Endothelin-receptor blockade improves endothelial vasomotor dysfunction in heart failure

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Abstract

Objectives: To elucidate the effect of selective endothelin ET\textsubscript{A} and mixed ET\textsubscript{A/B}-receptor antagonists on endothelial vasomotor dysfunction in rats with heart failure after myocardial infarction (MI).

Methods: Vasoreactivity and superoxide anion formation were determined in aortic rings from Wistar rats 12 weeks after extensive MI (>46% of left ventricle) compared to sham-operated animals. Rats were either treated with the selective ET\textsubscript{A}-receptor antagonist LU 135252 (30 mg/kg/day), the mixed ET\textsubscript{A/B}-receptor antagonist Bosentan (100 mg/kg/day) or placebo.

Results: In MI rats, the concentration–response curve of the endothelium-dependent, nitric oxide-mediated relaxation induced by acetylcholine was significantly shifted to the right and the maximum relaxation was attenuated. Long-term treatment with both ET antagonists significantly improved acetylcholine-induced relaxation in MI rats. LU 135252 was more effective than Bosentan. Endothelium-independent relaxations induced by sodium nitroprusside as well as endothelin- and phenylephrine-induced contractions were similar in all groups of rats. Plasma renin activity and aortic superoxide formation, which were enhanced in rats with heart failure, were normalized by LU 135252, but not by Bosentan treatment.

Conclusions: Long-term treatment with ET-receptor antagonists improves endothelial vasomotor dysfunction in rats with chronic MI. This mechanism may essentially contribute to the beneficial effects of ET receptor blockade in heart failure. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Endothelial function; Endothelins; Free radicals; Heart failure; Nitric oxide; Vasocostriction/dilation

1. Introduction

The pathophysiological events following myocardial infarction (MI) are modulated by the endogenous endothelin (ET) system. Development of heart failure is accompanied by an altered regulation of the two specific ET receptor subtypes characterized so far, the so-called ET\textsubscript{A} and ET\textsubscript{B} receptor (for review see Ref. [1]). In addition, increased plasma ET levels correlate with the severity of heart failure [2]. Long-term treatment with both mixed ET\textsubscript{A/B} antagonists and selective ET\textsubscript{A} antagonists, partially prevents left ventricular dilation and improves hemodynamics and survival in rats with chronic MI [3–6].

Endothelin, the most potent vasoconstrictor substance known, contributes to the increased peripheral resistance in humans and rats with chronic cardiac dysfunction by peripheral vasoconstriction [7,8]. A shift of the balance between dilator and constrictor influences may also be crucial for the development of endothelial dysfunction in heart failure. Reduced endothelium-dependent vasodilator capacity of coronary, large conductance and peripheral arteries contributes to reduced myocardial perfusion, increased peripheral vascular resistance and cardiac workload in patients with heart failure and experimental models of cardiac dysfunction [9–14]. Alteration of endothelial function has been attributed to a decreased production of endothelium-derived nitric oxide (NO) due to an attenuated expression of the endothelial NO synthase (eNOS) [15]. This may be the result of a chronically reduced blood flow and shear stress at the endothelial
surface [16]. In addition, an increased generation of superoxide (O$_2^-$) radicals may reduce the bioavailability of NO and contribute to the attenuation of endothelium-dependent dilations in heart failure [17].

The endothelium has been recognized as an important therapeutic target in heart failure, since the normalization of endothelial function reduces vascular resistance and enhances arterial compliance, tissue perfusion and exercise capacity [16,18,19]. However, the effect of ET-receptor blockade on endothelial dysfunction in chronic ischemic heart failure has not been elucidated yet. ET-receptor antagonists exert beneficial effects on endothelial function in other cardiovascular diseases such as hypertension (for review see Ref. [20]). Nevertheless, it is unclear, whether mixed ET$_A$/B antagonists or selective ET$_A$-receptor blockers are preferable. ET$_A$ receptors are primarily expressed on vascular smooth muscle cells and mediate vasoconstriction. ET$_B$ receptors are predominantly located on endothelial cells and mediate the release of dilator substances such as NO and prostacyclin. However, ET$_B$ receptors on smooth muscle cells have also been described which mediate vasoconstriction [1]. From these data, it is conceivable that profound differences may be expected between selective ET$_A$ antagonism and mixed ET$_A$/B, e.g. in angiotensin II-induced and chronic NO-deficient hypertension, mixed ET$_A$/B antagonists were not effective whereas selective ET$_A$-receptor blockade improved vascular dilator responses [21–23].

Therefore, we investigated the effect of chronic treatment with the selective ET$_A$-receptor antagonist LU 135252 and the mixed ET$_A$/B-receptor blocker Bosentan on endothelium-dependent and -independent dilator as well as contractile responses in the aorta from rats with chronic heart failure following experimental MI. Our hypothesis was that ET antagonists may improve NO-mediated vasodilation. In order to elucidate the potential mechanisms, by which the ET-antagonists ameliorate the attenuated endothelium-dependent relaxation in heart failure, we investigated the effect of exogenous superoxide dismutase as well as vascular O$_2^-$ formation.

2. Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. Study protocol, myocardial infarction, hemodynamic measurements

Left coronary artery ligations were performed in adult male Wistar rats (250–300 g) as previously described [3]. Briefly, the thorax was opened under ether anesthesia, the heart exteriorized and a ligature placed around the proximal left coronary artery. Subsequently, the heart was returned to its normal position and the thorax closed. On the seventh postoperative day, surviving rats were randomly selected for ET antagonist or placebo treatment and maintained with free access to standard rat chow and water. Bosentan (100 mg/kg body wt) and LU 135252 (30 mg/kg body wt) were prepared freshly every day as a microsuspension in 5% arabic gum and administered by gavage daily for 11 weeks. These doses were chosen since the pressor effect of ET-1 in Wistar rats at various oral doses of Bosentan was inhibited following application of a dose of 100 mg/kg body wt [3]. Furthermore, treatment with LU 135252 at a dose of 30 mg/kg body wt completely inhibited the pressor effect of ET-1. It did not affect the transient decrease in blood pressure induced by the ET$_B$ agonist Sarafotoxin S6c. Thus, both the selectivity and efficacy of the ET$_A$ receptor antagonist was confirmed [6].

Hemodynamic studies were performed 12 weeks after coronary artery ligation as described [3] under barbiturate anesthesia and controlled respiration. Hemodynamic measurements, vascular reactivity studies and sample collection were performed 36 h after the last administration of the study drugs.

2.2. Sample collection, determination of infarct size and ventricular remodeling

After hemodynamic measurement, a blood sample was collected from the right carotid artery into a chilled tube containing potassium EDTA (2 mg/ml blood) and plasma was separated by centrifugation at 3000×g for 10 min at 4°C and stored at −80°C. The heart was subsequently excised and dissected into atria, right, and left ventricle including septum, in ice-cold Krebs–Ringer solution. Histological slices (5 μm) were obtained and stained with van Gieson’s stain. The boundary length of the infarcted and non-infarcted endocardial and epicardial surfaces were traced with a planimeter digital image analyzer. Infarct size (fraction of the infarcted left ventricle) was expressed as a percentage of length and only rats with extensive infarcts (>46%) were included in the study of vascular reactivity. Left ventricular cavity area (area enclosed by left ventricular endocardial circumference) was used as index of left ventricular dilatation.

2.3. Vascular reactivity studies

The descending thoracic aorta was dissected following removal of the heart and cleaned of connective tissue. The lower section (10 mm) was used for measurement of superoxide anion production, while the remainder was cut into rings (3 mm in length) which were mounted in an organ bath (Föhr Medical Instruments, Seeheim, Germany) for isometric force measurement. The rings were equilibrated for 30 min under a resting tension of 2 g in oxygenated (95% O$_2$/5% CO$_2$) Krebs–Henseleit solution.
Global parameters in rats with heart failure 12 weeks following myocardial infarction (MI) as compared to sham-operated animals (Sham)

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Sham Plac</th>
<th>MI Plac</th>
<th>Sham LU</th>
<th>MI LU</th>
<th>Sham Bos</th>
<th>MI Bos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct size (%)</td>
<td></td>
<td>54.2±1.5</td>
<td></td>
<td>53.8±2</td>
<td></td>
<td>55.2±2</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>150±4</td>
<td>136±4*</td>
<td>138±5</td>
<td>118±6*</td>
<td>144±6</td>
<td>135±5</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.5±1.9</td>
<td>14.6±2*</td>
<td>3.3±2.3</td>
<td>11.2±2.7*</td>
<td>3.1±2.4</td>
<td>10.2±2.4*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>132±4</td>
<td>120±4</td>
<td>127±5</td>
<td>110±6*</td>
<td>137±5</td>
<td>123±5</td>
</tr>
<tr>
<td>LVCA (mm²)</td>
<td>9.2±0.9</td>
<td>38.4±2.8*</td>
<td></td>
<td>32.3±3.1</td>
<td></td>
<td>25.5±2.5*</td>
</tr>
</tbody>
</table>

Note: All biochemicals were obtained in the highest purity available from Sigma (Deisenhofen, Germany). Bosentan was provided by Actelion (Allschwil, Switzerland) and LU 135252 by Knoll AG (Ludwigshafen, Germany).

2.7. Statistics

Dilator responses were given as percentage dilatation relative to the preconstriction level, constrictions were expressed as percentage of the response to KCl. Values are expressed as mean±S.E.M. of n experiments with segments from different arteries. Statistical analysis was performed by two factor analysis of variance (ANOVA) or one-way ANOVA followed by a Bonferroni t-test or by the two-tailed Student’s t-test for unpaired data, where appropriate, with P values <0.05 considered statistically significant.

3. Results

3.1. Global parameters

Global parameters of rats with heart failure and sham-operated animals are shown in Table 1. Infarct size was matched between the placebo and the two treatment groups. Left ventricular systolic pressure was significantly lower in rats with chronic MI, whereas left ventricular end-diastolic pressure was elevated. The ET₄ antagonist LU 135252 further reduced left ventricular systolic pressure in infarcted animals as compared to the placebo group. The mixed antagonist Bosentan did not significantly affect hemodynamic parameters, but did reduce left ventricular cavity area, a measure for ventricular dilation, which was only slightly attenuated by LU 135252.

3.2. Vasoconstrictor responses in aortic rings

The tension developed by KCl (50 mmol/l) was similar among the various groups (Sham: 1.3±0.2 g; MI Plac: 1.4±0.1 g; MI LU 135252: 1.5±0.2 g; MI Bosentan: 1.5±0.2 g). Cumulative application of phenylephrine elicited a concentration-dependent constriction in aortic rings which was slightly but not significantly increased in rats with heart failure as compared to sham-operated
animals (Fig. 1A). ET-1-induced constriction was similar in both groups of rats (Fig. 1B).

Furthermore, neither long-term treatment with the \( \text{ET}_{A} \) antagonist LU 135252 nor with the mixed antagonist Bosentan significantly affected the contractile responses elicited by both agonists (Fig. 1).

3.3. Vasodilator responses in aortic rings

In phenylephrine-preconstricted aortic rings, acetylcholine induced a concentration-dependent relaxation which was blunted in aortae from rats with chronic MI (Fig. 2A and Table 2). The concentration–response curve of the endothelium-independent vasodilator sodium nitroprusside was not different between sham-operated animals and rats with heart failure, although it was slightly shifted to the right (Fig. 3A and Table 2). Chronic treatment with the \( \text{ET}_{A} \) antagonist LU 135252 lead to a nearly complete restoration of the acetylcholine-induced relaxation in aortic rings from rats with heart failure (Fig. 2B and Table 2).
Table 2

<table>
<thead>
<tr>
<th></th>
<th>Sham Plac</th>
<th>MI Plac</th>
<th>Sham LU</th>
<th>MI LU</th>
<th>Sham Bos</th>
<th>MI Bos</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh ED&lt;sub&gt;m&lt;/sub&gt; (−log)</td>
<td>7.12 ± 0.05</td>
<td>6.58 ± 0.07*</td>
<td>7.02 ± 0.09</td>
<td>7.01 ± 0.06*</td>
<td>6.97 ± 0.08</td>
<td>6.84 ± 0.06*</td>
</tr>
<tr>
<td>R&lt;sub&gt;max&lt;/sub&gt; (%)</td>
<td>97 ± 1</td>
<td>86 ± 3*</td>
<td>95 ± 1</td>
<td>96 ± 1*</td>
<td>92 ± 1</td>
<td>94 ± 2*</td>
</tr>
<tr>
<td>SNP ED&lt;sub&gt;m&lt;/sub&gt; (−log)</td>
<td>7.49 ± 0.07</td>
<td>7.30 ± 0.08</td>
<td>7.28 ± 0.09</td>
<td>7.37 ± 0.05</td>
<td>7.43 ± 0.07</td>
<td>7.32 ± 0.08</td>
</tr>
<tr>
<td>R&lt;sub&gt;max&lt;/sub&gt; (%)</td>
<td>98 ± 1</td>
<td>97 ± 1</td>
<td>98 ± 1</td>
<td>99 ± 1</td>
<td>99 ± 1</td>
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</table>

* Animals were either treated with placebo (Plac), with the ET<sub>A</sub>-receptor antagonist LU 135252 (LU) or with the mixed ET-receptor antagonist Bosentan (Bos). * P<0.05 vs. Sham Plac; † P<0.05 vs. MI Plac.

Moreover, the mixed antagonist Bosentan also improved acetylcholine-induced relaxation in aortic rings from rats with chronic MI, however, the dilator response remained slightly diminished as compared to the placebo-treated sham-operated animals (Fig. 2C and Table 2).

3.4. Effects of radical scavengers on vascular reactivity

In phenylephrine-preconstricted aortic rings, exogenous superoxide dismutase (SOD, 600 U/ml) elicited a dilator response, which was significantly enhanced in aortae from rats with chronic MI suggesting enhanced O<sub>2</sub><sup>−</sup> production (83 ± 3 vs. 58 ± 3%, P<0.01). Long term treatment with LU 135252 significantly reduced SOD-induced relaxation (62 ± 3%, P<0.01). In contrast, Bosentan had no effect on SOD-induced relaxation in rats with chronic MI (83 ± 3%).

Furthermore we evaluated the effect of exogenous SOD (200 U/ml) on the relaxation elicited by a median concentration of acetylcholine. In aortae from rats with chronic MI treated with placebo acetylcholine-induced relaxation was significantly enhanced in the presence of exogenous SOD, albeit not completely restored as compared to sham-operated animals (Fig. 2). Although long-term treatment with both ET-receptor blockers significantly improved acetylcholine-induced relaxation per se in the absence of SOD, LU 135252 was more effective (Fig. 4). Moreover, only in the Bosentan group, but not in the LU 135252-treated animals, exogenous SOD further enhanced the dilator response (Fig. 4).

3.5. Production of superoxide anions

O<sub>2</sub><sup>−</sup> generated by aortic rings was assessed by lucigenin-enhanced chemiluminescence. O<sub>2</sub><sup>−</sup> release was greater in aortae from rats with chronic MI (Table 3) and was significantly reduced in rats treated with LU 135252 but not in the Bosentan group.

3.6. Plasma endothelin levels

ET plasma level was markedly enhanced in rats with heart failure (3.8 ± 0.8 pg/ml) as compared to sham-operated animals (1.3 ± 0.3 pg/ml, P<0.05). Neither treatment with LU 135252 (3.8 ± 0.7 pg/ml) nor with Bosentan (3.9 ± 1.0 pg/ml) had a significant effect on plasma ET levels in rats with heart failure.

3.7. Plasma renin activity

Plasma renin activity (ng angiotensin I ml/h) was almost doubled in rats with heart failure (36.5 ± 3.6) as compared to sham-operated animals (19.9 ± 3.9, P<0.01). In rats with heart failure treated with LU 135252 plasma renin activity was significantly reduced (19.2 ± 4.4, P<0.01), whereas chronic Bosentan treatment did not significantly affect plasma renin activity (28.2 ± 4.4).

4. Discussion

In the present study, we demonstrate the improvement of NO-mediated relaxations in rats with heart failure following MI by chronic treatment with both selective ET<sub>A</sub>- and mixed ET<sub>A/B</sub>-receptor antagonists. While the selective ET<sub>A</sub> antagonist LU 135252 normalized plasma renin activity and vascular superoxide anion production, the ET<sub>A/B</sub> blocker Bosentan prevented left ventricular dilation.

Chronic heart failure is associated with endothelial dysfunction of coronary and peripheral arteries contributing to reduced myocardial perfusion, arterial compliance, and elevated peripheral vascular resistance [9–11]. The underlying mechanisms appear to be complex including an imbalance between dilation and constriction with a predominance of the contractile stimuli, and a defective production of endothelium-derived NO due to an attenuated expression of eNOS [15,25]. We demonstrated recently that in rats with ischemic heart failure endothelial dysfunction was present despite an augmented eNOS expression [17] and provided experimental evidence for an increased vascular release of superoxide anion. An imbalance between NO and O<sub>2</sub><sup>−</sup> production with a reduction of bioactive NO despite a normal or even increased generation of NO, has been associated with endothelial dysfunction in other cardiovascular diseases such as hypertension and atherosclerosis [24,26–28].

The effect of chronic treatment with ET antagonists on NO-mediated vasodilation and O<sub>2</sub><sup>−</sup> production in heart
failure has been evaluated for the first time in the present study. Both selective $\text{ET}_A$- and mixed $\text{ET}_{A/B}$ receptor antagonists enhanced NO-mediated relaxations. The selective $\text{ET}_A$ blocker LU 135252 appeared to be more effective than Bosentan. The mechanisms by which ET antagonism improves endothelial function may include direct beneficial actions on the vascular system or indirect effects on vasomotion secondary to an improvement of left ventricular remodeling and/or performance.

Our data show that Bosentan had only minor effects on blood pressure in rats with heart failure. In agreement with recent studies Bosentan significantly reduced left ventricular dilation [3,4]. Improved left ventricular function may enhance blood flow and shear stress at the endothelial surface of the vessels resulting in improvement of endothelial function [16]. In contrast, the $\text{ET}_A$ blocker LU 135252 significantly reduced blood pressure in rats with heart failure, but elicited only non-significant ameliorations in left ventricular dilation. A lack of effect of an $\text{ET}_A$ antagonist on cardiac remodeling has also recently been demonstrated in a pig model of pacing-induced heart failure [29]. However, Mulder et al. [6] found a small, albeit significant attenuation of left ventricular dilatation. Differences of results may be explained by the time when active treatment was started. This is supported by recent observations that when given immediately after the infarction procedure, $\text{ET}_A$-antagonists even aggravated left ventricular dilation [30,31]. Therefore, the beneficial effects of LU 135252 on endothelium-dependent dilation may not be secondary to a correction of left ventricular function.

As ET-induced constrictions are mediated predominantly by $\text{ET}_A$ receptors on vascular smooth muscle cells and ET potentiates the response to other vasoconstrictors, both selective $\text{ET}_A$ and mixed $\text{ET}_{A/B}$ antagonists may favorably modulate the sensitivity to contractile agonists. However, we did not observe a significant increase in phenylephrine-mediated contractions in rats with heart failure. Furthermore, neither LU 135252 nor Bosentan treatment significantly affected the phenylephrine- and ET-induced contractions in the aorta from rats with heart failure.

Although both ET antagonists improved endothelium-dependent relaxation, a considerable difference between mixed ET and selective $\text{ET}_A$ blockers consisted in their contrasting effects on aortic superoxide production. While Bosentan did not significantly affect aortic $\text{O}_2^-$ formation, the release of this NO scavenging radical was markedly attenuated by the selective $\text{ET}_A$-antagonist LU 135252. As a result, enhancement of NO-mediated relaxation was more pronounced by treatment with the selective $\text{ET}_A$ antagonist. This is supported by the observation, that exogenous SOD further improved acetylcholine-induced relaxation in Bosentan-treated rats with heart failure, while no additional effect of SOD was observed in LU 135252-treated animals. Similar observations have been made in other models of endothelial dysfunction associated with increased superoxide formation: in angiotensin II-induced
hypertension and chronic NO-deficient hypertension, mixed ET$_{A/B}$ antagonists were not effective whereas selective ET$_A$-receptor blockade improved vascular dilator responses [21–23] (for review see Ref. [20]). Although the underlying mechanisms are not clear, in rats with heart failure treatment with LU 135252 decreased plasma renin activity, an index of the stimulation of the renin–angiotensin system, whereas Bosentan had no effect. As angiotensin II constitutes an important stimulus for vascular radical generation [28,32], the normalization of renin activity by the ET$_A$ antagonist but not by Bosentan may lead to the reduction in O$_2^-$ production and increase in bioavailability of NO. Furthermore, NO formation may be enhanced by augmented stimulation of ET$_A$-receptors during chronic ET$_A$-antagonism [33]. Increased NO production may counteract the detrimental effects of cardiac dysfunction on vascular homeostasis and attenuate up-regulation of vascular radical formation.

The improvement of endothelial vasodilator function by ET receptor blockade in heart failure may be of considerable clinical relevance. Heart failure after extensive MI in the rat closely mimics the pathophysiological situation in patients and is a useful model for novel therapies [34,35]. Furthermore, the endothelium has been recognized as a therapeutic target in heart failure, since beneficial modulation of endothelial function will reduce vascular resistance and enhance arterial compliance and tissue perfusion [16,18,19]. These assumptions are supported by recent findings which suggest that in patients with heart failure improved endothelial function results in enhanced exercise capacity [16,36]. Due to the heterogeneity of vascular reactivity over the arterial bed, our results obtained in aortic rings cannot be easily extrapolated to other arteries such as skeletal muscle or coronary arteries. Nevertheless, we suppose that the correction of endothelial dysfunction by chronic ET blockade may improve quality of life and prognosis in patients with heart failure. Although our data would suggest that selective ET$_A$ antagonists may be preferable, further studies are required to clarify whether selective ET$_A$ or mixed ET$_{A/B}$ antagonists will provide more clinical benefit.

In conclusion, our data show that long-term treatment with ET antagonists improves NO-mediated dilation in rats with chronic MI. This mechanism may play an important role for beneficial effects of these drugs in heart failure. Selective ET$_A$ blockade markedly attenuates the activation of the renin–angiotensin system and reduces vascular superoxide formation. These results offer a promising additional therapeutical option for the treatment of patients with chronic heart failure.

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