Chronic endothelin receptor blockade prevents renal vasoconstriction and sodium retention in rats with chronic heart failure

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Abstract

Objective: Importance of endothelin in mediating the chronic renal alterations of chronic heart failure was studied in rats chronically treated with bosentan after myocardial infarction. Methods: Rats were subjected to coronary artery ligation and were treated for 8 weeks with placebo or bosentan, a dual ET\textsubscript{A} and ET\textsubscript{B} receptor antagonist, (\textasciitilde100 mg/kg/day) as food admix. Sham-operated rats served as normal controls. Cardiac and renal functions were measured at the end of 8-week treatment. Results: Bosentan significantly reduced the elevated left ventricular end-diastolic pressure (from 26.6±3.3 to 11.4±2.2 mmHg, \(P<0.001\)) and the increased heart-to-body-weight ratio seen in untreated rats with myocardial infarction. Bosentan prevented the marked increase in renal vascular resistance (bosentan, 7.7±0.6; untreated, 15.6±2.5 mmHg/ml/min; \(P<0.001\)). This led to a significant increase in renal plasma flow resulting in a decrease in filtration fraction. Bosentan furthermore increased urinary sodium excretion. Conclusions: Prolonged ET receptor blockade in rats with myocardial infarction has chronic renal vasodilatory effect and improves renal sodium excretory function. Thus, dual ET antagonists such as bosentan might be useful in the treatment of the progressive renal failure associated with human chronic heart failure. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Endothelins; Heart failure; Infarction; Renal function; Vasoactive agents

1. Introduction

Chronic heart failure (CHF) is characterized by left ventricular (LV) dysfunction, increased renal vascular resistance (RVR), sodium retention and neurohormonal activation [1,2]. There is evidence to suggest that endothelin (ET) may be involved in the pathophysiology of renal dysfunction associated with CHF. The kidney is one of the organs that is most sensitive to the profound vasoconstrictor effects of exogenously administered ET [3,4]. Administration of exogenous ET-1 at concentrations mimicking those observed in CHF constricts the renal vasculature, resulting in an increase in RVR and a decrease in renal blood flow (RBF) [4,5]. An elevation in local renal ET-1 concentration and a blunted renal response to ET-1 have been reported in experimental CHF [6–8]. Acute ET receptor blockade improves RBF in experimental CHF, as measured by Doppler flowmeter [7,9].

We recently found that acute ET receptor blockade with a dual parenteral ET receptor antagonist, tezosentan [10], in rats with CHF induced by myocardial infarction (MI) reversed the profoundly increased RVR and improved renal plasma flow and sodium excretion [11]. Therefore, activation of the endogenous ET system appears to contribute to the renal vasoconstriction and sodium retention seen in CHF. Chronic selective ET\textsubscript{A} receptor antagonism has been reported to suppress plasma atrial natriuretic peptide, attenuate the magnitude of sodium retention, and prevent the decline in creatinine clearance in experimental CHF [12,13]. The goal of the present study was to evaluate the

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effects of chronic dual ET\textsubscript{A}/ET\textsubscript{B} receptor blockade on renal alterations in CHF.

For this purpose, the effects of long-term ET receptor blockade with the dual ET receptor antagonist bosentan were assessed on renal hemodynamics and renal function in experimental CHF. Accordingly, we measured the effects of chronic bosentan treatment on cardiac and renal hemodynamics, as well as on 24-h urinary sodium excretion in rats with CHF following MI.

2. Methods

Studies were conducted on 33 male Wistar rats weighing 220–260 g (supplied by the Experimental Animal Center of Chinese Academy of Sciences, Shanghai, China, Grade II, Certificate No. 9904). All rats were housed in climate-controlled conditions with a 12-h light–dark cycle and free access to normal rat chow and drinking water. The animals were handled according to the Position of the American Heart Association on Research Animal Use adopted November 11, 1984, by the American Heart Association.

2.1. Induction of myocardial infarction

Myocardial infarction (MI) was produced by a method described elsewhere [14]. In brief, rats were anesthetized with a mixture of ketamine (Ketosol-100, Stäub, Bern, Switzerland) and xylazin (Rompun 2%, Bayer, Leverkusen, Germany) (50 mg/5 mg per kg, intraperitoneally). The trachea was intubated with a small metal cannula (20-G) and the rats mechanically ventilated with room air by means of a small rodent ventilator (Model 7025 Rodent Ventilator, Hugo Sachs Elektronik, March-Hugstetten, Germany) at a rate of 60 cycles/min and a tidal volume of 1 ml/100 g body weight. A left thoracotomy was performed, and the heart was exposed. The left coronary artery was ligated with a 6-0 silk suture approximately 2 mm from its origin between the pulmonary artery outflow tract and the left atrium. Then the chest was closed in three layers (ribs, muscles and skin). The air within the thorax was removed, allowing the rats to resume spontaneous respiration. The sham-operated rats were subjected to the same procedure, except that the coronary artery was not ligated. The rats were allowed to recover from the anesthesia and were then returned to their cages. The 24-h postoperative mortality rate was about 15% for the infarction group.

2.2. Experimental protocols

Twenty-four hours after ligation, infarcted rats were randomized to receive either placebo (untreated, Veh-CHF, n=11) or bosentan (Bos-CHF, n=13) for 8 weeks, and were housed individually. Nine untreated sham-operated rats (Sham, n=9) were used as controls. Treatment was given as food admix (120 mg bosentan per 100 g powdered food). Food intake was monitored daily and body weights were measured twice a week throughout the 8-week period. The average bosentan intake was 93±6 mg/kg body weight per 24 h during the second week and remained constant over time in individual animals. The average dose of bosentan of 100 mg/kg/day was chosen as the maximally effective dose on the basis of in vivo pharmacology studies by Clozel et al. [15]. At week 8, the rats were placed in individual metabolic cages overnight. Food but not water was withdrawn from the metabolic cages. Total urine volume was collected for determination of urinary sodium concentration, and 24-h urinary sodium excretion was calculated.

2.3. Measurements of cardiac and renal hemodynamics

The cardiac hemodynamic and renal clearance experiments were conducted as follows. Rats were anesthetized with 100 mg/kg, i.p. thiobutabarbital-Na (Inactin, Byk-Gulden, Konstanz, Germany) and placed on a thermostatically controlled heating table to maintain body temperature at 36–38 °C. A tracheotomy tube was put in place and a catheter was inserted into the left femoral vein for infusion of synthetic plasma, inulin and p-aminohippurate (PAH). A polyethylene cannula was placed in the left femoral artery and connected to a pressure transducer (MLT1050 precision BP transducer, AD Instruments, Hastings, UK) for recording of arterial blood pressure (BP) and periodic sampling of blood. The right carotid artery was cannulated with a high-fidelity microtip 2F catheter (SPR-249, Millar Instruments, Houston, TX, USA) that was inserted through the aorta into the left ventricle (LV) to continually record LV pressure, heart rate (HR) and the maximal rate of rise of LV pressure (dP/dt\text{max}). All tracings (LV pressure, BP, HR, mean arterial pressure and dP/dt) were recorded on a computer using a PowerLab system. The data acquisition system consisted of a PowerLab (ML780 PowerLab/8s and ML118 Quad amplifiers, AD Instruments, Hastings, UK) which was connected to an HP Pavilion 8565C computer with the chart software (version 3.4, AD Instruments). Through a small, suprapubic incision, a flanged catheter was placed in the urinary bladder for collection of urine. During surgery, the rat received an intravenous infusion of 1 ml synthetic plasma and a 0.5 ml bolus of 0.9% NaCl containing 40 mg/ml inulin (Sigma, St. Louis, MO, USA) and PAH (0.5%, Merck Sharp & Dohme, West Point, PA, USA). Subsequently, a continuous intravenous infusion of 0.9% NaCl containing the same concentrations of inulin and PAH at a rate of 10 μl/min/100 g body weight was initiated. After a 45-min equilibration period, renal clearance experiments were begun in which two consecutive 20-min urine collections were performed, with midpoint
arterial blood samples (~150 μl). Blood was centrifuged, plasma was kept for analysis (see below).

### 2.4. Morphometric evaluation of infarct size

On completion of the hemodynamic experiments, the rats were killed by exsanguination. The heart was removed and weighed. The right and left ventricle were dissected and weighed. The left ventricle, including the interventricular septum, was fixed in 10% buffered formalin. The left ventricles were cut from apex to base into four transverse segments. The middle two segments, representing the bulk of the left ventricle, were embedded in paraffin, sectioned, and stained. Four slices from these segments were projected onto a screen for morphometry. The entire length of the endocardial circumference and the segment of the endocardial circumference made by the infarcted segment of each of the four slices of the LV were measured. The entire length of the epicardial circumference and the segment of the epicardial circumference made by the infarcted segment of each of the four slices of the LV were measured. These were then averaged for each of the four slices. The fraction of infarcted ventricle was calculated as the average of the four slices expressed as a percent of the length of the circumference.

### 2.5. Chemical analyses

Urine volume was measured gravimetrically, and urine was analyzed for sodium, inulin, and PAH concentrations. Sodium concentrations in urine were measured using an ion-selective electrode (Automatic Biochemical Analyzer, Hitachi 7150, Japan). Inulin concentrations in urine and plasma were determined using the anthrone methods [16,17]. PAH concentrations in urine and plasma were measured colorimetrically, as described elsewhere [18].

These measurements allowed calculation of inulin clearance (equal to glomerular filtration rate, GFR), PAH clearance (equal to renal plasma flow (RPF) when factored for renal extraction of PAH), renal vascular resistance (RVR), filtration fraction (FF) and urinary excretion of sodium (U_n V). The calculations have been described elsewhere [19–21]. The results of the two clearance periods were averaged.

### 2.6. Statistical analysis

Data are expressed as mean±S.E.M. Statistical analyses were done by analysis of variance (ANOVA) using STATIS-TICA (Statsoft) to assess differences in parameters between groups. Significant differences were then subjected to post-hoc analysis using the Student–Newman–Keuls procedure. Statistical significance is defined where P<0.05.

### 3. Results

#### 3.1. Characterization of myocardial infarction-induced chronic heart failure

Data for heart weight, blood pressure and cardiac hemodynamics at the end of the 8-week treatment are summarized in Tables 1 and 2. Coronary artery ligation produced extensive MI with an infarct size of 43±2% of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effects of chronic bosentan treatment on organ weights and infarct size in rats with chronic heart failure after myocardial infarction</th>
</tr>
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<tbody>
<tr>
<td>Sham (n=9)</td>
<td>BW (g)</td>
</tr>
<tr>
<td>300±10</td>
<td>3.0±0.1</td>
</tr>
<tr>
<td>CHF+veh (n=7)</td>
<td>279±12</td>
</tr>
<tr>
<td>CHF+bos (n=11)</td>
<td>298±14</td>
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</table>

Myocardial infarction-induced CHF rats received placebo (vehicle, CHF+veh) or bosentan (CHF+bos, 100 mg/kg/day as food admix) for 8 weeks. Untreated sham-operated rats (Sham) were used as controls. BW, body weight; CHF, chronic heart failure; HW:BW, heart-to-body-weight ratio; RV:BW, right-ventricular-to-body-weight ratio; LV+S:BW, left-ventricle-plus-septum-to-body-weight ratio.

* P<0.05; ** P<0.01; *** P<0.001 vs. Sham; ‘ ‘ P<0.05; CHF+bos vs. CHF+veh.

<table>
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<th>Table 2</th>
<th>Effects of chronic bosentan treatment on blood pressure and cardiac hemodynamics in rats with chronic heart failure after myocardial infarction</th>
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<tr>
<td>Sham (n=9)</td>
<td>MAP (mmHg)</td>
</tr>
<tr>
<td>111.4±2.7</td>
<td>387±10</td>
</tr>
<tr>
<td>CHF+veh (n=7)</td>
<td>86.4±2.2***</td>
</tr>
<tr>
<td>CHF+bos (n=11)</td>
<td>89.6±3.8**</td>
</tr>
</tbody>
</table>

Myocardial infarction-induced CHF rats received placebo (vehicle, CHF+veh) or bosentan (CHF+bos, 100 mg/kg/day as food admix) for 8 weeks. Untreated sham-operated rats (Sham) were used as controls. MAP, mean arterial pressure; HR, heart rate; LVEDP, left ventricular (LV) end-diastolic pressure; dP/dt_max, the maximal rate of rise of LV pressure.

* P<0.05; ** P<0.01; *** P<0.001 vs. Sham; ‘ ‘ P<0.01; CHF+bos vs. CHF+veh.
the LV. Eight weeks after MI, untreated rats developed severe CHF, evidenced by a marked elevation in left ventricular end-diastolic pressure (LVEDP) (26.6±3.3 vs. 4.3±0.3 mmHg in control rats, \(P<0.001\)) and by significant decreases in the maximal rate of rise of LV pressure (dP/dt\(_{max}\)) (55%) and mean arterial pressure (MAP) (22%), when compared to the sham-operated rats. In CHF rats, heart and right ventricular weights increased significantly compared to sham-operated rats. There was no change in body weight (BW) but a significant increase was observed in heart weight:body weight (HW:BW), right ventricular:body weight (RV:BW) and left ventricular+septum:body weight (LV+S:BW) compared to sham-operated rats (Table 1).

As shown in Fig. 1, the renal hemodynamics of untreated CHF rats were characterized by a profound elevation in RVR, leading to a marked decrease in RPF but a proportionately lesser decline in GFR. Thus, the filtration fraction rose. Urine flow-rate was lower in CHF rats (data not shown) and sodium excretion (\(U_{Na}V\)) as measured during clearance periods was significantly lower in CHF rats, as compared to the sham group. The 24-h \(U_{Na}V\) was

![Graphs showing renal hemodynamic parameters, glomerular filtration rate and urinary sodium excretion measured in anesthetized CHF rats treated or not with bosentan and compared to sham-operated rats. GFR, glomerular filtration rate; RPF, renal plasma flow; RVR, renal vascular resistance; \(U_{Na}V\), urinary sodium excretion; CHF, chronic heart failure; veh, vehicle; bos, bosentan * \(P<0.05\), ***, \(P<0.001\).]
Effects of chronic bosentan treatment in rats with CHF after MI

After 8 weeks of treatment, bosentan tended to increase survival: 11 of 13 (84.6%) bosentan-treated CHF rats remained alive compared with 7 of 11 (63.6%) untreated CHF rats (*P* = 0.067). Chronic blockade of the endogenous ET system in CHF rats with bosentan led to a significant decrease in LVEDP (from 26.6±3.3 to 11.4±2.2 mmHg, *P* < 0.001), without significantly affecting MAP, heart rate and cardiac contractility (dP/dt max) (Table 2). This long-term treatment with bosentan also largely reduced the increased HW:BW and RV:BW seen in the untreated MI-induced CHF rats (Table 1), but heart weight remained significantly elevated compared to sham-operated rats. There was no significant difference in infarct size between bosentan-treated rats and vehicle-treated rats (41±2 vs. 43±2%) (Table 1).

Fig. 1 shows that chronic bosentan treatment significantly reduced the markedly elevated RVR in the rats with CHF induced by MI (7.7±0.6 vs. 15.6±2.5 mmHg/ml/min, *P* < 0.001). This led to a large increase in RPF (6.1±0.5 vs. 3.0±0.3 ml/min, *P* < 0.001), resulting in a significant decrease in filtration fraction (0.268±0.0155 vs. 0.454±0.037, *P* < 0.001) since the increase in GFR was moderate. This chronic treatment also significantly increased urinary sodium excretion as measured during clearance periods (Fig. 1). Furthermore, the 24-h *Un* V was also significantly increased in the bosentan-treated CHF rats (Fig. 2) as compared with untreated CHF animals (0.407±0.048 vs. 0.253±0.043 mmol/24-h, *P* < 0.05).

3.2. Effects of chronic bosentan treatment in rats with CHF after MI

4. Discussion

The rat model of coronary artery ligation is a well characterized model of CHF that shares many features with congestive heart failure in humans. In the present study, in which hemodynamic measurements were made at 8 weeks after coronary artery ligation, depressed cardiac performance in the untreated MI rats was documented by a profound increase of LVEDP with a mean value of exceeding 26 mmHg, mild hypotension and significant decrease in cardiac contractility (dP/dt max). This hemodynamic abnormality was associated with large infarct and left ventricular hypertrophy. These findings indicate that relatively severe CHF occurred in these animals, a condition typically associated with compromised renal function and sodium retention [22–24].

Indeed, we found that in this study, untreated CHF rats exhibited profound renal vasoconstriction associated with a large fall in RPF and a decline in GFR, resulting in a significant rise in FF. Also, the CHF rats had impaired renal ability to excrete sodium. Our findings are in agreement with previously reported studies from other laboratories [22–25]. Whole kidney GFR is minimally affected in mild to moderate CHF, but RPF and GFR are decreased, and FF is increased in severe CHF [1,25].

Although the present study was not designed to elucidate the renal microcirculatory changes in CHF, the elevated FF does suggest altered glomerular hemodynamics in CHF. A small number of previous reports have been concerned with the intrarenal hemodynamics in CHF [22,23,25]. At a single nephron level, single nephron GFR, glomerular plasma flow (*Qp*) and glomerular capillary ultrafiltration coefficient (*Kf*) are reduced. Single nephron FF and glomerular blood pressure (*Pgc*) are elevated, the latter resulting from the substantial increase in efferent arteriolar resistance (*Rg*) in CHF produced by myocardial infarction in rats [22,23,25]. In CHF rats, the increased single nephron FF was associated with augmented oncotic pressure in plasma leaving the glomerulus, thereby favoring enhanced proximal tubular sodium reabsorption [22]. Thus, the altered glomerular hemodynamics has been thought to be responsible for the common occurrence of sodium and fluid retention in CHF.

There is considerable evidence for a role of ET in the pathogenesis of renal dysfunction and sodium retention associated with CHF. The known renal vasoconstrictive and antinatriuretic actions of ET-1 mimic the alterations in renal hemodynamics and sodium excretion in CHF [1,4–6,20,22,25]. Furthermore, previous studies have documented high levels of plasma ET-1, and a blunted renal response to ET-1 in animals and humans with CHF [6,7,26–28].

An elevation in local renal ET-1 concentration has also recently been reported in experimental CHF [7,8]. Acute ET receptor blockade improves renal blood flow in experimental CHF, as measured by Doppler flowmeter [7,9].
We recently found that acute ET receptor blockade in MI-induced CHF rats with tezosentan reversed the profoundly increased RVR and improved renal plasma flow and sodium excretion [20]. Chronic selective ET$_A$ receptor antagonism suppressed plasma atrial natriuretic peptide, attenuated the magnitude of sodium retention, and prevented the decline in creatinine clearance in experimental CHF [12,13]. However, there are to our knowledge no reports concerning the effect of chronic dual ET$_A$/ET$_B$ receptor blockade on the renal alterations associated with CHF. Thus, this study was designed primarily to investigate the renal responses to prolonged dual ET receptor blockade in CHF rats with bosentan.

In the present study, chronic ET receptor blockade with bosentan largely prevented the severe renal vasoconstriction associated with CHF, leading to improvements in RPF, GFR, and renal sodium excretion. The natriuretic response to chronic bosentan treatment in CHF rats may be due to removal of its direct stimulatory effect on tubule sodium reabsorption or may be secondary to inhibition of ET$_B$-stimulated aldosterone production [20,29–31]. In this study, we did not assess the renal effects of bosentan in sham-operated rats. However, in a previous study, acute bosentan administration in normal conscious rats had no effect on BP, renal plasma flow, or RVR, although an increase in urinary sodium excretion and a small decline in GFR were observed [20]. The findings of the present study suggest that ET is implicated in the pathophysiology of renal vasoconstriction and sodium retention in animal models of CHF.

Chronic bosentan treatment also markedly decreased LVEDP without affecting heart rate and cardiac contractility. Although acute ET receptor blockade has been reported to improve renal blood flow in experimental CHF [7,9], our study is the first to describe the beneficial effects of long-term blockade of dual ET receptors on whole kidney function and sodium excretory function in the rat model of CHF induced by MI.

The precise mechanisms by which chronic bosentan treatment improves whole kidney functions in CHF are as yet unknown and not directly addressed in this study. However, our findings of significantly decreased FF by bosentan treatment suggest that chronic ET blockade causes complex glomerular hemodynamic changes in CHF rats. The decrease in FF might suggest that chronic ET blockade induces a predominant post-glomerular vasodilatation. This is consistent with the effect of the dual antagonists bosentan [32] and tezosentan [10] in the model of glycerol-induced rhabdomyolysis. Previous reports have shown that the pre-glomerular and efferent arterioles are highly sensitive to the vasoconstrictor effects of ET-1 for the treatment of renal failure in patients with CHF. However, we cannot directly extrapolate the data to the clinical condition, since patients with CHF are often treated with diuretics, ACE inhibitors and β-blockers, all of which have renal consequences. Bosentan may have a different effect when given in addition to these drugs.
Nevertheless, the present data suggest that dual endothelin receptor blockers such as bosentan, in addition to their beneficial effects on cardiac hemodynamics, may have beneficial effects on renal function. The ongoing large clinical trials testing the effects of bosentan and other ET receptor antagonists in patients with CHF will give some indication of the renal effects of this type of drug.

In conclusion, long-term ET receptor blockade not only improves cardiac hemodynamics, but also prevents the renal vasoconstriction leading to improvements in renal plasma flow, GFR, and sodium excretion. These observations suggest that chronic ET receptor blockade with dual ET receptor antagonists such as bosentan may be beneficial in the treatment of the progressive renal dysfunction and sodium retention associated with human CHF.

Acknowledgements

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References


