Hemodynamic and Coronary Effects of the Endothelin Antagonist Bosentan in Patients With Coronary Artery Disease

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Background—Endothelin is a potent endothelium-derived vasoconstrictor peptide with proliferative properties. Elevated levels of the peptide occur in coronary artery disease; however, its pathophysiological role as a regulator of coronary tone and structure is uncertain. Endothelin-receptor antagonists are specific tools to clarify this issue and might be useful in the treatment of coronary artery disease.

Methods and Results—In a double-blind, placebo-controlled randomized study, we investigated the effects of the ET A/ET B endothelin-receptor antagonist bosentan or placebo on systemic and coronary hemodynamics in 28 patients with angiographically documented stable coronary artery disease by quantitative coronary angiography and an intracoronary Doppler guidewire. Bosentan 200 mg IV decreased systolic blood pressure (P < 0.05), whereas heart rate increased slightly (P < 0.05). Coronary diameter increased, particularly in vessels with no or mild angiographic changes (P < 0.01). Glycerol trinitrate did not further dilate these segments, whereas coronary diameter increased significantly after nitrate in the placebo group. The increase in coronary diameter after bosentan correlated inversely with plasma LDL-cholesterol levels (P < 0.01) in both stenotic and angiographically normal coronary segments. Coronary flow velocity did not change. Bosentan was well tolerated.

Conclusions—Endogenous endothelin exerts a vasoconstrictor tone in epicardial coronary arteries of patients with coronary artery disease, as evidenced by the vasodilation exerted by the combined ET A/ET B endothelin-receptor antagonist bosentan under acute conditions. Bosentan can safely be given to these patients. Hence, further long-term studies are necessary to determine the therapeutic potential of endothelin-receptor antagonists in patients with coronary artery disease. (Circulation. 1998;98:2235-2240.)

Key Words: endothelin ■ bosentan ■ blood flow ■ coronary disease ■ angiography

Dysfunction of the vascular endothelium is an early event in cardiovascular disease; in coronary vessels with structural changes, a severe disequilibrium between vasoconstrictors and vasodilators occurs. The vascular endothelium produces substances such as nitric oxide, prostacyclin, and endothelin (ET)-1, which regulate vascular tone and structure via ET A or ET B receptors. In smooth muscle cells, both receptors mediate vasoconstriction, whereas endothelial ET B receptors cause vasodilation via nitric oxide and prostacyclin. Infusion of ET causes transient vasodilation followed by profound and long-lasting vasoconstriction. Elevated ET-1 levels occur in human atherosclerosis, pulmonary hypertension, and congestive heart failure. After acute myocardial infarction, plasma ET levels predict 1-year mortality. Although it is still unclear whether these elevated levels of ET are markers or mediators of the disease, it has been shown that such levels induce enhanced vasoconstriction. In addition to these extensively studied effects on vasomotion, however, ET seems to be a mediator (or at least comediator) of mitogenesis and thus to contribute to the proliferation of the vascular smooth muscles. In situations with chronically elevated plasma and/or local ET levels, this may lead to enhanced proliferation of the blood vessel wall.

Endothelin antagonists allow us to investigate the role of endogenous ET and may provide a new therapy for coronary artery disease. In vitro and in intact animals, ET antagonists are potent inhibitors of ET. In the human forearm circulation, the drugs inhibit ET-induced vasoconstriction and have mild vasodilator effects. In coronary artery disease, contractile ET A receptors on vascular smooth muscle cells are upregulated suggesting that combined ET A/ET B-receptor antagonists might be suited for this condition. Bosentan, an

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ET_{a}/ET_{b} antagonist, has been studied in animals and patients with congestive heart failure; it produced a marked vasodilation in both the peripheral and pulmonary circulation of these patients with very high plasma ET levels. The effects of an ET antagonist in patients with coronary artery disease are unknown. Because ET in these patients might contribute to myocardial ischemia and thus deteriorate myocardial oxygen supply, we investigated the effects of the ET antagonist bosentan on systemic and coronary hemodynamics in coronary artery disease.

**Methods**

**Study Population**

Thirty patients with coronary artery disease were investigated (Table 1). There were no differences regarding coronary risk factor profile, ejection fraction, or degree of the culprit stenosis (Table 1). Efficacy parameters were available in 28 patients (placebo, n=14; bosentan, n=14). All patients underwent angiography and had at least 1-vessel stable coronary artery disease. Patients with acute myocardial infarction within the previous 2 weeks or other severe diseases were excluded. The study design was double-blind, ie, neither the patients nor the investigator was aware of whether bosentan or placebo was infused. The study was approved by the Ethics Committee of the University Hospital Bern, Switzerland. All subjects gave written informed consent.

**Study Design**

Calcium channel blockers, ACE inhibitors, nitrates, diuretics, and other vasodilators were stopped at least 3 to 4 half-lives before the study. Coronary angiography was performed by the femoral approach. If target vessel stenosis was 50%, the patient was excluded. After completion of the diagnostic catheterization, heparin 20 000 IU IV was given and a 6F guiding catheter (Pink Power, Schneider-Pfizer) was introduced into the left or right coronary artery. A Doppler guidewire (Flowire, medium tip, Cardiometrics) was advanced into the stenotic artery and the tip placed beyond the stenosis. The area of the vessel was assessed by quantitative coronary angiography beyond the stenosis in the region of the tip of the Doppler wire, ie, where flow velocity was measured. An index for coronary vascular resistance was calculated from the ratio of the mean aortic blood pressure divided by the coronary flow index.

**Derived Parameters**

For estimating directional changes in coronary blood flow, a coronary blood flow index was calculated by multiplying the mean Doppler flow velocity with the cross-sectional area as described. The area of the vessel was assessed by quantitative coronary angiography beyond the stenosis in the region of the tip of the Doppler wire, ie, where flow velocity was measured. An index for coronary vascular resistance was calculated from the ratio of the mean aortic blood pressure divided by the coronary flow index.

**Drugs**

Bosentan or placebo (P. Hoffmann-La Roche) was prepared in a double-blind, randomized fashion by a trained nurse not involved in the study. Glycerol trinitrate (Perlinganit, Schwarz-Pharma) and adenosine (Krenosin, Sanofi Winthrop) were injected as an intracoronary bolus. All investigators performing the angiograms and their analysis were not aware of the randomization code (double-blind, placebo-controlled study design).

**Hormone Plasma Levels**

Plasma immunoreactivity for ET was measured by radioimmunoassay as previously described. Plasma catecholamines were determined by high-performance liquid chromatography (Hypertension Laboratory, University Hospital Bern, Switzerland).

**Analysis and Statistics**

Results are expressed as mean ± SEM; significances of differences were calculated by ANOVA or Student’s t test as appropriate; a value of P ≤ 0.05 was taken for statistical significance. For blood pressure and heart rate, the area under the time-response curve was calculated.

**Results**

**Systemic Hemodynamics**

**Blood Pressure**

At baseline, systolic and diastolic blood pressures were similar in the two groups (placebo, 143 ± 7 / 79 ± 3 mm Hg; bosentan, 139 ± 6 / 76 ± 3 mm Hg; P = NS). After bosentan, but not after placebo, systolic blood pressure decreased (Figure 1, Table 1).

**TABLE 1. Main Demographic Data of the Patients Studied**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bosentan (n=14)</th>
<th>Placebo (n=14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57±2</td>
<td>60±3</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79±2</td>
<td>84±3</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173±1</td>
<td>173±2</td>
<td>NS</td>
</tr>
<tr>
<td>Stenosis, %*</td>
<td>61±3</td>
<td>53±4</td>
<td>NS</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>65±3</td>
<td>62±2</td>
<td>NS</td>
</tr>
<tr>
<td>Stenotic coronary segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>6</td>
<td>10</td>
<td>...</td>
</tr>
<tr>
<td>RCA</td>
<td>4</td>
<td>5</td>
<td>...</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>Risk factors, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of cardiovascular disease</td>
<td>32</td>
<td>46</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hypertension</td>
<td>46</td>
<td>33</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>33</td>
<td>66</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td>60</td>
<td>73</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

LAD indicates left anterior descending coronary artery; RCA, right coronary artery. *% stenosis as assessed by quantitative coronary angiography.
left; \( P<0.05 \) versus baseline), whereas diastolic pressure did not change (Figure 1, left; \( P=NS \) versus baseline). After intracoronary glycerol trinitrate, systolic but not diastolic pressure tended to decrease further (Figure 1, left; \( P=NS \) versus control before nitrate).

**Heart Rate**

At baseline, heart rate was similar in the two groups (placebo, \( 63 \pm 2 \) bpm; bosentan, \( 65 \pm 2 \) bpm; \( P=NS \)). Heart rate tended to increase slightly after placebo, whereas it tended to decrease after placebo (Figure 1, right; \( P=NS \) versus baseline, \( P<0.05 \) versus placebo) and increased further when intracoronary glycerol trinitrate was injected on top of bosentan (Figure 1, right; \( P=0.05 \) versus control before nitrate, \( P<0.05 \) versus placebo).

**Coronary Hemodynamics**

**Epicardial Coronary Diameter**

Baseline coronary diameters were similar in the two groups in stenosed and control vessels (Table 2). After bosentan, maximal diameter increased in the control vessel (Table 2, \( P<0.01 \) versus baseline) and tended to increase in the diseased vessel (Table 2, \( P=NS \) versus baseline), whereas it did not change with placebo (Table 2). Within stenotic segments, there was no difference between placebo and bosentan (\( P=NS \)). After intracoronary nitrate, maximal diameter of diseased and control vessels increased in the placebo group (Table 2, \( P<0.05 \) versus placebo). In contrast, nitroglycerin did not further increase coronary diameter in the bosentan group (Table 2; \( P=NS \) versus control).

Changes in coronary diameter after bosentan, but not after placebo, correlated inversely with LDL cholesterol plasma levels in both the stenotic segment and the control vessel (Figure 2; \( r^2=0.52 \) to 0.56; \( P<0.01 \)). No such correlation was found with HDLs or triglycerides (\( r^2<0.1 \); \( P=NS \)). There was no correlation of the changes in coronary blood flow or the changes in plasma ET levels with LDL-cholesterol levels (\( r^2<0.1 \); \( P=NS \)).

**Coronary Blood Flow**

At baseline, both average and maximum flow velocities were similar in the two groups (Table 2; \( P=NS \)). Average and maximum flow velocity did not change after bosentan or placebo (Table 2; \( P=NS \)). Coronary flow index tended to increase in the bosentan group but not in the placebo group (\( P=NS \)). Coronary resistance tended to increase in the placebo group, whereas it did not change with bosentan (Table 2; \( P=NS \)). Coronary flow reserve (ratio of peak to baseline flow velocity after IC adenosine) was impaired in all patients (placebo, \( 2.0 \pm 0.2 \); bosentan, \( 1.8 \pm 0.1 \)) but not different in the placebo and bosentan groups (\( P=NS \)).

**Biochemical Parameters**

Baseline plasma ET levels were similar in the two groups (placebo, \( 3.8 \pm 0.2 \) pg/mL; bosentan, \( 3.4 \pm 0.2 \) pg/mL; \( P=NS \), Figure 3). After bosentan, plasma ET increased by 240% (\( P<0.0001 \) versus placebo, Figure 3) but not with placebo (\( P=NS \)). Plasma norepinephrine tended to increase in the two groups (placebo, from 23 to 28 \( \pm 4 \) ng/dL, \( P=NS \); bosentan, from 21 to 27 \( \pm 4 \) ng/dL, \( P=NS \)), whereas plasma epinephrine tended to decrease after bosentan (placebo, from 5.0 to 5.3 \( \pm 0.7 \) ng/dL, \( P=NS \); bosentan, from 5.5 to 1.0 to 4.7 \( \pm 0.5 \) ng/dL). No significant changes in hemoglobin, white blood cell count, creatinine, or liver enzymes were observed after bosentan or placebo.

**Discussion**

We investigated, for the first time, the effects of intravenous administration of an ET-receptor antagonist on systemic and coronary hemodynamics in patients with stable coronary artery disease. The ET₁/ET₂ antagonist bosentan 200 mg IV

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**TABLE 2. Main Coronary Hemodynamic Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bosentan</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>40 min After Drug Application</td>
</tr>
<tr>
<td>Mean blood flow velocity, cm/s</td>
<td>17.8±2</td>
<td>17.8±2</td>
</tr>
<tr>
<td>Maximum blood flow velocity, cm/s</td>
<td>29.6±3</td>
<td>28.9±2</td>
</tr>
<tr>
<td>Coronary diameter, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotic vessel (minimum luminal diameter)</td>
<td>1.2±0.2</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Stenotic vessel (maximum luminal diameter)</td>
<td>3.1±0.2</td>
<td>3.4±0.3</td>
</tr>
<tr>
<td>Control vessel</td>
<td>2.6±0.2†</td>
<td>2.8±0.2†</td>
</tr>
<tr>
<td>Coronary flow index</td>
<td>152±26</td>
<td>160±34</td>
</tr>
<tr>
<td>Coronary resistance index</td>
<td>1.4±0.5</td>
<td>0.9±0.2</td>
</tr>
</tbody>
</table>

\*\( P<0.05 \), †\( P<0.01 \) vs baseline.
decreased systolic blood pressure and increased heart rate. Bosentan increased coronary diameter, particularly in segments with no or mild angiographic changes, whereas coronary blood flow velocity did not increase significantly. Interestingly, the coronary vasodilator effects of bosentan were inversely related to plasma LDL levels.

Bosentan inhibits ET-induced vascular responses both in vitro and in intact animals. After blockade of ET receptors, plasma levels of the peptide increase. Accordingly, in this study, bosentan but not placebo led to a marked increase in plasma ET. Hence, the dosage of bosentan that was selected on the basis of previous experience in animals and humans effectively blocked ET receptors.

Baseline plasma ET levels were slightly above normal and increased by a factor of 2.4 after bosentan. Bosentan decreased systolic blood pressure and increased heart rate. Although diastolic blood pressure also tended to decrease, this effect was less pronounced. It is possible that ET contributes to or regulates vascular compliance of larger conduit arteries and, in turn, affects systolic blood pressure in particular. ET also has positive inotropic properties; hence, an ET antagonist might reduce stroke volume. The small increase in heart rate must be due to unloading baroreceptors, with activation of the sympathetic nervous system.

The coronary effects of bosentan were assessed in a diseased segment (>50% stenosis, which was later dilated) and a control segment with angiographically mild or no disease. Bosentan increased coronary artery diameter in control but not in diseased segments. This suggests that endogenous ET contributes to coronary vascular tone. The fact that vasodilation to bosentan was less pronounced in stenotic segments indicates that the vasodilator capacity was impaired or that too much ET is produced in these segments and hence that the antagonist was less active than in control segments. Alternatively, it is possible that in severely diseased coronary segments that led to angina and therefore underwent PTCA, ET production, like that of the other local mediators, is downregulated. Under these conditions, an ET antagonist also would have no effect; however, several
studies clearly show that especially in the atherosclerotic vessel wall, a high immunostaining for ET indicates large amounts of the peptide. Nitroglycerin did not exert an additional vasodilator effect in the bosentan group, whereas in the placebo group, it markedly increased coronary diameter; thus, in the bosentan group, the segments already had reached their dilator capacity as a result of the application of bosentan, whereas coronary segments of the placebo group remained responsive to the nitrate. The vasodilatation to bosentan was inversely related to plasma LDL, whereas no such correlation was found with placebo. Other lipid fractions did not correlate with the effects of bosentan. It is possible that vasodilatation in diseased vessels is impaired and LDL levels only reflect this phenomenon. However, changes in coronary diameter induced by nitroglycerin were not related to plasma LDL, unlike those to bosentan, demonstrating that the relation was specific for bosentan. The definitive interpretation of these results is difficult in this in vivo study. Experimental data as well as in vivo studies in pigs show high levels of ET in hypercholesterolemia, suggesting that an ET antagonist should be particularly effective under these circumstances. Most likely, with higher local levels of ET, a higher concentration of the competitive antagonist, eg, bosentan, would also be needed to inhibit ET-induced vasoconstriction.

We studied the effects of bosentan 40 minutes after start of the infusion of the drug; we choose this time point on the basis of experience obtained in previous clinical studies with the drug, in which there was a marked effect of bosentan after this period of time. A dose-response assessment would have been the ideal design to assess the effects of bosentan. However, to minimize the risk and discomfort for the patients associated with the long duration of this invasive study in the catheter laboratory, we decided to assess coronary hemodynamics 40 minutes after administration of a single dose of the drug. Because we observed a clear time-response relation in the parameters assessed continuously during the 40 minutes of observation, ie, blood pressure and coronary blood flow, we are confident that there was a marked effect of bosentan. This is also reinforced by the changes in plasma ET during bosentan infusion. We cannot fully exclude the possibility that we might have seen a stronger effect of the drug if we had been able to wait 50 minutes, which, however, was not possible for ethical reasons. Blood pressure and heart rate decreased in the placebo group, indicating that there was not a completely steady-state situation in this study. However, the design was identical in the two groups, ie, placebo and bosentan; thus, it is unlikely that this influenced the outcome of the study.

Coronary blood velocities did not increase in patients receiving bosentan. Given the vasodilator effects of bosentan in epicardial coronary arteries in which flow was measured, this is not surprising, because flow velocity actually tends to decrease (with a similar coronary vascular resistance) if epicardial vessels dilate. It is possible, however, that we missed vasodilator effects of bosentan in the coronary resistance circulation, because velocities were measured in diseased vessels beyond the stenosis. Measurement of intracoronary Doppler velocity is a widely used method and has been shown to reliably assess coronary blood flow velocity. It is possible, however, that in diseased segments in which flow was measured, the vasodilator capacity of the resistance vessel is reduced because of the stenosis. We gave heparin to our patients before beginning coronary catheterization; this drug can interact with mediators of the vascular endothelium, ie, ET, and decreases plasma ET-1 levels, possibly by a nitric oxide-mediated mechanism. However, because of the experimental protocol, it was not possible to do the study without heparinization of the patients. Because the study was placebo-controlled and all patients received the same amount of heparin, this effect is not likely to influence the results.

These results are important for the pathophysiology of coronary artery disease and for future drug development. Indeed, the vasodilator effects of bosentan suggest that vascular ET contributes to coronary vascular tone. Thus, ET-receptor antagonists might be useful to treat myocardial ischemia. It is possible that during chronic therapy, inhibition of the proliferative effects of ET may be beneficial for structural changes. Hence, long-term studies with ET-receptor antagonists in coronary artery disease are necessary to determine their clinical potential.

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References


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