Suppression of Endothelial Progenitor Cells in Human Coronary Artery Disease by the Endogenous Nitric Oxide Synthase Inhibitor Asymmetric Dimethylarginine

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OBJECTIVES We tested the hypothesis that asymmetric dimethylarginine (ADMA) may be an endogenous inhibitor of endothelial progenitor cells (EPCs).

BACKGROUND Endothelial progenitor cells play a pivotal role in regeneration of injured endothelium, thereby limiting the formation of atherosclerotic lesions. Reduced numbers of EPCs may affect progression of coronary artery disease. Regulation of EPC mobilization and function is mediated in part by nitric oxide (NO). Endogenous inhibitors of NO synthases, such as ADMA, contribute to endothelial dysfunction and injury.

METHODS We used flow cytometry and in vitro assays to investigate the relationship between EPC number and function with ADMA plasma levels in patients with stable angina.

RESULTS The plasma concentration of ADMA was related to the severity of coronary artery disease and correlated inversely with the number of circulating CD34+/CD133+ progenitor cells (r = −0.69; p < 0.0001) and endothelial colony forming units (CFUs) (r = −0.75; p < 0.0001). Adjusting for all patient characteristics, we confirmed these findings in multivariate regression analyses. In vitro differentiation of EPCs was repressed by ADMA in a concentration-dependent manner. Compared with untreated cells, ADMA reduced EPC incorporation into endothelial tube-like structures to 27 ± 11% (p < 0.001). Asymmetric dimethylarginine repressed the formation of CFUs from cultured peripheral blood mononuclear cells to 64 ± 6% (p < 0.05) when compared with controls. Co-incubation with the hydroxymethyl glutaryl coenzyme A reductase inhibitor rosvastatin abolished the detrimental effects of ADMA.

CONCLUSIONS Asymmetric dimethylarginine is an endogenous inhibitor of mobilization, differentiation, and function of EPCs. This contributes to the cardiovascular risk in patients with high ADMA levels and may explain low numbers and function of EPCs in patients with coronary artery disease. (J Am Coll Cardiol 2005;46:1693–701) © 2005 by the American College of Cardiology Foundation

A functional and intact endothelium plays a crucial role in the maintenance of vascular tone and structure. Endothelial lesions initiate atherosclerotic processes (1). Endothelial progenitor cells (EPCs) are mobilized from bone marrow into the circulation and contribute to replacement of diseased endothelial cells, thereby limiting atherosclerotic plaque formation (2–4). Impaired mobilization of EPCs from bone marrow may lead to cardiovascular disease; indeed, reduced numbers of circulating EPCs have been found in patients with coronary artery disease (CAD) (4–6). Moreover, patients with dysfunctional EPCs suffer from inadequate coronary collateral development (7). Bone marrow–derived mononuclear cells from patients with CAD have reduced capacity to induce neovascularization (8). The underlying mechanism has not been determined.

Mobilization and differentiation of EPCs is known to be modified by nitric oxide (NO) (9,10), and bone marrow–expressed endothelial NO synthase (eNOS) is essential for the mobilization of stem and progenitor cells in vivo (9). Endogenous NO synthase inhibitors, such as asymmetric dimethylarginine (ADMA), contribute to endothelial dysfunction and inhibition of angiogenesis in vivo (11,12). Asymmetric dimethylarginine is also a marker for cardiovascular risk and is correlated with the incidence of major adverse cardiovascular events or deaths (13). Thus, NO impacts on important steps during differentiation of EPCs.

In a clinical study, we have investigated the relationship between plasma concentrations of ADMA and the number

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of circulating CD34+/CD133+ progenitor cells and endothelial colony forming units (CFUs) in patients with stable angina admitted for coronary angiography. The hypothesis was that ADMA may be an important endogenous inhibitor of EPC mobilization and function.

**METHODS**

**Study subjects.** Approval from the ethical committee of the University of Würzburg was obtained, as was informed written consent from patients. Eighty consecutive patients admitted to the hospital because of stable angina (49 men) aged 39 to 81 years (mean age, 66.1 ± 1.1 years) were enrolled. All patients underwent cardiac catheterization, and CAD was excluded (CAD 0) or quantified by the number of coronary arteries involved (CAD 1 to 3). For a stenosis to qualify, the luminal diameter of the coronary vessel had to be reduced by 50%. Patient characteristics are shown in Table 1.

**Measurement of ADMA in plasma samples.** Blood (5 ml) was drawn from the antecubital vein of patients using syringes containing ethylenediaminetetraacetic acid and immediately put on ice. Blood samples were centrifuged at 1,500 g and 3°C for 15 min. The plasma obtained was used immediately or stored at −80°C until further analysis. Ultrafilters (approximately 0.7 to 0.8 ml) from 1-ml aliquots of plasma samples were obtained by centrifugation at 7,500 g and 20°C for 20 min using the Amicon Ultra Millipore ultrafiltration cartridges (Millipore, Schwabach, Germany). For detection and measurement of ADMA, a gas chromatography-tandem mass spectrometry method was used (14). The measurements were performed on a triple-trap quadruple mass spectrometer ThermoQuest TSQ 7000 (Finnigan MAT, San Jose, California) directly interfaced with a Trace 2000 series gas chromatograph equipped with an autosampler AS 2000 (CE Instruments, Austin, Texas). The gas chromatograph was equipped with a fused-silica capillary column Optima 17 (30 m × 0.25 mm ID, 0.25 μm film thickness) from Macherey-Nagel (Düren, Germany). Synthetic ADMA served as the internal standard.

**Isolation of peripheral blood mononuclear cells (PBMCs).** The PBMCs were isolated by Ficoll density centrifugation as described (4). For in vitro assays, PBMCs were harvested by blood withdrawal or leukapheresis (Cobe Spectra device, Gambro, Germany) of healthy volunteers (n = 5).

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 20)</th>
<th>CAD 1 (n = 20)</th>
<th>CAD 2 (n = 20)</th>
<th>CAD 3 (n = 20)</th>
<th>All (n = 80)</th>
<th>p Value CAD 0 vs. CAD 3</th>
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<tr>
<td><strong>Age (yrs)</strong></td>
<td>64.6 ± 1.8</td>
<td>63.7 ± 1.0</td>
<td>66.7 ± 2.0</td>
<td>69.5 ± 3.0</td>
<td>66.1 ± 1.1</td>
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<tr>
<td><strong>Male (%)</strong></td>
<td>55</td>
<td>60</td>
<td>65</td>
<td>65</td>
<td>61.3</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>CD34+/CD133+ (%)</strong></td>
<td>0.22 ± 0.03</td>
<td>0.18 ± 0.04</td>
<td>0.11 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>CFU (number/well)</strong></td>
<td>16.7 ± 1.2</td>
<td>16.8 ± 2.1</td>
<td>10.7 ± 1.2</td>
<td>8.3 ± 0.2</td>
<td>13.1 ± 1.6</td>
<td>&lt;0.0001</td>
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<tr>
<td><strong>ADMA (μmol/l)</strong></td>
<td>0.47 ± 0.02</td>
<td>0.45 ± 0.02</td>
<td>0.51 ± 0.03</td>
<td>0.58 ± 0.02</td>
<td>0.50 ± 0.01</td>
<td>&lt;0.0001</td>
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<tr>
<td><strong>Hypertension (%)</strong></td>
<td>70</td>
<td>65</td>
<td>90</td>
<td>85</td>
<td>77.5 ± 1.3</td>
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<tr>
<td><strong>Diabetes (%)</strong></td>
<td>5</td>
<td>30</td>
<td>25</td>
<td>35</td>
<td>23.8 ± 1.5</td>
<td>0.004</td>
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<td><em><em>Medication</em> (%)</em>*</td>
<td></td>
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<td>Aspirin</td>
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<td>85</td>
<td>75</td>
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<td>ACE inhibitors/ARB</td>
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<td>60</td>
<td>55</td>
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<td>Beta-blocker</td>
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<td>Statin</td>
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<td>55</td>
<td>55</td>
<td>50</td>
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<td>Nitrates</td>
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<td>15</td>
<td>30</td>
<td>18.8</td>
<td>≤0.015</td>
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<td>Creatinine (mg/dl)</td>
<td>0.84 ± 0.06</td>
<td>0.92 ± 0.04</td>
<td>0.99 ± 0.06</td>
<td>0.98 ± 0.06</td>
<td>0.93 ± 0.03</td>
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<td>Cholesterol (mg/dl)</td>
<td>187.1 ± 7.8</td>
<td>204.6 ± 9.9</td>
<td>214.7 ± 10.8</td>
<td>180.9 ± 10.5</td>
<td>196.4 ± 4.9</td>
<td>≤0.048†</td>
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<td>LDL (mg/dl)</td>
<td>105.9 ± 6.9</td>
<td>118.1 ± 8.2</td>
<td>126.2 ± 9.3</td>
<td>96.5 ± 7.1</td>
<td>111.3 ± 4.0</td>
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<td>HDL (mg/dl)</td>
<td>54.4 ± 3.2</td>
<td>50.9 ± 3.4</td>
<td>51.6 ± 3.5</td>
<td>48.6 ± 3.7</td>
<td>51.4 ± 1.7</td>
<td>0.24</td>
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<td>Hemoglobin (g/dl)</td>
<td>14.3 ± 0.4</td>
<td>14.1 ± 0.4</td>
<td>14.2 ± 0.3</td>
<td>13.3 ± 0.3</td>
<td>14.0 ± 0.2</td>
<td>0.07</td>
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<tr>
<td>CRP (mg/dl)</td>
<td>0.84 ± 0.23</td>
<td>0.60 ± 0.16</td>
<td>0.52 ± 0.10</td>
<td>0.83 ± 0.20</td>
<td>0.70 ± 0.09</td>
<td>0.97</td>
</tr>
<tr>
<td>Leukocytes (10³/mm³)</td>
<td>6.9 ± 0.4</td>
<td>7.0 ± 0.4</td>
<td>7.9 ± 0.5</td>
<td>7.9 ± 0.6</td>
<td>7.4 ± 0.2</td>
<td>0.15</td>
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</table>

Values are mean ± standard error of the mean. *Medication at admission to hospital. †p < 0.05 versus CAD 2.

ACE = angiotensin-converting enzyme; ADMA = asymmetric dimethylarginine; ARB = angiotensin-receptor blockers; CAD = coronary artery disease; CAD 0–3 = number of involved major coronary vessels; CFU = colony-forming units; CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
Determination of progenitor cells. A variety of assays were used to determine the amount, differentiation, and function of EPCs as detailed in the subsequent text.

ENDOTHELIAL PROGENITOR CELL CULTURE ASSAY. We cultured 2 × 10⁵ PBMCs on fibronectin-pre-coated eight-well chamber slides (Lab-Tek, Wiesbaden, Germany) in endothelial basal medium-2 (EBM2) culture medium supplemented with EGM SingleQuots (Cambrex, Verviers, Belgium) for four days. To exclude contamination with mature circulating endothelial cells, we carefully removed culture supernatant 8 h after initial seeding and placed it to new fibronectin-pre-coated chamber slides. After dilution of 1,1'-dioctadecyl-3,3,3',3-tetramethyl-indocarbocyanine perchlorate labeled acetylated low-density lipoprotein (diL-ac-LDL) (Molecular Probes, Carlsbad, California) and fluorescein isothiocyanate (FITC)-conjugated lectin from Ulex europeus (UEA-1; Sigma, Deisenhofen, Germany) in serum-free EBM2 medium, cells were washed twice and incubated for 4 h at 37°C in EBM2 medium containing 10 µg/ml diL-ac-LDL and 10 µg/ml UEA-1. After washing, cells were observed by fluorescence microscopy. Only double-positive (diL-ac-LDL and UEA-1) cells were counted in at least eight independent randomly selected high-power fields.

DETECTION OF CD34⁺/CD133⁺ CELLS. The PBMCs were isolated by Ficoll density centrifugation as described previously (4). Thereafter, we incubated a volume of 60 µl (about 1 × 10⁵ PBMCs) with appropriate amounts of FITC-labeled monoclonal mouse anti-human CD34 and allophycocyanin-labeled monoclonal mouse anti-human CD133/2 (293C3) antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) for 10 min at 6°C. In parallel experiments, we added appropriate isotype controls (FITC-conjugated mouse immunoglobulin [Ig] G2a monoclonal Ig and allophycocyanin-conjugated mouse IgG2b monoclonal Ig). We acquired at least 20,000 cells using a FACSCalibur cytometer (Becton Dickinson, Heidelberg, Germany). The number of progenitor cells was expressed as a percentage of all PBMCs. Two investigators independently assessed the number of progenitor cells in blinded experiments.

CFUS. The PBMCs were isolated by Ficoll density gradient centrifugation, and 5 × 10⁶ cells from leukapheresis donors were plated on fibronectin-coated six-well plates in EndoCult medium (StemCell Technologies, St. Katharinen, Germany) according to the manufacturer’s instructions. In case of the patients study, 5 × 10⁶ PBMCs were cultured on fibronectin-coated 12-well plates. After 48 h, non-adherent cells were collected and plated in replicate fibronectin-coated 24-well plates. Colonies were evaluated and quantified three days later. A colony was defined as a central core of “round” cells with more elongated “sprouting” cells at the periphery and are referred to as early outgrowth CFU-endothelial cells (4). The endothelial lineage of these cells has been confirmed previously by immunocytochemical staining for von Willebrand factor, vascular endothelial growth factor receptor 2, and CD31 (4).

INCORPORATION INTO TUBE-LIKE STRUCTURES. We measured incorporation of EPCs during endothelial tube formation as described (15). Briefly, diL-ac-LDL pre-labeled EPCs (2 × 10⁵ cells) were mixed with human umbilical vein endothelial cells (4 × 10⁵) on an eight-well glass slide pre-coated with 200 µl Matrigel (BD Bioscience, Heidelberg, Germany) in 500 µl EBM-2 medium with supplements (Cambrex) with the addition of 0, 1.0, 5.0, or 10.0 µmol ADMA. Further, the 10-µmol group was co-treated with rosuvastatin (10 µmol). The concentration of rosuvastatin was based on a previous in vitro study (16). After eight hours of incubation in a 5% CO₂ humidified atmosphere at 37°C, cells were examined under a fluorescence microscope. The amount of incorporated diL-ac-LDL-labeled EPCs in formed endothelial tubes was determined. Two investigators in blinded experiments examined eight randomly selected high-power fields.

DETECTION OF eNOS ACTIVITY IN EPCs. Cultured EPCs were pretreated with 0, 1.0, 5.0, or 10.0 µmol ADMA, as well as ADMA (10 µmol) + rosuvastatin (10 µmol) for 24 h. The EPCs were then incubated at 37°C with 5 mmol L-[guanidino-¹⁵N₂]arginine for 8 h. Then, supernatant was removed and eNOS activity was determined by assessing the conversion of L-[guanidino-¹⁵N₂]arginine to ¹⁵N-nitrate with gas chromatography-mass spectrometry, according to a previously described method (17,18).

Statistical analysis. Data are expressed as mean values ± standard error of the mean. A statistical analysis was performed by one-way analysis of variance followed by multiple comparisons using the Fisher protected least-significant difference test. To analyze relationships between variables, simple, polynomial, and multivariate regression analyses were performed. Statistical analysis was performed using the StatView 5.0 statistic program (Abacus Concepts, Berkeley, California). Statistical significance was assumed at p < 0.05.

RESULTS

Clinical study. We first investigated whether there was a correlation between the plasma concentration of ADMA and the number of circulating progenitor cells. Patients were divided into four groups: patients in whom coronary lesions were <50% of diameter (CAD 0) and patients with one, two, or three involved coronary vessels (CAD 1 to 3). There was no statistical difference in age among the different patient groups, as shown in Table 1. Total cholesterol levels were higher in CAD patients (CAD 0: 187.1 ± 7.8 mg/dl vs. CAD 2: 214.7 ± 10.8 mg/dl; p < 0.05; Table 1), but not correlated with the severity of disease (Table 1) or the amount of circulating CD133⁺/CD34⁺ progenitor cells (r = −0.01). Circulating CD133⁺/CD34⁺ progenitor cells correlated well with endothelial CFU (r = 0.83, p <
The ADMA plasma concentrations in patients increased with the severity of CAD (CAD 0: 0.47 ± 0.02 μmol/l vs. CAD 3: 0.58 ± 0.02 μmol/l; p < 0.001) (Table 1, Fig. 1A). There was an inverse correlation between ADMA plasma levels and the amount of CD133+/CD34+ progenitor cells (r = -0.69, p < 0.0001) (Fig. 1B) or endothelial CFUs (r = -0.75, p < 0.0001) (Fig. 1D) in isolated PBMCs of patients, which were reduced by 77.3% and 50.3% in patients with three-vessel disease (CD133+/CD34+ cells: CAD 0: 0.22 ± 0.03% vs. CAD 3: 0.05 ± 0.01%, p < 0.0001; CFU: CAD 0: 16.7 ± 1.2 colonies/well vs. CAD 3: 8.3 ± 0.7 colonies/well, p < 0.0001) (Table 1). Diabetes correlated with the number of vessels with CAD (p ≤ 0.004), whereas hypertension did not (Table 1). Patients with severe CAD were more commonly treated with a statin (p < 0.001) or nitrates (p < 0.05), whereas treatment with other drugs was not different between the groups (Table 1).

In stepwise multivariate regression analysis, we found that ADMA (standardized coefficient, −0.51; p < 0.0001) and CAD (standardized coefficient, −0.43, p < 0.01) independently related to the number of circulating CD34+/CD133+ progenitor cells. In this analysis the following factors were not significant predictors: age, plasma creatinine, leukocytes, hemoglobin, C-reactive protein, hypertension, diabetes mellitus, drug treatment (aspirin, clopidogrel, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers, statins, nitrates), total cholesterol, low-density lipoprotein and high-density lipoprotein. In a similar analysis, we found that only ADMA significantly predicted endothelial CFUs (standardized coefficient, −0.67, p < 0.0001).

**ADMA inhibits differentiation and function of cultured EPCs.** To test the hypothesis that ADMA may be an endogenous inhibitor of EPCs, we undertook further in vitro studies. ADMA reduced the amount of adherent dil-ac-LDL/UEA-1 double-positive cells after four days in culture to 37 ± 4% (p < 0.0001; n = 5) (Fig. 2) in a concentration-dependent manner. The number of CFUs formed after five days of cell culture was repressed after treatment with increasing concentrations of ADMA (Fig. 3A). Co-treatment with the 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor rosuvastatin (10 μmol/l) normalized CFU numbers. We also assessed the size of CFUs as a general proliferation marker of EPCs. ADMA treatment resulted in repression of the colony sizes to 29 ± 9% (p < 0.0001; n = 5), whereas rosuvastatin attenuated the anti-proliferative effects of ADMA (Fig. 3B). Likewise, the capability to incorporate into tube-like structures in co-culture experiments with human umbilical vein endothelial cells was repressed to 27 ± 11% (p < 0.001; n = 5) of untreated cells after 24 h preincubation of EPCs with ADMA (10 μmol/l). This inhibitory action was reversed with rosuvastatin treatment (Fig. 4). Because the function of EPCs is, in part, regulated by NO, we also determined eNOS activity, which was reduced on ADMA
treatment to 64 ± 6% (p < 0.05; n = 5) of untreated cells in culture supernatants, whereas co-treatment with rosvavastatin completely normalized eNOS activity (Fig. 5).

DISCUSSION

In the present study, we show an inverse correlation between the number of circulating progenitor cells, the severity of coronary heart disease, and ADMA plasma levels in patients admitted to the hospital because of stable angina. In vitro studies extend and support these clinical observations. ADMA markedly repressed EPC differentiation and function. Thus, the clinical findings were likely to be mediated by reduced eNOS activity in ADMA-treated EPCs. eNOS-derived NO plays a key role in the process of EPC mobilization and differentiation (9,10) as well as angiogenesis (12). eNOS expression and activity in vitro and in vivo is increased by statins (10,19,20). In the present study, the HMG-CoA reductase inhibitor rosuvastatin normalized eNOS activity in ADMA-treated EPCs, and reversed the inhibitory effects of ADMA on EPC differentiation and function.

In apolipoprotein E-deficient hypercholesterolemic mice, increased plasma ADMA levels and impaired angiogenesis are closely correlated (12). When ADMA was directly added to a subcutaneously implanted disk angiogenesis system, inhibition of basal and fibroblast growth factor-induced angiogenesis was observed in a dose-dependent manner, showing that ADMA is an endogenous inhibitor of angiogenesis (12). Recent findings have shown that EPCs play a pivotal role in neoangiogenesis (reviewed in Losordo and Dimmeler [21]) in keeping with our findings. The ADMA-mediated impairment of EPCs may explain, at least in part, reduced angiogenesis as observed by the reduced proliferation capacity of ADMA-treated CFUs in this study. Neovascularization is impaired in diabetes, and EPCs from diabetic patients show decreased proliferation capacity, adhesion, and incorporation into vascular structures (5). The close association between CAD and diabetes (22) as well as increased ADMA levels in this study cohort may suggest a contribution of ADMA-mediated reduction of circulating EPCs and their function to CAD progression, especially in diabetes. Interestingly, although total cholesterol concentrations were higher in our cohort of CAD patients, there was no significant correlation with the severity of CAD or the amount of circulating progenitor cells. Moreover, in multivariate regression analyses we could
show that ADMA was the strongest predictor of circulating progenitor cells (both CD34+/CD133+ cells and endothelial CFUs) independently of disease (diabetes), basic laboratory parameters, and treatment status, all of which have previously been shown to regulate EPCs (5,23–26). Although increasing age was previously reported to be correlated with reduced EPC levels in patients with CAD (27), this was not seen in our study, probably because of the minimal variation of age among the patients.

Our study identifies enhanced ADMA concentrations in patients with CAD as a predominant repressor of circulating progenitor cells in humans. The in vitro data further support a mechanistic link between increased ADMA levels and decreased EPCs, although we completely rule out that this is, in part, explainable by co-correlation because both ADMA and EPC levels are related to CAD.

The HMG-CoA reductase inhibitors are known to increase EPC mobilization and function (10,28,29). Statin treatment of patients with stable CAD for four weeks led to a three-fold increase in circulating progenitor cells (28). This treatment also augmented the migratory capacity of progenitor cells. Some statins may also reduce ADMA plasma concentrations. Recently, the effects of rosvastatin on plasma levels of ADMA in patients with hypercholesterolemia were investigated in a multicenter, randomized, double-blind, placebo-controlled trial (30). Treatment for six weeks resulted in significant reductions of ADMA plasma levels and increased flow-mediated vasodilation (30). In contrast, treatment with either simvastatin or atorvastatin did not reduce plasma ADMA levels, suggesting that the ADMA-lowering effect may be specific for rosvastatin (31), although more and larger studies are needed for conclusive evidence. In the present study, exposure to rosvastatin normalized EPC differentiation and function. Because patients suffering from CAD have reduced numbers, colony-forming capacity, and migratory response of EPCs, rosvastatin may be an interesting drug for both quantitative and qualitative improvement of EPCs in patients with cardiovascular disease, as previously suggested (29). There may be ADMA-dependent and -independent effects of statins. In future studies it will be possible to determine which class of statins is superior in improving EPC mobilization, function, and ultimately cardiovascular outcome.

We used a relatively high dose of rosvastatin in our in vitro experiments. The concentration was based on mul-
Multiple previous reports showing that in vitro, higher concentrations than in vivo are needed to show the pleiotropic effects of statins, especially when modulation of eNOS activity is assessed (16,32,33). The concentrations of ADMA used in vitro were higher than the plasma levels of the patients. However, systemic ADMA levels in general seem to be lower compared with concentrations achieved in tissues (34). Therefore, the ADMA concentrations used in our in vitro assays are likely to reflect levels in local target tissues (34). We could show a significant positive correlation between CD34+/H11001+/CD133+/H11001 cells and endothelial CFUs, which have been shown to express von Willebrand factor, vascular endothelial growth factor receptor 2, and CD31 (4). ADMA repressed both CD34+/CD133+ cells and endothelial CFUs, which supports the view that not only hematopoietic and EPCs but also other progenitor cell types may be inhibited by high ADMA plasma levels. This remains to be investigated in the future.

In rats with heart failure after myocardial infarction, we have shown improvement of left ventricular remodeling and cardiac function by statin treatment (35). The present study suggests that improved EPC function by statins might contribute to this favorable effect. In patients with an acute coronary syndrome, an intensive lipid-lowering statin regimen provides greater protection against death or major cardiovascular events (36). In the present study more patients with the diagnosis of CAD were being treated with statins at the time of admission to the hospital when...
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compared with patients without CAD (p < 0.001; Table 1). Although statins increase the number of circulating EPCs (10,28,29), this was not observed in our study cohort. There are two possible reasons. First, dose-dependent effects of statin treatment regarding the amount of mobilized EPCs are currently not known. Higher doses of certain statins might result in stronger improvement of EPC mobilization and function. It is likely that if patients with severe CAD were not being treated with statins, EPC levels could be even lower. Second, the inhibitory effects of ADMA may be far stronger compared with statin effects, and thus the development of novel drugs that effectively reduce ADMA levels could lead to a novel therapeutic concept in the prevention and treatment of atherosclerosis based on improved mobilization and function of EPCs.

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