Angiotensin II Type 1 Receptor Antagonism Improves Hypercholesterolemia-Associated Endothelial Dysfunction

Sven Wassmann, Stefan Hilgers, Ulrich Laufs, Michael Böhm, Georg Nickenig

Objective—Hypercholesterolemia-induced angiotensin II type 1 (AT₁) receptor overexpression is thought to be a key event in the development of endothelial dysfunction.

Methods and Results—The effect of a 6-week treatment with the AT₁ receptor antagonist candesartan (16 mg/d) on endothelial function and serum inflammation markers was compared with the effect of treatment with placebo or the calcium channel antagonist felodipine (5 mg/d) in 47 hypercholesterolemic patients (low density lipoprotein cholesterol >160 mg/dL). Endothelial function was assessed by measurement of forearm blood flow (FBF) by venous occlusion plethysmography. FBF during reactive hyperemia was significantly improved by candesartan, whereas felodipine and placebo exerted no effect. Nitroglycerin-induced vasorelaxation and basal FBF were not altered significantly. Blood pressure and cholesterol levels were not affected significantly by any drug. Serum concentrations of 8-isoprostane, monocyte chemotactic protein-1, and soluble intercellular adhesion molecule-1 were significantly reduced by candesartan treatment but not by placebo or felodipine (ELISA assays). Levels of high-sensitivity C-reactive protein and tumor necrosis factor-α were not altered significantly by any treatment.

Conclusions—These data suggest that AT₁ receptor antagonism improves endothelial function during hypercholesterolemia and that this applies not only to endothelium-dependent vasodilatation but also to oxidative stress and events involved in monocyte attraction and adhesion. AT₁ receptor blockade may potentially represent a novel approach for the prevention of vascular dysfunction associated with hypercholesterolemia that is independent of lipid-lowering and blood pressure–lowering interventions. (Arterioscler Thromb Vasc Biol. 2002;22:1208-1212.)

Key Words: angiotensin II ■ angiotensin II type 1 receptors ■ endothelial function ■ hypercholesterolemia ■ oxidative stress

Hypercholesterolemia is an important risk factor in the development of atherosclerosis. The latter is preceded by endothelial dysfunction, a state of abnormal vasomotion, procoagulant processes, and inflammation. Although the detailed mechanisms remain undetermined, enhanced oxidative stress seems to be essential for the induction of endothelial dysfunction by risk factors such as hypercholesterolemia.

Angiotensin II type 1 (AT₁) receptor activation is a predominant source of free radical release in the vessel wall. Previous studies in cell cultures, animal models, and humans have shown that hypercholesterolemia induces AT₁ receptor overexpression associated with enhanced vasoconstriction and cell growth, the propagation of oxidative stress, and endothelial dysfunction and, ultimately, progressive atherosclerosis. These interactions could explain the association of hypercholesterolemia with hypertension and atherosclerosis, because AT₁ receptor overexpression may account for enhanced release of free radicals as well as increased vasoconstriction and cell proliferation. However, it has not been determined whether the blockade of AT₁ receptor activation may cause an improvement of hypercholesterolemia-induced endothelial dysfunction. Therefore, the present study evaluated whether short-term treatment with the AT₁ receptor antagonist candesartan influenced endothelium-dependent vasorelaxation as well as inflammation events known to be involved in early and advanced atherosclerosis.

Methods

Patients and Study Protocol
Forty-seven middle-aged, normotensive, white patients with LDL cholesterol >160 mg/dL were enrolled. The exclusion criteria were as follows: treatment with ACE inhibitors or statins, left ventricular ejection fraction <60%, hypertension (>145/90 mm Hg), acute coronary syndromes, revascularization procedures within the last 8 weeks, any severe disease at present or in the past, history of drug or alcohol abuse, gravidity, and current treatment with any investigational drug. Patients gave written consent, and a physical examination, an ECG recording, and blood pressure measurements (at least 3 times in supine position) were undertaken. Measurements of fasting serum cholesterol and triglyceride concentrations were performed. Patients were randomized in a double-blind fashion to 1 of the 3 treatment groups, either placebo, 5 mg/d felodipine, or 16 mg/d candesartan, according to a random-number code list (block size 6, treatments 3). Treatment was engaged for 6 weeks. Measurements

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were performed before and after treatment. Eight patients withdrew before completion of the study (1 patient taking placebo, 1 patient taking candesartan, and 6 patients taking felodipine). The study was approved by the ethics committees of the University of Homburg/Saar, Homburg/Saar, Germany.

The study design was a prospective, double-blind, placebo-controlled, randomized monocenter study.

### Measurement of FBF

All studies were performed between 9:00 and 11:00 AM in a temperature-controlled room (at 23°C) with patients in the postabsorptive state. Subjects were asked to refrain from drinking alcohol and smoking cigarettes for 12 hours before the study. All subjects rested at least 30 minutes to establish a stable baseline, and measurements were performed with patients in the supine position. Forearm blood flow (FBF) was assessed by noninvasive venous occlusion plethysmography (model EC6 plethysmograph, D.E. Hokanson). Hand circulation was excluded by a wrist cuff inflated to suprasystolic pressure. A mercury-in-silastic strain gauge was fixed 5 cm below the antecubital crease. A cuff situated around the upper arm was connected to a rapid cuff inflator (model E20, D.E. Hokanson). FBF was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating the upper arm cuff to a venous-occlusion pressure of 50 mm Hg. Flow measurements were recorded for 9 seconds every 15 seconds, and an average of 4 measurements was used for analysis. Endothelium-dependent vasodilatation was assessed by ischemia-induced reactive hyperemia. After a baseline recording of 4 minutes, ischemia was induced for 5 minutes by inflating the upper arm cuff to 200 mm Hg. Immediately after cuff deflation, maximal hyperemic FBF was measured (peak flow), followed by continuous measurement for 3 minutes. Endothelium-independent vasorelaxation was assessed after administration of nitroglycerin. After a baseline recording of 4 minutes, 0.8 mg nitroglycerin (Pohl Boskamp) was given sublingually, and FBF was measured for 5 minutes. FBF was analyzed by NIVP 4.25 software (D.E. Hokanson) and is expressed in milliliters per minute per 100 mL of forearm volume.

### Reproducibility of FBF Measurements

Before initiation of the present study, we performed FBF measurements in 12 subjects on 3 consecutive days to validate the reproducibility of this method. Values for basal blood flow (in mL/min per 100 mL forearm volume: day 1, 2.4±0.2; day 2, 2.5±0.2; and day 3, 2.4±0.2) and postischemic hyperemic blood flow increase (in mL/min per 100 mL forearm volume: day 1, 22.4±1.2; day 2, 23.7±1.3; and day 3, 23.4±1.0) were highly reproducible (data not shown). In addition, we compared FBF increase during reactive hyperemia between 7 healthy control subjects and 7 hypercholesterolemic subjects (LDL >160 mg/dL). Endothelium-dependent FBF increase was significantly reduced in the hypercholesterolemic subjects (25.4±2.0 [control] versus 17.0±1.1 [hypercholesterolemia] mL/min per 100 mL forearm volume, *P*<0.05 versus control; data not shown).

### Measurement of Serum Parameters

Venous blood samples were taken before and after treatment. Serum concentrations of monocyte chemoattractant protein (MCP)-1, soluble intercellular adhesion molecule (sICAM)-1, and tumor necrosis factor (TNF)-α (IBL) and of 8-isoprostane (Cayman Chemical) were assessed in at least duplicate by ELISA assays according to the manufacturers’ protocols and quantified in an ELISA reader (Bio-Rad). High-sensitivity C-reactive protein (hs-CRP) was determined by using a latex-enhanced turbidimetric assay (Roche).

### Statistical Analysis

All results were compared before and after therapy and calculated as change in percentage. All results are expressed as mean±SE. Statistical analysis was performed by ANOVA. Post hoc comparisons were performed by using the Neuman-Keuls test. A multivariate regression analysis was performed to evaluate the influence of blood pressure or lipid levels on FBF. The SPSS software package (SPSS Inc) was used for statistical analysis. A value of *P*<0.05 indicates statistical significance.

### Results

Forty-seven middle-aged, normotensive, hypercholesterolemic patients were included in the present study. Detailed characteristics of the enrolled patients are given in Table 1. There were no significant differences with respect to lipid and blood pressure levels, smoking habits, diabetes, serum parameters, or FBF.

The Figure (panel A) illustrates enhancement of FBF increase during reactive hyperemia in the 3 treatment groups after 6 weeks of treatment (expressed as change in percentage). In contrast to felodipine, hyperemic FBF was significantly improved by candesartan. Nitroglycerin-induced blood flow increase was not significantly influenced by any regimen (Figure, panel B). Basal FBF was not significantly different between groups (change in percentage: placebo, 10.8±5.3%; felodipine, 7.1±7.1%; and candesartan, 15.8±8.1%). Neither blood pressure levels nor cholesterol levels were altered significantly by any drug (Table 2). A multivariate analysis revealed that the changes in blood pressure or lipid levels had no influence on endothelium-dependent FBF.

Serum concentrations of inflammation markers were assessed before and after therapy. As shown in Table 2 (values reflect changes in percentage after therapy), treatment with candesartan significantly reduced MCP-1, sICAM-1, and 8-isoprostane levels, whereas felodipine had no significant effect. There was a trend toward lower levels of hs-CRP in the candesartan group compared with placebo or felodipine treatment groups; however, this effect was not statistically significant (Table 2). Serum concentrations of TNF-α were not altered significantly by the study medications (Table 2).

### Discussion

The state of early atherosclerosis is characterized by attraction of monocytes to the vessel wall by factors such as MCP-1. Adhesion of these mononuclear cells to the dysfunctional endothelium is mediated in part by adhesion molecules such as ICAM-1. Induction of MCP-1 as well as ICAM-1 is preceded by free radical release, which is induced after, for example, AT1 receptor activation. Elevated levels of those and other participating factors may predict progression of atherosclerotic diseases. Moreover, oxidative stress, which is associated with increased concentrations of 8-isoprostane in the serum, is known to cause endothelial dysfunction. Hypercholesterolemia, a fundamental risk factor of atherosclerosis, is also associated with increased attraction and adhesion of monocytes and impaired endothelium-dependent vasorelaxation. One possible underlying mechanism could be the hypercholesterolemia-induced AT1 receptor overexpression in the vasculature, leading to increased free radical release.

Our findings indicate that AT1 receptor antagonism with candesartan, compared with placebo or felodipine treatment, leads to an improvement of endothelium-dependent vasodilatation in hypercholesterolemic subjects, as demonstrated by enhancement of ischemia-induced hyperemic FBF. Reactive...
hyperemia-induced increase in FBF is a frequently used marker of endothelium-dependent vasorelaxation, especially because of the noninvasive approach.\(^8\)\(^9\)\(^{10}\) However, there are certain limitations compared with the reference method of FBF measurement during intra-arterial infusion of acetylcholine. Hyperemic blood flow is not exclusively dependent on the endothelium, because in addition to endothelium-derived vasoactive agents, other local metabolic factors may contribute to vasodilatation after ischemia. Furthermore, the placement of the arm occlusion (upper versus lower arm) and the age of the investigated subjects may influence the correlation of hyperemic FBF with endothelium-mediated vasorelaxation.

Vasodilatation exerted by nitroglycerin was not significantly different between study groups, indicating that endothelium-independent vasorelaxation is not affected by candesartan or felodipine treatment. Furthermore, certain markers of oxidative stress and inflammatory processes (8-isoprostane, MCP-1, and ICAM-1) are significantly reduced by treatment with candesartan. Other serum parameters of inflammation that were investigated in the present study (hs-CRP and TNF-\(\alpha\)) were not significantly altered by any treatment.

TNF-\(\alpha\) may induce the production of CRP. These factors have been described as predictors of the progression of atherosclerosis.\(^4\)\(^5\)\(^6\)\(^7\) There are no human studies available at present that demonstrate a clear interaction between AT\(_1\) receptor activation and circulating levels of TNF-\(\alpha\) or CRP.

The presented data indicating candesartan-induced improvement of endothelial function may have several implications. First, the present study, involving hypercholesterolemic humans, provides novel insights into the pathophysiological role of AT\(_1\) receptor activation in endothelial dysfunction. AT\(_1\) receptor antagonism may reduce oxidative stress, as assessed by decreased 8-isoprostane levels. This may lead to an increased bioavailability of NO, causing enhanced endothelium-dependent vasodilatation.\(^2\)\(^3\) The reduced oxidative stress may potentially decrease MCP-1 and ICAM-1 production, which could ultimately diminish the adhesion of white blood cells to the vessel wall. Thus, hypercholesterolemia-induced AT\(_1\) receptor overexpression may possibly be an important event in the pathogenesis of lipid-associated vascular dysfunction.

Second, the provided data are in agreement with previously published investigations demonstrating that AT\(_1\) receptor antagonists improve endothelial function in hypertensive patients or in patients with atherosclerosis.\(^{20}\)\(^{21}\) Of note, the patients enrolled in those studies were not hypercholesterolemic but instead displayed hypertension or diabetes. Interestingly, hyperinsulinemia, which is frequently present in diabetic individuals, has been shown to upregulate AT\(_1\) receptor expression, possibly resulting in blood pressure elevation.

**TABLE 1. Patient Characteristics and Baseline Data**

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=17)</th>
<th>Felodipine (n=13)</th>
<th>Candesartan (n=17)</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>55.2±1.8</td>
<td>58.4±1.7</td>
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<tr>
<td>Men</td>
<td>16</td>
<td>12</td>
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<td>Women</td>
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<td>1</td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>Smokers</td>
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<td>2</td>
<td>2</td>
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<td>Blood pressure, mm Hg</td>
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<tr>
<td>Systolic</td>
<td>130.0±2.9</td>
<td>131.7±3.9</td>
<td>138.1±3.8</td>
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<td>Diastolic</td>
<td>77.2±2.1</td>
<td>80.8±2.0</td>
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<td>Lipid levels, mg/dL</td>
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<tr>
<td>Cholesterol</td>
<td>261.1±7.1</td>
<td>259.6±7.0</td>
<td>256.7±4.4</td>
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<tr>
<td>HDL</td>
<td>48.8±3.3</td>
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<td>45.4±2.0</td>
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<tr>
<td>LDL</td>
<td>186.8±7.0</td>
<td>181.6±6.6</td>
<td>183.9±3.8</td>
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<td>Triglycerides</td>
<td>168.7±17.2</td>
<td>196.0±14.5</td>
<td>181.8±17.9</td>
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<td>Serum markers</td>
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<td>8-isoprostane, pg/mL</td>
<td>199.5±42.6</td>
<td>256.1±50.2</td>
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<td>MCP-1, pg/mL</td>
<td>126.4±23.4</td>
<td>142.8±29.2</td>
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<td>sICAM-1, ng/mL</td>
<td>108.6±18.9</td>
<td>137.8±26.5</td>
<td>138.7±25.2</td>
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<td>hs-CRP, mg/L</td>
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<td>7.6±2.7</td>
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<tr>
<td>TNF-(\alpha), pg/mL</td>
<td>16.3±1.4</td>
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<td>15.9±1.6</td>
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<tr>
<td>Blood flow, mL · 100 mL forearm (^{-1}) (\min^{-1})</td>
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<td></td>
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<td>Basal blood flow</td>
<td>2.1±0.2</td>
<td>2.1±0.1</td>
<td>2.3±0.2</td>
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<tr>
<td>Reactive hyperemia</td>
<td>20.5±1.3</td>
<td>22.7±1.8</td>
<td>20.4±0.9</td>
</tr>
</tbody>
</table>

Forty-seven patients were randomly assigned to the indicated treatment regimen with placebo, felodipine (5 mg/d), or candesartan (16 mg/d). Data are expressed as mean±SE. There were no statistically significant differences among the candesartan versus placebo versus felodipine groups.
oxidative stress, endothelial dysfunction, and progressive vascular lesions. Therefore, AT_1 receptor activation may potentially be of general significance in the development of atherosclerosis independent of the preceding risk factors. For other antihypertensive drugs, it was also shown that endothelial function may be beneficially influenced in hypertensive patients. In a study investigating the effect of irbesartan and atenolol in patients with untreated hypertension, both drugs tended to be improved after amlodipine treatment, whereas atenolol had no effect. In several other studies, calcium channel antagonists exerted no beneficial effect on endothelial function compared with ACE inhibitors or AT_1 receptor antagonists. In most studies, patients with untreated hypertension were enrolled, whereas in the present study, normotensive patients were investigated. Importantly, reduction of elevated blood pressure levels in hypertension may, per se, improve endothelial function. This was excluded in the present study because blood pressure levels remained unaffected by treatment.

Third, the data of the present study may provide a potential therapeutic rationale. AT_1 receptor antagonism is capable of improving endothelial function during hypercholesterolemia with respect to endothelium-dependent vasorelaxation and monocyte attraction and adhesion, regardless of lipid lowering or blood pressure reduction. Presumably, AT_1 receptor blockade may represent a novel approach for the prevention of vascular dysfunction associated with hypercholesterolemia.

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