Cardiac mast cell-mediated activation of gelatinase and alteration of ventricular diastolic function

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MATERIALS AND METHODS

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Cardiac mast cells have been implicated in the pathophysiology of several cardiovascular disorders. In fact, increased numbers of mast cells have been reported in human hearts with end-stage cardiomyopathy (30, 31) and in animal models of hypertension (28, 29), myocardial infarction (10, 21), and volume overload secondary to infrarenal aortocaval (AV) fistula and mitral regurgitation (8, 13). Mast cells store and release a variety of biologically active mediators including tumor necrosis factor-α and proteases such as tryptase, chymase, and stromelysin (25, 26, 43). Furthermore, it has been demonstrated that enzymes from noncardiac mast cells are capable of in vitro activation of matrix metalloproteinases (MMPs) (22, 26, 40). While MMPs function in the normal turnover of the extracellular matrix (ECM), they are also involved in the myoccardial remodeling contributing to congestive heart failure and cardiomyopathy (4, 5, 12, 16, 37, 42). Mast cell density in the normal heart is relatively low, ranging between 1.5 and 5.3 cells/mm² (10, 31, 34), and it is not known if the degranulation of this resident cardiac mast cell population would have an impact on MMP activity. Therefore, the goal of this study was to determine whether cardiac mast cells are present in sufficient numbers in the normal heart to affect MMP activity if degranulated. To this end, compound 48/80 was used to induce degranulation of cardiac mast cells. Compound 48/80 is a well-documented mast cell-degranulating agent that has been shown to liberate histamine from mast cells while having no effect on other histamine-containing cells such as macrophages and lymphocytes (44). The effects of cardiac mast cell degranulation on plasma histamine levels, ventricular function, myocardial water content, and coronary flow were also assessed. The results indicate that chemically induced degranulation of cardiac mast cells leads to an increase in MMP activity, collagen degradation, and altered ventricular diastolic function.

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

Experimental design. The effects of cardiac mast cell degranulation on coronary flow and ventricular function were determined in 10 hearts using an isolated, blood-perfused Langendorff preparation as previously described (3). Briefly, the ascending thoracic aorta in the anesthetized rat was cannulated for continuous retrograde perfusion of the heart...
via an apparatus consisting of a pressurized perfusion reservoir (105–105 mmHg) and a collection reservoir connected in circuit with a support rat. The extirpated heart attached to the perfusion apparatus was perfused with oxygenated blood obtained from the support rat via a carotid artery catheter. The coronary venous effluent was collected in a reservoir and returned to the support rat via a jugular vein catheter. After removal of the left atrium, a latex balloon was inserted into the ventricular chamber to obtain left ventricular (LV) function. The proximal end of the balloon was connected via a short piece of tubing to a three-way stopcock, which was used to adjust the balloon volume through one port while measuring LV pressure using a pressure transducer (Transpac IV, Abbott Critical Care Systems; North Chicago, IL) attached to the remaining port. Once the heart developed stable isovolumetric contractions, the balloon volume that produced an LV end-diastolic pressure (LVEDP) of 0 mmHg (V₀) was determined. The volume in the balloon was then increased in 10- to 20-μl increments until an LVEDP of 25 mmHg was attained, and the end-diastolic and peak isovolumetric pressures were recorded after each increase in balloon volume. Three or four data sets were recorded to ensure that the preparation was stable. These data sets were used to construct pressure-volume (P-V) curves fitted to a third-order linear regression (SigmaPlot, SPSS; Chicago, IL). The volumes corresponding to pressure values at 2.5-mmHg increments were determined and averaged to obtain the volumes corresponding to pressure values at 2.5-mmHg increments until an LVEDP of 25 mmHg was attained. The volume in the balloon was then increased in 10- to 20-μl increments until an LVEDP of 25 mmHg was attained, and the end-diastolic and peak isovolumetric pressures were recorded after each increase in balloon volume. Three or four data sets were recorded to ensure that the preparation was stable. These data sets were used to construct pressure-volume (P-V) curves fitted to a third-order linear regression (SigmaPlot, SPSS; Chicago, IL). The volumes corresponding to pressure values at 2.5-mmHg increments were determined and averaged to obtain the final P-V relationship for each group. These P-V relationships were obtained for each heart before and 30 min after an infusion of compound 48/80. Administration of 1 ml of 0.9% sterile saline containing 7.2 mg/ml compound 48/80 (Sigma; St. Louis, MO) to the isolated heart was accomplished by introducing the solution into the pressurized perfusion reservoir, allowing the solution to mix with the reservoir blood and perfuse through the beating heart. To assess the effect of mast cell degranulation on coronary flow, coronary venous effluent was collected for 3 min immediately before and after compound 48/80 administration. The blood containing compound 48/80 was not returned to the support rat. Control hearts underwent the same blood-perfused Langendorff procedure; however, saline, not compound 48/80, was introduced into the perfusion reservoir.

After the functional studies were completed, the atria and great vessels were removed, and the LV (plus septum) and right ventricle were separated. A complete transmural section of the LV at midventricle was placed in buffered formalin for fixation, and the remainder was minced into ~1-mm cubes, snap-frozen in liquid nitrogen, and stored at ~80°C. Portions of this frozen LV tissue were used to determine the wet-to-dry weight ratio of both control and compound 48/80-treated hearts to assess differences in percent myocardial water (%H₂O). The formalin-fixed tissue was processed for routine histopathology, and 5-μm-thick paraffin-embedded sections were stained with either toluidine blue for visualization of mast cell morphology or picrosirius red for quantification of collagen volume fraction (CVF), presented as the percentage of collagen area relative to total myocardial area as previously described (37).

**MMP activity.** MMP activity in cardiac tissue extracts was analyzed using gelatin zymography performed by standard procedures using a SDS-PAGE matrix containing gelatin (1 mg/ml) (4, 15). Briefly, samples (10–20 μg/lane) were electrophoresed on a 10% gel at 20°C. After electrophoresis, the gels were washed to remove the SDS and permit enzyme renaturation and incubated in substrate buffer on a shaker for 18 h at 37°C. The gels were then stained with 0.1% Coomassie brilliant blue and destained in distilled deionized H₂O, and the activity of the bands was quantified by densitometry (ImageQuant, Molecular Dynamics). All of the zymograms 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). 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, and, to compare results from different gels, a single extract from the same control heart was used as a standard on all gels. The activity of the lytic bands in the other lanes of the gel was expressed as a percentage of this standard’s activity. Once normalized in this fashion, the percent activities from hearts belonging to each group (i.e., control and compound 48/80) were averaged.

**Measurement of plasma histamine.** In a separate set of experiments, hearts from four animals were isolated and perfused as described above for administration of 7.2 mg compound 48/80; however, functional studies were not performed. In these hearts, the coronary effluent was collected before and after compound 48/80 administration as previously described. In addition to %H₂O and MMP activity, these hearts were also assayed for plasma histamine concentration using a fluorometric technique to assess histamine content of the effluent as a marker of mast cell degranulation (36).

**Statistical analysis.** Statistical analyses were performed with Systat 9.0 software (SPSS). All group data are expressed as means ± SD. Grouped data comparisons were made by one-way ANOVA, with intergroup comparisons analyzed using Bonferroni post hoc testing. Statistical significance was taken to be P < 0.05. P-V curves were analyzed using a two-factor repeated-measures ANOVA.

**RESULTS**

In contrast to the normal appearance of mast cells in control hearts, evidence of extensive mast cell degranulation in the compound 48/80-treated hearts was found upon histological examination (Fig. 1). Consistent with this finding, myocardial %H₂O in hearts exposed to compound 48/80 was significantly increased above that of control hearts (80.1 ± 3.4% vs. 77.4 ± 1.08%, respectively; P ≤ 0.03; Fig. 2).

Similarly, mast cell degranulation resulted in the subsequent activation of MMPs. Figure 3A shows a representative zymography gel demonstrating a 21% decrease in latent MMP-2 relative to control hearts after compound 48/80-induced mast cell degranulation, whereas compound 48/80-treated hearts had a corresponding 126% increase in active MMP-2 above that of control hearts. These average relative changes in latent and active MMP-2 between control and compound 48/80-treated hearts are summarized in Fig. 3B. Mast cell degranulation and the subsequent increase in MMP activity also produced a significant reduction in CVF, with CVF being decreased below control in all compound 48/80-treated hearts (0.46 ± 0.10% vs. 0.97 ± 0.33%, P = 0.001).

After compound 48/80 infusion, coronary flow increased in 7 of 10 animals in which this parameter was measured (Fig. 4). The compound 48/80-induced increase in coronary flow observed in these seven animals ranged from 8% to 196% of baseline flow with a mean increase of 73%. Although these changes in cor-
Coronary flow did not attain significance, the increase (2.56 ± 1.07 vs. 3.28 ± 1.67 ml/min before and after compound 48/80, respectively; P = 0.27) reflects the vasodilatory effects of histamine released by degranulating mast cells. Concurrent with the increase in coronary flow, histamine levels in the coronary venous effluent increased 15% within 6 min after administration of compound 48/80 (from 69.3 ± 5.3 to 79.8 ± 3.7 ng/ml, P ≤ 0.02).

The average LVEDP-LV end-diastolic volume (LVEDV) relationships before and after compound 48/80 are depicted in Fig. 5. Although LVEDV at a pressure of 0 mmHg (V₀) before and after compound 48/80 increased (277 ± 48 vs. 309 ± 71 μl, P ≥ 0.09), this difference did not reach the level of statistical significance. Myocardial compliance, assessed by the volume required to increase LVEDP from 0 to 25 mmHg, was not different between groups (112.7 ± 48.7 vs. 104.4 ± 44.5 μl before and after compound 48/80, respectively, P ≥ 0.20). These data indicate an essentially parallel shift to the right, consistent with modest LV dilatation, despite the presence of significant myocardial edema after compound 48/80 administration. This rightward shift after compound 48/80 was observed in 8 of 10 hearts. Of the two hearts that did not develop this rightward shift, one heart showed no change from basal measurements and the other heart had a very slight parallel shift to the left after compound 48/80. Consistent with these hearts being relatively stiffer, myocardial %H₂O in these hearts averaged 84.7%, which was markedly higher than the group average.

The effects of mast cell degranulation on systolic function were assessed using the slope of the peak isovolumetric pressure-LVEDV (Pₘₐₓ-V) relationship. This measurement has previously been demonstrated...
to be an accurate index of LV contractility (39) and was calculated as described elsewhere (3, 4). The relationship between $P_{\text{max}}$ and LVEDV was highly linear, as evidenced by correlation coefficients that were $>0.94$ in all hearts. The slopes of the $P_{\text{max}}$-V relationships for hearts before and after compound 48/80 were not significantly different ($0.806 \pm 0.234$ vs. $0.916 \pm 0.341$, respectively, $P \geq 0.49$). However, a transient average increase of 53% (range of 5–146% increase) in $P_{\text{max}}$ at a constant LVEDV occurred after administration of compound 48/80 but had returned to baseline values within 30 min after compound 48/80.

**DISCUSSION**

While it is known that mast cell numbers are increased in diseased human hearts (21, 30, 31) and in various animal models of cardiovascular disease (8, 10, 13, 29), the role of mast cells in myocardial pathophysiology has yet to be elucidated. We previously reported herein are significant in that they for the first time clearly establish that the relatively small number of mast cells present in the myocardium represent an in vivo element whose secretory products are capable of activating cardiac MMPs.

Compound 48/80 induced extensive mast cell degranulation and led to increased coronary flow in the majority of the hearts studied. Histamine is known to be a potent vasodilator (11), and the potential pathological role of histamine in relation to the heart has been the subject of extensive study (11, 19, 20). Accordingly, histamine has long been thought to be involved in the progression of adriamycin-induced cardiomyopathy (2) and has also been shown to increase vascular permeability and cause cardiac edema (23, 32). As mast cells are the principle source of preformed histamine in tissues (6, 25), it is not unexpected that degranulation of mast cells would cause histamine release, leading to coronary vasodilation and increased coronary flow. Confirmation of histamine release as a result of compound 48/80 exposure was demonstrated by the nearly complete degranulation of virtually all cardiac mast cells, together with a significant increase in plasma histamine levels in the coronary effluent occurring in the first 6 min after exposure to compound 48/80. Likewise, the observation of significant edema in compound 48/80-treated hearts is consistent with the previous qualitative findings of Dvorak (9), indicating that mast cell degranulation is accompanied by histological evidence of edema in human hearts.

One observation from this study that on the surface would be considered to be unusual was that administration of compound 48/80 produced a parallel shift to the right in the P-V relationship despite the concurrent presence of significant myocardial edema. This is in contrast to several previous studies that have demonstrated that myocardial edema comparable to that occurring in this study typically results in a marked nonparallel leftward shift in the P-V relationship (1, 7, 33, 38). For example, Cross et al. (7) found that decreased ventricular distensibility in dogs was proportional to the extent of fluid accumulation, with abnormal LV diastolic pressures occurring when the edematous accumulation reached 4–5% of heart weight. Similarly, Amirhamzeh et al. (1) found that a 4.5% increase in myocardial water content in rat hearts resulted in a nonparallel leftward shift in the P-V relationship. Therefore, it would appear that the expected increase in diastolic stiffness due to edema induced by treatment with compound 48/80 was offset by ventricular dilatation and increased myocardial compliance secondary to significant MMP activation and the accompanying degradation of the interstitial collagen network. An interesting observation is that the extent of LV dilatation occurring within 30 min after compound 48/80 in the present study approached 55% of the

**Fig. 4.** Demonstration of the change in coronary flow induced after infusion of compound 48/80.

**Fig. 5.** Average left ventricular end-diastolic pressure-end-diastolic volume relationships for hearts before and after compound 48/80.
increase in LV volume occurring after 1 wk of chronic volume overload in the AV fistula model (3). Furthermore, the significant LV dilatation postfistula has also been shown to be associated with a 40% decrease in interstitial collagen (14). Accordingly, the finding that LV dilatation is occurring in the compound 48/80-treated hearts despite significant myocardial edema is indicative of rapidly induced changes in the ECM, similar to that occurring in animal models of congestive heart failure.

That significant MMP activation can be induced by cardiac mast cell degranulation represents an important new concept. These findings are also novel in that they identify mast cells as potential regulators of a mechanism contributing to the adverse cardiac remodeling associated with heart failure. Ventricular diastolic stiffness is known to be dependent on the integrity of the extracellular collagen matrix (17), with dilation of the ventricle being associated with collagen degradation (14). Several studies have identified MMPs as being responsible for collagen degradation. Rapid activation of MMPs associated with subsequent degradation of the fibrillar collagen matrix in the heart has been shown to precede LV dilatation in the AV fistula model (14). Increased MMP activity has also been observed soon after initiation of rapid pacing in the pig (37) and after myocardial infarction in rats and pigs (35, 41). In each of these studies, increased MMP activity resulted in disruption of the collagen matrix as measured by either a reduction in CVF or hydroxyproline. Other studies have also correlated MMP activity with the onset of collagen degradation and ventricular dilatation associated with cardiomyopathy and heart failure (14, 42). However, none of these studies have identified or even speculated as to how this activation of MMPs occurs. Zymographic analysis of those hearts exposed to compound 48/80 revealed significant increases in levels of active MMP-2, with a corresponding decrease in latent MMP-2 compared with control hearts. Thus, whereas mast cell secretory products such as trypsin and stromelysin (MMP-3) have previously been shown to be potent activators of MMPs in vitro (26), this is the first study to show direct evidence of mast cell-induced MMP activation in the intact, albeit isolated, heart.

Degradation of the ECM after marked activation of MMPs can have almost immediate effects on diastolic stiffness. This was demonstrated by two previous studies in which bacterial collagenase was used to alter the functional characteristics of isolated hearts. O’Brien and Moore (27) demonstrated that a 163-min incubation of rabbit hearts with collagenase resulted in increased diastensibility of the ventricle, as evidenced by a rightward shift of the P-V relationship. MacKenna et al. (24) obtained similar results in isolated rat hearts perfused with bacterial collagenase. They demonstrated a parallel rightward shift of the P-V relationship and a 36% decrease in CVF after bacterial collagenase perfusion. The significant rightward shift of the P-V relationship after collagenase perfusion in this study was progressive, reaching a maximum after 60 min when LV volume was increased by an average of 55 μl. In the present study, we observed a comparable decrease in CVF of 52% in hearts treated with compound 48/80, although the rightward shift in the P-V relationship induced by compound 48/80 was only one-half of that reported by MacKenna et al. (average increase in LV volume of 28 ± 3 μl). The similar rightward shifts in the P-V relationship reported herein and in the aforementioned studies are consistent with extensive degradation of the ECM by MMPs, with the rapidity of this process being evidenced by alteration of the P-V relationship within minutes.

There are several factors that could explain why the rightward shift of the P-V relationship did not achieve significance in this study. First, degradation of the ECM by MMPs is progressive and time dependent. The endogenous activation of MMPs in the heart may have been unable to digest the ECM sufficiently to be manifested in a significant rightward shift in only 30 min. Second, the bacterial collagenase that was used in the aforementioned studies (24, 27) is highly concentrated and also contains other nonspecific proteases. In contrast, our preparation only allowed for the release of specific mast cell proteases and activation of endogenous MMPs. Third, perfusion with bacterial collagenase has not been found to result in the degree of edema that was found in the present study. Degranulation of mast cells with compound 48/80 resulted in notable histamine release and significant myocardial edema, which had offsetting effects on the rightward shift in the P-V relationship. Finally, as opposed to bacterial collagenase incubation or continuous bacterial collagenase perfusion, our study employed a single bolus of compound 48/80 to determine the acute effects of mast cell degranulation in the isolated heart. Regardless, all the animals that received compound 48/80 had significant MMP-2 activation and a significant reduction in collagen density as assessed by CVF, and the majority of the animals that underwent functional studies showed modest LV dilatation after compound 48/80 despite the presence of myocardial edema.

In summary, compound 48/80-induced mast cell degranulation caused cardiac edema in beating, blood-perfused hearts. In addition to increased coronary flow, alterations in ventricular function were also observed. Perhaps most surprising was the modest ventricular dilatation occurring after compound 48/80. Given the extent of myocardial edema in these hearts, they should have been stiffer rather than more compliant. However, this observation is likely explained by an offsetting increase in compliance secondary to significant increases in MMP activation and accompanying degradation of the ECM. This is also the first demonstration that MMP activation in the intact heart may be due to mast cell degranulation. Furthermore, we propose that rapid MMP activation mediated by cardiac mast cell degranulation is likely to contribute to
the adverse ventricular remodeling contributing to the development of congestive heart failure. We therefore conclude that mast cell density in the normal heart is sufficient to produce rapid activation of MMPs, degradation of the ECM, and alterations in ventricular function if suddenly degranulated.

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