Beneficial Cardiovascular Effects of Endothelin ET_A Receptor Blockade in Established Long-Term Heart Failure After Myocardial Infarction

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Although experimental prevention studies have suggested therapeutic potential of endothelin (ET) antagonists for the treatment of heart failure, the results of clinical trials using ET antagonists on top of standard heart failure medications have been largely disappointing. This experimental study investigated the effects of chronic ET_A receptor blockade in long-term survivors of myocardial infarction who had developed stable chronic heart failure in the absence of other treatments. Systolic blood pressure, heart rate, organ weights of the right atrium and ventricle, and the lungs were determined, and tissue ET-1 peptide levels were measured in cardiac tissue, lung, and aorta. The results show that chronic blockade of ET_A receptors stabilizes systolic blood pressure and reverses the heart failure–induced weight increases of right heart chambers and lung. The changes observed occurred independently of tissue ET-1 concentrations and heart rate, suggesting mechanisms independent of local cardiac or pulmonary ET-1 synthesis, which are yet to be identified. Exp Biol Med 231:857–860, 2006

Key words: chronic; heart; blood pressure; remodeling; right atrium; right ventricle; lung

Introduction

The role of endothelin (ET) for the pathogenesis and therapy of congestive heart failure (CHF) remains controversial (1). Although an activation of the ET system, reflected by increased circulating levels of ET-1, has been demonstrated in patients and animals with acute and chronic heart failure (2–4), clinical trials using ET receptor antagonists in patients with advanced CHF have been largely disappointing (5–7). Experimental studies in the mid-1990s using rat models of postinfarction heart failure suggested potent and beneficial effect of these drugs in the treatment of heart failure (8, 9); however, in these particular studies, treatment was initiated at the time or shortly before myocardial infarction of previously healthy hearts was induced by coronary ligation. This approach, though methodologically sound and reproducible, does not reflect the pathophysiology of CHF development in patients in whom structural damage of the myocardium is present and in whom disease typically has worsened during years and decades. Moreover, in all clinical studies conducted up to now, ET antagonists were added on top of standard CHF therapy, thus masking a potential therapeutic benefit of ET inhibition (1, 10).

The goal of the present experimental study was, therefore, to determine whether, and by what possible mechanisms, ET_A blockade affects cardiovascular parameters in established, long-term CHF as a consequence of myocardial infarction.

Materials and Methods

Heart Failure Model, Treatment, and Tissue Harvesting. Myocardial infarction in male Sprague Dawley rats (291 ± 40 g) was induced by coronary ligation. This method has been described in detail elsewhere (11, 12). Using this method, the 24-hr mortality rate in the study animals was 40%, as was late mortality (4 months after infarction; Ref. 12). Surviving animals were kept on standard rat chow. Six months after myocardial infarction, long-term survivor animals were randomly assigned to

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Table 1. Physiology and ET-1 Tissue Levels in the Study Animals[a]

Group	CTL	CHF	CHF DAR
Body weight increase, Week 0 to 6 (g)	27.3 ± 1.2	48.3 ± 3.4*	25.0 ± 5.6**
SBP, Week 0 (mm Hg)	142 ± 1	135 ± 2*	131 ± 3*
SBP, Week 6 (mm Hg)	141 ± 4	126 ± 1*,***	135 ± 2**
Heart rate, Week 0 (bpm)	362 ± 4	376 ± 4*	375 ± 8
Heart rate, Week 6 (bpm)	381 ± 11	364 ± 8	363 ± 5
Right atrial ET-1 (pg/g tissue)	n.d.	61 ± 9	68 ± 4
Right ventricular ET-1 (pg/g tissue)	n.d.	42 ± 2	40 ± 3
Pulmonary ET-1 (pg/g tissue)	1353 ± 222	2128 ± 135*	2265 ± 166*
Aortic ET-1 (pg/g tissue)	432 ± 69	526 ± 103	435 ± 126

[a] CTL, sham-operated controls; CHF, long-term CHF; CHF DAR, established heart failure animals treated with darusentan for 6 weeks; SBP, systolic blood pressure; n.d., not determined.
*$P < 0.05$ versus control; **$P < 0.05$ versus CHF; ***$P < 0.05$ Week 6 versus Week 0.

either 50 mg/kg/d darusentan treatment (administered by chow; $n = 9$) or placebo ($n = 9$) for 6 weeks, as described (13). Sham-operated age- and gender-matched animals ($n = 4$) were used as controls. At the end of drug treatment, animals were anesthetized with intraperitoneal administration of 50 mg pentobarbital, and killed by exsanguination. Heart, lungs, and aorta were removed and dissected. Right ventricle, right atrium, lungs, and aorta were weighed, rapidly snap-frozen in liquid nitrogen, and kept at –80°C until further analyses were performed. Experimental procedures were in accordance with the institutional guidelines for care and use of laboratory animals.

Measurement of Physiologic Parameters. Body weight, blood pressure (tail-cuff method; Letica LE 5000; Barcelona, Spain), and heart rate were measured in conscious animals at the beginning and at the end of the 6-week treatment period.

ET Tissue Content. ET-1 content in lung and cardiac tissue was determined by radioimmunoassay using a specific rabbit antibody against synthetic ET-1 (Peninsula Laboratories, Bachem, St. Helens, UK; Ref. 13). Reverse-phase high-performance liquid chromatography was used for ET-1 identification, and ET-1 tissue content was related to tissue weight (pg/g). The extraction procedure and quantification has been described in detail elsewhere (13).

Calculations and Statistical Analysis. Data are given as means ± SEM. For multiple comparisons, results were analyzed using analysis of variance (ANOVA), followed by Bonferroni's correction. For comparison between two values, the unpaired Student's t test or the nonparametric Mann-Whitney U test were used, when appropriate. $P < 0.05$ was considered significant.

Results

Physiologic Parameters. During the study period, the body weight increase in animals with CHF was 1.8-fold greater than in controls ($P < 0.05$; Table 1). This increase in body weight was significantly attenuated by darusentan treatment ($P < 0.05$; Table 1). Blood pressure was significantly lower in CHF animals ($P < 0.05$ vs. control; Table 1). During the study, blood pressure further decreased in animals with CHF ($P < 0.05$), but not in sham-treated animals. Treatment of animals with CHF with darusentan for 6 weeks prevented a further drop in blood pressure, as seen in untreated animals ($P < 0.05$ vs. CHF untreated), effects that were independent of heart rate (Table 1).

Organ Weights and Tissue ET-1 Levels. As previously described for short-term experimental models of CHF (8, 12, 14), heart failure was associated with increased tissue weights of lungs, right ventricle, and right atrium ($P < 0.05$ vs. control; Fig. 1). Treatment with darusentan reduced organ weights of lungs and cardiac chambers in CHF animals ($P < 0.05$ vs. CHF untreated; Fig. 1), but no effect in controls were observed (not significant [n.s.], data not shown). In the lung, tissue levels of ET-1 were 3-fold higher than in the aorta of animals with CHF ($P < 0.05$). With heart failure, ET-tissue levels increased only in the lungs ($P < 0.05$ vs. controls; Fig. 1) but not in the heart or aorta (n.s.; Table 1). Treatment with darusentan had no significant effect on tissue ET-1 levels in any of the organs investigated (n.s. vs. CHF untreated; Table 1).

Discussion

The results of the present study demonstrate novel and distinct effects of prolonged ET_A receptor blockade in established experimental CHF. Treatment was initiated 6 months after myocardial infarction had occurred; given the lifespan of laboratory rats, this compares to approximately 10–15 years in humans. ET blockade stabilized systolic blood pressure, improved right heart remodeling, and reduced lung weight, the latter being an important indicator of pulmonary congestion caused by left ventricular failure. The observed effects were independent of heart rate and ET-1 tissue levels, suggesting other mechanism(s) underlying the beneficial effects. Possibly, given the observed decrease in body weight, the secondary effects of ET blockade, such as increased natriuresis (15) or effects on pulmonary interstitial fluid accumulation, may have been present.

To the best of our knowledge, the present study is the

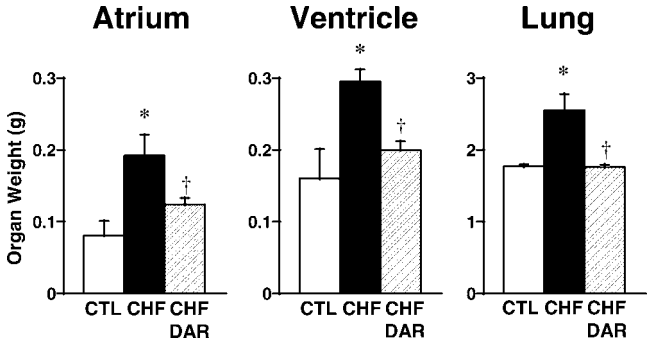

Figure 1. Organ weights of right atrium (left), right ventricle (middle), and lungs (right) in sham-operated control animals (CTL), in untreated rats with CHF (CHF), and animals with established heart failure treated with the ET_A receptor antagonist, darusentan, for 6 weeks (CHF DAR). Note the 10-fold difference on *y* axis units in right panel (lung). Data are given as means ± SEM; $*P < 0.05$ versus CTL; $\dagger P < 0.05$ versus CHF untreated.

first to address the question of whether ET-1 plays a role in established heart failure and its consequences in the absence of other factors, including additional heart failure medications or comorbidities, such as atherosclerosis, hypertension, or dyslipidemia—conditions almost invariably found in patients with established heart failure (1, 6, 7). Our results are in agreement with previous prevention studies by Sakai and colleagues, Thuillez's laboratory (8, 9), as well as with those by Nyugen *et al*., which indicated that blockade of the ET_A receptor ameliorates disease progression in CHF. Our data extend these previous observations and now provide evidence to suggest that—at least experimentally and in the absence of other vasoactive medications (7)—ET receptor blockade may indeed be beneficial for the treatment of CHF and/or other diseases affecting the lung and/or the right heart, by reversing established structural alterations in the cardiovascular system. Indeed, studies by Dupuis and coworkers have elegantly shown that blockade of ET_A receptors in experimentally induced pulmonary hypertension reduces pulmonary pressure (16, 17). These investigators, as well as Sakai *et al*. (8), observed that early ET blockade also had beneficial effects on right atrial and ventricular hypertrophy (17).

In a prevention study using Wistar rats, Nguyen *et al*. previously reported beneficial effects of ET blockade during postmyocardial infarction heart failure on organ weights of lung and right ventricle (14). The same investigators also found a reduction of RV hypertrophy using identical doses of darusentan (16). Our findings are limited by the lack of information on the histology of the tissues investigated because all of the tissue was required for the biochemical analyses. Whether the beneficial effect on right heart structure and pulmonary circulation extend to a survival benefit remains to be addressed in future studies.

In summary, the present study provides the first evidence of a beneficial effect of ET receptor blockade after long-term experimental CHF is established. The reversal of established heart failure–induced changes shown in the present study is in line with previous studies using ET antagonists in other pathologies; these studies on monocrotaline-induced pulmonary hypertension (18), age-dependent renal disease (19), or vascular calcification (20), and the present study, indicate therapeutic potential for ET antagonists. Given the reversal of established disease, ET antagonists may be suitable for secondary prevention of cardiovascular disease, possibly also in humans.

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