or any severe disease of other origin at present or in the past, any cardiovascular medication, any long-term medical treatment, lipid-lowering drugs, history of drug or alcohol abuse, and current treatment with any investigational drug. Patients gave written consent, and a physical examination, an ECG, and blood pressure measurements were performed. The study was approved by the ethics committees of the University of Cologne.

Blood Pressure

Basal blood pressure was evaluated after 30 minutes resting time in supine position. 0.5 to 20 ng · kg⁻¹ · min⁻¹ of angiotensin II was infused stepwise via cubital vein (6 minutes per concentration). Blood pressure was automatically assessed in 2-minute intervals. Thirty minutes later, the norepinephrine infusion was started (0.6 to 4.8 μg · kg⁻¹ · min⁻¹). Drug administration was ceased after reaching 190 mm Hg systolic or 110 mm Hg diastolic blood pressure, occurrence of chest pain, or arrhythmias.

Radioligand Binding Assays

Blood (60 mL) was drawn and stored on ice. Platelet-rich supernatant was collected and centrifuged (4°C for 10 minutes) at 1313g.

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The platelet pellet was resuspended and washed twice. Platelets were incubated with increasing concentrations of 125I-angiotensin II (0.2 to 2 nmol/L). Nonspecific binding was assessed in the presence of 10 μmol/L losartan. Incubation was performed for 60 minutes at room temperature. Reaction were terminated by aspiration with ice-cold buffer containing 0.5% BSA and 10 mmol/L Tris-HCl through GF/C Whatman filters with a Brandel cell harvester.

Statistics
All results are given as mean ± SEM. Statistical procedures were performed using SSPS 7.0 software. For comparisons, nonparametric tests (Mann-Whitney U test, Wilcoxon test) were applied. Correlations were calculated using the Pearson test.

Results
Detailed characteristics of the enrolled patients are given in Table 1. Men with cholesterol plasma levels ≥200 mg/dL and LDL concentrations ≥130 mg/dL were included into the normocholesterolemic subset, whereas patients with cholesterol levels ≥200 mg/dL and LDL ≥130 mg/dL were considered hypercholesterolemic.

Figure 1A illustrates basal blood pressure levels in both groups, displaying no significant differences between groups. Figure 1B compares relative systolic blood pressure elevation in normo- and hypercholesterolemic patients. Angiotensin II caused a more profound blood pressure increase in hypercholesterolemic individuals. Hypercholesterolemic patients (n=8) were treated for 6 weeks with 20 to 40 mg atorvastatin or simvastatin to lower cholesterol plasma levels (LDL ≥200 mg/mL, 40 mg/day; LDL <200 mg/mL, 20 mg/day). Cholesterol decreased from 293±15 to 228±14 mg/dL, LDL from 223±13 to 151±16 mg/dL. HDL concentrations was comparable between groups (48±3 versus 49±4 mg/dL). Triglycerides amounted to 192.2±35.5 before and 186.6±32.2 mg/dL after treatment. Cholesterol-lowering therapy caused a significant decrease in angiotensin II-induced blood pressure increase (Figure 1C).

Norepinephrine led to a similar increase of blood pressure in both groups, suggesting a specific effect of hypercholesterolemia on angiotensin II-driven blood pressure control (Table 2).

AT1 receptor expression was quantified by radioligand binding assays in isolated intact platelets. Figure 2A shows a saturation binding experiments with 125I-angiotensin II. Figure 2B illustrates data of AT1 receptor density in 19 normo- and 20 hypercholesterol-
Hypercholesterolemia-Induced AT1 Receptor Regulation

TABLE 2. Blood Pressure Increase (mm Hg) on Angiotensin II and Norepinephrine

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Normocholesterolic (n=14)</td>
</tr>
<tr>
<td></td>
<td>Systolic</td>
</tr>
<tr>
<td>Angiotensin II, mg · kg⁻¹ · min⁻¹</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.5±2.3</td>
</tr>
<tr>
<td>5</td>
<td>5.5±1.8</td>
</tr>
<tr>
<td>10</td>
<td>12.0±1.9</td>
</tr>
<tr>
<td>20</td>
<td>19.0±1.6</td>
</tr>
<tr>
<td>Norepinephrine, μg · kg⁻¹ · min⁻¹</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>0.7±1.8</td>
</tr>
<tr>
<td>1.2</td>
<td>4.1±2.2</td>
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<tr>
<td>2.4</td>
<td>5.4±2.7</td>
</tr>
<tr>
<td>4.8</td>
<td>10.2±2.5</td>
</tr>
<tr>
<td>9.6</td>
<td>26.5±4.3</td>
</tr>
</tbody>
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Increasing concentrations of angiotensin II were infused. Each concentration of angiotensin II was applied for 6 minutes. Blood pressure was measured in 2-minute intervals (3 times per concentration). Mean blood pressure values/infused concentration were calculated. Statin indicates therapy with 20–40 mg atorvastatin or simvastatin for 6 weeks; ND, not done. *P<0.05.

Olemic patients. Whereas the ligand affinity was not significantly different between subsets (Kᵦₛ=1.6 nmol/L [95%CI 0.3 to 2.9 nmol/L] versus 2.4 nmol/L [95%CI 0.9 to 3.9 nmol/L]), the AT1 receptor density was increased in hypercholesterolemic patients (Bₘₐₓ=5.2±0.7 fmol/mg protein) in comparison to normocholesterolic men (Bₘₐₓ=2.1±0.2 fmol/mg protein; P<0.05).

Lipid-lowering therapy led to a significant decrease in AT1 receptor density from Bₘₐₓ=5.6±1.4 to Bₘₐₓ=1.5±0.4 fmol/mg protein in individuals on statins (n=8) (Figure 2C, P<0.05). Receptor affinity was unchanged (Kᵦₛ=1.8 nmol/L [95%CI 0.5 to 4.2 nmol/L] versus 1.6 nmol [95%CI 0.4 to 2.7 nmol/L]) between groups.

Figure 3 shows the statistically significant correlation between AT1 receptor density and LDL plasma concentration. The results suggest that increasing cholesterol plasma levels induces closely dependent elevations of AT1 receptor expression, which causes a more profound angiotensin II-induced blood pressure increase.

Discussion

Hypercholesterolemia is associated with significant overexpression of AT1 receptors in humans, leading to a profound increase of angiotensin II-induced blood pressure elevation.

Elevated LDL plasma levels are a major risk factor for the development of coronary heart disease, the leading cause of death in the western world.1 LDL and especially oxidized LDL have been implicated in impaired release of nitric oxide, damage of endothelial and VSMC, adhesion of mononuclear cells on the vessel wall, blood coagulation, cytokine release, and enhanced efficacy of vascular growth factors.2–12 Nevertheless, it is well-known, but mechanistically unsettled, that hypertension is frequently attended by hypercholesterolemia and vice versa, both potentiating each other with respect to the development of coronary heart disease.4

The AT1 receptor mediates many biological effects of the renin-angiotensin system, including vasoconstriction, cell growth, water and electrolyte homeostasis, and sympathetic activation.6 In view of the pathogenesis of hypertension and atherosclerosis and their interactions, hypercholesterolemia-induced AT1 receptor overexpression may have major implications.1 The angiotensin II-induced vasoconstriction is enhanced during hypercholesterolemia. In the beginning, this may not have tremendous impact on basal conditions, because negative feedback regulation of the renin-angiotensin system may occur. Nevertheless, situations of enhanced neurohumoral activation such as mental stress and exercising or additional risk factors like smoking or increased salt intake may, under these circumstances, lead to greater blood pressure elevations that may chronically accumulate to established hypertension.2 The AT1 receptor induces growth of VSMC, an event thought to be of central relevance for the pathogenesis of atherosclerosis.6,9 AT1 receptor overexpression during hypercholesterolemia may accelerate this process.3 The AT1 receptor is a major source of reactive oxygen species (ROS) in the vessel wall.12 These free radicals are also potentially involved in the development of cardiovascular diseases.13 Interestingly, ROS production is enhanced in hypercholesterolemia.14 Moreover, this ROS excess was normalized in hypercholesterolemic animals through AT1 receptor antagonism,15 suggesting that AT1 receptor overexpression is a decisive mechanism in the pathogenesis of lipid-induced atherosclerosis.4 In men with coronary heart disease, ACE inhibitors improve endothelial dysfunction closely related to ROS production and atherosclerosis.16 This effect was especially pronounced in hypercholesterolemic individuals.3 Treatment with statins causes a decrease of mortality and morbidity in normo- and hypercholesterolemic patients.2–5 There is increasing evidence that these drugs exert this beneficial effect only in part by lowering of plasma cholesterol concentrations. Beside other cellular effects, statins directly downregulate AT1 receptor expression in isolated VSMC.17 The present data support the notion that this effect may play an important role in vivo; AT1 receptor density was reduced in the statin treatment group to an extent that could not be predicted in view of the changes in cholesterol levels. Namely, AT1 receptor density was reduced...
by statins to 26% compared with levels before treatment, although LDL levels were only reduced to 70% of the pretreatment level. In contrast, in the untreated groups, LDL plasma concentrations were 218 versus 106 mg/dL (48%), but AT1 receptor density was only decreased to 40% in normocholesterolemic compared with hypercholesterolemic patients.

Thus, the hypercholesterolemia-induced overexpression of AT1 receptor expression resembles a novel concept that advances our understanding of the pathogenesis of chronic cardiovascular disease. Intervention trials are warranted; these will test the effect of AT1 receptor antagonists or ACE inhibitors on atherosclerosis and hypertension related to hypercholesterolemia.

Acknowledgments
This work was supported by the Deutsche Forschungsgemeinschaft, the Köln Fortune Program/Faculty of Medicine, University of Cologne, and by the Deutsche Herzstiftung.

References
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_Circulation_. 1999;100:2131-2134
doi: 10.1161/01.CIR.100.21.2131

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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