Acute effects of an endothelin-1 receptor antagonist bosentan at different stages of heart failure in conscious dogs

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Abstract

Objective: Inhibition by endothelin antagonist is a potential therapy in heart failure. However, the effect of endothelin inhibition during the development of heart failure has not been evaluated. The goal of our study was to examine the acute hemodynamic effects of the mixed endothelin receptor antagonist bosentan in the control state and at different stages of heart failure induced by right ventricular pacing (250 bpm) in conscious dogs. Methods: Nine dogs were chronically instrumented for the measurements of left ventricular pressure and its first derivative (dP/dt), cardiac output, left ventricular regional wall thickness and aortic pressure. Bosentan (3 mg/kg, i.v. bolus) and placebo were given at control, at 1 week of pacing (stage of left ventricular dysfunction with preserved cardiac output) and at 3 weeks of pacing (phase of heart failure with low cardiac output). Results: With the development of heart failure, baseline plasma endothelin level increased progressively. Placebo did not induce hemodynamic and plasma endothelin changes during the 30 min recording at any stage. At control, bosentan did not change hemodynamics. At 1 and 3 weeks of pacing, bosentan did not modify left ventricular myocardial function indices but reduced mean arterial pressure (by 7±2 and 8±1 mm Hg respectively, p < 0.005). Bosentan increased stroke volume at 3 weeks of pacing only. Conclusions: Endothelin inhibition by endothelin antagonist bosentan, decreases arterial pressure in both early left ventricular dysfunction and in heart failure in contrast with the control state. In the phase of heart failure with low cardiac output, bosentan increases stroke volume. In the early left ventricular dysfunction, bosentan, by reducing arterial pressure, may limit the deterioration of cardiac function through a reduction of the workload imposed on the heart. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Conscious dogs; Endothelin; Endothelin inhibitors; Heart failure; Ventricular function

1. Introduction

Endothelin-1 is one of the three isoforms of endothelin (ET) that can cause potent vasoconstriction [1]. ET-1 can be produced by endothelial cells, vascular smooth muscle cells and other cell types such as cardiac myocytes and may contribute to the development of myocardial hypertrophy [2,3]. Two ET receptors (ie, ET-A and ET-B) mediate the vascular effects of ET [4,5]. ET-A receptors which mediate most of the vasoconstrictor action of ET-1 [4,5] are present on vascular smooth muscle cells and cardiac myocytes. ET-B receptors are present on vascular endothelium, mediating the transient vasodilator response to ET [4,5] and on vascular smooth muscle, participating in the vasoconstrictor effects of ET [6–8].

The pathophysiological importance of ET in experimental and human heart failure (HF) is suggested by its plasma elevation [9,10]. In HF, recent studies showed that systemic vasoconstriction was mediated in part by ET-1 and the ET-1 plasma levels were correlated with the degree of HF [10]. The blockade of ET receptors, by decreasing...
afterload, may thus be beneficial in HF. Indeed, the use of a mixed ET receptor antagonist in patients with HF with low cardiac output was shown to induce an improvement of the hemodynamic profile [11]. In contrast, little is known concerning the contribution of ET in a less severe phase of HF and the effect of ET blockade has never been examined at this stage. Therefore, the goal of this study was to examine the acute hemodynamic effects of a mixed ET receptor antagonist, bosentan, at different stages of HF induced by rapid ventricular pacing in chronically instrumented conscious dogs.

2. Material and methods

2.1. Surgery and instrumentation

Experiments were performed in dogs in accordance with the position of the American Heart Association on Research Animal Use. Animal facilities have received the agreement of the French Ministère de l’Agriculture and the investigation conforms with the Guide for the Care and Use of Laboratory Animals published by US national Institutes of Health (NIH Publication No. 85-23, revised 1985). Nine mongrel dogs of either sex, weighing between 19 and 32 kg were sedated with sodium thiopental (Specia, Paris, France, 20 mg/kg, i.v.) and anesthetized using halothane (1% vol.). Under sterile conditions and through an incision in the fifth left intercostal space, catheters (Tygon, Norton Plastics, Akron, OH) were implanted in the ascending aorta and the left atrium. A solid-state pressure gauge (P22, Konigsberg Instruments, Pasadena, CA) was implanted in the left ventricle (LV) through the apex to measure LV pressure and the first derivative of LV pressure (LV dP/dt). In eight dogs, one pair of 5 MHz piezoelectric crystals was implanted to measure posterior LV full wall thickness. For each pair, one crystal was placed on the epicardium and the other was advanced obliquely to the endocardial layer. To further characterize the changes in left ventricular and systemic hemodynamics induced by one week of pacing and to dissect further the effects of bosentan, an aortic flow probe (Transonic Systems, Ithaca, NY) was implanted around the root of the ascending aorta to measure cardiac output in five dogs. Five dogs only were instrumented because of the risk of aortic rupture which usually increases with prolongation of chronic instrumentation (over 8 weeks in the current study). Stainless-steel pacing wires were implanted on the right ventricle. A permanent drainage tygon catheter (Norton Plastics, Akron, OH) was placed in the left thoracic cavity. Catheters and leads were externalized infra- and supracardially, and the thoracotomy was closed in layers. Burprenorphine (3 mg) was given subcutaneously when the dog had restored autonomic respiration. The animals were given daily post-operative care. All dogs were allowed to recover for 2–3 weeks.

2.2. Experimental protocol

For each dog, the experimental protocol consisted of three steps: first, before the induction of HF (control state), second, after one week of pacing (LV dysfunction with preserved cardiac output) and third after 3 weeks of pacing (HF with low cardiac output). During the three states, experiments were performed in dogs fully awake, lying quietly on their right side.

2.2.1. Experiments in the control state

Bosentan (Hoffmann-La Roche Ltd., Basel, Switzerland), dissolved in 7 ml of distilled water, was injected as an intravenous bolus (3 mg/kg). Hemodynamic parameters were recorded in the basal state and every 5 min during 30 min after drug injection.

2.2.2. Experiments during the development of heart failure

After completion of the studies in the control state, ventricular pacing was initiated at a rate of 250 beats/min, using a programmable miniature pacemaker (model 5320, Medtronic Inc, Minneapolis, Min, U.S.A) connected to the right ventricular pacing wires and placed in a pocket of a jacket on the back of the animal. Dogs were examined daily to confirm the continuous pacing. At 1 week and 3 weeks of pacing, after a 15-min stabilization period subsequent to deactivation of the pacemaker, baseline measurements in sinus rythm were taken, then, placebo or bosentan were injected. Placebo and bosentan were given on different days with a separation period of 24 h. The first day, placebo (7 ml of distilled water) was injected as an intravenous bolus and, 24 h later, bosentan was injected also as an intravenous bolus (3 mg/kg). Hemodynamic parameters were recorded in the basal state and every 5 min during the 30 min period following drug or placebo injection. After 3 weeks of pacing, 45 min after the intravenous injection of 3 mg/kg of bosentan, an additional bolus of 10 mg/kg of bosentan was injected intravenously. The dose of 3 mg/kg of bosentan was based on the study of Teerlink et al [12] which demonstrates in normal anesthetized dogs, that an intravenous injection of 3 mg/kg of bosentan prevents nearly completely the systemic vasoconstriction induced by big-ET-1 and the vasodilation induced by an ET-B receptor agonist sarafotoxin S6c. Considering the volume of distribution of bosentan in dog: 0.27–0.38 l/kg (Hoffman-La-Roche, unpublished data), it can be estimated that an intravenous injection of 3 mg/kg of bosentan results in a concentration of 1–2 10−5 M in dog.

2.3. Data collection and analysis

Absolute values of LV pressure were obtained by calibrating the micromanometer in 37ºC water against a Statham P23ID transducer (Gould Inc., Valley View, Ohio,
USA) before implantation. The shift of zero pressure during the study was corrected by comparing LV pressure with left atrial pressure and/or aortic pressure simultaneously recorded with a Statham pressure transducer. All signals were recorded and calculated with a computer (Vectra 486/66XM, Hewlett-Packard, Palo Alto, CA) using a software (HEM v1.4, Notocord systems, Croissy-sur-Seine, France) and also recorded on a multi-task graphic recorder (MT-95000, Astro-Med Inc, RI) at a paper speed of 50 mm/sec. Left ventricular measurements and aortic pressures obtained from the computer were verified by one of us (RC). For this purpose, 9–12 beat measurements were averaged and maximal and minimal rates of LV pressure changes (LV dP/dt max and LV dP/dt min) were derived from the LV pressure by use of operational amplifiers connected as differentiators with a frequency response of 700 Hz. To directly calibrate the differentiator, a triangular wave signal was substituted for the pressure signals. End diastole was defined as the time point when LV pressure began to rise just after the atrial contraction. End systole was defined as the time point occurring at LV dP/dt min. Stroke volume was calculated as the ratio of cardiac output/heart rate. Total peripheral resistance was calculated as the ratio of (mean arterial pressure−right atrial pressure)/ cardiac output. Cardiac output measurements were obtained in 5 dogs. LV end-diastolic wall thickness was measured at the onset of LV contraction, indicated by the initial increase in LV dP/dt. LV end-systolic wall thickness was measured at the time point occurring at the peak negative LV dP/dt. Adequate LV wall thickness measurements were obtained in 7 of the 8 dogs instrumented for that measurement. LV wall thickness measurement was rejected for analysis in one dog with a control systolic LV wall thickening below 15%.

2.4. Plasma ET-1 and big-ET-1 assays

To measure arterial plasma ET-1 and big-ET-1 levels, blood samples were withdrawn from the aortic catheter at baseline, after 15 and 30 min of bosentan or placebo injection in the control state and after one and three weeks of pacing. The samples were collected in tubes containing EDTA and were immediately placed on ice, and centrifuged at 4°C; plasma was then stored at −70°C until assay. All samples from a single dog were analyzed in the same assay to avoid inter-assay variability. ET-1 was determined with the polyclonal rabbit antiserum RAS 6901 developed in our laboratory. The sensitivity of the assay was 0.2 pg/tube. For measurement of big-ET-1, an Elisa assay was used (RPN 229, Amersham Int, obtained from Life Science, France) according to the manufacturer’s instructions.

2.5. Statistical analysis

Values are presented as means±s.e.m. All statistical computations were accomplished using commercially available software (SuperANOVA V1.11, Abacus Concepts, Berkeley, CA, USA). One-way analysis of variance for repeated measures over time was used for intragroup parameter changes after drug injection. Two-way analysis of variance for repeated measures of the same parameters over time was used to compare intergroup drug interactions. After analysis of variance, comparisons between means were performed with contrast analysis. When only two means were compared, a t-test was used. Statistical significance was defined as p<0.05.

3. Results

3.1. Baseline measurements before, one week and three weeks after pacing

Representative illustrations of waveforms from the same animal in the control state, after one week and three weeks of pacing are displayed in Fig. 1. Baseline hemodynamics in the same 9 dogs studied in sinus rhythm in the three stages are shown in Tables 1–3. One week of pacing induced a LV dysfunction characterized by a significant increase in LV end-diastolic pressure (p<0.005) as compared with the control state and significant decreases in mean aortic pressure (p<0.005), LV dP/dt max (p<0.005) and LV systolic wall thickening (p<0.005). At this stage, heart rate, cardiac output and total peripheral resistance were not modified significantly.

After three weeks of pacing, dogs developed HF which was characterized by peripheral signs of congestive HF (exertional dyspnea, ascites) and by a low cardiac output associated with greater alterations in LV regional wall thickness (p<0.05 as compared with control state or 1 week of pacing; Fig. 1 and Table 3). Heart rate increased significantly (p<0.005 as compared with control state and p<0.05 as compared with 1 week of pacing). Total peripheral resistance was significantly increased as compared with one week of pacing (p<0.05).

Baseline hormonal characteristics are shown in Table 4. After one week of pacing, mean plasma ET-1 level raised significantly (Table 4). Between one and three weeks of pacing, plasma ET-1 levels increased further (but not significantly as compared with 1 week of pacing). Plasma big-ET-1 did not increase at one week of pacing but increased significantly after three weeks of pacing as compared with the control state and with one week of pacing (Table 4).

3.2. Cardiovascular effects of bosentan

In the control state, bosentan administration (3 mg/kg, i.v.) did not produce hemodynamic changes throughout the 30 minute period of recording (Table 1). Baseline hemodynamic measurements were similar be-
Before the injection of bosentan or placebo both after one week and three weeks of pacing (Tables 2 and 3).

After one week of pacing, placebo infusion did not produce hemodynamic changes demonstrating the validity of the experimental procedure (Table 2), whereas bosentan administration (3mg/kg; i.v.) produced a decrease in mean aortic pressure (Table 2 and Fig. 2, left panel). The effect occurred progressively, was apparent 3 min after the injection, became maximal at 15 min (from 81±3 mm Hg at baseline to 73±2 mm Hg, \( p<0.001 \)) and persisted until the end of the experiment (Fig. 2, left panel). LV systolic pressure decreased in parallel. The other measured cardiovascular parameters remained unchanged.

After three weeks of pacing, placebo infusion did not produce hemodynamic changes (Table 3). Bosentan (3mg/kg; i.v.) decreased mean aortic pressure (Table 3 and Fig. 2, right panel). The effect was apparent 1 minute after the injection, progressively increased and became maximal at 25 min of infusion (from 84±2 mm Hg at baseline to 73±2 mm Hg, \( p<0.001 \)). It persisted until the end of the experiment. After bosentan, heart rate decreased significantly; stroke volume increased by 17% (from 12.8±0.6
End-diastolic wall thickness (mm) 7 9.8
Total peripheral resistance (mmHg / l / min) 5 42
Stroke volume (ml) 5 13.6
Cardiac output (l / min) 5 1.83
% of systolic wall thickening (%) 7 14.3

End-diastolic wall thickness (mm) 7 10.5
Total peripheral resistance (mmHg / l / min) 5 33
Stroke volume (ml) 5 24
Cardiac output (l / min) 5 2.44
% of systolic wall thickening (%) 7 22.5

LV, left ventricular.

Table 2
Hemodynamic and dimensional parameters in response to placebo and to bosentan (3 mg/kg, i.v.) at the stage of left ventricular dysfunction (1 week of right ventricular pacing)

<table>
<thead>
<tr>
<th>n</th>
<th>Placebo</th>
<th></th>
<th>Bosentan</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>30 min</td>
<td>Baseline</td>
<td>30 min</td>
</tr>
<tr>
<td>Mean aortic pressure (mmHg)</td>
<td>9</td>
<td>80±3</td>
<td>81±3</td>
<td>75±2*</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mmHg)</td>
<td>9</td>
<td>18±3</td>
<td>18±3</td>
<td>17±2</td>
</tr>
<tr>
<td>LV systolic pressure (mmHg)</td>
<td>9</td>
<td>97±3</td>
<td>100±3</td>
<td>99±3</td>
</tr>
<tr>
<td>LV dP/dt max (mmHg/s)</td>
<td>9</td>
<td>1563±36</td>
<td>1592±56</td>
<td>1504±32</td>
</tr>
<tr>
<td>LV dP/dt min (mmHg/s)</td>
<td>9</td>
<td>−1862±94</td>
<td>−2036±120</td>
<td>−1881±75</td>
</tr>
<tr>
<td>Cardiac output (l / min)</td>
<td>5</td>
<td>2.44±0.21</td>
<td>2.34±0.18</td>
<td>2.41±0.23</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>5</td>
<td>19±1</td>
<td>20±1</td>
<td>20±2</td>
</tr>
<tr>
<td>Total peripheral resistance (mmHg/l/min)</td>
<td>5</td>
<td>33±4</td>
<td>35±4</td>
<td>33±4</td>
</tr>
<tr>
<td>End-diastolic wall thickness (mm)</td>
<td>7</td>
<td>10.5±0.6</td>
<td>10.5±0.6</td>
<td>10.5±0.6</td>
</tr>
<tr>
<td>% of systolic wall thickening (%)</td>
<td>7</td>
<td>14.3±1.6</td>
<td>13.8±1.3</td>
<td>14.0±2.1</td>
</tr>
</tbody>
</table>

LV, left ventricular. *, p<0.005 as compared with the corresponding placebo values.

ml at baseline to 14.3±0.6 ml) and total peripheral resistance decreased by 17% (from 45±5 mm Hg/l/min at baseline to 39±4 mm Hg/l/min). The other measured cardiovascular parameters remained unchanged throughout the study (Table 3).

In order to verify that blockade of endothelin receptors after the dose of 10 mg/kg. During the same time frame, mean arterial pressure decreased from 86±3 to 78±3 mm Hg after the dose of 3 mg/kg and to 77±2 mm Hg 30 min after the dose of 10 mg/kg. During the same time frame, heart rate decreased from 133±4 to 120±4 and to 126±6 beats/min respectively. Similarly, LV dP/dt and cardiac

Table 3
Hemodynamic and dimensional parameters in response to placebo and to bosentan (3 mg/kg, i.v.) at the stage of heart failure (3 weeks of right ventricular pacing)

<table>
<thead>
<tr>
<th>n</th>
<th>Placebo</th>
<th></th>
<th>Bosentan (3 mg/kg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>30 min</td>
<td>Baseline</td>
<td>30 min</td>
</tr>
<tr>
<td>Mean aortic pressure (mmHg)*</td>
<td>9</td>
<td>79±4</td>
<td>79±4</td>
<td>84±2</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mmHg)*</td>
<td>9</td>
<td>32±2</td>
<td>32±3</td>
<td>31±3</td>
</tr>
<tr>
<td>LV systolic pressure (mmHg)*</td>
<td>9</td>
<td>97±3</td>
<td>96±3</td>
<td>101±2</td>
</tr>
<tr>
<td>LV dP/dt max (mmHg/s)*</td>
<td>9</td>
<td>1345±151</td>
<td>1345±152</td>
<td>1542±66</td>
</tr>
<tr>
<td>LV dP/dt min (mmHg/s)*</td>
<td>9</td>
<td>−1555±77</td>
<td>−1616±99</td>
<td>−1788±96</td>
</tr>
<tr>
<td>Cardiac output (l / min)</td>
<td>5</td>
<td>1.83±0.20</td>
<td>1.74±0.12</td>
<td>1.80±0.15</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>5</td>
<td>13.6±1.3</td>
<td>13.5±1.0</td>
<td>12.8±0.7</td>
</tr>
<tr>
<td>Total peripheral resistance (mmHg/l/min)</td>
<td>5</td>
<td>42±7</td>
<td>44±6</td>
<td>45±5</td>
</tr>
<tr>
<td>End-diastolic wall thickness (mm)</td>
<td>7</td>
<td>9.8±0.4</td>
<td>9.8±0.4</td>
<td>9.8±0.4</td>
</tr>
<tr>
<td>% of systolic wall thickening (%)</td>
<td>7</td>
<td>7.9±3.6</td>
<td>7.6±3.5</td>
<td>6.8±2.9</td>
</tr>
</tbody>
</table>

* n=8 for the placebo study; LV, left ventricular; * p<0.05 and † p<0.005 as compared with the corresponding baseline values.
Baseline concentrations and responses to bosentan and saline injections of endothelin-1 and big-endothelin-1 at different stages of heart failure

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline</th>
<th></th>
<th>Response</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Saline</td>
<td></td>
<td>Bosentan</td>
<td></td>
</tr>
<tr>
<td>Control state</td>
<td>8</td>
<td>1.02±0.27</td>
<td>–</td>
<td>7.58±0.38**</td>
<td></td>
</tr>
<tr>
<td>ET-1 (pg/ml)</td>
<td>8</td>
<td>4.76±0.26</td>
<td>–</td>
<td>0.12±0.18</td>
<td></td>
</tr>
<tr>
<td>LV dysfunction (1 week after pacing)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET-1 (pg/ml)</td>
<td>8</td>
<td>2.46±0.55*</td>
<td>0.09±0.20</td>
<td>7.98±0.74**</td>
<td></td>
</tr>
<tr>
<td>Big-ET-1 (pg/ml)</td>
<td>8</td>
<td>5.29±0.26</td>
<td>0.17±0.15</td>
<td>–0.21±0.21</td>
<td></td>
</tr>
<tr>
<td>Heart failure (3 weeks after pacing)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET-1 (pg/ml)</td>
<td>8</td>
<td>3.26±0.61*</td>
<td>–0.05±0.24</td>
<td>7.84±0.89**</td>
<td></td>
</tr>
<tr>
<td>Big-ET-1 (pg/ml)</td>
<td>8</td>
<td>8.82±1.35**</td>
<td>–0.01±0.35</td>
<td>–0.96±1.42</td>
<td></td>
</tr>
</tbody>
</table>

LV, left ventricular; Δ, difference between the value obtained 30 min after injection of bosentan or saline and the corresponding baseline value. Values are presented as mean ± s.e.m. –, no value available; *, p<0.05 as compared with control values; †, p<0.05 as compared with LV dysfunction state; **, p<0.05 as compared with corresponding baseline value.

3.3. Hormonal effects of bosentan

Plasma ET-1 levels were similar at baseline before the administration of placebo or bosentan and did not vary after placebo injection but increased significantly to a similar extent after bosentan injection (3 mg/kg) in all 3 stages, suggesting a similar level of receptor blockade (Table 4). The additional dose of 10 mg/kg of bosentan did not increase further plasma ET-1 levels, confirming the adequacy of the endothelin blockade by the dose of 3 mg/kg of bosentan. Plasma big-ET-1 levels which were similar at baseline before the administration of placebo or bosentan remained unchanged after placebo or bosentan injection (Table 4).

4. Discussion

The present study determined the hemodynamic effects of acute administrations of a mixed ET receptor antagonist, bosentan, at control and at different stages of development of HF induced by rapid ventricular pacing in chronically instrumented conscious dogs. In the control state, bosentan did not induce any change in hemodynamic parameters but at a stage of LV dysfunction, bosentan decreased the aortic pressure and consequently the workload of the heart. This result demonstrates the vasomotor effect of endothelin in the early phase of HF. At the stage of HF associated with low cardiac output, the hemodynamic effects of bosentan persisted and were associated with an increase in stroke volume.

4.1. Heart failure model and ET plasma levels

The model used in this study, particularly in the phase of HF, has been characterized in detail in previous studies [13–20]. In contrast, the phase of LV dysfunction has been less characterized in the literature [21]. The present study characterizes further the early phase of HF showing, after one week of pacing, a marked alteration in LV systolic wall thickening associated with a maintained cardiac output, two typical features of a state of LV dysfunction.

In accordance with previous studies, endothelin plasma levels increased with the development of HF. In the phase of LV dysfunction, ET-1 plasma level doubled without significant increases in big-ET-1 plasma concentration. In the phase of HF, big-ET-1 increased only 2 folds while ET-1 increased 3 folds. Among the mechanisms which
may explain a larger increase in ET-1 than in big-ET-1 in our study, one can quote changes in the conversion rate of big-ET-1 to ET-1, an increased substrate availability and/or a decreased clearance in renal and pulmonary circulations.

4.2. Hemodynamic effects of bosentan in the control state

Haynes and Webb [8] found, in healthy subjects, that local intra-arterial administration of the ET-1 receptor antagonist BQ-123 which selectively blocks ET-A receptors, induced a forearm blood flow increase, suggesting that ET-1 contributes to the maintenance of basal vascular tone. In our study, in the control state, bosentan was devoid of hemodynamic effect (Table 1). This suggests that ET-1 plays a minor role (if any) in the maintenance of vascular tone. Our results are in agreement with those of Teerlink et al [22] who found in anesthetized rats no significant change in blood pressure after administration of bosentan. The discrepancy between these studies is probably due to the different blockade produced by bosentan (both ET-A and ET-B receptors) and by BQ-123 (ET-A receptors only). It is possible that the blockade of ET-A receptors alone increases ET plasma level which may in turn stimulates ET-B receptors, inducing a vasodilatation which is known with the stimulation of these receptors [4,5].

4.3. Hemodynamic effects of bosentan in heart failure

One of the major findings of our study is the demonstration of the vasodilatory effect of bosentan in the phase of LV dysfunction. Our results show that bosentan produces marked decreases in aortic and ventricular systolic pressures with a trend towards a decrease in total peripheral resistance while cardiac output, heart rate and indices of myocardial performance, such as LV dP/dt max or LV systolic wall thickening remained unchanged (Table 2, Fig. 2 left panel). The decrease in mean aortic pressure clearly suggests that, in contrast with the control state, endogenous ET-1 plays a role in the maintenance of the vascular tone at the stage of LV dysfunction. In the phase of heart failure, bosentan decreased mean aortic pressure, left ventricular systolic pressure and total peripheral resistance. Our results contrast with those of a past study in dogs with chronic HF induced by multiple intracoronary emboliizations [23], where bosentan reduced systemic vascular resistance without affecting mean aortic blood pressure and increased indices of ventricular performance. Our results are, however, in accordance with the results of Teerlink et al [22] who reported that the acute administration of bosentan lowered blood pressure in rats with chronic HF induced by myocardial infarction. Our results are also in agreement with those of Kiowski et al [11] who provided the first evidence that ET-1 contributes to vasoconstriction in patients with severe chronic HF. However, a direct comparison between our results and those obtained in patients must be made with caution, since patients with heart failure receive additional therapies, particularly angiotensin-converting enzyme inhibitors which may modify the endothelin system due to potential interaction between the endothelin and the renin–angiotensin systems [22,24].

In addition to its potent vasocontractile effects, in vitro ET-1 is a positive inotropic agent on isolated heart muscle [25]). This effect is mediated in a large part by ET-A receptors [26]. ET-A density was shown to be increased in HF in rats [27]. The up-regulation of ET-A receptors and the increase in plasma ET levels may provide an inotropic support of the failing heart. It is thus possible to speculate that, in HF, blockade of these receptors may produce a decrease in contractility. This was found in one study in rats with HF treated with BQ-123 [27]. In contrast with this hypothesis, a study with bosentan in dogs with HF showed an increase in LV performance indices. In our study, an increased stroke volume was observed in HF, probably due to an improvement in systemic hemodynamics because no change in LV myocardial function was found with bosentan in HF (since peak LV dP/dt and LV systolic wall thickening did not change with an unchanged end-diastolic pressure). The absence of change in LV myocardial function indices found in our study may be due to opposite factors acting on ventricular function (loading conditions, heart rate changes and contractility) leading to an unchanged LV myocardial function indices. After bosentan, in association with decreased mean aortic pressure, heart rate decreased in the phase of HF. Since endothelin exerts positive chronotropic effect as shown in isolated tissues [28,29], its receptor blockade by bosentan can be expected to reduce heart rate by blocking the effects of endogenous ET. This was the case in our study. The absence of tachycardia in spite of a decrease in aortic pressure in response to bosentan was also probably due to a blunted baroreflex and a decreased sympathetic sensitivity which are known in this model [13,17,19,21] and to an absence of sympathetic stimulation after ET antagonist administration in heart failure [23,30,31].

4.4. Plasma ET-1 and big-ET-1 changes induced by bosentan

After bosentan, ET-1 plasma levels increased to a similar extent at the three stages without any effect on big-ET-1 plasma levels. This is related to the direct competitive displacement of ET-1 from ET receptors by bosentan [32]. This similarity in ET receptor blockade level at three stages was, however, associated with increased hemodynamic effects of bosentan. This suggests an increased role of ET in the vasoconstriction occurring during the development of HF.
4.5. Study limitations

The importance of endothelin in the pulmonary circulation in HF has been extensively studied [11,33,34]. Cody et al and Tsutamoto et al have shown in patients with HF that plasma ET correlates with the extent of pulmonary hypertension [33,34], a major determinant of survival in HF. The present study did not examine the direct effects of bosentan on this circulation and therefore cannot determine the contribution of the pulmonary circulation modification in the hemodynamic and hormonal effects of bosentan. However, since Kiowski et al showed parallel hemodynamic changes in both circulations after bosentan in patients with HF [11], similar modifications of both pulmonary and systemic circulations in response to bosentan can be expected.

Recent studies have reported protective effects of the selective ET-A antagonists in pacing-induced heart failure in dogs and rabbits [31,35]. A comparison between the effects of bosentan and those of a selective ET-A or ET-B antagonist would have lead to important informations. However, since our study was designed to examine the effects of a mixed endothelin ET-A and ET-B receptor antagonist at different phases of HF, we cannot determine the difference in the effects of a non selective ET-A and ET-B receptor antagonist and a selective ET-A receptor antagonist in HF.

4.6. Possible clinical implications

Our results may have clinical implications since our study describes a progressive increased contribution of ET in the maintenance of vascular tone with a progressive increase in plasma ET-1 levels and the evolutive effects of ET antagonism during the development of heart failure. Our data which show that bosentan decreases aortic pressure in the phase of LV dysfunction when cardiac output is preserved suggest that endothelin inhibitors may represent a potential therapy in the early developing HF to hemodynamics and plasma noradrenaline levels in conscious dogs with heart failure. Furthermore, data in the phase of HF strengthen the concept that ET antagonism may produce a beneficial effect in patients with HF.

References


