

Correlation Between Mast Cell Density and Myocardial Fibrosis in Congestive Heart Failure Patients

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ABSTRACT

Besides the well-established role of mast cells in allergic reactions as an important source of vasoactive and proinflammatory products, they have been related to tissue fibrosis and remodelling processes. In a heart failure (HF) animal model, mast cells were shown to synthesize transforming growth factor $\beta 1$ and basic fibroblast growth factor in myocardial tissue and were localized to an area with fibrosis. Our objective was to quantify mast cell density in left ventricles from patients with congestive heart failure who were candidates for transplantation and to analyze whether they showed a correlation with the fibrosis level of the same area.

Methods. We obtained myocardial biopsies from 20 patients with end-stage HF secondary to idiopathic dilated cardiomyopathy (iDCM) undergoing heart transplantation and 15 controls (donors without cardiopathy). Mast cells were detected by immunohistochemistry with a human mast cell chymase antibody and fibrosis levels measured with picrosirius red staining of collagen fibrils with later quantification by morphometry.

Results. The patients mean age was 51 ± 3 years. Fibrosis levels in the myocardial sections from patients with congestive HF was three-fold higher than those in control myocardium ($12.41 \pm 1.7\%$ vs $3.98 \pm 0.63\%$, $P < .001$). Mast cell density correlated with the collagen fraction and could be fitted to a linear regression curve: collagen fraction = $0.78 + 0.05$ mast cell density ($n = 33$, $P < .005$, $R^2 = 0.28$).

Conclusion. The elevated collagen fraction present in failing hearts may be the cause of increased stiffness and loss of elasticity that is detected in patients with end-stage HF. Due to the mast cells capacity to synthesize vasoactive and fibrogenic products and the correlation between their density and fibrosis levels, they probably play a role in the ventricular remodelling in HF.

INCREASED DEPOSITION of collagen fibers, together with left ventricular dilatation, have been documented in the ventricular remodelling that leads to heart failure (HF). The increased collagen in HF is most probably due to a disturbed balance between its synthesis and degradation. In animal models of cardiomyopathy, such as the Syrian hamster, there is higher expression of collagens I and III in the last stages of cardiomyopathy.¹ Mast cells have an important role in allergic reactions, but there is growing evidence that they also play a role in chronic inflammatory reactions as well as in tissue remodelling.² Mast cells contain a variety of biological mediators such as histamine, serotonin, heparin, and tryptase and chymase proteins. They are already synthesized and stored in the cell ready to

be released upon activation. In other cases, such as prostaglandin D₂ and leukotriene C₄, they are synthesized when the mast cell is activated. Mast cells also produce a myriad

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Supported by grants from Sociedad Española de Cardiología (SEC) and from Fondo de Investigación Sanitaria U-2004-FS040534-O, Redes Temáticas V-2003-REDC01-O.

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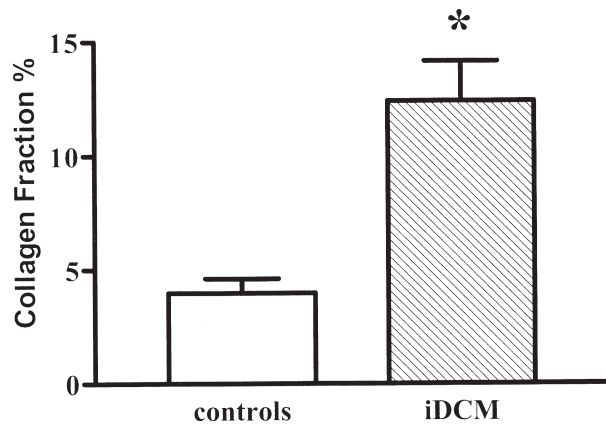


Fig 1. Collagen fraction increase in HF. Collagen fraction in control ventricles (controls, white bar) and in ventricles from idiopathic dilated cardiomyopathy patients (iDCM, stripped bar). * $P < .001$.

of cytokines and growth factors, such as interleukin (IL)-1, IL-3, IL-4, IL-5, IL-6, IL-8 transforming growth factor (TGF)- β , tumor necrosis factor (TNF)- α , and fibroblast growth factor (FGF). Various mast cell populations have been identified in humans, depending on their content of chymase, or tryptase proteins, or both. If mast cells store only tryptase they are classified as MC_T and if they store both tryptase and chymase are classified as MC_{TC}; both populations differ in their cytokine contents as well.³ An increase in mast cells has been reported in an HF animal model with spontaneously hypertensive rats (SHR) compared to normotensive littermates, together with increased TGF- β 1, bFGF, and myocardial fibrosis.⁴ Also, in a reperfused canine infarct model, mast cells numbers were higher in the fibrotic areas.⁵

In a previous study we observed a greater content of chymase-containing mast cells in ventricles from congestive HF patients.⁶ Therefore we sought to analyze whether there was a correlation with the collagen fraction that could imply a role of mast cells in human myocardial fibrogenesis.

METHODS

We analyzed left ventricle biopsies from 20 patients with iDCM who had undergone cardiac transplantation and 15 control samples from organ donors. The protocol was approved by the ethical committee of the affiliated institution.

To quantify the collagen fraction, ventricular tissue was fixed with formaldehyde and embedded in paraffin. Four-micron sections were stained with picrosirius red. The interstitial collagen fraction was quantified by morphometric analysis with exclusion of perivascular collagen. The immunohistochemical detection of mast cells with the antihuman mast cell chymase antibody ab2377 (Abcam, Cambridge, UK) was done as previously described.⁶ Mast cell density was calculated as number of mast cells per cm².

All results are expressed as the mean values $\pm$ SEM, except for the clinical characteristics of the subjects, which are expressed as mean values $\pm$ standard deviations. Statistics were calculated with the SPSS software, version 10.0 (SPSS Institute, Cary, NC, USA). A two-tailed, unpaired Student's *t* test was applied to compare the

mean values of collagen fraction between the control and HF populations. The bilateral parametric Pearson test was used for regression analysis. $P < .05$ was considered to be statistically significant.

RESULTS

Clinical Characteristics of the Studied Subjects

The patients with idiopathic dilated cardiomyopathy (iDCM) who underwent heart transplantation showed a mean age of 51 ± 3 years, a left ventricular end-diastolic diameter of 77 ± 12 mm, a left ventricular end-systolic diameter of 65 ± 12 mm, and an ejection fraction of $21 \pm 5\%$.

Myocardial Fibrosis Levels

The collagen fraction was three-fold higher among failing ($12.41 \pm 1.73\%$) than control hearts ($3.98 \pm 0.63\%$; $P < .001$; Fig 1). The percentage of fibrosis in the iDCM sections analyzed ranged from 1.99% to 29.04%. Analysis of a different region of the left ventricle in some failing hearts showed high variations (up to 10%) in the collagen fraction (data not shown). In control sections the values ranged from 0.84% to 8.67%.

Correlation Between Mast Cell Density and Collagen Fraction

A correlation was observed between mast cell density in the myocardium and the collagen fraction ($P < .005$, $R^2 = 0.28$) that could be fitted to the linear regression curve; collagen fraction = $0.78 + 0.05$ mast cell density (Fig 2).

DISCUSSION

Analysis of the collagen fractions in left ventricle sections demonstrated a threefold increase in failing hearts when

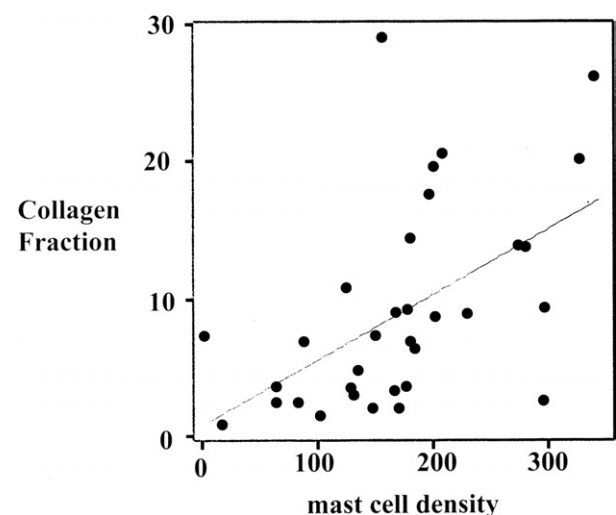


Fig 2. Correlation between mast cell density and collagen fraction. Linear regression fit between mast cell density and collagen fraction, collagen fraction = $0.78 + 0.05$ mast cell density. * $P < .005$.

compared with control hearts. Strikingly, values of collagen fractions showed a wide range of 27.05% among hearts from patients with iDCM: the lowest value was 1.99% and the highest, 29.04%. The collagen fraction in control sections also spanned a wide range, 7.83%, but presented less variability than in the failure sections. When collagen values were extreme, we evaluated the collagen content of sections from another region of the heart. Unexpectedly, we observed large differences (up to 10%) in two different regions of the same heart. Because all pathological hearts analyzed were from nonischemic origin, we expected a global uniform change in left ventricle remodelling.

Several lines of evidence link mast cell presence and fibrogenesis.^{2,5,7} In this report we found that the percentage of interstitial myocardial collagen highly correlated with mast cell density. The mechanism by which mast cells promote collagen synthesis has not been established. Because of the diversity of proinflammatory and fibrogenic factors that are synthesized and stored in mast cells, many different molecular pathways are plausible. In this work, we detected a mast cell subpopulation that contains chymase, a proteolytic enzyme that synthesizes angiotensin II (Ang II). Increased Ang II amounts could induce fibrogenesis. Ang II has been implicated in fibrosis induction in vitro and in animal models. Ang II infusions in rats enhanced fibroblast proliferation as well as augmented collagen content in the myocardium. These effects could be partially reduced with ACE inhibition with losartan.⁸ Mast cells have also been shown to activate fibroblasts. When they differentiate to

myofibroblasts they become the main source of procollagen synthesis.⁷

Further analysis of the mast cell population and its content in failing human myocardium will be necessary to better understand the molecular pathways that result in left ventricle fibrosis and the consequent loss of elasticity.

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