Effects of nonselective endothelin-1 receptor antagonism on cardiac mast cell-mediated ventricular remodeling in rats

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Murray DB, Gardner JD, Brower GL, Janicki JS. Effects of nonselective endothelin-1 receptor antagonism on cardiac mast cell-mediated ventricular remodeling in rats. Am J Physiol Heart Circ Physiol 294: H1251–H1257, 2008. First published January 4, 2008; doi:10.1152/ajpheart.00622.2007.—The objective of this study was to investigate the effect of a nonselective endothelin-1 (ET-1) receptor antagonist (bosentan) on the acute myocardial remodeling process including left ventricular (LV) mast cells and matrix metalloproteinase (MMP) activity secondary to volume overload. Additionally, we investigated the overall functional outcome of preventative endothelin receptor antagonism during 14 days of chronic volume overload. LV tissue from sham-operated (Sham), untreated-fistula (Fist), and bosentan (100 mg·kg⁻¹·day⁻¹)-treated animals (Fist + Bos) was analyzed for mast cell density, MMP activity, and myocardial collagen volume fraction at 1 and 5 days after the creation of an aorticaval fistula. When compared with untreated fistulas, bosentan treatment prevented the marked increase in LV mast cell density at 1 day postfistula (3.1 ± 0.3 vs. 1.3 ± 0.3 LV mast cells/mm², Fist vs. Fist + Bos, P = 0.01). Additionally, the substantial increase in MMP-2 activation in the untreated fistula at 1 day was prevented following bosentan treatment (1.6 ± 0.3 vs. 0.9 ± 0.1 arbitrary activity units, Fist vs. Fist + Bos, P = 0.01). The marked decrease in collagen volume fraction seen in the Fist group (1.4 ± 0.1 vs. 0.8 ± 0.1% myocardial tissue, Sham vs. Fist, P = 0.01) was significantly attenuated following bosentan treatment at both the 1- and 5-day time points. Lastly, a 2 wk preventative treatment with bosentan resulted in significant attenuation of the increase in LV end-systolic and -diastolic volumes compared with those in untreated fistula hearts. In summary, nonselective ET-1 antagonism prevents the acute increases in cardiac mast cell density and MMP activation induced secondary to chronic volume overload. By preventing these events, ET-1 antagonism was efficacious in attenuating ventricular dilatation and limiting the development of structural and functional deficits in the first 2 wk of chronic volume overload. Accordingly, these results are the first to demonstrate that cardiac mast cells are responsive to the endogenous endothelin system in vivo. Another novel finding from this study is that chronic nonspecific endothelin antagonist may inadvertently potentiate ET-1-mediated signaling.

bosentan; heart; extracellular matrix; cardiac function

DISCOVERED ONLY 16 years ago, the neurohormone endothelin-1 (ET-1) has been intensely investigated due to its potent cardiovascular effects and strong association with the pathophysiological worsening of cardiac function in congestive heart failure (7, 13, 16, 29, 35). ET-1 is a 21 amino acid peptide predominantly synthesized and secreted by endothelial cells. The effects of ET-1 are dictated by binding to one of two G protein-linked receptor subtypes A or B (ET_A or ET_B) (16). In the heart and vasculature, binding and activation of ET_A is known to mediate powerful vasoconstrictor and pressor effects, in addition to chronotropic and inotropic effects (7, 19). The ET_B receptor subtype is responsible for systemic clearance of circulating ET-1 and nitric oxide (NO)-dependent vasodilatation (1). Previous studies from our laboratory using the aortocaval (AV) fistula model of volume overload established a causal role for cardiac mast cells in the activation of matrix metalloproteinases (MMPs) (3, 8, 22, 31). The role of MMPs in mediating the degradation of the extracellular matrix leading to ventricular dilatation has been well documented (3, 6, 9, 20, 38). However, the potential for ET-1 to directly impact myocardial remodeling was recently established by our study, demonstrating that ET-1 causes degranulation of resident cardiac mast cells (31). ET-1 receptor antagonism has also been shown to attenuate MMP activation following myocardial infarction (37). However, to date, our understanding of the in vivo regulation of cardiac mast cell activation by endothelin receptors in the normal or diseased heart is limited. Therefore, the aim of the present study was to determine whether ET-1 receptor antagonism is efficacious in attenuating mast cell-mediated myocardial remodeling and systolic dysfunction in the AV fistula model of volume overload. To this end, we evaluated changes in left ventricular (LV) mast cell density, MMP activity, and extracellular matrix content, as well as diastolic and systolic function. The findings herein demonstrate that endothelin receptor antagonism prevented the increase in cardiac mast cell density during the acute stages of volume overload. Furthermore, sustained ET-1 antagonism was able to prevent mast cell-mediated interstitial collagen degradation and adverse ventricular remodeling.

MATERIALS AND METHODS

Animal Welfare

All experiments were performed using 9 wk (200–250 g) male Sprague-Dawley rats housed under standard environmental conditions and maintained on commercial rat chow and tap water ad libitum. All studies conformed to the principles of the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and were approved by our Institutional Animal Care and Use Committee. Anesthesia for the experimental procedure was effected by pentobarbital sodium (50 mg/kg ip).

Experimental Design

Animal groups. Three separate study periods (1, 5, and 14 days following the creation of an AV fistula) were investigated. The

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nonselective (ET<sub>A</sub>/ET<sub>B</sub>) endothelin receptor antagonist, bosentan (100 mg·kg<sup>−1</sup>·day<sup>−1</sup>), was dissolved in gum arabic and delivered via oral gavage once a day, initiated 24 h before surgery, and continued for the duration of the specified experimental time group. Sham-operated (Sham), untreated fistula (Fist), and bosentan-treated (Fist + Bos) groups at the 1 and 5 day time points (n = 6–8 animals/group) were used to determine whether bosentan treatment could prevent the acute increase in mast cell density and MMP-2 activation, as well as prevent the myocardial extracellular collagen matrix degradation typical of the initial phase of remodeling in this model (22). Corresponding bosentan-treated sham-operated groups (Sham + Bos, n = 4–6 animals/time point) were also evaluated at the 1- and 5-day time points. To assess the structural and functional effects of ET-1 receptor antagonism, Sham (n = 7), Fist (n = 8), and Fist + Bos (n = 7) groups were analyzed at 14 days postsurgery to obtain LV systolic and diastolic pressures and volumes via high-fidelity Millar conductance catheterization. These time points were chosen based on our previous characterization studies demonstrating significant acute increases in the number of cardiac mast cells and MMP activity occurring following the creation of an AV fistula (within 1 day postfistula), preceding significant collagen degeneration (3 to 5 days postfistula) producing changes in ventricular morphology. Subsequent to this, the remodeling consists of return-to-normal-normal collagen values in the myocardium between 7 to 14 days. Concomitant chamber dilatation developing in this initial period rapidly stabilizes and does not progress further until beyond 4 to 5 wk postfistula (3–5).

**Infrarenal abdominal aorta-inferior vena cava: AV fistula.** An infrarenal AV fistula was created in rats as described previously (4). Briefly, a ventral abdominal laparotomy was performed to expose the aorta and caudal vena cava. Both vessels were temporarily occluded proximal and distal to the intended puncture site, and an 18-gauge needle was inserted into the abdominal aorta ~1.5 cm below the renal arteries and advanced through the medial wall into the vena cava (4, 5). The needle was withdrawn and the puncture site sealed with surgical glue. Creation of a successful AV fistula was evident by the pulsatile flow of oxygenated blood into the vena cava.

**Assessment of ventricular volume and function.** In vivo LV volume and function was assessed in anesthetized rats using a high-fidelity Millar SPR-838; Millar Instruments, Houston, TX) conductance catheter inserted into the LV via the right carotid artery at 2 wk postsurgery as previously described (32, 43). This measurement technique requires that a small amount (0.1 ml) of 15% saline be introduced into the external jugular vein of the anesthetized animal at the end of the experimental protocol as a correction factor for the blood-LV tissue interface. Data were analyzed using proprietary software (PVAN 3.5; Millar Instruments).

**Assessment of mast cell density and fibrillar collagen concentration.** At the end of the experimental period, a transmural section of LV was taken from the midventricle and placed in buffered formalin for fixation. The tissue was then processed for routine histopathology, using sequential 5-µm paraffin-embedded sections stained with either pinacyanol erthrosinate for the visualization of mast cell morphology or picrosiris red for quantification of myocardial interstitial collagen volume fraction (CVF) as previously described (8, 31). Mast cell density was determined from the total number of mast cells per LV cross section normalized for the total myocardial area determined from the digitized image (ImageQuant, Molecular Dynamics). CVF was obtained using a Bio-Rad MRC-1024 confocal laser-scanning microscope with a ×40 objective lens magnification. Twenty random fields per slide were digitized, and image analysis was performed using Scion-image software. The pixel count of highlighted interstitial collagen fibers was expressed as a percentage of the total number of pixels in the field to derive the percent area of collagen. Perivascular collagen was excluded from the analysis. Tissue sections were analyzed in a blinded fashion.

**MMP activity.** MMP activity in cardiac tissue extracts was analyzed using gelatin zymography performed by standard procedures using a sodium dodecyl sulfate-polyacrylamide gel electrophoresis matrix containing gelatin (1 mg/ml) (8). The activity of the bands was quantified by densitometry (ImageQuant, Molecular Dynamics). All of thezymograms had two lytic bands corresponding to standards for the proenzyme (68 kDa) and activated (62 kDa) forms of gelatinase A (MMP-2) (Chemicon International, Temecula, CA). Thesezymograms were also analyzed for two lytic bands corresponding to standards for the proenzyme (95 kDa) and activated (82 kDa) forms of MMP-9; however, no differences between groups were noted. The values obtained for MMP activity for each sample were normalized for their protein concentration using a Bio-Rad protein assay. Each gel was run in duplicate. To compare results from different gels, a single extract from the same control heart was used as a standard on all gels. The activities of the lytic bands in the other lanes of a gel were expressed as a percentage of the activity of this standard. Once normalized in this fashion, the percent activities from hearts belonging to each group (i.e., control and fistula) were averaged.

**Enzyme-linked ImmunoSorbent assay.** ET-1 and TNF-α myocardial tissue levels were analyzed using commercially available kits (Alpco Diagnostics and Quantikine by R & D Systems, respectively). Briefly, precoated wells were treated with assay buffer and 50 μl of extracted protein sample for 1 h and washed five times with wash buffer. Wells were then incubated with appropriate monoclonal antibody directed against ET-1 or TNF-α, followed by horseradish peroxidase conjugated antibody after washing. They were then read by spectrophotometry at 450 nm, according to manufacturer’s specifications.

**Statistical analysis.** Statistical analyses were performed with SPSS 11.5 software (SPSS, Chicago, IL). All grouped data are expressed as means ± SE, unless otherwise noted. Grouped data comparisons were made by one-way analysis of variance with intergroup comparisons analyzed using Fisher protected least significant difference post hoc testing. Statistical significance was taken to be p ≤ 0.05.

**RESULTS**

**Acute Studies: 1- and 5-day Time Points**

**Control groups.** No significant differences in body, LV or right ventricular (RV) weights, mast cell density, MMP activity, or CVF were observed between the 1- and 5-day sham-operated control groups. Likewise, comparison of bosentan-treated 1- and 5-day sham-operated groups revealed no significant differences. Therefore, the data for these time points were combined and considered as untreated and drug treated surgical control groups for comparison purposes.

**Mast cell density and MMP activation.** We investigated the influence of ET-1 antagonism on LV mast cell density, MMP activity, and extracellular matrix components at 1 and 5 days following the creation of an AV fistula. As seen in Fig. 1, LV mast cell density was significantly increased (72%) in the untreated fistula hearts at 1 day postfistula relative to control, and bosentan treatment prevented this increase. LV mast cell density remained significantly elevated (28% above that of the Sham group) in the untreated fistula group at 5 days postfistula, whereas bosentan-treated sham-operated and fistula rats were not significantly different from sham-operated rats (Fig. 1).

Similar to the increase in mast cell density, Fig. 2 depicts the marked increase in MMP-2 activity induced in the untreated fistula hearts at 1 day (1.6 ± 0.3 vs. 0.9 ± 0.1 arbitrary activity units; Fist vs. Sham-operated, respectively, P ≤ 0.01). Bosentan treatment also prevented MMP-2 activation at 1 day postfistula (1.6 ± 0.3 vs. 0.8 ± 0.1 arbitrary activity units; Fist vs. Fist + Bos, respectively, P ≤ 0.01). However, this inhibitory effect was not sustained at 5 days postfistula (1.7 ± 0.5 vs. 1.8 ± 0.5 arbitrary activity units; Fist vs. Fist + Bos, respect-
tively, $P = \text{not significant (NS)}$. In contrast to MMP-2, densiometric evaluation of zymography gels for the MMP-9 active (82 kDa) and latent (95 kDa) bands revealed no changes in the level of activity between any of the experimental groups.

**Extracellular matrix content and composition.** As can be seen in Fig. 3, MMP-2 activation in the untreated fistula hearts produced a marked degradation of ventricular collagen at 1 day postfistula compared with Sham (0.8 ± 0.1% vs. 1.4 ± 0.1%, Fist vs. Sham, $P = 0.05$), which persisted at 5 days postfistula (1.0 ± 0.1% vs. 1.4 ± 0.1%, Fist vs. Sham, $P = 0.05$). Consistent with the acute inhibition of MMP activity, bosentan treatment prevented this decrease in CVF at both 1- and 5-days postfistula (Fig. 3).

**Neurohormone/cytokine interaction.** LV myocardial ET-1 levels were significantly elevated 5.4-fold above sham-operated levels in the 1-day untreated fistula group (Fig. 4). While bosentan treatment significantly attenuated this acute increase postfistula, ET-1 levels were still elevated relative to sham-operated values. However, at the subsequent 5-day time point, ET-1 levels in untreated fistula hearts were comparable with the time-matched bosentan-treated fistula group. Furthermore, consistent with the observation that bosentan inhibited ET-1 clearance, LV ET-1 levels were significantly increased in sham-operated animals after 5 days of bosentan treatment relative to untreated sham-operated values.

**Chronic Function Studies**

**Fourteen-day group.** We also investigated the overall functional effects of a 2-wk prevention study with bosentan initiated 24 h before the creation of chronic volume overload. This group of animals, together with an untreated fistula group and sham-operated group, subsequently underwent an in vivo functional assessment using a Millar pressure/volume conductance catheter at 2 wk postsurgery. Table 1 details the functional parameters for these three groups. When compared with sham-operated animals, LV, RV, and lung weights were significantly increased in both the treated and untreated AV fistula groups. Although no difference in heart rate was evident between any of the groups, cardiac output and stroke volume were increased to a similar extent in both the bosentan-treated and untreated fistula group. However, ejection fraction (end-diastolic volume/end-systolic volume/end diastolic volume), which was diminished in the untreated fistula group, was substantially preserved with bosentan treatment. The marked increase in LV end-systolic volume in the untreated fistula group relative to sham-operated values was significantly attenuated by bosentan. Likewise, the considerable increase in LV end-diastolic volume in the untreated fistula compared with that in the sham-operated group was also significantly attenuated by bosentan treatment (Table 1). Maximum first derivative of pressure ($dP/dt$) was moderately enhanced in both groups postfistula, whereas minimum $dP/dt$ was not markedly different among the groups.

![Fig. 1. Effect of bosentan (Bos) treatment on left ventricular (LV) mast cell density in sham-operated, untreated fistula (Fist), and bosentan-treated fistula rats. Values are reported as means ± SE. *$P = 0.05$ compared with sham; †$P = 0.05$ compared with untreated fistula.](image1)

![Fig. 2. Effect of bosentan treatment on matrix metalloproteinase-2 activity. Values are reported as means ± SE. *$P = 0.05$ compared with sham; †$P = 0.05$ compared with untreated fistula.](image2)

![Fig. 3. Effect of nonselective endothelin receptor blockade on LV myocardial collagen content. Values are reported as means ± SE. *$P = 0.05$ compared with sham; †$P = 0.05$ compared with untreated fistula.](image3)

![Fig. 4. ELISA analysis of endothelin-1 in LV tissue in sham-operated, fistula, and bosentan-treated fistula hearts at 1 and 5 day experimental end points. Values are reported as means ± SE. *$P = 0.05$ vs. sham; †$P = 0.05$ vs. time matched untreated fistula; #$P = 0.01$ for 1 vs. 5 day untreated fistula groups.](image4)
effects of nonselective (ETA and ETB) ET-1 receptor antago-

Fig. 5. ELISA analysis of TNF-α in LV tissue in sham-operated, fistula, and bosentan-treated fistula hearts at 1 and 5 day experimental end points. Values are reported as means ± SE. *P ≤ 0.01 vs. sham.

**DISCUSSION**

Previous work using the AV fistula model of chronic volume overload established a causal role for cardiac mast cells in the regulation of MMP activity (3, 8). Mast cell-mediated MMP activation, leading to degradation of the extracellular matrix and ventricular dilatation, are well-documented features in this model of heart failure (3, 6, 8, 9, 20). The stimulus initiating this mast cell-mediated remodeling is unknown; however, we recently demonstrated that ET-1 is capable of causing cardiac mast cell degranulation and the subsequent activation of MMPs ex vivo (31). Accordingly, the current study investigated the effects of nonselective (ETA and ETB) ET-1 receptor antagonism on the acute myocardial remodeling process associated with ventricular volume overload.

**Mast Cell Density Postfistula**

The significant, sustained increase in cardiac mast cell density in the first 5 days postfistula is consistent with our previous report (4). However, bosentan treatment effectively prevented the acute increase in myocardial mast cell density. Similar findings have been documented in a diabetic model, with bosentan preventing ET-1-mediated mast cell accumulation in the intestine (17). In their studies of ischemia-reperfusion, Frangogiannis et al. (15) reported an increased number of mast cells in the reperfused region of the heart. This led them to hypothesize that chemotaxis of circulating mast cell precursors may be responsible for the mast cell accumulation in the healing myocardium. However, another potential source of this increased mast cell density is the rapid maturation of immature, resident mast cells. We recently found chronic volume overload induced maturation/differentiation of cardiac mast cells in rats with an AV fistula (14). Four stages (I–IV) of mast cell maturation have been identified using differential staining of mast cells. Stages I and II are identified as being capable of mitosis, whereas stages III and IV are mitotically inactive. A significant decrease in immature cells (i.e., stages I and II), coupled with a marked increase in mature cardiac mast cells (stage III and IV), was observed within 24 h postfistula relative to sham-operated rats. The findings herein that bosentan prevented the fistula-induced increase in mast cell density suggest that ET-1 may stimulate maturation/differentiation of immature, resident cardiac mast cells. Accordingly, these are the first in vivo results to demonstrate responsiveness of cardiac mast cells to the endogenous endothelin system.

One mechanism by which ET-1 receptor antagonism could prevent increases in cardiac mast cell density or activity is through the prevention of oxidative stress in the myocardium. Extracellular oxidative stress has been implicated in mast cell activation following ischemia and reperfusion (18). The findings from that study showed corresponding increases in mast cell secretory products (i.e., histamine or TNF-α) and elevated myocardial oxidants. Furthermore, in vitro studies have identified intracellular reactive oxygen species as the initiator of mast cell cytokine expression and histamine release (2, 40, 44). Recent reports have demonstrated that treatment of ischemic myocardium with either selective (ETA, BQ-123) or nonselective (ETα/ETβ, bosentan) receptor antagonist significantly reduced catalase and superoxide dismutase activity as well as glutathione content (21, 36). Interestingly, NO was shown to possess an inhibitory influence over intracellular mast cell reactive oxygen species generation and subsequent recruitment of inflammatory mediators (33, 41). Taking into account the linkage of the ETβ receptor subtype to NO production and ET-1 influence over the oxidant/antioxidant balance in myocardial tissue (21, 36), it is reasonable to expect ET-1 receptor antagonism to inhibit mast cell activation.

**MMP Activation Postfistula**

Numerous studies have implicated a direct contribution of MMPs in the pathophysiological dilatation that occurs in the failing heart (22, 39). However, the findings here implicate ET-1 in causing the acute stimulation of cardiac mast cell-mediated activation of MMPs in chronic volume overload. The ability of bosentan to prevent the increase in mast cell density as well as the initial increase in MMP activity, together with our previous findings, establishes a pathway by which ET-1-mediated cardiac mast cell activation results in increased MMP activity (3, 4). Interestingly, a recent study demonstrated that ETβ-specific receptor antagonism was capable of preventing

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**Table 1. Comparison of body, LV, RV, and lung weights in 2 wk postsurgery sham-operated, untreated, and bosentan treated fistulas**

<table>
<thead>
<tr>
<th>Parameters, In Vivo Conductance Catheter</th>
<th>Sham-Operated</th>
<th>2 wk Untreated Fistula</th>
<th>2 wk Bosentan-Treated Fistula</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>297±4</td>
<td>304.7±5</td>
<td>315±8</td>
</tr>
<tr>
<td>LV wt, mg</td>
<td>635±12</td>
<td>870±23*</td>
<td>883±37*</td>
</tr>
<tr>
<td>RV wt, mg</td>
<td>174±5</td>
<td>280±7*</td>
<td>267±15*</td>
</tr>
<tr>
<td>Lung wt, mg</td>
<td>1,376±17</td>
<td>1,714±65*</td>
<td>1,737±68*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>350±11</td>
<td>356±8</td>
<td>348±5</td>
</tr>
<tr>
<td>Stroke volume, μl</td>
<td>168±12</td>
<td>239±17*</td>
<td>218±7</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>60±5</td>
<td>84±25*</td>
<td>75±3</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>57±3</td>
<td>39±2*</td>
<td>49±6</td>
</tr>
<tr>
<td>End-systolic volume, μl</td>
<td>124±17</td>
<td>312±30*</td>
<td>194±34†</td>
</tr>
<tr>
<td>End-diastolic volume, μl</td>
<td>279±24</td>
<td>514±35*</td>
<td>387±30†</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/s</td>
<td>8,195±571</td>
<td>10,275±446</td>
<td>10,510±909</td>
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<tr>
<td>dP/dtmax, mmHg/s</td>
<td>-7,387±497</td>
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<td>-8,257±1,299</td>
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</tbody>
</table>

Body, LV, RV, and lung wts are reported as means ± SD. Conductance catheter values are reported as means ± SE. Comparison of body, LV, RV, and lung wts in 2 wk postsurgery sham-operated, untreated, and bosentan-treated fistulas are shown. Systolic and diastolic parameters in 2 wk postsurgery sham-operated, untreated fistula, and bosentan-treated fistulas via in vivo conductance catheter are shown. *P ≤ 0.05 compared with sham; †P ≤ 0.05 compared with untreated fistula.
MMP activation following a myocardial infarction (37). Unfortunately, this study did not investigate the potential effect of ET\textsubscript{A} antagonism on mast cells. Another study by Deschamps et al. (12) demonstrated that ET-1 mediates increased interstitial membrane type 1-MMP activity induced in ischemia-reperfusion. However, in the present study the subsequent significant increase in MMP activity at 5 days postfistula points to alternative or redundant pathways capable of inducing myocardial remodeling that are eventually induced in response to chronic, sustained increases in myocardial stress. Clearly, the upregulation of neurohormonal pathways or the induction of oxidative stress can result from a variety of stimuli.

Myocardial Collagen Content Postfistula

The total levels of myocardial collagen were significantly decreased in untreated fistula groups at both 1 and 5 days. In contrast, treatment with bosentan prevented the decrease in CVF at both acute time points postfistula, despite MMP-2 activity becoming markedly elevated on day 5. This preservation of the extracellular matrix suggests that collagen degradation is not occurring despite the increase in MMP-2 activity. One limitation of the measurement of MMP activity using zymography is that this method specifically analyzes gelatinase activity and does not directly reflect collagenases that are involved in extracellular matrix degradation and LV remodeling. Initial cleavage of collagen by collagenase is necessary in order for gelatinases to complete degradation. Thus it is possible that other MMPs, including collagenases, may continue to be affected in the bosentan-treated AV fistula hearts (10, 12). Regardless, it would appear that this relative protection against adverse extracellular matrix remodeling persisted beyond the acute effects of ET-1 antagonism on MMP activation, given the attenuated LV chamber dilatation seen in the 14-day bosentan-treated group.

Our previous findings demonstrate that extracellular matrix synthesis and degradation is dynamic, with interstitial collagen density recovering rapidly after the initial degradation (23). In that study after collagen degradation peaked at 5 days postfistula, with CVF at its lowest by day 5 (61% below control), interstitial collagen then rebounded and slightly exceeded control levels at between 7 and 14 days (3). Accordingly, the resultant remodeling is dependent on a shift toward degradation that, if left unhindered, results in adverse myocardial remodeling consisting of an inadequate fibrillar collagen matrix, progressive ventricular dilatation and sphericization with wall thinning, and eventual congestive heart failure (23). Thus bosentan could have sufficiently delayed the loss of fibrillar collagen during the initial injury phase in response to volume overload, such that a balance in synthesis and degradation was achieved, which effectively attenuated further ventricular dilatation.

Neurohormonal Cytokine Axis

Recent debate as to the direct or indirect mechanism of action of neurohormones on the cells of the heart has fostered the concept of a neurohormonal-mediated inflammatory response in the initial adaptation of the stressed heart. Based on the findings between 1- and 5-day-treated and untreated groups in regard to LV mast cell density, MMP activity, and collagen degradation, we sought to investigate the potential link between ET-1-mediated neurohormonal regulation of remodeling and the induction of proinflammatory cytokines. Previous studies have documented an ET-1-induced upregulation of TNF-\alpha in mast cells (11, 27), and it should be noted that TNF-\alpha in the unstressed heart is predominantly localized in the mast cell (15, 18). Elevated ET-1 levels in the untreated fistula were matched with corresponding significant increase in myocardial TNF-\alpha at the 1-day time point. Also, the administration of bosentan to sham-operated animals resulted in elevated ET-1 levels (possibly due to limited clearance by the blockade of the ET\textsubscript{B} receptor), which was associated with a similar increase in TNF-\alpha. However, myocardial TNF-\alpha was still elevated on day 5 in the untreated fistula, whereas ET-1 levels were similar to that in bosentan-treated fistula hearts. This reduction in ET-1 levels in the untreated-fistula group on day 5 could possibly be accounted for by the similar findings of Maurer et al. (28) and Metzatine et al. (30), demonstrating that the ET-1 peptide was sensitive to chymase-mediated degradation. The sustained mast cell activation reflected by increased MMP activity would be expected to produce increased chymase levels at the 5-day time point in the fistula model (3, 4).

Effect of ET Receptor Antagonism on Postfistula Remodeling and LV Contractile Function

The extent of myocardial remodeling as reflected by increases in LV, RV, and lung weights was comparable in both the bosentan-treated and untreated-fistula groups after 2 wk of volume overload. However, the significant increase in LV end-diastolic volume in the untreated group was markedly attenuated in the bosentan-treated hearts. Thus, although most assessments of contractile function, including dP/dt and cardiac output, reflect the fistula groups remaining functionally compensated, this adverse myocardial remodeling in the untreated-fistula group produced a noticeable depression in ejection fraction. In contrast, the ejection fraction in the bosentan-treated group is reduced, but not significantly different, from the sham-operated control group. The efficacy of bosentan treatment in attenuating ventricular dilatation and maintaining ejection fraction is synonymous with preservation of contractile function. The beneficial role of mixed and selective ET-1 receptor antagonists in maintaining cardiovascular function in animal models and human heart failure has been intensely investigated. Originally, ET-1 was thought to have positive inotropic and chronotropic effects in vitro (16). This remains controversial, however, since a deleterious role for ET-1 has been observed in isolated cardiomyocytes chronically treated with ET-1 (45). These findings of ET-1-induced diminished contractile function are parallel with previously reported in vivo effects (26). Also consistent with our findings, several clinical trials [Endothelin Antagonism with Bosentan and Lowering Events (ENABLE), Endothelin A Receptor Antagonist Trial in Heart Failure (EARTH), Randomized Intravenous Tezosentan Study (RITZ-4), and Heart Failure ET\textsubscript{A} Receptor Blockade Trial (HEART)] have shown that systolic function and cardiac contractility are maintained by ET-1 receptor blockade, yet unfortunately morbidity and mortality were not improved (25, 34, 42). These studies evaluated patients with extensive myocardial remodeling (New York Heart Association class II to
IV) with the intent of ameliorating progressive deterioration of existing ventricular dysfunction. However, while our investigation clearly demonstrates that ET-1 contributes to the initiation of adverse remodeling in the early response of the heart to volume overload, a reversal of the advanced existing pathology by ET receptor inhibition is improbable. Nevertheless, a timely publication by Kelland and Webb (24) cautions against discounting endothelin antagonism as a viable treatment strategy. An assumption that is inherent to all studies investigating bosantan is the principle that this nonselective endothelin receptor antagonist effectively prevents ET-1-mediated signaling. However, our results support the possible potentiation of ET-1 effects resulting from blocking the clearance receptor and, thereby, increasing the endogenous concentration of this neurohormone. This “potentiation phenomenon” argument could actually shed light on the reason clinical trials with nonselective endothelin antagonists did not succeed and account for the apparent escape from inhibition seen in the current study.

In summary, nonselective ET-1 antagonism prevents the acute increases in cardiac mast cell density and MMP activation induced secondary to chronic volume overload. By preventing these events, ET-1 antagonism was efficacious in attenuating ventricular dilatation and limited the development of structural and functional deficits in the first 2 wk of chronic volume overload. Accordingly, these results are the first to demonstrate that cardiac mast cells are responsive to the endogenous endothelin system in vivo. Another novel finding from this study is that chronic nonspecific endothelin antagonism may inadvertently potentiate ET-1-mediated signaling.

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