Effects of Endothelin A Receptor Blockade on Endothelial Function in Patients With Chronic Heart Failure

Rudolf Berger, MD; Brigitte Stanek, MD; Martin Hülsmann, MD; Bernhard Frey, MD; Sandra Heher, MD; Richard Pacher, MD; Thomas Neunteufl, MD

**Background**—Chronic heart failure (CHF) is associated with impaired endothelium-dependent vasodilation and increased basal vascular tone due, in part, to elevated endothelin-1 plasma levels. In the present study, we investigated whether a reduction of vascular tone using an endothelin A receptor blocker attenuates the impairment of endothelium-dependent, flow-mediated vasodilation (FMD).

**Methods and Results**—Twenty-one patients with CHF randomly received either the endothelin A receptor blocker LU 135252 (30 mg/d, n = 7; 300 mg/d, n = 7) or a placebo (n = 7). Using high-resolution ultrasound, FMD and endothelium-independent, nitroglycerin-induced dilation of the brachial artery were assessed at baseline in the 21 patients with CHF and in 11 controls and after 3 weeks treatment in the 21 patients with CHF. FMD at baseline was impaired in all 21 patients with CHF (3.2 ± 2%) when compared with the 11 controls (9.7 ± 4.9%; P = 0.0005). In comparison with baseline, FMD significantly improved after 3 weeks of treatment with LU 135252 in all 14 patients receiving it (from 3.0 ± 2.0% to 4.9 ± 2.9%; P = 0.04), but FMD remained unchanged with placebo. Subgroup analysis, according to different dosages, revealed a significant increase of FMD compared with baseline (from 2.4 ± 1.5% to 5.5 ± 2.4%; P = 0.03) in the patients treated with the low-dose (30 mg/d), whereas a high dose of 300 mg/d failed to increase FMD significantly. Improvement in the high-dose group, however, may have been masked by reduced vasodilator capacity due to a significant increase in vessel size (from 4.8 ± 0.4 to 5.1 ± 0.7 mm; P = 0.03).

**Conclusions**—These results suggest that endothelin A receptor blockade improves FMD in CHF patients. *(Circulation. 2001;103:981-986.)*

**Key Words:** heart failure • endothelin • endothelium

Patients with chronic heart failure (CHF) are characterized by increased vasoconstriction and a reduced vasodilatory response to exercise. In addition to various systemic mechanisms, including increased sympathetic activation and stimulation of the renin-angiotensin system, there is evidence that the endothelium plays an important role in the regulation of vascular tone.

With regard to abnormal vasodilator response, previous clinical studies have demonstrated endothelial dysfunction of both large conduit arteries and small resistance arteries in patients with CHF. In particular, the role of the endothelium-derived relaxing factor nitric oxide (NO) has received considerable attention. Impaired smooth muscle responsiveness to NO stimulation, impaired L-arginine availability or use, increased NO degradation, reduced NO synthase activity, and endothelial release of vasoconstricting prostanoids have all been implicated in this impaired response. Moreover, elevated plasma levels of endothelin-1 (ET-1) and its precursor big ET-1 in patients with advanced heart failure suggest that the vasoconstrictor activity of this peptide contributes to the increased basal vascular tone in CHF.

Because vascular tone is a result of various, simultaneously acting vasodilators and vasoconstrictors, impaired endothelium-dependent vasodilation may result not only from the reduced effectiveness of the NO system, but also from the increased basal vascular tone counteracting vasodilation. Therefore, an ET receptor antagonist may improve endothelium-dependent flow-mediated vasodilation (FMD) in patients with CHF by reducing increased basal vasoconstriction.

ET-1 exerts a variety of effects via the activation of specific receptors. Type A receptors (ET_{A}) are expressed by vascular smooth muscle cells and cause vasoconstriction. Type B receptors (ET_{B}) have dual functions depending on their location: when expressed by vascular smooth muscle cells, they mediate vasoconstriction, but when expressed by endothelial cells, they are linked to vasodilation via NO and/or prostacyclin synthesis. Because a nonselective ET receptor blocker inhibits both ET-mediated vasoconstriction and vasodilation, selective ET_{A} receptor blockade may be more effective than nonselective blockade in improving endothelium-dependent vasodilation.
In the present study, we sought to determine the effects of 2 different dosages of LU 135252, a specific ETA receptor antagonist, on the FMD of the brachial artery in patients with advanced heart failure.

Methods

Design
LU 135252 (Knoll AG) is an orally active, ETα receptor-selective antagonist that is currently being investigated in a multicenter, prospective, randomized, placebo-controlled dose-finding trial of patients with CHF. A study was designed in patients who were recruited locally at the University of Vienna, Austria, to investigate FMD and nitroglycerin-induced dilation (NMD), as modified by endothelial function were performed on all available patients who were randomly assigned to receive placebo (7 patients), 30 mg of LU 135252 per day (7 patients), or 300 mg of LU 135252 per day (7 patients) during the double-blind study period.

Patients
Patients were enrolled in the study if they met the following criteria: (1) age ≥18 years; (2) present or recent NYHA class III heart failure, on the basis of ischemic heart disease or idiopathic dilated cardiomyopathy; (3) left ventricular ejection fraction ≤35%; (4) pulmonary capillary wedge pressure ≥12 mm Hg; and (5) cardiac index ≤2.6 L·min⁻¹·m⁻². The protocol was approved by the institutional ethics committee, and all patients gave written informed consent before inclusion.

Twenty-one patients (3 women and 18 men; mean age, 57 years) with CHF (due to idiopathic dilated cardiomyopathy in 12 patients and ischemic cardiomyopathy in 9 patients) who had echocardiographic signs of cardiomegaly (mean left ventricular end-diastolic diameter of 69±9 mm) were studied. All patients were in NYHA functional class III. Systolic left ventricular dysfunction was documented as an average left ventricular ejection fraction of 20±6%, which was determined by radionuclide angiography. Mean pulmonary capillary wedge pressure was 19±5 mm Hg, and mean cardiac index was 2.0±0.3 L·min⁻¹·m⁻², as assessed by right heart catheterization. Characteristics of patients with CHF, according to subgroups, are presented in Table 1. No differences were found with respect to age, sex, diagnosis, body mass index, cholesterol levels, distribution of diabetics or smokers, left ventricular ejection fraction, or left ventricular end-diastolic diameter.

All 21 patients received background heart failure therapy with digitalis, furosemide, and high-dose ACE-inhibitors. In addition, 11 patients were treated with β-blockers and 2 patients received additional angiotensin II receptor blockers, as shown in Table 2. Patients followed a stable dose of their CHF therapies (ACE-inhibitors, β blockers, and angiotensin II receptor blockers) for at least 3 months before the present study.

The CHF patient group included 6 smokers (29%), 6 patients with diabetes mellitus (29%), and 5 patients with hypercholesterolemia (24%). The distribution of risk factors according to the underlying disease was as follows. The patient group with ischemic cardiomyopathy (n=9) included 2 smokers (22%), 3 patients with diabetes mellitus (33%), and 1 patient with hypercholesterolemia (11%). Patients with idiopathic cardiomyopathy (n=12) included 4 smokers (33%), 3 patients with diabetes mellitus (25%), and 4 patients with hypercholesterolemia (33%) (Table 3).

Controls
Eleven age- and sex-matched subjects without CHF or coronary artery disease (excluded by angiography) volunteered to be controls. Because the CHF patients displayed numerous risk factors, sex- and age-matched controls with several risk factors were included for adequate comparison.

The demographic and clinical characteristics of the 21 CHF patients in comparison with the 11 controls are given in Table 3. No differences between CHF patients and controls were observed with

### TABLE 1. Characteristics of CHF Patients According to Subgroups

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=7)</th>
<th>All (n=14)</th>
<th>30 mg/d (n=7)</th>
<th>300 mg/d (n=7)</th>
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</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57±9</td>
<td>56±8</td>
<td>60±7</td>
<td>52±8</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>6/1</td>
<td>13/1</td>
<td>6/1</td>
<td>7/0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7±2.4</td>
<td>27.8±3.5</td>
<td>26.9±1.7</td>
<td>28.7±4.7</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>167±37</td>
<td>178±44</td>
<td>176±59</td>
<td>180±27</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>95±29</td>
<td>112±35</td>
<td>111±48</td>
<td>113±21</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>43±10</td>
<td>39±7</td>
<td>39±5</td>
<td>38±9</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>2/7 (29)</td>
<td>4/14 (29)</td>
<td>0/7 (0)</td>
<td>4/7 (57)</td>
</tr>
<tr>
<td>Diabetes mellitus II, n (%)</td>
<td>2/7 (29)</td>
<td>4/14 (29)</td>
<td>1/7 (14)</td>
<td>3/7 (43)</td>
</tr>
<tr>
<td>Dilated/ischemic cardiomyopathy, n</td>
<td>4/3</td>
<td>8/6</td>
<td>5/2</td>
<td>3/4</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>21±7</td>
<td>19±6</td>
<td>16±4</td>
<td>22±6</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>66±7</td>
<td>71±10</td>
<td>71±14</td>
<td>71±6</td>
</tr>
<tr>
<td>Cardiac index, L·min⁻¹·m⁻²</td>
<td>2.0±0.4</td>
<td>2.0±0.3</td>
<td>1.9±0.3</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>19±6</td>
<td>19±4</td>
<td>20±3</td>
<td>18±5</td>
</tr>
</tbody>
</table>

LVEDD indicates left ventricular end-diastolic diameter; PCWP, pulmonary capillary wedge pressure.

### TABLE 2. Concomitant Medication

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>All</th>
<th>30 mg/d</th>
<th>300 mg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digitalis, n</td>
<td>7</td>
<td>14</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Enalapril or equivalent, n</td>
<td>7</td>
<td>14</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Enalapril dose, mg/d</td>
<td>36±8</td>
<td>38±7</td>
<td>40±0</td>
<td>36±11</td>
</tr>
<tr>
<td>Angiotensin II receptor blocker, n</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>β-Blocker, n</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Furosemide dose, mg/d</td>
<td>53±43</td>
<td>32±27</td>
<td>34±28</td>
<td>29±27</td>
</tr>
</tbody>
</table>
TABLE 3. Characteristics of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=11)</th>
<th>CHF (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>55±10</td>
<td>57±8</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>9/2</td>
<td>18/3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8±3.4</td>
<td>27.2±2.2</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>229±47</td>
<td>178±44</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>150±36</td>
<td>107±34</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>37±15</td>
<td>41±10</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>6/11 (55)</td>
<td>6/21 (29)</td>
</tr>
<tr>
<td>Diabetes mellitus II, n (%)</td>
<td>2/11 (18)</td>
<td>6/21 (29)</td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>4.6±0.7</td>
<td>5.0±0.7</td>
</tr>
<tr>
<td>FMD, %</td>
<td>9.7±4.9</td>
<td>3.2±2.0*</td>
</tr>
<tr>
<td>NMD, %</td>
<td>11.7±4.6</td>
<td>10.9±4.9</td>
</tr>
<tr>
<td>Big ET-1 plasma levels, fmol/mL</td>
<td>2.3±0.7</td>
<td>5.0±2.4*</td>
</tr>
</tbody>
</table>

*P=0.0005 vs controls.

regard to body mass index, cholesterol, or distribution of diabetics or smokers.

Protocols

Baseline FMD and NMD were measured 8 hours after routine morning medications. On the following day, blood sampling was performed 2 hours after routine morning medications. The randomized study drug was then administered and continued for the next 3 weeks. One day before the end of the study, FMD and NMD measurements were repeated 8 hours after morning medications, which included the study drug. The next day, blood sampling was performed 2 hours after morning medications, which included the study drug.

Assessment of FMD and NMD

Ultrasound measurements were performed according to the method described by Celermajer et al16 using high-resolution ultrasound (Hewlett Packard Sonos 2500) with a 7.5-MHz linear array transducer. Diameter measurements of the right brachial artery were taken at rest after supine rest for at least 10 minutes, after cuff deflation completing suprasystolic compression (240 mm Hg) of the right upper arm for 4.5 minutes, and after sublingual application of 0.8 mg of nitroglycerin. Scans were taken of the brachial artery proximal to the bifurcation of the radial and the ulnar artery by the same ultrasound operator. After optimal transducer positioning, the arm was kept in the same position and the skin was marked. Lumen diameters were measured from one media-adventitia interface to the other at least 3 times at baseline, every 20 seconds after reactive hyperemia, and subsequent to the administration of nitroglycerin. The maximum FMD and NMD diameters were taken as the average of the 3 consecutive maximum diameter measurements. Vasodilation was then calculated as the percent change in diameter over the baseline value.

To verify that suprasystolic compression of the brachial artery produced adequate increases in blood flow, flow velocity was measured at rest and within 15 seconds after cuff deflation. Blood flow was calculated by multiplying the velocity time integral by the heart rate and the vessel cross-sectional area [3.14×(D/2)²]; D indicates diameter. Reactive hyperemia was then calculated as the percent change in flow during hyperemia over the baseline value.

Big ET-1

Our assay quantified the sum of big ET-1 and C-terminal big ET-1 without discrimination between the 2 peptides.17 We evaluated circulating big ET-1 and the C-terminal fragment on the basis of the assumption that they may reflect ET overproduction more accurately than circulating ET-1. The C-terminal fragment of big ET-1 is released during a proteolytic step to produce ET. Because ET-1, like other peptides with high biological activity, is rapidly cleared18 and acts mainly paracrine,19 the majority of it may never reach the circulation. However, precursor elements without biological activity often circulate in higher concentrations, integrate the secretory activity of endothelial cells, and open an analytic window.20 In addition, there is no evidence for an increase in ET-converting enzyme activity in patients with heart failure compared with normal patients.21,22

To determine plasma levels of big ET-1, venous blood samples were drawn from an indwelling catheter after at least 30 minutes of rest. Test tubes were placed on ice and centrifuged immediately. Plasma samples were stored at −70°C until they were analyzed. Plasma big ET-1 levels were measured by an extraction-based radioimmune assay (BioMedica). Our reference range is 0.8 to 1.8 fmol/mL.

Statistical Analyses

Results are expressed as mean±SD. The Student’s test for continuous variables and Fischer’s exact test for categorical data were used to compare baseline characteristics, vessel size, FMD, NMD, hyperemia, and big ET-1 levels between groups. Paired t tests were performed to estimate the effects of 3 weeks of treatment on vessel size, FMD, NMD, hyperemia, and big ET-1 levels within the LU 135252 group, the LU 135252 subgroups, and the placebo group. The nonparametric correlation coefficient (Spearman’s r) was used to quantify the relationship between the change of vessel size and NMD by treatment with different doses of LU 135252 or placebo. Differences were considered significant at P<0.05.

Results

Vasodilation and Blood Flow Responses

Vessel size and hyperemia were similar in CHF patients and age- and sex-matched controls. Thus, it can be assumed that the stimulus for FMD was similar in both study groups. However, FMD values were significantly impaired in patients with CHF when compared with controls (9.7±4.9% versus 3.2±2.0%; P=0.0005), whereas NMD values were similar in both groups (Table 3) and comparable to those reported in patients with similar vessel sizes and risk factor profiles.16,23

When comparing all patients receiving LU 135252 with those taking the placebo, vessel size, hyperemia, FMD, and NMD were not different between groups at baseline (Table 4). However, within these 2 groups, a significant improvement of FMD (from 3.0±2.0% to 4.9±2.9%; P=0.04) was found after 3 weeks of ETA receptor blockade but not after placebo intake (Figure 1 and Table 4). NMD, vessel size, and hyperemia remained unchanged in both the placebo and the LU 135252-treated groups (Table 4).

The analyses of subgroups with regard to different dosages are as follows. In patients treated with a low dose of LU 135252 (30 mg/d), no changes were found with respect to vessel diameter at rest or hyperemia. FMD increased from 2.4±1.5% to 5.5±2.4% (P=0.03), but NMD remained unchanged (Figure 2 and Table 4). In contrast, a daily dose of 300 mg of LU 135252 resulted in a significant increase in the vessel size of the brachial artery (from 4.8±0.4 to 5.1±0.7 mm; P=0.03). However, FMD did not change in this group. This was likely due to the significant increase in baseline diameter. Remarkably though, NMD was significantly reduced (from 13.5±9.0% to 7.1±6.8%; P=0.04).
Big ET-1 Plasma Levels

The mean big ET-1 plasma concentration in the CHF patients was 5.0±2.4 fmol/mL. This was markedly higher than the cut point of 4.3 fmol/mL, indicating a very poor short-term prognosis.8,24 In contrast, the controls had a significantly lower big ET-1 plasma concentration compared with the CHF patients (2.3±0.7 fmol/mL versus 5.0±2.4 fmol/mL; P=0.0005) (Table 3). Three weeks of treatment with LU 135252 (or placebo) had no influence on the elevated big ET-1 plasma levels (Table 4).

**Discussion**

The present study demonstrates that the ET\(_A\) receptor antagonist LU 135252 has the potential to attenuate the impairment of FMD in patients with CHF. An improvement in the FMD of the brachial artery after 3 weeks of treatment was observed specifically in patients treated with 30 mg of LU 135252 per day; 300 mg/d did not influence endothelium-dependent vasodilation. In contrast, brachial artery diameter remained unaffected by the low-dose treatment but increased significantly with the higher dose.

Physiologically, ET-1 may interact with short-acting regulatory systems such as NO, thereby maintaining vascular tone and simultaneously allowing flexibility of the vascular tone on demand.10 In addition to myogenically or neurogenically induced contraction,10 ET-1 is responsible for basal vasoconstrictive tone.25 NO, however, mediates the vasodilation of peripheral conduit arteries due to an increase in blood flow.26

CHF patients are characterized by impaired endothelium-dependent vasodilation.3,27 Moreover, plasma concentrations of ET-1 and its precursor big ET-1 are elevated in patients with CHF, and ET-1 contributes to increased basal vasomotor tone.6–8 Thus, CHF patients in this study were characterized by both impairment of FMD and highly elevated big ET-1 plasma levels. The major hypothesis for improvement of the endothelial function in CHF patients with ET receptor blockers is a reversal of the imbalance between basal vasoconstrictive and vasodilating forces on demand by reducing the increased basal tone.

Recently, Krum and colleagues38,29 observed a reduction in absolute forearm blood flow responses to peak reactive hyperemia, as well as in metabolic vasodilatation during exercise in healthy humans exposed to ET-1 infusion, although basal and stimulated NO production remained unchanged. Thus, it may be suggested that an imbalance of the endothelial regulatory system by increased ET-1 plasma concentrations contributes to the impairment of vasodilator response to shear stress. Furthermore, in an animal model characterized by increased vascular ET-1 generation (spontaneously hypertensive rats), an improvement of FMD and acetylcholine-induced vasodilation was documented after
long-term ETₐ receptor blockade. The results of the present study in humans with CHF are in accordance with the concept that impaired FMD, accompanied by increased ET-1 production, can be improved by ETₐ receptor blockade.

ETₐ receptors expressed by endothelial cells mediate vasorelaxation due to the release of NO or prostacyclin. Thus, an improvement of FMD in this study may have been partly due to selective ETₐ receptor blockade, thus focusing the effects of flow-induced ET release on ETₐ receptors. Previously, it has been shown that increased ET-1 reduces metabolic vasodilation in normal individuals through mechanisms presumably unrelated to altered NO production. In our study, however, the inability of ET-1 to increase endothelial NO production may have been abrogated by specific ETₐ receptor blockade. Moreover, although the pressor effect of ET-1 does not seem to be modified by cyclooxygenase inhibition, vasoconstrictor prostanoids (thromboxane) seem to potentiate the regional vasoconstriction induced by ET-1. In view of the fact that long-term ETₐ receptor blockade increases the participation of cyclooxygenase products in the FMD of animals, we think it is possible that LU 135252 in our patients may have prevented the vasoconstrictor prostanoid-dependent activity and thus favored the effect of vasodilator prostanoids on flow stimulation.

The lack of improvement in FMD with a high dose of LU 135252 seems to be a limitation of this study. However, an improvement in FMD with higher doses of LU 135252 could have been masked by vasodilation, because an increase in vessel size caused by ETₐ receptor blockade is closely related to reduced vasodilator capacity. FMD was preserved even under high-dose treatment, whereas NMD was substantially blunted. Moreover, our results are in accordance with previous studies, indicating an inverse correlation between vessel size and vasodilation after hyperemia and nitroglycerin, respectively.

In vitro experiments have demonstrated that LU 135252 has a 100-fold higher affinity to human ETₐ than to human ETₐ receptors (A.G. Knoll and L. Unger, unpublished data, 2000). Despite the preserved selectivity of ETₐ receptor blockade at higher doses of LU 135252 in rats (29 mg · kg⁻¹ · d⁻¹ equals 2100 mg/d for a human weighing 70 kg), these results cannot be directly transferred to humans. Theoretically, it is possible that LU 135252 is a selective blocker of ETₐ receptors at a lower dose (30 mg/d) but that at higher dosage (300 mg/d), it also...
blocks ET₆ receptors. Therefore, it might be possible that the higher concentration of the ET₆ receptor blocker inhibits, at least in part, NO production and thus shows little effect on FMD.

In conclusion, this study indicates that the ET₆ receptor blocker LU 135252 attenuates the impairment of FMD seen in patients with CHF. Our findings support the concept that endothelial dysfunction in CHF is caused by an imbalance between the increased, continuously acting vasoconstrictor ET-1 and short-acting regulatory systems like NO.

References
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