Protective role of mast cells in homocysteine-induced cardiac remodeling


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Joseph, Jacob, Richard H. Kennedy, Sulochana Devi, Junru Wang, Liжа Joseph, and Martin Hauer-Jensen. Protective role of mast cells in homocysteine-induced cardiac remodeling. Am J Physiol Heart Circ Physiol 288: H2541–H2545, 2005. First published December 9, 2004; doi:10.1152/ajpheart.00806.2004.—Recent reports including those from our laboratories indicate that hyperhomocysteinemia (Hhe) is an independent risk factor for cardiac dysfunction and clinical heart failure. Mast cell accumulation is a prominent feature in our model of Hhe-induced cardiac dysfunction. Because mast cell-derived mediators can potentially attenuate cardiac remodeling, we investigated the possible protective role of mast cells in Hhe-induced cardiac remodeling using a mast cell-deficient rat model that in our recent report did not demonstrate any adverse cardiac function at younger age (6 mo) than mast cell-competent control animals. Mast cell-deficient (Ws/Ws) rats and mast cell-competent (+/+) littermate control animals (3 mo of age) were treated with a Hhe-inducing diet for 10 wk. Cardiac remodeling was assessed structurally utilizing histomorphometric methods and functionally using an isolated Langendorff-perfused heart preparation. The Hhe-inducing diet caused similar elevations of homocysteine levels in the two groups. Compared with Hhe +/+ rats, the Hhe Ws/Ws rats demonstrated strikingly exacerbated adverse cardiac remodeling and myocardial fibrosis. Cardiac function measurement showed worsened diastolic function in Hhe Ws/Ws rats compared with Hhe +/+ rats. The absence of mast cells strikingly exacerbates Hhe-induced adverse cardiac remodeling and diastolic dysfunction. These findings indicate a potential dual rather than sole deleterious role for mast cells in cardiac injury.

myocardial fibrosis; diastolic dysfunction; collagen

EPIDEMIOLOGICAL AND CLINICAL studies demonstrate an association between hyperhomocysteinemia (Hhe) and cardiovascular disease, specifically, coronary artery disease and stroke (7). Moreover, studies from our laboratory and the laboratories of others (10, 11, 19, 29) indicate that in addition to its purported atherothrombotic effects, Hhe is also a powerful stimulus for cardiac remodeling and dysfunction and clinical heart failure. Mast cell accumulation is one of the most important cellular features of Hhe-induced cardiac remodeling (10, 11) and accompanies significant myocardial fibrosis. Although initial studies have focused on the role of mast cells in promoting cardiac remodeling and dysfunction (3, 5), several mast cell-derived mediators have the potential to attenuate tissue remodeling and fibrosis. Hence, we examined the hypothesis that mast cells may be protective in Hhe-induced cardiac remodeling utilizing a mast cell-deficient rat model. Our results show conclusively that the absence of mast cells exacerbates Hhe-induced adverse cardiac remodeling and diastolic dysfunction. This protective role of mast cells indicates a potential dual role of mast cells in cardiac remodeling as opposed to a sole deleterious role.

MATERIALS AND METHODS

Animals. All procedures in this study were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences. Three-month-old mast cell-deficient (Ws/Ws) rats and normal littermate control animals (+/+; n = 10 rats/group; body wt, 300–325 g) were purchased from Japan SLC (Hamamatsu, Japan) and maintained in our institutional Division of Laboratory Animal Medicine on a 12:12 light-dark cycle with free access to diet and water. The Ws/Ws rats have a 12-base deletion in the tyrosine kinase domain of the c-kit receptor and, for all practical purposes, are devoid of mast cells in various organs including the heart. Their phenotype is otherwise normal except for white spotting of the skin and a mild macrocytic anemia that is spontaneously ameliorated by 10 wk of age (21). These rats do not exhibit the severe anemia seen in mast cell-deficient mice. The animals were randomized into two groups after acclimatization and were fed one of two purified amino acid diets (Harlan Teklad; Indianapolis, IN) for 10 wk, namely, an amino acid-defined control or a homocystine-supplemented Hhe-inducing diet (10, 11). After the 10-wk treatment period, arterial blood pressure was measured in anesthetized animals by carotid artery cannulation, body weight was obtained, blood was drawn for measurement of plasma homocysteine, and hearts were procured for functional and structural analysis and assessment of collagen content. In addition, age-matched +/+ and Ws/Ws rats (n = 3 or 4 per group) were killed for histomorphometric measurements.

Langendorff-perfused hearts. Cardiac function measurements were performed as previously described (10, 11). Briefly, hearts were perfused via the aorta with an oxygenated Krebs-Henseleit solution (37°C) with the flow rate set at 8.0 ml·g heart−1·min−1; coronary pressure was monitored continuously. Ventricles were paced electrically at 250 beats/min, and intraventricular pressure was measured by a fluid-filled balloon catheter connected to a pressure transducer. Contractile function was monitored by measuring peak pressure, +dp/dt max, and −dp/dt max at various preload balloon volumes. In addition to a polygraph recording, all data were digitized and analyzed with the use of CODAS acquisition and analysis software (DataQ Instruments; Akron, OH). The response of coronary pressure to adenosine infusion was determined at the end of the experiment as a measure of coronary flow reserve.

Histomorphometric analysis. Coronal sections of ventricular myocardium (at the equator of the heart) were fixed in 10% neutral-buffered formalin and embedded in paraffin. Serial sections (5 μm) including both left and right ventricles were stained with hematoxylin

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and eosin for examining myocyte size, picrosirius red for identifying fibrillar collagen, and toluidine blue for signifying mast cells. Myocyte size, coronary arteriolar remodeling including perivascular collagen, interstitial collagen volume fraction, and total myocardial mast cell number were estimated as described previously (10, 11).

**Assessment of total collagen by Sirius red/fast green spectrophotometry.** The relative content of total collagen was measured spectrophotometrically by a method adapted from Junqueira et al. (12) and used in our prior studies (10). This method is based on the preferential staining of fibrillar collagen with Sirius red and noncollagenous protein with fast green. The collagen content determined by this method correlates well with fibrosis as determined by morphometry (13, 15). Briefly, paraffin-embedded tissue was cut into 15-μm sections, and deparaffinized sections were treated with a saturated solution of picric acid in distilled water that contained 0.1% fast green FCF and 0.1% Sirius red F3BA. The dyes were then eluted with methanol, and absorbance values were measured in a spectrophotometer at wavelengths of 540 and 605 nm (corresponding to maximum absorbance values of Sirius red and fast green, respectively). An estimate of collagen content, expressed as a percentage of total protein, was obtained using a previously described formula (10, 12).

**Statistical analysis.** Data were evaluated by ANOVA with a Student-Newman-Keuls post hoc test or by t-test as appropriate using SigmaStat software (SPSS; Chicago, IL). The criterion for significance was a P value of <0.05. Data are reported as means ± SE.

**RESULTS**

After 10 wk of Hhe, homocysteine levels rose similarly in the moderate-Hhe range (15–30 μmol/l) in both Ws/Ws and +/- control animals. There were no significant differences in body weight, heart weight, or heart weight-to-body weight ratios (Table 1). Systolic and diastolic blood pressures (measured while rats were under anesthesia at the end of the 10-wk dietary treatment period) were significantly lower in Ws/Ws rats than in control animals. Homocysteine levels were in the normal range in age-matched +/- and Ws/Ws rats fed normal diet (+/-, 7.85 ± 2.4; Ws/Ws, 8.3 ± 2.5 μM).

Table 2 shows cardiac remodeling parameters in both groups. There were significant increases in coronary arteriolar wall thickness, perivascular collagen, and interstitial collagen measurements in the Ws/Ws group compared with the +/- animals; there was no change in myocyte transverse diameter (+/+, 20.0 ± 0.7; Ws/Ws, 20.5 ± 1.6 μM). Figure 1 shows representative left ventricular sections from Ws/Ws and +/- rats. Coronary arteriolar wall thickening was secondary to vascular smooth muscle cell accumulation as indicated by immunostaining for α-smooth muscle actin. Spectrophotometric assessment of the collagen-to-total protein ratio in the entire myocardium by the Sirius red elution technique (10) confirmed the quantitative histological studies as shown in Table 2. Interestingly, these changes developed despite a lower blood pressure in the Ws/Ws group. Age-matched mast cell-competent and -deficient rats on normal diets showed no difference in interstitial collagen, although mean values in these groups were significantly lower than both Hhe groups (+/+, 0.60 ± 0.02; Ws/Ws, 0.63 ± 0.04%).

Figure 2A shows the effects of Hhe on diastolic function in the Langendorff-perfused heart preparation. There was a significant upward and leftward shift of the diastolic pressure-volume curve in the Ws/Ws group compared with control animals, which indicates diastolic dysfunction. The rates of myocardial relaxation (~dP/dtmax) were similar in the two groups (+/+, 1,441 ± 172; Ws/Ws, 1,282 ± 106 mmHg/s), which indicates that the difference in diastolic function resulted from a decrease in myocardial compliance (6). There were no significant differences in developed pressure (Fig. 2B) or +dP/dtmax (+/+, 2,634 ± 189; Ws/Ws, 2,385 ± 110 mmHg/s) between the two groups.

**DISCUSSION**

Mast cells accumulate in areas of tissue injury and repair including in the myocardium. The precise role of mast cells in cardiac remodeling has not been well defined, because mast cell mediators can have a positive or negative effect on molecular mechanisms of cardiac remodeling. Our present study demonstrates that Hhe-induced cardiac remodeling and diastolic dysfunction is significantly worsened by the absence of mast cells. Therefore, our results strongly suggest a protective role for mast cells in Hhe-induced cardiac remodeling. These findings in concert with prior studies showing a deleterious role for mast cells in cardiac remodeling indicate a potential dual role for mast cells. Because Hhe is highly prevalent in the general population and has been linked to clinical heart failure, and because mast cell function is readily modulated, these results may have important clinical implications for patients with Hhe.

A role for mast cells has been implicated in a variety of disease states associated with abnormal tissue remodeling (9, 22). Mast cells contain a wide array of mediators including proteases, cytokines, proteoglycans, and arachidonic acid metabolites that influence cellular function during injury and repair and can be anti- or profibrogenic (18). Mast cell-derived chymase may exert antifibrogenic effects by directly activating collagen degrading pro-matrix metalloproteinase (MMP)-1 to its active form (24), whereas tryptase can activate pro-MMP-3 and initiate a cascade of activation of multiple MMPs (6).
Chancey et al. (5) showed that chemically induced degranulation of mast cells in Langendorff-perfused rat heart preparations led to activation of MMP-2. On the other hand, various mast cell-derived mediators promote fibroblast proliferation and collagen production (15, 18). Mast cell mediators, specifically chymase, prevent smooth muscle cell proliferation and collagen production and promote apoptosis (30, 31). Hence, they may decrease medial hypertrophy and possibly perivascular fibrosis of coronary arterioles that are seen in many forms of cardiac remodeling including Hhe. In summary, present knowledge regarding mast cells and tissue remodeling indicates that mast cell mediators have the potential to both promote as well as interfere with tissue remodeling.

Normal myocardium contains mast cells in the perivascular space and between myocytes. Panizo et al. (22) demonstrated the association of mast cell accumulation with extensive perivascular and interstitial fibrosis in hypertensive rats. Hara et al. (9) showed that the transition from compensated pressure overload-induced cardiac hypertrophy to cardiac failure is decreased in a mast cell-deficient mouse model (that has severe anemia) compared with control animals. Boerma et al. (2), in their histopathological study of rat hearts subjected to irradiation, observed that mast cell density correlated with worsened myocardial injury. A recent study done by Akgul et al. (1) examined the role of mast cells in patients with end-stage ischemic cardiomyopathy and the effect of unloading the ventricles with mechanical ventricular support. They demonstrated that although mast cell numbers were increased in failing myocardium compared with control hearts, there was a secondary increase in chymase-negative mast cells associated with a decrease in myocardial fibrosis. The same group published a recent article (27) examining the effects of these

Fig. 1. Representative left ventricular sections. Picrosirius-stained sections from mast cell-competent control (+/+) (A) and mast cell-deficient (Ws/Ws; B) rats show greater perivascular collagen accumulation and arteriolar wall thickening in the Ws/Ws group. Similarly stained sections from the +/- (C) and Ws/Ws (D) groups show increased interstitial collagen in the Ws/Ws group compared with control animals. Toluidine blue-stained sections of left ventricle from +/- (E) and Ws/Ws (F) groups show an absence of mast cells in the Ws/Ws animals (arrows indicate mast cells). α-Smooth muscle actin immunostaining of representative vessels shows increased medial thickening in the Ws/Ws (H) compared with the +/+ group (G). See Table 2 for total mast cell numbers per left ventricle. Original magnification: A-F, ×200; G and H, ×400.

Fig. 2. Effects of balloon volume on diastolic left intraventricular pressure (A) and left intraventricular developed pressure (peak systolic-diastolic; B) in Langendorff-perfused hearts isolated from +/+ control and Ws/Ws rats (n = 5 rats/group). Values for diastolic pressure (A) were not detectable at balloon volumes <100 μL. Mast cell-deficient rats were significantly different from mast cell-competent control animals (ANOVA; P < 0.05). There were no significant differences in developed pressure between the groups (B). Vertical bars represent SE.
phenotypically different mast cells on fibroblast function. This study showed that mast cells from failing human myocardium promoted collagen synthesis by cultured fibroblasts, whereas the phenotypically altered (chymase-negative) mast cells from myocardia unloaded by mechanical ventricular assistance decreased collagen synthesis. Our results support these observations indicating that mast cells can play an antifibrotic role and decrease myocardial fibrosis. Additionally supporting our observation of a protective role for mast cells is the recent report (20) that renal interstitial fibrosis in response to puromycin administration was worse in mast cell-deficient rats compared with mast cell-competent controls.

We utilized the mast cell-deficient rat model (21), which is similar to the mast cell-deficient mouse model (9, 16) in that there is the same degree of mast cell deficiency, with a total absence of mast cells in internal organs and <1% of the number of mast cells in skin of wild-type animals. Both the mouse and the rat models are the result of spontaneous mutations in the tyrosine kinase domains of the mast cell receptor c-kit, and although the mutation is in the same domain, there appear to be minor but important phenotypic differences. Most importantly, mast cell-deficient mice have severe anemia, whereas mast cell-deficient rats have a mild anemia that is ameliorated by 10 wk of age. Our recent report on cardiac function in this model (14) indicates that there are no differences in cardiac function at the younger age comparable to this study, whereas the absence of mast cells worsens age-associated decreases in diastolic function. Based on the above report and our results from this study, it is possible that resident cardiac mast cells are important for preventing age-related adverse changes in myocardium and in attenuating cardiac remodeling in response to injury.

The effects of Hhe on blood pressure are controversial. Animal studies with methionine-supplemented diets have shown that Hhe results in hypertension (23). However, our data with homocystine-supplemented diets to induce Hhe did not reveal a hypertensive effect of Hhe in either spontaneously hypertensive or Wistar-Kyoto rats (10, 11). Supporting this premise are recent data from the Framingham Heart Study (28), which show that there is no relation between Hhe and hypertension in the general population. Hence, our model of diet-induced Hhe allows for examination of the effects of Hhe on cardiac remodeling independent of the proposed hypertensive effects.

Mast cells may also be expected to influence blood pressure. Mast cell chymase-mediated ANG II generation has been shown to be involved in blood pressure regulation (16). In addition, mast cells were recently shown to be the major source of renin in myocardium (26). Interestingly, rat mast cell chymase has both ANG II-generating (α-chymase) and degrading (β-chymase) properties and thereby influences the balance between ANG II production and degradation (4, 25). Lundequist et al. (17) have also demonstrated that the net effect of mast cells on ANG II levels depends on relative activity of mouse mast cell protease 4 (the human analog of chymase), which catalyzes ANG II generation, and carboxypeptidase A, which promotes ANG II degradation. Because in our study, mast cell-deficient rats exposed to Hhe had significantly lower systolic and diastolic blood pressures than +/− control animals, these results may suggest an interaction of mast cells, the renin-angiotensin system, and homocysteine in the maintenance of normal blood pressure. This intriguing possibility needs to be investigated.

In conclusion, our data demonstrate that mast cells are protective against Hhe-induced cardiac remodeling, in contrast to the solely deleterious role postulated for mast cells in cardiac remodeling. Additional studies will address the precise mechanisms of the protective role of mast cells in cardiac remodeling and the potential phenotypical changes that may determine the balance between the protective or deleterious roles of mast cells in cardiac remodeling.

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