Cardiac diastolic dysfunction in conscious dogs with heart failure induced by chronic coronary microembolization

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Table 1. LV contractile and diastolic function in conscious dogs before and after chronic coronary microembolization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>122±2</td>
<td>101±2*</td>
</tr>
<tr>
<td>LV dP/dtmax, mmHg/s</td>
<td>2.798±0.107</td>
<td>2.249±0.65*</td>
</tr>
<tr>
<td>LV fractional shortening, %</td>
<td>21.1±2.3</td>
<td>12.4±1.3*</td>
</tr>
<tr>
<td>LV stroke work, ( \times 10^3 ) dyn/cm²</td>
<td>125±11</td>
<td>56±9*</td>
</tr>
<tr>
<td>LV minute work, ( \times 10^5 ) dyne·cm⁻¹·min⁻¹</td>
<td>10.5±0.8</td>
<td>5.5±0.8*</td>
</tr>
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</table>

LV systolic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>8±1</td>
<td>19±1*</td>
</tr>
<tr>
<td>LV end-diastolic dimension, mm</td>
<td>41.7±2.5</td>
<td>46.5±2.9</td>
</tr>
<tr>
<td>LV end-diastolic wall stress, g/cm²</td>
<td>22±3</td>
<td>61±6*</td>
</tr>
<tr>
<td>LV dP/dtmax, mmHg/s</td>
<td>2.467±0.62</td>
<td>1.851±0.49*</td>
</tr>
<tr>
<td>LV relaxation ( \tau ), s⁻¹</td>
<td>24.5±0.9</td>
<td>28.9±1.3</td>
</tr>
<tr>
<td>LV isovolumic relaxation duration, s</td>
<td>22.3±0.5</td>
<td>27.8±1.2*</td>
</tr>
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</table>

Data are means ± SE for \( n = 13 \) dogs. LV, left ventricular; dP/dtmax, maximum positive and negative change of pressure with time; \( \tau \), time constant. *\( P < 0.05 \) vs. control.

systolic LV dimensions, respectively. LV stroke work was measured as the integral area of LV pressure-dimension loops that were constructed from LV pressure and internal diameter obtained simultaneously. LV minute work was calculated as the product of LV stroke work and HR. Myocardial contractile parameters included wall thickening and end-systolic elastance (Ees). LV active relaxation indexes are LV maximum negative dP/dt (dP/dtmax), and isovolumic relaxation duration and time constant (\( \tau \)) (5, 14, 15).

LVEDV (ml) was calculated with an ellipsoidal model (8) as EDV = \((\pi/6) \times [(E_{D\alpha}/2)^2 \times (E_{D\beta}/2)]/1,000\), where E_Dα is end-diastolic short-axis dimension measured from LV dimension crystals and E_Dβ is LV end-diastolic long-axis dimension, calculated based on the end-diastolic short axis-to-long axis ratio, which was measured

from the echocardiogram. LV chamber stiffness was evaluated from the LV EDPVR. The EDPVR was assumed to be exponential and was analyzed by curve fitting in the form EDP = be^{EDV}, where \( b \) is a chamber stiffness coefficient (3, 9). The chamber stiffness (dP/dV) was derived from the relation dP/dV = kEDP, where dP/dV is the change in EDP related to a change in EDV, and \( k \) is a chamber stiffness constant (3, 9).

LV end-diastolic circumferential wall stress (\( \sigma \)) was calculated with an ellipsoidal model (8) as \( \sigma = (1.36 \times EDP \times E_{D\alpha} \times E_{D\alpha}/(2 \times EDWT) \times (E_{D\alpha} + E_{D\beta} + EDWT), \) where EDWT is end-diastolic wall thickness. LV wall regional myocardial end-dia-stolic strain (\( \varepsilon \)) was determined according to the Lagrangian strain: \( \varepsilon = (l - l_0)/l_0 \), where \( l \) is end-diastolic wall thickness and \( l_0 \) is end-diastolic wall thickness at zero or the lowest wall stress (LVEDP was \( \sim0 \)). The regional myocardial end-diastolic stiffness was estimated from the regional myocardial EDSSR in LV anterior and posterior walls, which was assumed to be exponential and was analyzed with curve fitting in the form \( \sigma = be^{k\varepsilon} \), where \( b \) is the myocardial stiffness coefficient (3, 9). Both LV EDPVR and EDSSR were constructed from the data collected from a brief inferior vena caval (IVC) occlusion and an acute volume loading.

**Chronic coronary microembolization.** CHF was induced by CCE with microspheres (Bang Laboratories; 90- to 120-μm diameter) for 24 ± 4 days with an average of total 1.2 million microspheres, until LVEDP was \( \leq 18 \) mmHg and LV maximum dP/dt (dP/dtmax) reduced by 20% of control value (5, 19).

**Experimental protocols.** To determine the impact of cardiac preload on LV isovolumic relaxation, five dogs were studied with a brief IVC occlusion that created a matching level of cardiac preload attained in the control state and heart failure. To determine LV chamber and regional myocardial stiffness, seven dogs were studied with both brief IVC occlusion and acute volume loading, in which 1,000 ml of saline was infused rapidly \((\sim100 \text{ ml/min})\) under 250–300 mmHg of pressure with a pressure bag (16).

Fig. 1. Left ventricular (LV) diastolic function in control and congestive heart failure (CHF) were examined under matching LV end-diastolic pressure (LVEDP) and heart rate (HR) during a brief inferior vena caval (IVC) occlusion. There was a significant decrease in LV minimum change of pressure with time (dP/dtmax), increase in LV relaxation time constant \( \tau \) and isovolumic relaxation duration, and decrease in LV maximum change of pressure with time (dP/dtmax) in CHF when LVEDP and HR were similar. *\( P < 0.05 \), control vs. CHF.
Western blot analysis. The methods for Western blot analysis were described previously (15). Protein was extracted from LV myocardial biopsy specimens. Collagen I and III and PAI-1 were detected by monoclonal antibodies, and bands were quantified by densitometry. Calsequestrin was used as the internal control.

Statistical analysis. For all hemodynamic and cardiac functional data, values are expressed as means ± SE. The difference between the studied dogs before and after chronic coronary microembolization was determined by Student’s t-test for paired comparison. All changes were considered significant when P < 0.05 with a two-tailed t-distribution.

RESULTS

Systemic hemodynamics and LV contractile function. Compared with the control, CCE reduced mean arterial pressure from 96 ± 2 to 82 ± 2 mmHg (P < 0.05) and increased HR from 86 ± 3 to 100 ± 5 beats/min (P < 0.05), accompanied by elevation of mean LAP from 3 ± 1 to 13 ± 1 mmHg (P < 0.05). Furthermore, CCE decreased LV systolic pressure by 26 ± 3 mmHg and depressed LV dP/dtmax by 18 ± 4%, LV fractional shortening by 39 ± 5%, LV stroke work by 53 ± 7%, and minute work by 47 ± 7% (all P < 0.05) (Table 1).

Local myocardial contractile function was examined with LV anterior and posterior wall crystals. CCE reduced LV anterior wall thickening and Ean by 19 ± 11% and 11 ± 3% (P < 0.05) respectively, and reduced LV posterior wall thickening and Eap by 64 ± 13% and 17 ± 3% (both P < 0.05), respectively.

LV diastolic function. LVEDD and LVEDP were significantly increased after CCE, accompanied by an elevation of LV end-diastolic wall stress (Table 1). Furthermore, LV dP/dtmin decreased by 25 ± 2% (P < 0.05) and LV isovolumic relaxation τ and duration increased by 19 ± 5% and 25 ± 6%, respectively (both P < 0.05), compared with their control values.

LV diastolic function with matched loading conditions. The impact of preload on LV isovolumic relaxation in heart failure was assessed in five dogs before and after CCE under a matched cardiac preload with a brief IVC occlusion. Figure 1 shows that, with similar LVEDP (3.6 ± 1.1 vs. 4.2 ± 1.2 vs. mmHg) and HR (101 ± 5 vs. 91 ± 7 beats/min), compared with the control LV dP/dtmin remained lower by 27 ± 6% (P < 0.05) and both LV isovolumic relaxation τ and duration remained higher by 53 ± 16% and 27 ± 6%, respectively (both P < 0.05), after CCE. Thus the impairment of LV isovolumic relaxation after CCE was preload independent.

LV chamber stiffness. Figure 2A illustrates a representative LV EDPVR in an individual dog studied before and after CCE. Before embolization, the increase in LVEDP was small despite a relative large increase in LVEDV, and this relationship became steeper only when LVEDP was >10 mmHg. After CCE, LV EDPVR was not only significantly shifted to the right, indicating a great enlargement of the LV chamber, but also became much steeper, with an increase in LV chamber stiffness coefficient α by 62 ± 10% (P < 0.05) (Table 2), suggesting a marked reduction of chamber compliance. Figure 2B further demonstrates a representative LV chamber stiffness (dP/dV) and LV EDPVR relationship. LV chamber stiffness increased linearly with LVEDP and chamber stiffness constant k, where the slope of this relationship was significantly increased by 66 ± 13% after CCE (Table 2). Together, our results suggest a significant increase in LV chamber stiffness in CHF induced by CCE.

Myocardial diastolic stiffness. Myocardial diastolic stiffness was evaluated by a regional myocardial EDSSR in LV anterior and posterior walls. Figure 3 demonstrates a representative change in myocardial diastolic stiffness in an individual dog studied before and after CCE. In the control, the regional myocardial end-diastolic stress was only slightly elevated despite a large increase in regional myocardial end-diastolic strain during an increase in LV chamber volume. After CCE, this curve became much steeper, indicating a greater elevation of regional myocardial end-diastolic stress in response to an increase in regional myocardial end-diastolic strain. The summarized data (Table 2) from seven dogs showed that the regional myocardial stiffness was increased by 70 ± 25% in the LV anterior wall and by 63 ± 24% in the LV posterior wall (both P < 0.05) after CCE.

Myocardial fibrosis and increased PAI-1 protein expression. Western blot analysis showed significant increases in myocardial collagen I and III by 2- and 10-fold (both P < 0.05), respectively. The LV anterior wall and by 63% after CCE (6% (both P < 0.05), compared with their control values.

Table 2. LV myocardial and chamber stiffness in conscious dogs before and after chronic coronary embolization

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<tr>
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<th>Control</th>
<th>Heart Failure</th>
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<tbody>
<tr>
<td>Anterior wall stiffness coefficient (n = 6)</td>
<td>17.5 ± 1.4</td>
<td>31.1 ± 6.7*</td>
</tr>
<tr>
<td>α, mm⁻¹</td>
<td>18.4 ± 1.6</td>
<td>31.3 ± 6.1*</td>
</tr>
<tr>
<td>Posterior wall stiffness coefficient (n = 7)</td>
<td>0.11 ± 0.02</td>
<td>0.17 ± 0.04*</td>
</tr>
<tr>
<td>Chamber stiffness coefficient (n = 7)</td>
<td>0.09 ± 0.01</td>
<td>0.14 ± 0.02*</td>
</tr>
<tr>
<td>α, ml⁻¹</td>
<td>0.04 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Chamber stiffness constant (n = 7)</td>
<td></td>
<td></td>
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<tr>
<td>b, mmHg</td>
<td></td>
<td></td>
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<tr>
<td>Chamber stiffness constant (n = 7)</td>
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Values are means ± SE for n dogs. b, intercept value. *P < 0.05 vs. control.
respectively, and a significant increase in myocardial PAI-1 level by ~2.3-fold \((P < 0.05)\) in the failing heart with CCE (Fig. 4) compared with normal hearts.

**DISCUSSION**

CCE resulted in significant LV diastolic functional abnormalities that covered both active and passive aspects of diastolic function, with a depressed LV systolic function. Importantly, the impairment of LV isovolumic relaxation after CCE was independent of the alteration of the cardiac preloading condition, and increases in LV chamber and regional myocardial diastolic stiffness were accompanied by development of tissue fibrosis and activation of PAI-1 after myocardial injury.

Ventricular isovolumic relaxation is an active diastolic process resulting from the sequestration of calcium and cross bridge uncoupling and can be quantified by measurements of LV \(dP/dt_{\text{min}}\) and isovolumic relaxation \(\tau\) and duration \((3, 8, 9)\). In an earlier investigation of the canine pacing CHF model, Komamura et al. \((8)\) showed that impaired LV isovolumic relaxation in CHF was associated with increased cardiac preload and could be reversed by normalization of the loading condition on the heart. In the present study, we found that, with the significant impairment of LV isovolumic relaxation by CCE, LV \(dP/dt_{\text{min}}\) remained lower and LV isovolumic relaxation duration and relaxation \(\tau\) remained longer even under the condition of reduced cardiac preload during a brief IVC occlusion (Fig. 1). Thus our results suggest that, different from the canine pacing model of CHF, the abnormalities of active diastolic relaxation in CHF following myocardial injury were load independent and therefore could not be reversed by normalization of the loading condition.

Abnormality in ventricular passive diastolic properties (the component determined by the distensibility of the myocardium) is one of the fundamental aspects responsible for the hemodynamic and symptomatic abnormalities of heart failure \((7, 13, 20)\). LV passive diastolic properties could be well demonstrated by EDPVR \((2)\). It is important to recognize that EDPVR of the LV chamber in the intact heart is nonlinear, the curve is flat in the low pressure-volume range, and the curve rises sharply as the ventricle becomes distended \((3, 8, 16)\). The EDPVR constructed from nonlinear curve fitting may appear very different if the chamber volume data are collected from different end-diastolic pressure ranges (as commonly occurs when comparing a normal state to a heart failure state), although the data may delineate an identical EDPVR in the overlapping pressure-volume ranges. Therefore, it becomes critical to cover both low and high pressure-volume ranges in constructing the entire EDPVR. In the present study, using the combination of brief IVC occlusion and acute volume loading to dramatically change LV chamber volume, we constructed...
both low and high parts of the EDPVR and demonstrated a right upward shifting in the EDPVR after CCE (Fig. 2A). In contrast to the nonlinear relationship of EDPVR, the relationship of \( \frac{dp}{dv} \) and LVEDP is linear, and the slope of this relationship, termed the chamber stiffness constant, has been used by many investigators as an index of LV diastolic chamber properties (3, 9). In the present study, we showed a significant increase in the slope of the LV \( \frac{dp}{dv} \) and LVEDP relationship by \( 66 \pm 13\% \) after CCE (Fig. 2B). Altogether, our analysis clearly demonstrated a significant increase in LV chamber stiffness in the canine model of CHF induced by CCE.

Passive myocardial properties greatly impact the chamber stiffness and can be characterized by myocardial EDSSR (3, 9). Analogous to the EDPVR, this relationship is nonlinear. In the present study, the myocardial diastolic stiffness was estimated from myocardial regional EDSSRs in LV anterior and posterior walls before and after CCE. The significant shifting of myocardial EDSSRs following CCE indicates an increased myocardial diastolic stiffness, which could contribute to the change in LV chamber stiffness in CHF.

Alteration of the extracellular matrix is a major mechanism contributing to the change of myocardial and chamber passive diastolic properties (4, 7, 13, 20). As a consequence of the myocardial injury following CCE, our histological examination revealed an interstitial and perivascular fibrosis (5), associated with an accumulation of collagen I and III, consistent with these earlier reports (10, 13, 17). Furthermore, we showed an increase in the protein expression of PAI-1, the principal inhibitor of plasminogen activation. PAI-1 could promote fibrosis by preventing the activation of matrix metalloproteinases, enzymes that degrade collagenous proteins, and the degradation of extracellular matrix by plasminogen activators and plasmin (1, 18). Thus the significant development of myocardial fibrosis, related to the activation of PAI-1, could be an important underlying molecular alteration contributing to the impairment of myocardial passive diastolic properties.

In summary, CCE resulted in significant abnormalities in both active and passive diastolic function, with depressed LV systolic dysfunction. The impairment of LV isovolumic relaxation was preload independent and could not be reversed by normalizing the LV loading condition. The LV chamber stiffness and regional myocardial diastolic stiffness were significantly increased and may relate to the development of tissue fibrosis, associated with increase in PAI-1 activity after myocardial injury. The canine model of CHF induced by CCE could be uniquely useful for the study of cardiac diastolic dysfunction.

ACKNOWLEDGMENTS

We are grateful to Dr. Gerald D. Smith, Jennifer K. Hochstetler, and Allison Renee Cook for excellent veterinary care throughout the course of this study.

REFERENCES


14. Shannon RP, Konamura K, Stambler BS, Bigaud M, Manders WT, Hawkins ET, and Goldstein S. Alteration of the extracellular matrix is a major mechanism contributing to the change of myocardial and chamber passive diastolic properties (4, 7, 13, 20). As consequence of the myocardial injury following CCE, our histological examination revealed an interstitial and perivascular fibrosis (5), associated with an accumulation of collagen I and III, consistent with these earlier reports (10, 13, 17). Furthermore, we showed an increase in the protein expression of PAI-1, the principal inhibitor of plasminogen activation. PAI-1 could promote fibrosis by preventing the activation of matrix metalloproteinases, enzymes that degrade collagenous proteins, and the degradation of extracellular matrix by plasminogen activators and plasmin (1, 18). Thus the significant development of myocardial fibrosis, related to the activation of PAI-1, could be an important underlying molecular alteration contributing to the impairment of myocardial passive diastolic properties.

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