Effect of Experimental Hyperhomocysteinemia on Cardiac Structure and Function in the Rat

Ernest Walker,1 Jason Black,2 Cordel Parris,3 Elizabeth C. Bryda,2 Silvestre Cansino,3 Lisa Hunt,3 Jean Chappell,4 Paulette Wehner,3 Mark Studeny,3 and Gary L. Wright 4
Departments of Pathology,1 Microbiology, Immunology and Molecular Genetics,2 Cardiology,3 and Physiology,4 The Joan Edwards School of Medicine, Marshall University, and St. Mary Medical Center, Huntington, West Virginia

Abstract. Male Sprague-Dawley rats were subjected for 2 weeks to daily injections of homocysteine (Hcy), which increased plasma Hcy approximately 2-fold. Echocardiography indicated significant increases in left ventricular diastolic (13%) and systolic (31%) dimensions and decreases in posterior wall thickness (diastolic, -17%; systolic, -20%) in Hcy-treated animals. Slight changes were noted in the ejection fraction, systolic fractional shortening, and maximal aortic valvular blood flow velocity, but they were not statistically significant or were similar to those in vehicle controls. The results suggest that an initial effect of Hcy administration involves loss of myocardial structure without a direct influence on myocardial contractile function. Consistent with this conclusion, in vitro evaluation of the myocardial ring contractile response showed no significant difference in left ventricular maximal isometric force between the control (13.9 ± 2.7 g/g tissue) and Hcy-injected (11.0 ± 2.8 g/g tissue) animals. (received 25 September 2003, accepted 24 December 2003)

Keywords: echocardiography, myocardial contractility, homocysteine, cardiac remodeling

Introduction

Research on familial hyperhomocysteinemia first suggested that elevated circulating levels of homocysteine (Hcy) were linked to atherosclerotic vascular disease [1]. Subsequent studies confirmed and extended this observation to indicate that even moderate increases of plasma Hcy concentrations pose an independent risk factor for premature development of atherosclerosis [2-4]. Recent evidence suggests that increase of plasma Hcy directly impairs coronary vascular relaxant function and contributes to restenosis following angioplasty [5,6]. The mechanisms of Hcy actions on vascular function and pathology remain uncertain. However, the primary Hcy-induced lesion may involve overt endothelial cell damage, which leads to dysfunction of endothelial contractile responses and disturbed regulation of smooth muscle cell growth [7-11].

Recent interest has focused on the effects of Hcy on cardiac function. In addition to indirect effects on myocardial performance that stem from Hcy-induced alterations in coronary blood flow, there is evidence Hcy may have a direct effect on the myocardium. Pacher et al [12] showed that acute exposure of isolated papillary muscle to Hcy at concentrations of 10^{-6} M decreased the rate of rise and the duration of the action potential, without detectable changes in the resting membrane potential or the early phase of repolarization. Tyagi and co-workers [13] demonstrated that acute treatment of cardiac ring preparations with Hcy impaired endocardial endothelium-dependent activity and acted synergistically with other agonists to enhance contractile responsiveness. They further showed that the folic acid-mediated lowering of plasma Hcy in homocysteinemic hypertensive rats reduced myocardial matrix metalloproteinase activity and
collagen expression, while increasing TGF-β1 expression [14]. Left ventricular hypertrophy of these animals was improved, with no change in arterial pressure suggesting a direct effect of Hcy on myocardial structure. Joseph et al [15] reported echocardiographic and in vitro contractile measures of cardiac structure and function in rats made hyper-homocysteinemic for 10 wk. They noted left ventricular hypertrophy characterized by increased myocyte size, increased perivascular and interstitial collagen, coronary arterial wall thickening, and mast cell infiltration of the myocardium. Consistent with these observations, in vitro assessment of left ventricular function indicated an abnormal diastolic pressure-volume curve, probably related to reduced compliance. These findings suggest that Hcy could be a cardiac hypertrophic and remodeling agent.

In the present study, we used echocardiographic and ex vivo cardiac ring assessment to evaluate the effects of subacute exposure (2 wk) to Hcy on cardiac structure and function in rats. The results show mild to moderate loss in left ventricular structure with little or no change in functional parameters. These results are consistent with a previous speculation [15] that ventricular hypertrophy after long-term exposure to Hcy could represent a compensatory response to early loss of myocytes.

Materials and Methods

Animals. All experimental treatment of animals was approved by the Marshall University Animal Care Committee. Male Sprague-Dawley rats (12 wk old) were divided into groups (6 rats/group) receiving daily ip injections of distilled water (vehicle controls) or DL-homocysteine (1.8 mmol/rat/day, Sigma, St. Louis, MO). Because there was evidence of acclimation to echocardiographic measurements during the experimental interval, a third group of untreated rats was subjected to a single echocardiographic evaluation at the end of the experiment (naïve controls). The rats were housed on wood chip bedding at 23±2°C with a 12hr/12 hr light/dark cycle.

Echocardiographic evaluation. Rats were evaluated immediately before the start of injections (baseline control) and on the 7 and 14 days of treatment. After a rat was anesthetized with a ketamine/xylazine mixture, the ventral thorax was shaved and covered with ultrasonic transmission gel. Echocardiographic measurements were made with a Phillips Sonos 5500 echocardiogram system using a 12 megahertz transducer. Measurements were performed at a minimum of 3 hr after control or Hcy injections. Two-dimensional measurements were utilized to image cardiac structures in the parasternal long- and short-axis views. These echocardiographic views were used to position the M-mode echocardiographic line. In the long axis procedures, the probe was oriented toward the base of the heart projecting toward the apex (x-axis) with depth along the y-axis, thus allowing pulse wave doppler evaluation of valvular blood flow velocities. In the short axis procedures, the probe was oriented toward the left ventricle and across the heart for evaluation of wall structure, which was utilized in the calculation of ejection fraction and fractional shortening during systole. M-mode displays were analyzed by a digital echocardographic analysis system.

Six measurements were selected for each assessment of cardiac structure and function. Structural parameters included diastolic (IVSd) and systolic (IVSs) left ventricular septal thickness, diastolic (LVIDd) and systolic (LVIDs) left ventricular internal dimension, diastolic (LVPWd) and systolic (LVPWs) left ventricular posterior wall thickness, and right ventricular diastolic internal dimension (RV). Function measurements included ejection fraction (EF), left ventricular fractional shortening during systole (FS), maximal aortic (AVmax), pluomnary (PVmax), mitral (MVmax), and tricuspid (TVmax) valvular blood flow velocity.

Contractility measurements. In vitro measurement of cardiac ring contractility was conducted by the method of Tyagi et al [13]. Hearts were excised and placed in warmed Krebs buffer (in mM: NaCl, 118; KCl, 4.7; CaCl2, 1.2; NaHCO3, 24; MgCl2,1.1; KH2PO4, 1.2; glucose, 5.6; pH 7.4) aerated with 5% CO2 in O2. Two left ventricular rings were prepared by 3 mm cross-sections with a razor blade, followed by severing of the right ventricular wall,
leaving the septum intact. Tissue rings were immediately suspended between a metal tissue holder and an L-hook and then placed in a 25 ml organ bath containing aerated (5% CO₂ in O₂) Krebs buffer maintained at 37°C. Rings were equilibrated for 30 min at a passive tension of 8 g, which was shown in a preliminary analysis to result in maximal isometric contraction. The rings were isometrically contracted by addition of 20 mM CaCl₂ to the bath. Preliminary studies indicated that this concentration resulted in a maximal contractile response. To ensure maximal contraction, a second addition of 20 mM CaCl₂ was added to the bath prior to termination of the experiment. The tissues were then removed from the bath and weighed. Isometric force was measured with a Grass FT03D force transducer and Grass Model 7 polygraph (Grass Instruments, Quincy, MA). Active force development was calculated as g tension/g tissue (wet weight) and the values for the two rings from each rat were averaged.

**Homocysteine measurement.** Blood taken from small tail incisions into heparinized capillary tubes was centrifuged at 3200 rpm for 10 min and the plasma stored at -70°C until analysis. Plasma total homocysteine concentration was assayed using an homocysteine immunoassay kit and IMX Immunoanalyzer (Abbott Laboratories, Abbott Park, IL) [16].

**Statistics.** Two-tailed analysis of variance and the Student-Neuman-Keuls test were used for between-group comparisons and Student’s paired t-test was used for within-group comparisons. Contractility and plasma homocysteine data were analyzed by Student’s unpaired t-test. A p value <0.05 was considered statistically significant. Values are reported as mean±SEM.

**Results**

Plasma homocysteine levels were 4.6±0.5 µmol/L in control rats and 8.4±1.3 µmol/L in those injected with 1.8 mmol of Hcy/day for 2 wk (p <0.05).

Echocardiographic evaluation of vehicle control rats at wk 1 and 2 showed no consistent changes in structural features of the left ventricle, although the left ventricular internal dimension (LVID) was increased in these rats at wk 1 compared to values obtained at the initiation of the study prior to vehicle injection (Table 1). By comparison, Hcy-injected animals showed a progressive increase in systolic and diastolic LVID and significant decrease in left ventricular posterior wall thickness during the 2-wk interval of injections. Left intraventricular septal thickness (IVS) in these rats was unchanged, suggesting selective cardiotoxic effects of Hcy. There was an unexplained increase of the diastolic right ventricular dimension in both the control and the Hcy-injected groups (Table 1).

In control rats, the ejection fraction (EF) and the left ventricular shortening fraction (FS) both decreased during treatment (Table 2), suggesting
acclimation responses to the treatment and/or echocardiograph protocol. Consistent with this explanation, untreated (naive) rats exhibited high EF and FS values similar to those obtained for baseline values of treated rats prior to injections (Table 3).

Maximal aortic valvular blood flow velocity (AVmax) in controls was increased throughout the period of injections, whereas, pulmonary (PVmax), mitral (MVmax), and tricuspid (TVmax) valvular blood flow velocities were unchanged (Table 2). Similar to controls, the EF and FS of Hcy-treated animals decreased during the period of injections. Unlike the control AVmax, which increased, the AVmax of Hcy-injected rats was stable. The PVmax of these rats tended to decrease during treatment. The MVmax and TVmax were both similar to control values in the Hcy group.

Direct measurements of left ventricular maximal isometric force under in vitro conditions indicated a tendency for decreased (-21%) contractility (control, 13.9±2.7 g/g tissue; Hcy, 11.0±2.8 g/g tissue). However, the differences between these groups were not statistically significant.

**Discussion**

The present study examined the effects of sub-acute (2 wk) exposure to pharmacologic levels of Hcy (1.8 mmole/day) on the echocardiographic profile and in vitro left myocardial contractility in rats. As a central element of the study design, rats were subjected to serial echocardiographic measurements beginning in the week before and thereafter at 7-day intervals during the period of treatment. This protocol allowed a more robust evaluation of Hcy-induced changes in cardiac geometry and function through comparison of each animal with its pre-treatment baseline value.

As a major observation, Hcy-treated rats showed progressive, significant increase in left ventricular diastolic (13%) and systolic (31%) dimensions and decrease in posterior wall thickness (diastole, -17%; systole, -20%), which were not seen in vehicle-

---

Table 2. Echocardiographic evaluation of cardiac functional parameters in control (vehicle) and homocysteine-injected rats. Results are reported as mean ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>EF %</th>
<th>FS %</th>
<th>AVmax (cm/sec)</th>
<th>PVmax (cm/sec)</th>
<th>MVmax (cm/sec)</th>
<th>TVmax (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 wk</td>
<td>6</td>
<td>76.5±2.2</td>
<td>40.6±2.1</td>
<td>81.5±4.1</td>
<td>100.4±7.1</td>
<td>59.9±3.6</td>
<td>65.8±4.8</td>
</tr>
<tr>
<td>1 wk</td>
<td>6</td>
<td>75.0±1.4</td>
<td>39.1±1.3</td>
<td>94.1±4.1†</td>
<td>105.4±1.9</td>
<td>61.2±4.1</td>
<td>53.9±4.2†</td>
</tr>
<tr>
<td>2 wk</td>
<td>6</td>
<td>69.8±1.7†</td>
<td>34.8±1.2†</td>
<td>89.6±3.5†</td>
<td>99.9±2.1</td>
<td>61.4±4.5</td>
<td>57.1±4.5</td>
</tr>
<tr>
<td>Homocysteine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 wk</td>
<td>6</td>
<td>79.6±1.7</td>
<td>43.4±1.6</td>
<td>84.2±5.5</td>
<td>97.4±6.3</td>
<td>59.9±2.9</td>
<td>65.5±4.6</td>
</tr>
<tr>
<td>1 wk</td>
<td>6</td>
<td>71.8±3.2†</td>
<td>36.8±2.6†</td>
<td>80.2±6.0</td>
<td>86.7±4.1†</td>
<td>61.4±5.1</td>
<td>56.7±3.7†</td>
</tr>
<tr>
<td>2 wk</td>
<td>6</td>
<td>73.1±3.8†</td>
<td>38.0±3.0†</td>
<td>86.9±2.4</td>
<td>94.4±3.7</td>
<td>64.7±3.5</td>
<td>57.1±3.9</td>
</tr>
</tbody>
</table>

* p <0.05 vs the corresponding vehicle control group.
† p <0.05 vs the corresponding untreated zero week control.

Table 3. Echocardiographic evaluation of structural and functional parameters in untreated (naive) rats. Values are reported as mean ± SEM, N = 24.

<table>
<thead>
<tr>
<th>Structural Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSd (cm)</td>
</tr>
<tr>
<td>0.12±0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Functional Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%)</td>
</tr>
<tr>
<td>78.8±5.0</td>
</tr>
</tbody>
</table>
injected controls (Table 1). Hcy-injected rats also exhibited a slight but nonsignificant reduction in ejection fraction (-8%) and a similar (-9%) but statistically significant decrease in fractional shortening; they showed little or no change in aortic valvular blood flow velocity (Table 2). However, a similar pattern of reduction in these parameters in vehicle controls suggests that apparent decreases in function could likely reflect acclimation of animals to the measurement protocol. This explanation is supported by the further finding that untreated rats subjected to a single echocardiographic evaluation showed elevated values for left ventricular functional parameters (Table 3) similar to those recorded in baseline measurements of experimental groups prior to initiation of injections.

These results suggest that the in vivo evaluation of cardiac function by echocardiogram may be significantly influenced by extrinsic factors that could themselves vary with treatment. Hence, the interpretation of echocardiographic results may be complicated in studies that employ single echocardiogram determinations at the end of treatment. Taken together, the results suggest that sub-acute exposure to Hcy results in mild to moderate alteration in cardiac structure without significant functional involvement. The finding of insignificant change in the in vitro force generating capacity of myocardial rings from Hcy-treated rats supports the argument for lack of overt damage to myocardial contractile function.

Two other studies have examined potential direct effects of Hcy on cardiac structure or function in the rat model. Tyagi et al [13] reported that acute in vitro exposure to Hcy (10^{-7}M to 10^{-3}M) induced contraction of cardiac tissue in which the maximum tension achieved was 3-fold greater in rings that were chemically denuded of endothelium. They further showed that, in the presence of sub-threshold concentrations of either endothelin or angiotensin II, the contractile response to Hcy addition was markedly enhanced. Their data suggest that Hcy exerts a direct contractile effect on the myocardium, while also acting to impair endothelial endothelial function. They speculated that longer-term Hcy exposure could alter endocardial endothelial function in synergy with or in antagonism to other endothelium-derived factors. In contrast, the present results provide little or no evidence for significant change in myocardial contractility after in vivo exposure for 2 wk to elevated Hcy levels.

Our findings are consistent with those of Joseph et al [15] who fed normotensive rats a hyperhomocysteinemia-inducing diet for 10 wk, after which they evaluated the echocardiographic profile and in vitro left ventricular functional status. They reported significant alterations in cardiac structure including left ventricular hypertrophy with increased myocyte size, increased perivascular and interstitial collagen, arteriolar wall thickening, and mast cell infiltration of the myocardium. However, left ventricular systolic function determined in vitro was not different from controls. They concluded that Hcy may act as an independent stimulus for myocardial remodeling while suggesting that both the deposit of interstitial collagen and the hypertrophic response of cardiomyocytes could reflect compensatory responses to early myocyte loss. The present findings support the conclusions of Joseph et al [15], suggesting that Hcy primarily affects cardiac geometry without a direct effect on myocardial contractility. In contrast to hypertrophic effects of Hcy [15], we observed changes in geometry consistent with loss of structure. The differences in results may reflect the fact that our study subjected the rats to less severe hyperhomocysteinemia (2-fold vs 5-fold increases in plasma levels) for a shorter duration (2 wk vs 10 wk).

In summary, the present findings suggest that a relatively short-term exposure to elevation of plasma Hcy results in alterations in left ventricular geometry without evidence for a direct effect on myocardial contractile function. The results are consistent with the conclusions of Joseph et al [15] that Hcy may act as stimulus for cardiac remodeling and that an initial Hcy-induced loss in structure may lead to a secondary compensatory hypertrophic response of the ventricle.

References


