Stimulation of Endothelial Progenitor Cells
A New Putative Therapeutic Effect of Angiotensin II Receptor Antagonists

Ferdinand H. Bahlmann, Kirsten de Groot, Ottfried Mueller, Barbara Hertel, Hermann Haller, Danilo Fliser

Abstract—The number of circulating endothelial progenitor cells (EPCs) correlates with endothelial dysfunction and cardiovascular risk in humans. We explored whether angiotensin II receptor antagonist therapy affects the number of regenerative EPCs in patients with type 2 diabetes. In a prospective double-blind parallel group study, we randomly treated 18 type 2 diabetics with olmesartan (40 mg) or placebo for 12 weeks. We analyzed circulating CD34+ hematopoietic progenitor cells (flow cytometry) and EPCs (in vitro assay) before and after therapy. We verified the results in a second open trial treating 20 type 2 diabetics with 300 mg of irbesartan for 12 weeks. The number of EPCs was significantly lower in diabetic patients as compared with 38 age-matched healthy subjects (210±10 versus 258±18 per high-power field; P<0.05), whereas there was no significant difference with respect to hematopoietic progenitor cells. Treatment with olmesartan (n=9) significantly increased EPCs from 231±24 to 465±71 per high-power field (P<0.05), but not hematopoietic progenitor cells. In contrast, placebo treatment (n=9) did not affect EPCs and hematopoietic progenitor cells. With irbesartan therapy, EPC number increased significantly from 196±15 to 300±23 per high-power field (P<0.05) already after 4 weeks of treatment. At the end of 12-week therapy, patients had 310±23 EPCs per high-power field (P<0.05 versus baseline). Angiotensin II receptor antagonists increase the number of regenerative EPCs in patients with type 2 diabetes mellitus. This action may be of therapeutic relevance contributing to their beneficial cardiovascular effects. (Hypertension. 2005;45:526-529.)

Key Words: receptors, angiotensin II ▪ endothelium ▪ blood vessels ▪ cardiovascular diseases

In experimental studies using different animal models of cardiovascular injury and repair, bone marrow-derived endothelial progenitor cells (EPCs) have been shown to be responsible for endothelial and hence vascular repair.1–6 Circulating EPCs incorporate into sites of active neovascularization, where they orchestrate re-endothelialization of damaged vessel walls, also by secreting a large number of cytokines that attract and govern cells that are indispensable in the process of vascular repair.7,8 Human data are even more intriguing, because in patients with coronary artery disease the number of EPCs correlates with the number of cardiovascular risk factors, and this correlation exists even in apparently healthy subjects without manifest atherosclerosis.9,10 In the latter population, the number of EPCs also significantly correlated with the degree of endothelial dysfunction.10 Moreover, in patients with clinical conditions known to be associated with increased cardiovascular risk such as type 2 diabetes mellitus or renal failure, the number and/or function of EPCs is significantly altered.11–13

Theoretically, EPCs can be expanded in vitro for therapeutic use,5,14 but currently this procedure is laborious and expensive. Experimental work and studies in humans have revealed that the number of functionally active EPCs can be increased by pharmacological intervention, however, eg, administration of statins15,16 and recombinant human EPO (rHuEPO) or its analogue darbepoetin.12,17,18 This finding could be of therapeutic relevance, because persistent stimulation of EPCs by targeted pharmacological intervention could, at least theoretically, repair endothelial injury and progression of atherosclerotic vascular disease in patients at risk. We have therefore explored the effect of angiotensin II subtype 1-receptor antagonist therapy on EPCs in patients with type 2 diabetes mellitus in a prospective double-blind parallel group study. We verified the results in a second open study.

Patients and Methods

The studies were approved by the Hannover Medical School Ethics Committee. We have obtained written informed consent from all participants. In a prospective double-blind parallel group trial, 18 patients with type 2 diabetes mellitus randomly received placebo (n=9; age, 60.4±2.6 years; body mass index, 29.4±1.2 kg/m²) or 40 mg of the angiotensin II receptor antagonist olmesartan (n=9; 61.7±2.4 years; 30.0±1.6 kg/m²) for 12 weeks. They were normotensive, ie, sitting diastolic blood pressure <90 mm Hg or had mild
to moderate hypertension, ie, sitting diastolic blood pressure after washout between 90 and 105 mm Hg. Subjects with malignant disease, past cardiovascular events, or active inflammation were not studied. Antihypertensive drugs (if present) were completely washed out for at least 2 weeks before study entry. To achieve blood pressure control during the study period, ie, a sitting diastolic blood pressure <90 mm Hg, hydrochlorothiazide (up to 25 mg per day) and atenolol (up to 100 mg per day) were added to the double-blind medication in both treatment arms. In a second open study, we treated 20 patients (up to 100 mg per day) were added to the double-blind medication in both treatment arms. In a second open study, we treated 20 patients with type 2 diabetes mellitus (age, 64.5 ± 1.4 years; body mass index, 32.7 ± 1.4 kg/m²) who were normotensive or had mild to moderate hypertension, ie, sitting diastolic blood pressure after washout between 90 and 105 mm Hg. To achieve blood pressure control during the study period, ie, a sitting diastolic blood pressure <90 mm Hg, hydrochlorothiazide (up to 25 mg per day) and atenolol (up to 100 mg per day) were added to the double-blind medication in both treatment arms. In a second open study, we treated 20 patients with type 2 diabetes mellitus (age, 64.5 ± 1.4 years; body mass index, 32.7 ± 1.4 kg/m²) who were normotensive or had mild to moderate hypertension with 300 mg of the angiotensin II receptor antagonist, ie, olmesartan and irbesartan (n = 29).

Patients data are shown at study entry (n = 38) and after 12 weeks of treatment with both angiotensin II receptor antagonist, ie, olmesartan and irbesartan (n = 29). BMI indicates body mass index, DBP, diastolic blood pressure; SBP, systolic blood pressure.

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>Healthy Subjects</th>
<th>Before Therapy</th>
<th>After Therapy</th>
</tr>
</thead>
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<tr>
<td>Gender, M/F</td>
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<td>22/16</td>
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<tr>
<td>Age, y</td>
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<td>62.9 ± 1.1</td>
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<tr>
<td>BMI, kg/m²</td>
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<td>SBP, mm Hg</td>
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<td>144 ± 4</td>
<td>131 ± 5†</td>
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<td>DBP, mm Hg</td>
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<td>97 ± 2</td>
<td>87 ± 3†</td>
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<td>Total cholesterol, mg/dL</td>
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<td>Triglycerides, mg/dL</td>
<td>128 ± 7*</td>
<td>185 ± 13</td>
<td>191 ± 15</td>
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<tr>
<td>HbA1c, %</td>
<td>...</td>
<td>6.6 ± 0.2</td>
<td>6.6 ± 0.2</td>
</tr>
</tbody>
</table>

*P < 0.05, comparison between patients and healthy subjects. †P < 0.05, comparison between pretreatment and post-treatment values in patients with type 2 diabetes mellitus.

The clinical data of 38 patients with type 2 diabetes mellitus and in 38 age- and gender-matched healthy subjects are presented in the Table. Patients with type 2 diabetes had significantly higher body mass index, systolic and diastolic blood pressure, and serum triglyceride levels. Moreover, as shown in Figure 1, the number of EPCs was significantly lower in patients with type 2 diabetes mellitus as compared with healthy subjects. In contrast, the number of CD34⁺ HPCs was not significantly different in diabetic patients compared with healthy subjects (1.88 ± 0.13 versus 1.82 ± 0.14 /μL; not significant).

Treatment with olmesartan markedly increased EPC number (Figure 2), whereas it had no effect on circulating CD34⁺ HPCs (1.89 ± 0.32 versus 1.85 ± 0.28 /μL; not significant).

Table. Patients with type 2 diabetes had significantly higher body mass index, systolic and diastolic blood pressure, and serum triglyceride levels. Moreover, as shown in Figure 1, the number of EPCs was significantly lower in patients with type 2 diabetes mellitus as compared with healthy subjects. In contrast, the number of CD34⁺ HPCs was not significantly different in diabetic patients compared with healthy subjects (1.88 ± 0.13 versus 1.82 ± 0.14 /μL; not significant). In

**Results**

The clinical data of 38 patients with type 2 diabetes and 38 age- and gender-matched healthy subjects are presented in the Table. Patients with type 2 diabetes had significantly higher body mass index, systolic and diastolic blood pressure, and serum triglyceride levels. Moreover, as shown in Figure 1, the number of EPCs was significantly lower in patients with type 2 diabetes mellitus as compared with healthy subjects. In contrast, the number of CD34⁺ HPCs was not significantly different in diabetic patients compared with healthy subjects (1.88 ± 0.13 versus 1.82 ± 0.14 /μL; not significant).

**EPCs (per high power field)**

![Figure 1](http://hyper.ahajournals.org/lookup/doi/10.1161/01.HYP.0000619984.85493.5d)

Figure 1. Absolute number of endothelial progenitor cells (EPCs) in 38 patients with type 2 diabetes mellitus and in 38 age- and gender-matched healthy subjects. The number of EPCs was significantly lower in diabetic patients as compared with age-matched healthy subjects. Data are presented as 95% confidence interval of the mean.
Our best knowledge, angiotensin II receptor antagonists are the third drug group in cardiovascular medicine for which such evidence now exists. So far, an effect on EPCs has been proven only for statins\textsuperscript{15,16} and rHuEPO, and its analogue darbepoetin.\textsuperscript{12,18} Similarly, as for statins and rHuEPO, so-called pleiotropic effects of angiotensin II receptor antagonists attract increasing interest in the cardiovascular community, including their antidiabetogenic and anti-inflammatory action.\textsuperscript{20,21} It is even hypothesized that ancillary effects of angiotensin II receptor antagonists not related to their main action, ie, lowering of blood pressure, may contribute to their beneficial cardiovascular effects, particularly in patients with the metabolic syndrome.\textsuperscript{20–22} In this respect, we have to point out that the effect of angiotensin II receptor antagonists on EPCs in our diabetic patients was independent from their blood pressure-lowering action as documented by comparable blood pressure reduction achieved in the olmesartan and placebo (ie, cotherapy with hydrochlorothiazide and atenolol) treatment arms. In contrast, in patients treated with standard antihypertensives we did not observe an effect on EPCs.

Emerging data on the beneficial role of EPCs for cardiovascular repair make our results all the more relevant. Patients with type 2 diabetes mellitus have high cardiovascular morbidity and mortality, and most die of complications related to atherosclerosis.\textsuperscript{20,22} Several cardiovascular risk factors are thought to play a role, but the idea that impaired vascular repair mechanisms as a result of impaired function and/or reduced number of EPCs may contribute to the problem has been proposed only recently. Tepper et al\textsuperscript{11} have shown that EPCs from type 2 diabetes exhibit impaired proliferation, adhesion, and incorporation into vascular structures in vitro. Our finding of significantly reduced EPC numbers in patients with type 2 diabetes mellitus further supports this hypothesis. Stimulating regenerative EPCs may therefore contribute to the beneficial cardiovascular effects of angiotensin II antagonists in these patients as well as in other populations with high cardiovascular risk. However, increasing the number of circulating endothelial progenitors might have also untoward effects. EPCs incorporate into the damaged vessel wall and can even promote vasculogenesis, ie, the spreading of new capillaries. Because we do not have detailed knowledge on EPC homing, this process may theoretically also destabilize plaques.

Interestingly, treatment with angiotensin II receptor antagonists induced an increase of EPC numbers above that found in healthy subjects, similarly as it has been shown for statin and rHuEPO therapy.\textsuperscript{12,15} The effect was evident already after 4 weeks of irbesartan treatment, and it was clearly demonstrable after 12 weeks of therapy with both angiotensin II receptor antagonists. Long-term studies have to prove whether such a marked stimulation of EPCs can be observed during chronic therapy with angiotensin II receptor antagonists. In addition, intracellular mechanisms involved in EPC stimulation by angiotensin II receptor antagonist have yet to be explored. Both statins and rHuEPO modulate EPC proliferation and differentiation via activation of the surviving intracellular Akt pathway.\textsuperscript{12,15–18} Elucidating the intracellular mechanisms of the effect of angiotensin II receptor antagonists on EPCs will generate additional knowledge on their pleiotropic effects.

![Figure 2. Effect of olmesartan (n=9) and placebo (n=9) treatment on endothelial progenitor cells (EPCs) in patients with type 2 diabetes mellitus. Treatment with olmesartan significantly increased EPCs by ~50%, whereas placebo treatment did not affect EPCs. Data are presented as 95% confidence interval of the mean.](http://hyper.ahajournals.org/)

By contrast, placebo treatment did not affect EPCs (Figure 2) and HPCs (1.66±0.19 versus 2.00±0.23 /μL; not significant). Systolic blood pressure decreased significantly (P<0.05) and comparably in both treatment groups, ie, from 144±5 to 130±3 mm Hg in the olmesartan treatment group, and from 143±5 to 127±3 mm Hg in the placebo treatment group. Similarly, we observed a comparable decrease in diastolic blood pressure with olmesartan (from 98±2 to 86±2 mm Hg; P<0.05) and with placebo therapy (from 96±2 to 86±2 mm Hg; P<0.05).

We confirmed these results in the second open trial with irbesartan. Angiotensin II blockade significantly increased EPCs in 20 patients with type 2 diabetes mellitus from 196±17 to 300±28 per high-power field (P<0.05) already after 4 weeks of therapy. At the end of 12-week therapy, patients had 310±33 EPCs per high-power field (P<0.05 versus baseline), ie, EPC number increased to >50% above the pretreatment value. In contrast, we did not observe a significant effect of irbesartan on circulating CD34+ HPCs. Their number was 2.37±0.33 /μL at the start of irbesartan treatment, 2.05±0.31 /μL after 4 weeks, and 2.12±0.28 /μL at the end of treatment.

**Discussion**

The results of the present study document that angiotensin II subtype 1-receptor blockade increases the number of EPCs in patient with type 2 diabetes mellitus. The effect seems to be a class effect of angiotensin II receptor antagonists, because we have demonstrated it with standard daily doses of 2 long-acting substances, namely olmesartan and irbesartan. To our best knowledge, angiotensin II receptor antagonists are
It is not clear whether the increase of EPC number with angiotensin II receptor antagonists observed in our patients is a result of EPC mobilization from the bone marrow, or whether angiotensin II receptor antagonists stimulate EPC proliferation and differentiation, or both. This issue can be clarified only in experimental studies examining the effect of angiotensin II receptor antagonists on EPC differentiation within the bone marrow and on mobilization of circulating stem cells directly from the bone marrow. In this respect, we did not observe an effect of angiotensin II receptor blockade on CD34+ HPCs in our diabetic patients. However, CD34+ HPCs give rise to endothelial cells as well as to peripheral blood cells, eg, erythrocytes, leukocytes, and thrombocytes. In conclusion, a standard therapy with angiotensin II receptor antagonists increases the number of regenerative EPCs in patients with type 2 diabetes mellitus. This action may be of therapeutic relevance contributing to their beneficial cardiovascular effects.

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References
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