The Effect of Statins on Angiotensin II–Induced Hemodynamic Changes in Young, Mildly Hypercholesterolemic Men

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Background: Angiotensin II type 1 (AT1) receptors are well known to mediate angiotensin II (Ang II)–induced pro-atherosclerotic effects. It has been found that hypercholesterolemia influences the expression of AT1 receptors on vascular smooth muscle cells and that increased density of AT1 receptors exaggerates the hemodynamic response to Ang II. We analyzed to what extent statins and AT1 receptor antagonists diminish the vasoconstrictive response to Ang II infusion in hypercholesterolemic patients.

Methods: A total of 24 male patients with LDL cholesterol levels $\geq 130$ mg/dL were enrolled in a randomized, cross-over study. After baseline evaluation, 12 patients received first cerivastatin (0.3 mg/day) and the other 12 patients initially received candesartan (8 mg/day) for 3 weeks, with subsequent cross-over of the medication for the second 3-week drug period. The vascular response was analyzed by the increase in mean arterial pressure (MAP) and total peripheral resistance (TPR) during infusion of increasing doses of Ang II at baseline and the end of each treatment period. Hemodynamic changes were also compared with those in 24 normocholesterolemic subjects without any therapy.

Results: At baseline, Ang II provoked a similar increase of MAP and TPR in patients and control subjects. Treatment with cerivastatin did not affect the response to Ang II compared with baseline. By contrast, treatment with candesartan attenuated significantly the response to Ang II compared with baseline and cerivastatin.

Conclusions: Our hemodynamic data indicate the hypothesis that statins do not reduce the responsiveness to Ang II in resistance arteries of young, mildly hypercholesterolemic patients.

Key Words: Angiotensin II, statins, hypercholesterolemia, blood pressure, AT1 receptor.

Statins were originally developed to treat hypercholesterolemia, a risk factor for atherosclerosis. Recently these substances have been associated with pleiotropic effects independent of lowering cholesterol by reversible blockage of the 3-hydroxyl-3–methylglutaryl coenzyme A (HMG-CoA) reductase. Statins ameliorate endothelial function and increase bioavailability of nitric oxide in patients with hypercholesterolemia.1

There are also data indicating that statins have immunomodulatory properties by interfering with the CD40/CD154 (CD40 ligand) system on macrophages2 or modulating inflammation,3 findings that could be beneficial in the treatment of atherosclerosis. In addition, there are pathophysiologic links between hypercholesterolemia and the renin-angiotensin system.1 Low-density lipoproteins (LDL) induce up-regulation of angiotensin II type 1 (AT1) receptor gene expression in isolated smooth muscle cells in vitro.4 Similarly, hypercholesterolemic rabbits exhibit enhanced vascular expression of AT1 receptors on thrombocytes.5,6 Angiotensin II (Ang II) induces oxidative stress via the AT1 receptor activating NADPH/H$^+$ oxidases.7,8 This effect is the most important source for superoxide in the vasculature.9 Treatment with statins actually reduced the number of AT1 receptors on thrombocytes of hypercholesterolemic patients.10 We recently demonstrated that blood pressure (BP) response to Ang II infusions was related to LDL cholesterol concentration even in normocholesterolemic or mildly hypercholesterolemic patients.11 This effect could be ameliorated by...
administration of a statin in patients with overt coronary artery disease. Beneficial effects of statins have been also observed in rats during cardiac remodeling after myocardial infarction and after Ang II–induced cardiac injury. These findings suggest an effect of statins on the regulation of the renin-angiotensin system and its pleiotropic effects in patients with established atherosclerosis. It is unknown whether these effects already exist in early stages of atherosclerosis, such as in patients with hypercholesterolemia without clinical manifestation of atherosclerosis. Therefore, we investigated whether statins reduce Ang II–mediated increases in mean arterial blood pressure (MAP) and total peripheral resistance (TPR) in a group of young men with mildly elevated cholesterol levels. In addition, the effect of statins on Ang II–induced hemodynamic changes are compared with those provoked by the blockade of AT1 receptors and to hemodynamic effects of phenylepinephrine, a vasoconstrictive substance, acting independently of the renin-angiotensin system. By its design, this study provides insight into whether the described interaction between hypercholesterolemia and AT1 receptors could be a pathogenetic relevant mechanism in the early stages of atherosclerosis.

**Methods**

**Patients and Control Subjects**

A total of 24 male patients with mildly elevated hypercholesterolemia were enrolled in a randomized cross-over study. Inclusion criteria were as follows: patient age 18 to 45 years, male sex, persistent LDL cholesterol >130 mg/dL over 4 weeks during a run-in period with dietary intervention, and no history or clinical sign of any cardiovascular disease (in particular, atherosclerotic lesions). Patients were excluded if they were smokers, had stopped smoking <1 year before, or had diabetes mellitus or sustained arterial hypertension (systolic BP >160 mm Hg or diastolic BP >95 mm Hg). Patients with any renal or liver disease, history of drug abuse, or allergy or intolerance to the study drugs were also excluded.

Patients were not allowed to use angiotensin converting enzyme (ACE) inhibitors or AT1 receptor antagonists, lipid lowering agents, diuretics, or vitamins 4 weeks before as well as during the study. Informed consent was obtained before study inclusion.

A group of male volunteers served as the control group. Inclusion criteria were age 18 to 45 years and LDL cholesterol <130 mg/dL.

The study complied with the Declaration of Helsinki and was approved by the local ethics committee of the University Erlangen-Nuremberg. The study was designed and performed before cerivastatin was withdrawn from the market by the manufacturing company. During the time that the study was performed, no warning letters had been issued about the side effects of cerivastatin.

**Study Design**

After baseline evaluation 24 patients were randomly allocated to receive cerivastatin at a dose of 0.3 mg/day for 3
weeks and candesartan (8 mg/day) for the next 3 weeks consecutively, or vice versa. To investigate Ang II–mediated effects in human patients, changes in MAP, TPR, cardiac output (CO), and stroke volume (SV) were selected as easily accessible and the most reproducible indications of changes in Ang II. In patients as well as in 24 control subjects, Ang II was administered intravenously at week 0 (before therapy) and at 3 weeks and 6 weeks. Each phase starting with 0.5 ng/kg/min, doses were increased consecutively, or vice versa. To investigate Ang II–mediated effects in human patients, changes in MAP, TPR, CO, and stroke volume were calculated each minute. Impedance cardiography is subject to criticism with respect to the assessed absolute values. However, we and other research groups have found that changes in cardiac output within one session or within patients have been repeatedly validated as reliable.

### Statistical Analysis

All data were analyzed using SPSS version 10.0 software (SPSS Inc., Chicago, IL). Continuous, normally distributed data are presented as mean ± SD. Nominal data are presented in percent. Two-sided t tests for independent values were used to compare patients with control subjects. One-factor analysis of variance for repeated measurements was used to test for significant differences between baseline and the two treatment phases, followed by a t test with Bonferroni correction as a post hoc test. A \( \chi^2 \) test was used for nominal data. Significance was set at a value of \( P < .05 \) (two-sided).

By assuming an \( \alpha \) error of 0.05, a standard \( \beta \) error of 0.2, and an average standard deviation of 10, a 10% increase in MAP response, compared with 20% during baseline, became detectable if data from 24 patients were investigated. Thus, by including 24 subjects, our study has the statistical power to detect a difference in MAP response of 10% between the two study groups. The statistical power to detect a difference in MAP response, compared with 20% during baseline, became detectable if data from 24 patients were investigated.

### Results

#### Study Population

All patients and control subjects were male and were similar in age (28 ± 3 years vs. 30 ± 5 years) and MAP at baseline (131 ± 12 mm Hg vs. 124 ± 7 mm Hg). By definition, patients compared with control subjects had significantly greater total cholesterol (270 ± 65 mg/dL vs. 170 ± 31 mg/dL, \( P < .01 \)) and LDL cholesterol (178 ±
49 mg/dL v 82 ± 23 mg/dL, P < .01), as well as greater triglyceride concentrations (171 ± 77 g/L v 102 ± 81 g/L; P < .01); however, patients had lower HDL cholesterol than control subjects (46 ± 9 mg/dL v 53 ± 11 mg/dL; P < .02).

**Blood Pressure Response**

Blood pressure responses are summarized in Table 1. The resting MAP was not different among control subjects, patients at baseline, and patients after administration of cerivastatin. As expected, candesartan lowered resting MAP compared with baseline and cerivastatin (P < .03). The response to Ang II showed a dose-dependent increase of MAP in all study subjects, becoming significant when Ang II concentrations >3.0 ng/kg/min were infused (P < .01). The Ang II–induced increase in MAP was not different between control subjects and hypercholesterolemic patients at baseline. Compared with values in control subjects and baseline values in hypercholesterolemic subjects, the Ang II–induced response of MAP was mitigated by candesartan (P < .001) but not by cerivastatin. By a distinct contrast with our hypothesis, patients treated with cerivastatin had a trend of higher BP response to Ang II infusion, although this was not significant.

There was a dose-dependent increase of MAP after PE infusion, which became significant after infusion of PE at concentrations of 0.5 μg/kg/min and greater (P < .01). Similarly to the findings with Ang II infusion, candesartan administration attenuated the increase of MAP to PE compared with baseline and cerivastatin therapy (P < .01).

Thus, candesartan but not cerivastatin lowered the increase in MAP to both Ang II and PE infusion when compared with the baseline values in patients and values in control subjects.

These results have been confirmed with the relative increases of MAP from baseline (ΔMAP%) after infusion of Ang II and PE used for statistical analysis instead of absolute values (Fig. 2).

**Response of Total Systemic Resistance and Heart Rate**

Responses of total systemic resistance and heart rate (HR) are shown in Table 2. The TPR was lower in control subjects than in patients at baseline, after cerivastan, and after cerivastatin (P < .01). Use of Ang II led to an increase of TPR in all participants (P < .01). Therapy with candesartan resulted in a lower response of TPR (P < .01) at Ang II doses of ≥5 ng/kg/min than the TPR response observed after therapy with cerivastatin.

Infusion of PE increased TPR dose dependently in all groups without any significant difference among control subjects and patients at baseline, after cerivastatin, and after candesartan.

When the increase in TPR (ΔTPR) was expressed in percent change from baseline, an analysis similar to that of absolute values emerged (Fig. 3).

**Effect of Treatment With Cerivastatin or Candesartan on Lipids**

Treatment with cerivastatin lowered total cholesterol (218 ± 58 mg/dL) and LDL cholesterol (128 ± 38 mg/dL) compared with baseline (P < .01) and treatment with candesartan (P < .001). This corresponds to a reduction in total cholesterol by 18.0% ± 0.1% and a reduction in LDL cholesterol by 25.9% ± 0.1%. Both HDL cholesterol and triglyceride levels (210 ± 337 g/dL) were unchanged.

Candesartan treatment had no influence on cholesterol (total cholesterol 272 ± 65 mg/dL v 270 ± 65 mg/dL without treatment) and LDL cholesterol levels (LDL cholesterol 179 ± 51 v 178 ± 49 mg/dL without treatment).

**Discussion**

The current study was designed to analyze the effects of the HMG-CoA reductase inhibitor cerivastatin on hemo-
Table 2. Total peripheral resistance (TPR) after infusion of angiotensin (Ang) II at 0, 0.5, 1.0, 3.0, 5.0, 10.0, and 20.0 ng/kg/min and phenylepinephrine (PE) at 0, 0.25, 0.5, 1.0 and 2.0 ng/kg/min in control subjects and in patients at baseline, after cerivastatin, and after candesartan

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<tr>
<th>Total Peripheral Resistance (mm Hg/L/min)</th>
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<td>Control Subjects</td>
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<td>Angiotensin II (ng/kg/min)</td>
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<td>Phenylephrin (ng/kg/min)</td>
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<td>2.0</td>
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Data are mean (±SD).

* P < .01 v control subjects; † P < .05 v control subjects; ‡ P < .05 v cerivastatin; § P < .01 v cerivastatin.

dynamic changes in Ang II in mildly hypercholesterolemic young male patients. By analyzing the change in MAP and TPR, we found a hemodynamic response to Ang II infusion in hypercholesterolemic patients treated with cerivastatin that was similar to the response observed at baseline and to that in our normocholesterolemic control group. Thus, our results do not support the notion that in early stages of atherosclerosis there is an Ang II–mediated up-regulation of AT1 receptors.4–6

The hemodynamic response to Ang II in the candesartan treated subjects was reduced compared with baseline and compared with the response in the cerivastatin treated group. By contrast, treatment with statins was not effective in attenuating the hemodynamic response to Ang II. Our data differ therefore from those in a previous report10 in which a reduced BP response to Ang II was observed after treatment of middle-aged hypercholesterolemic patients with atorvastatin.

There are important differences that might explain these contrasting results. First, our patients were approximately 15 years younger than patients examined by Nickenig et al10 (29 v 45 years). Age is established as an important covariate of the BP response to Ang II infusion. This has been demonstrated, for example, by Vuagnat et al in hypertensive sibling pairs.17 Whether age is an independent covariate of the hemodynamic response to Ang II itself, or whether age is simply correlated with the extent of atherosclerosis that has been found to progress with advancing age in the western hemisphere, is not known. Of note, age persisted as an independent predictor of BP response to Ang II in the multivariate analysis performed by Vuagnat et al.17 Consistently, the increase of MAP or TPR was not different between patients with mildly elevated cholesterol levels but without atherosclerosis and healthy control subjects, with both groups being similar in age.

Second, patients in the two study cohorts differed in LDL concentrations. Patients included in the study by Nickenig10 had more severe hypercholesterolemia than patients in the current study (LDL cholesterol 215 mg/dL v 178 mg/dL). This may imply that these patients had more marked atherosclerotic lesions, thereby suggesting more marked expression of AT1 receptors than in early atherosclerosis. The latter is supported by the correlation of Ang II–induced BP response to plasma LDL cholesterol.10 Thus, the AT1 receptor modulating effects of statins on hemodynamic responses to Ang II are more evident in patients with severe hypercholesterolemia, at a later stage of atherosclerosis than at the early stage observed in the current study.

Third, different statins were administered in the two studies: atorvastatin and cerivastatin. It is well known that statins have different pharmacologic profiles depending to their hydrophobic or hydrophilic properties. However, it is most unlikely that these differences may explain the diverging study results, because according to Ichiki et al,18 the hydrophilic compound cerivastatin has an even stronger effect on the AT1 receptor regulation than other HMG CoA reductase inhibitors, including atorvastatin.

Fourth, the genetic background of the two study populations might be different, as indicated by the different phenotype of the patients. Van Geel19 and Vuagnat found a link between the A1166C gene polymorphism and increased BP response to Ang II infusion, but our group
could not find any link between this polymorphism and the hemodynamic effects due to Ang II infusion. Thus, there are conflicting results published with regard to the relation of AT$_1$ receptor polymorphism with the response to Ang II infusion.

Maybe the most important difference between the two studies is the different design concerning duration of treatment with statins. Although study subjects were treated for 6 weeks in the study of Nickenig et al, we applied a protocol with treatment for 3 weeks. One can argue that effects of statins on hemodynamic reactivity to Ang II might take place only after period of >3 weeks. On the other hand, the statin-induced lowering of cholesterol (22.2% v 18%) and LDL cholesterol (32.3% v 25.9%) was quite comparable, the cholesterol concentrations were similar or lower in our study group at the end of treatment with statins. Although study subjects were treated for 6 weeks in the study of Nickenig et al, we applied a protocol with treatment for 3 weeks. On the other hand, the statin-induced lowering of cholesterol (22.2% v 18%) and LDL cholesterol (32.3% v 25.9%) was quite comparable, the cholesterol concentrations were similar or lower in our study group at the end of treatment. However, it cannot be excluded that structural vascular changes need more time to take place than functional changes such as an up-regulation of AT$_1$ receptors. Structural vascular changes cannot be excluded as a confounding factor of our results.

In conclusion, our hemodynamic data indicate the hypothesis that statins do not reduce the responsiveness to Ang II in resistance arteries of young, mildly hypercholesterolemic patients. In elderly patients with hypercholesterolemia, this has been conclusively reported. Thus, our data do not indicate that in young, mildly hypercholesterolemic patients there is an AT$_1$ receptor up regulation leading to an exaggerated hemodynamic response to Ang II, as found in elderly patients with hypercholesterolemia. By contrast, treatment with the AT$_1$ receptor antagonist candesartan was able to attenuate the hemodynamic response to Ang II in these patients with mildly elevated LDL cholesterol.

References


