ACE inhibitors and statins acutely improve endothelial dysfunction of human coronary arterioles

Christiane P. Tiefenbacher,1 Stefanie Friedrich,1 Tina Bleeke,1 Christian Vahl,2 Xiaobo Chen,1 and Feraydoon Niroomand1

1Department of Cardiology, University of Heidelberg, 69115 Heidelberg; and 2Department of Cardiac Surgery, University of Heidelberg, 69120 Heidelberg, Germany

Submitted 14 August 2003; accepted in final form 20 November 2003

Tiefenbacher, Christiane P., Stefanie Friedrich, Tina Bleeke, Christian Vahl, Xiaobo Chen, and Feraydoon Niroomand. ACE inhibitors and statins acutely improve endothelial dysfunction of human coronary arterioles. Am J Physiol Heart Circ Physiol 286: H1425–H1432, 2004. First published November 26, 2003; 10.1152/ajpheart.00783.2003.—Long-term treatment with angiotensin-converting enzyme (ACE) inhibitors as well as angiotensin II type 1 (AT1) receptor antagonists and statins reduces cardiovascular mortality in patients with coronary artery disease as well as chronic heart failure. Little is known about the acute effects of these compounds on vascular reactivity of coronary resistance vessels. Coronary arterioles were obtained from patients undergoing coronary bypass operation (atherosclerosis group) or valve replacement (control group). Responses to endothelium-dependent agonists (histamine, serotonin, and acetylcholine) as well as to the endothelium-independent agonist sodium nitroprusside (SNP) were investigated under baseline conditions and after incubation (15 min) with lisinopril (ACE inhibitor), candesartan (AT1 receptor antagonist), or fluvastatin. In atherosclerotic vessels, vasorelaxation was significantly reduced to all endothelium-dependent agonists but not, however, to SNP (77 ± 8, −24 ± 16, −46 ± 24, and 98 ± 8% relaxation for histamine, serotonin, acetylcholine, and SNP, respectively). Lisinopril and fluvastatin but not candesartan significantly improved the responses to the endothelium-dependent agonists (lisinopril: 94 ± 4, 17 ± 22, and −20 ± 13%; fluvastatin: 96 ± 8, 23 ± 21, and −25 ± 18% relaxation for histamine, serotonin, and acetylcholine, respectively). The effect of lisinopril was prevented by pretreatment with a bradykinin antagonist (HOE-130) and dichloroisocoumarine, an inhibitor of kinine-forming enzymes. Pretreatment with a nitric oxide (NO) synthase inhibitor abolished the improvement of endothelial function by lisinopril and fluvastatin. Vascular reactivity in the control group was not influenced by any of the pharmacological interventions. The data demonstrate that in atherosclerosis, endothelium-dependent relaxation of coronary resistance arteries is severely compromised. The impairment can acutely be reversed by ACE inhibitors and statins via increasing the availability of NO.

ACE inhibitors and statins reduce the isoprenylation of various membrane proteins, which, for example, in the case of the small GTP-binding protein rho, may lead to enhanced endothelial NO synthesis because there is an alternative pathway using chymase (34). Thus it has been proposed that blockade of the AT1 receptor may be more effective regarding protection from vasotoxic ANG II. AT1 receptor antagonists have been shown to reduce infarct size via activation of AT2 receptors, bradykinin, and prostaglandins (11).

Statins enhance the generation of NO by increasing the expression of NO synthase (NOS) and by exerting radical scavenging properties (3, 5, 14, 20, 22, 29, 33). In addition, statins reduce the isoprenylation of various membrane proteins, which, for example, in the case of the small GTP-binding protein rho, may lead to enhanced endothelial NO generation (15).

Long-term treatment with ACE inhibitors as well as AT1 receptor antagonists and statins reduces cardiovascular mortality in patients with coronary artery disease as well as chronic heart failure partially independent from effects on blood pressure and cholesterol reduction. Furthermore, a number of clinical and experimental studies have demonstrated protection of the endothelium by chronic treatment with ACE inhibitors, AT1 receptor antagonists, and statins (4, 20, 23, 26, 29, 33, 35, 37). Endothelial dysfunction has been shown to be of prognostic relevance for patients with coronary artery disease. It is unknown, however, whether treatment of endothelial dysfunction improves the prognosis and when treatment should be started. In addition, it is largely unknown whether ACE inhibitors, AT1 receptor antagonists, and statins can acutely improve endothelial function of coronary arterioles. The basis for our study was, therefore, to investigate whether administration of lisinopril, candesartan, or fluvastatin would rapidly improve endothelial dysfunction of human coronary resistance arteries.

Address for reprint requests and other correspondence: C. P. Tiefenbacher, Dept. of Cardiology, Univ. of Heidelberg, Bergheimerstrasse 58, 69115 Heidelberg, Germany (E-mail: ctiefenbacher@med.uni-heidelberg.de).
METHODS

General Preparation

**Human tissue.** The right atrial appendage was obtained from patients receiving coronary artery bypass graft surgery (CABG), valve replacement, or repair of septum defects. Patients were recruited from the outpatients clinic and from the coronary catheterization laboratory of the University of Heidelberg. Informed consent was obtained before enrollment in the study. The study was approved by the local ethics committee. Only patients who had not been on therapy with statins, ACE inhibitors, or AT1 blockers for at least 2 wk before enrollment were included. Immediately after removal, the tissue was placed in cold (4°C) saline.

**In vitro measurements of coronary arteriolar diameters.** Coronary arterioles 80–100 μm in diameter were dissected carefully from the myocardial tissue at 4°C and were transferred to an acrylic resin vessel chamber containing physiological salt solution (PSS)-albumin solution at pH 7.4. Both ends of each arteriole were cannulated with a glass micropipette with an external tip of ~40 μm and secured with 11-0 ophthalmic suture. Blood in the vessels was flushed out at low pressure (20 cmH2O), and the other end of the microvessel was secured to a second pipette.

After the vessels were cannulated, the chamber was transferred to the stage of an inverted microscope (IM35, Carl Zeiss; objective × 40, numerical aperture 0.75) fitted with a Cohu television camera and videomicrometer (Texas A&M Microcirculation Research Institute). Arterioles were pressurized to 60 cmH2O by adjusting the height of a reservoir connected to each micropipette. By setting both reservoirs to the same height, the vessels were pressurized without flow. Leaks were detected by closing off the system to the reservoirs and examining for a decline in intraluminal pressure. Vessels with leaks were excluded from further study. Internal diameters were recorded continuously during steady-state conditions after each intervention. The microvessels were set to their in situ length and were bathed in PSS-albumin solution with the temperature maintained at 36–37°C with the use of an external heat exchanger. Maximal diameter was defined as the diameter obtained at maximal relaxation before the development of tone or pharmacological preconstriction. All arterioles developed a spontaneous tone of 10–20% of maximal diameter and were further preconstricted with endothelin (10^{-10} M) to 25–30% of their maximal diameter. Changes in vessel diameter were expressed as percent relaxation based on the preconstricted diameter (0% relaxation) up to the maximal diameter (100% relaxation).

**Isolated Microvessel Protocol**

After the arterioles were allowed to equilibrate in the bath and were preconstricted, dose-response curves to the endothelium-independent vasodilator sodium nitroprusside (10^{-9}–10^{-5} M) and to the endothelium-dependent vasodilators histamine (10^{-9}–10^{-5} M), serotonin (10^{-9}–10^{-5} M), and acetylcholine (10^{-9}–10^{-5} M) were measured during steady-state conditions. Thereafter, microvessels were investigated in six groups: 1) vessels from the control group after incubation with lisinopril (1 μM, n = 8); 2) vessels from the control group after incubation with fluorastatin (1 μM, n = 8); 3) vessels from the control group after incubation with candesartan (1 μM, n = 7); 4) vessels from the atherosclerosis group after incubation with lisinopril (1 μM, n = 8); 5) vessels from the atherosclerosis group after incubation with fluorastatin (1 μM, n = 9); and 6) vessels from the atherosclerosis group after incubation with candesartan (1 μM, n = 8). Dose-response curves to the different agonists were repeated after incubation with either lisinopril, fluorastatin, or candesartan for 15 min. In an additional group of experiments (n = 6), vessels were exposed to a 30-min incubation with candesartan. To elucidate possible signal transduction pathways, lisinopril-treated vessels were additionally incubated with the bradykinin antagonist HOE-130 and diisoucomaraine (DCI), an inhibitor of kinine-forming enzymes. To demonstrate NO-dependent effects, vessels were pretreated with the NOS inhibitor Nα-nitro-l-arginine methyl ester (l-NNAME).

Lisinopril, fluorastatin, and candesartan, all kindly provided from ASTRA-ZENECA, were dissolved in DMSO. Two microliters of stock solution were added to the bath to obtain a final concentration of 1 μM. Administration of 2 μM DMSO alone to the bath had no vasoactive effect. The concentration of the compounds was similar to the concentration reported in previous studies.

**Data Analysis**

Measurements of microvascular diameters during interventions are expressed as percent relaxation, with 100% representing the maximal baseline diameter before vessels were pressurized from the diameter elicited after preconstriction with endothelin-1. Statistical comparisons of dose-response curves to different interventions and baseline diameters before each intervention were made with the use of two-way ANOVA with repeated measures, followed by the Bonferroni test to detect individual differences. All statistics were computed with the use of Statview 4.1 on a Macintosh 8100 computer. A probability level of 95% was used in all experiments as the criterion of statistical significance. All data are reported as means ± SE.

RESULTS

**Baseline Characteristics**

In total, vessels from right atrial appendage from 48 patients were investigated. Twenty-six patients received CABG surgery for severe coronary atherosclerosis; 22 patients received surgery for either mitral valve replacement (11 patients), aortic valve replacement (9 patients), or ventricular septum repair (2 patients). The latter group of patients was considered to be the control group because by preoperative coronary angiography, the presence of significant coronary artery disease (stenoses <30% in diameter) had been excluded. In addition, in the control group, the incidence of coronary risk factors was low [no patients with hypercholesterolemia (total cholesterol level >200 mg/dl), diabetes (fasting glucose >110 mg/dl; glycosylated hemoglobin >6%), or nicotin abuse (current smokers or smokers within the last 5 yr); five patients had a history of hypertension (RR >140/90 mmHg)]. Vessels from patients with diabetes were not included in the study. Patient characteristics are presented in Table 1.

**Pharmacological Studies**

**Control group.** In vessels from patients without atherosclerosis, there was near-maximal vasorelaxation to sodium nitro-

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of patients from the control and atherosclerosis groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Total no. of patients</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>No. of men</td>
</tr>
<tr>
<td>History of hypertension</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
</tr>
<tr>
<td>Mean total cholesterol level, mg/dl</td>
</tr>
<tr>
<td>Mean LDL-cholesterol level, mg/dl</td>
</tr>
<tr>
<td>Mean triglyceride level, mg/dl</td>
</tr>
<tr>
<td>Current smoker</td>
</tr>
<tr>
<td>Mean HbAlc, %</td>
</tr>
</tbody>
</table>
prusside and histamine (99 ± 7 and 96 ± 8% relaxation, respectively). Serotonin caused some vasorelaxation (24 ± 10%), and acetylcholine caused predominantly vasoconstriction (−13 ± 14%). After application of lisinopril (n = 8), fluvastatin (n = 8), or candesartan (n = 7), the response to the different agonists was unaltered (Figs. 1-3).

Atherosclerosis group. In vessels from humans with coronary artery disease, there was a significant reduction of vasodilatation to histamine compared with the control group. Serotonin induced vasoconstriction instead of vasorelaxation, and there was a significant enhancement of acetylcholine-induced vasoconstriction, indicating endothelial dysfunction. As ex-

Fig. 1. Summary of the responses of isolated coronary arterioles from the lisinopril control group (n = 8). Data were obtained from arterioles during baseline conditions and after incubation with lisinopril alone or in addition to disocoumarine (DCI), N-nitro-l-arginine methyl ester (l-NAME), or HOE-130 (HOE). SNP, sodium nitroprusside. Data are presented as means ± SE.

Fig. 2. Summary of the responses of isolated coronary arterioles from the fluvastatin control group (n = 8). Data were obtained from arterioles during baseline conditions and after incubation with fluvastatin alone or in addition to l-NAME. Data are presented as means ± SE.
pected, the effect of sodium nitroprusside was unaltered compared with vessels from patients without significant atherosclerosis (77 ± 8, −24 ± 16, −46 ± 24, and 98 ± 8% relaxation for histamine, serotonin, acetylcholine, and sodium nitroprusside, respectively; Figs. 4-6).

**Lisinopril.** After pretreatment with lisinopril, the responses to histamine, serotonin, and acetylcholine were significantly improved. In contrast, lisinopril did not influence vasorelaxation to sodium nitroprusside (94 ± 4, 17 ± 22, −20 ± 13, and 94 ± 8% relaxation for histamine, serotonin, acetylcholine, and sodium nitroprusside, respectively; Fig. 4) Additional pretreatment with the bradykinin antagonist HOE-130 and the kininase antagonist DCI significantly decreased the effect of lisinopril (HOE-130: 77 ± 9, −23 ± 18, −50 ± 12, and 97 ± 9%; DCI: 75 ± 7, −18 ± 17, −42 ± 11, and 99 ± 8% relaxation for histamine, serotonin, acetylcholine, and sodium nitroprusside, respectively; Figs. 4-6).
nitroprusside, respectively; Fig. 4). After pretreatment with L-NAME, the protective effect of lisinopril was completely abolished (25 ± 8, −26 ± 6, −50 ± 12, and 95 ± 7% relaxation for histamine, serotonin, acetylcholine and sodium nitroprusside, respectively; Fig. 4).

Fluvastatin

In a similar manner, pretreatment with fluvastatin significantly improved the effects of the endothelium-dependent agonists without altering vasorelaxation to sodium nitroprusside, respectively; Fig. 4).

Fluvastatin

In a similar manner, pretreatment with fluvastatin significantly improved the effects of the endothelium-dependent agonists without altering vasorelaxation to sodium nitroprusside, respectively; Fig. 4).

Fig. 5. Summary of the responses of isolated human coronary arterioles from patients with atherosclerosis (n = 9). Data were obtained from arterioles during baseline conditions and after incubation with fluvastatin alone or in addition to L-NAME. Data are presented as means ± SE. *P < 0.05 vs. baseline.

Fig. 6. Summary of the responses of isolated coronary arterioles from candesartan-treated atherosclerotic vessels (n = 8). Data were obtained from arterioles during baseline conditions and after incubation with candesartan.
side (96 ± 8, 23 ± 21, −25 ± 18, and 99 ± 8% relaxation for histamine, serotonin, acetylcholine, and sodium nitroprusside, respectively; Fig. 5). After pretreatment with 1-NAME, the effect of fluvastatin was completely abolished, (24 ± 5, −23 ± 18, −45 ± 13, and 99 ± 8% relaxation for histamine, serotonin, acetylcholine, and sodium nitroprusside, respectively; Fig. 5).

Candesartan. Although there was a trend for improvement in the response to acetylcholine after pretreatment with candesartan, there was no statistically significant difference in vascular responses to all tested endothelium-dependent agonists by candesartan compared with untreated vessels (77 ± 7, −25 ± 12, −37 ± 16, and 99 ± 8% relaxation for histamine, serotonin, acetylcholine, and sodium nitroprusside, respectively; Fig. 6). Prolongation of pretreatment with candesartan to 30 min induced further improvement of endothelial function (80 ± 8, −21 ± 11, −33 ± 15, and 98 ± 9% relaxation for histamine, serotonin, acetylcholine, and sodium nitroprusside, respectively; however, again, not a significant benefit).

**DISCUSSION**

Endothelial function of atherosclerotic coronary arterioles is severely compromised (1, 12, 32). This is confirmed by the findings of this study: that the response to endothelium-dependent vasodilators, and not, however, an endothelium-independent vasodilator, is greatly attenuated in vessels from patients with atherosclerosis compared with vessels from patients without. In our experiments, pretreatment of the vessels with either lisinopril, an ACE inhibitor, or fluvastatin significantly improved endothelial function. This effect was found after an incubation period as short as 15 min.

**Control Groups**

Because of the difficult preparation and the difficulty in investigating true control vessels, experiments in human coronary resistance arteries are challenging. However, because there is a strong species dependency of many endothelial agonist, the results of studies investigating endothelial function in animal models cannot necessarily be transferred to human pathophysiology. As in previous studies (32), we used as controls vessels from patients without significant atherosclerosis, i.e., vessels from patients undergoing open heart surgery for valve replacement or repair of septum defects in whom the presence of significant atherosclerosis had preoperatively been excluded by coronary angiography. To further diminish the risk of endothelial dysfunction of our control vessels, we collected tissue from patients without severe atherosclerotic risk factors. There were only five cases of mild hypertension and no case of hyperlipidemia or nicotin abuse in patients from the control group. However, we cannot completely rule out endothelial dysfunction in our control vessels. A certain alteration of endothelial function is to be expected, for example, due to an altered profile of pressure and flow, both influencing endothelial and smooth muscle cell function (13). Nevertheless, there was significantly greater vasorelaxation of the control vessels to all tested endothelium-dependent agonists compared with vessels from atherosclerotic individuals without differences in the response to the endothelium-independent agonist. Thus we propose that endothelial function of our control vessels was preserved in contrast to vessels from patients with atherosclerosis.

**Choice of Agonists**

In the present study, we used histamine, serotonin, and acetylcholine to determine endothelium-dependent vasorelaxation. All three agonists have been shown to induce NO-dependent vasorelaxation in human coronary vessels (32). By pretreatment with 1-NAME, an inhibitor of NOS, the effect of all three compounds was significantly reduced. Acetylcholine is widely used as a standard to test endothelial function in both human coronary arteries and arterioles (2, 27, 38). Under physiological conditions, acetylcholine causes vasorelaxation of coronary vessels. However, in vessels with atherosclerosis or in the presence of coronary risk factors, acetylcholine has been shown to induce paradoxical vasoconstriction (30). In our experiments, acetylcholine induced vasoconstriction both in “control” vessels and in atherosclerotic vessels. This could either indicate endothelial dysfunction of the control vessels or indicate that acetylcholine is a vasoconstrictor of human arterioles with a diameter of <100 μm, as shown in previous investigations (32). In addition, acetylcholine has a direct constrictive effect on vascular smooth muscle cells that is endothelium independent. Because histamine, a strongly NO-dependent dilator of human vessels, causes maximal vasodilatation in our controls, we propose that endothelial function of our control vessels was grossly normal. Serotonin caused minor vasodilatation in the control vessels. Interestingly, there was a great standard deviation of the effects of both acetylcholine and serotonin. This may result from a heterogeneity in the distribution of receptor subtypes and/or indicate early endothelial dysfunction of some of the control vessels.

**Physiological and Pathophysiological Implications**

Endothelial dysfunction represents an early finding in atherosclerotic vascular diseases (1, 12, 24) and is of prognostic value for patients with coronary artery disease (6, 25, 30). To date, it is unknown whether treatment of endothelial dysfunction results in an improvement of cardiovascular outcome. The results of large clinical trials provide evidence for a prognostic benefit by therapy with statins and ACE inhibitors (7, 8a). Primarily, the benefit of statin treatment has been related to the reduction of cholesterol levels and that of treatment with ACE inhibitors and AT1 receptor antagonists to blood pressure-lowering properties. However, there is no association of cholesterol reduction and the beneficial effect of statins in patients with stroke, and the protective effect of ramipril in the Heart Outcomes Prevention Evaluation study was independent from blood pressure reduction.

Statins have recently been shown to exert a number of endothelium-protective actions such as scavenging of oxygen free radicals, anti-inflammatory effects, and stimulation of NOS as well as tetrahydrobiopterin synthesis and NO release (10, 14, 28, 29). In clinical trials, an improvement of endothelial dysfunction by statins after several days of treatment has been found in different vascular beds (4, 20, 37). The present study extends these findings by demonstrating that fluvastatin improves endothelial dysfunction within 15 min. As previously described, the effect depends on the release of NO, because it is completely prevented by pretreatment with 1-NAME.
ACE inhibitors have been shown to improve endothelial dysfunction both in vitro and in vivo (8, 9). In the Trial on Reversing Endothelial Dysfunction study, patients receiving 6 mo of quinapril treatment showed a significant improvement of endothelium-dependent vasorelaxation (18). Similar to our findings, an acute improvement of endothelial function of the coronary macro- and microvasculature has been described after intracoronary application of quinapril (21). The underlying mechanism of the protective effect of ACE inhibitors on endothelial function is partially mediated via inhibition of the breakdown of bradykinin, an endogenous vasodilator, and partially via an antioxidative capacity (9, 16, 19). Prevention of kinin breakdown and increase of NO availability are also likely to be responsible for the acute effects of ACE inhibitors obtained in vitro. In our study, the effect of lisinopril could be inhibited by a bradykinin receptor antagonist and an inhibitor of kininase-producing enzymes as well as application of L-NAME.

AT1 receptor antagonists have been shown to possess anti-inflammatory, antithrombotic, and radical scavenging activities as well as to increase the release of NO in a number of experimental studies (21, 23, 35). Prasad and colleagues showed reversal of endothelial dysfunction after acute (minutes) intracoronary application of losartan in patients with atherosclerotic risk factors. An improvement of endothelial function of the forearm has been reported by Wassmann et al. (35) using candesartan over a treatment period of 6 wk in patients with hypercholesterolemia. Both studies were performed in small groups of patients and over a brief treatment period; long-term results are to be awaited. In our study, the AT1 receptor antagonist candesartan did not significantly influence endothelial dysfunction after pretreatment for 15 and 30 min. From these observations and from the data in the literature (21, 36), the effect of AT1 receptor blockade on endothelial function may not be as acute and as strong as that of ACE inhibitors or statins.

In conclusion, in the present study, endothelial dysfunction of coronary resistance arteries from patients with atherosclerosis was reversed 15 min after the application of losinopril or fluvastatin. The endothelium-protective effect of both was abolished in the presence of L-NAME. The effect of losinopril could additionally be prevented by cotreatment with HOE-130 or DCI, both of which inhibit kinin metabolism. The data provide evidence that losinopril and fluvastatin acutely improve endothelial function by increasing the bioavailability of NO. In the future, it needs to be shown whether acute treatment of endothelial dysfunction in the setting of acute myocardial infarction or unstable angina reduces cardiovascular mortality and which treatment strategies are most (cost) effective.

REFERENCES


