Regression of Hypertensive Myocardial Fibrosis by Na\textsuperscript{+}/H\textsuperscript{+} Exchange Inhibition

Horacio E. Cingolani, Oscar R. Rebolledo, Enrique L. Portiansky, Néstor G. Pérez, María C. Camilión de Hurtado

Abstract—We have recently reported that the inhibition of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger (NHE) during 1 month in spontaneously hypertensive rats (SHR) is followed by regression of cardiomyocyte hypertrophy but not of myocardial fibrosis. The aim of this study was to evaluate whether a treatment of longer duration could reduce myocardial fibrosis and stiffness. SHR received 3.0 mg/kg per day of the specific NHE-1 inhibitor cariporide; the effect on cardiomyocyte cross-sectional area, myocardial collagen volume fraction, collagen synthesis, and myocardial stiffness (length-tension relation in left papillary muscles) was evaluated at several time points (after 1, 2, or 3 months). A slight decrease of \( \approx 5 \) mm Hg in systolic blood pressure was observed after 1 month of treatment with no further changes. After 2 and 3 months of treatment, the size of cardiomyocytes remained within normal values and myocardial fibrosis progressively decreased to normal level. Accordingly, myocardial stiffness and the serum levels of the carboxyterminal propeptide of procollagen type I, a marker of collagen type I synthesis, were normalized after 3 months. Left ventricular weight decreased from 910±43 (in untreated SHR) to 781±21 mg (treated SHR) after 3 months of treatment. No difference in body weight between treated and untreated SHR was observed after this period of treatment. The present data allow us to conclude that in the SHR the administration of an NHE-1 inhibitor for 2 or 3 months leads to the normalization of collagen type I synthesis, myocardial collagen volume fraction, and stiffness. (Hypertension. 2003;41:373-377.)

Key Words: fibrosis ■ myocardium ■ extracellular matrix ■ signal transduction

Fibrous tissue accumulation is a feature of the structural remodeling of the myocardium seen in hypertensive heart disease.\textsuperscript{1} An exaggerated accumulation of collagen type I and type III occurs in the hypertrophied myocardium and also in the nonhypertrophied right ventricle and in the atria of animals and humans with arterial hypertension. This collagen accumulation has adverse effects. It results in the development of increased myocardial stiffness that leads to diastolic dysfunction and ultimately to the development of systolic dysfunction.

Recently, a new therapeutic strategy against hypertension has emerged from the use of Na\textsuperscript{+}/H\textsuperscript{+} exchanger (NHE) inhibitors.\textsuperscript{2-5} The inhibition of NHE-1 reduces the hypertrophy of the surviving myocytes after myocardial infarction\textsuperscript{6} and of the hearts of spontaneously hypertensive rats (SHR)\textsuperscript{2} and mice overexpressing \( \beta\)-adrenergic receptors.\textsuperscript{7} Taken together, these findings seem to indicate a key role of NHE activity in the development of myocardial hypertrophy. Even though our recent study showed that neither myocardial fibrosis nor stiffness was normalized in the SHR after 1 month of cariporide treatment,\textsuperscript{2} the regression of fibrosis was observed after 6 months of NHE inhibition in another model of cardiac hypertrophy, the transgenic mice overexpressing \( \beta\)-adrenergic receptors.\textsuperscript{8} Considering that the regression of myocyte hypertrophy and collagen accumulation might have a different time course, the present study sought to determine whether a more prolonged treatment with the NHE-1 blocker would cause a reduction in myocardial fibrosis in the SHR model of cardiac hypertrophy. A possible link between NHE-1 activity and fibrosis may rely on the fact that the antiporter is a downstream effector of several fibrosis-related signaling systems such as transforming growth factor-\( \beta\) (TGF\( \beta\)) and the renin-angiotensin-aldosterone system.\textsuperscript{8-8}

Methods

Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No 85-23, revised 1996) with 50 male SHR and 31 normotensive Wistar (NT) rats (4 months old). At this age and after recording baseline body weight (BW) and systolic blood pressure (SBP) by the tail-cuff method,\textsuperscript{3} the rats were assigned to either (a) death, to assess baseline cardiac parameters; (b) no drug treatment; or (c) cariporide treatment (3.0 mg/kg per day). Baseline BW in SHR was lower than in NT\textsuperscript{10} (mean values were 296±8 and 246±5 g in NT and SHR, respectively). There was no difference in BW gain during the experiment; BW reached 367±7, 323±9, and 325±10 g in NT, SHR, and cariporide-treated SHR 3 months later, respectively. Animals were euthanized after 1, 2, or 3 months of treatment under ether anesthesia, and their
Baseline and Cariporide-Induced Changes in SBP, LVW, LVW/BW Ratio, and LVCVF Values

<table>
<thead>
<tr>
<th>Groups</th>
<th>SBP, mm Hg</th>
<th>LVW, mg</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Basal 1 Month 2 Month 3 Month</td>
<td>Basal 1 Month 2 Month 3 Month</td>
</tr>
<tr>
<td>Untreated NT</td>
<td>121±3 (10) 119±2 (7) 118±2 (8) 121±2 (6)</td>
<td>608±20 (10) 670±34 (7) 700±58 (8) 726±17 (6)</td>
</tr>
<tr>
<td>Untreated SHR</td>
<td>179±1 (10)* 179±1 (10)* 173±2 (5)* 180±2 (7)*</td>
<td>676±17 (10)* 843±20 (10)* 956±44 (5)* 910±43 (7)*</td>
</tr>
<tr>
<td>Cariporide-treated SHR</td>
<td>179±1 (10)* 170±2 (7)* 168±3 (4)* 174±1 (7)*</td>
<td>676±17 (10)* 717±50 (7)† 762±26 (4)† 781±21 (7)†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Numbers within parentheses indicate the number of determinations.

*p<0.05 vs age-matched untreated NT rats, one-way ANOVA; †P<0.05 vs age-matched untreated SHR rats, one-way ANOVA.

Hearts removed. The left ventricle (LV) with the septum was weighed (LVW) and normalized by BW to determine cardiac hypertrophy.

Myocardial stiffness was determined in LV papillary muscles as previously described. Briefly, the muscles were stretched stepwise until the twitch-developed force reached the maximum. At each step, the muscle length was increased by 10% of the muscle length at zero resting tension (L0). The stress-strain relation of each muscle was fitted to an exponential equation of the form σ=A×e^K×L, where K represents the elastic stiffness constant. From each fitting, the stress values at given values of strain were estimated by interpolation and plotted as a function of strain.

Although circulating cariporide levels were not determined in our study, it was shown that rats treated with a similar or even lower dosage than ours had cariporide plasma levels higher than that in untreated SHR at the end of the study, it was shown that rats treated with a similar or even lower dosage than ours had cariporide plasma levels higher than that in untreated SHR at the end of the study. The analysis showed a statistical, highly significant difference (P<0.0001) between the adjusted LVW means, implying that there was not an overestimation of the antihypertrophic effect of NHE inhibition caused by the lighter BW of SHR.

The increased CSA of cardiomyocytes returned to a value within the normal range after 1 month of cariporide treatment (Figure 1A), and their size remained stable throughout the rest of the treatment. The normalization of cardiomyocyte size after 1 month of NHE-1 inhibition as well as the lack of effect on myocardial fibrosis at this time point (Figure 1B) were previously reported by us. Left ventricular collagen volume fraction (LVCVF) in untreated SHR was increased compared with age-matched NT at any time point during the treatment (Table). NHE-1 inhibition for 2 to 3 months induced a decline in myocardial fibrosis (not detected after 1 month), so that LVCVF reached values not different from those found in age-matched NT rats (Figure 1B and the Table). The changes in serum PIP paralleled the changes in myocardial fibrosis: PIP levels were significantly elevated in untreated SHR compared with NT rats and returned to normal values after 2 or 3 months of cariporide treatment (Figure 1C).

Figure 2 shows representative images of the increased interstitial fibrosis seen in LV of SHR and the progressive involution to normal content induced by 2 and 3 months of cariporide treatment.

Muscles from hypertensive hypertrophied hearts were stiffer than those from NT, as denoted by the leftward shift of the length-tension curves shown in Figure 3A. Myocardial stiffness was restored to normal after 2 or 3 months of cariporide treatment (Figures 3B and 3C). Considered as a whole, the data presented herein would indicate that treatment with an NHE-1 inhibitor should last for more than 1 month to interfere with collagen synthesis, normalize myocardial collagen content, and restore myocardial distensibility in SHR.

Discussion

Our results demonstrate the ability of the NHE-1 inhibitor cariporide to regress cardiac fibrosis in SHR with established...
hypertension. The decrease in fibrous tissue was accompanied by a decrease in the serologic marker of collagen type I synthesis, PIP. Of importance, the regression of fibrosis occurred in the absence of (or minimal) reduction in arterial pressure, indicating the hemodynamic independence of the mechanism. According to the regression in collagen content, a major determinant of tissue stiffness, there was an improvement in myocardial distensibility. Even though this is the first demonstration of the regression of myocardial fibrosis in the hypertensive heart induced by NHE-1 inhibition, an effect of cariporide on the fibrotic hypertrophied heart of mice overexpressing H$_9252^1$ receptors has been recently reported. 4

A recent study from our laboratory reported that 1 month of cariporide treatment induced the regression of cardiomyocyte hypertrophy in SHR without a detectable effect on fibrosis, myocardial stiffness, or serum PIP levels.3 The present study shows that extending cariporide treatment results in complete regression of myocardial fibrosis associated with the normalization of myocardial distensibility and serum PIP concentration. It has been recognized before that myocyte size declines, with antihypertensive therapy, more rapidly than the amount of collagen, a stable protein with 80 to 120 days half-life in myocardium.12

Signaling pathways leading to myocardial fibrosis are complex, yet incompletely defined. Diastolic dysfunction accompanies those models of cardiac hypertrophy in which myocardial fibrosis is also present,13 but no sign of diastolic dysfunction has been reported in cardiac hypertrophy when fibrosis is absent.14–16 Our results show that the regression of cardiomyocyte hypertrophy is not enough to normalize myocardial distensibility. The restoration of normal distensibility accompanied the decline in the amount of collagen, suggesting that fibrosis and not muscle mass per se is the major determinant of diastolic stiffness in the hypertrophied heart of SHR. In connection with this, the study of Brilla et al17 showed that the reduction of collagen content and the improvement of diastolic dysfunction in hypertensive patients were independent of the regression of cardiomyocyte hypertrophy. Regression of myocardial fibrosis and improvement of diastolic distensibility after long-term interruption of the renin-angiotensin system were previously reported in both SHR and hypertensive humans.17–19

It has been proposed that myocardial stiffness is a function of cross-linked collagen.20 Whether NHE-1 inhibition decreased cross-linked collagen was not explored in this study, but the normalization of myocardial distensibility after cariporide treatment may suggest an effect on insoluble collagen.

In our experiments we cannot exclude that changes in collagen degradation after NHE-1 blockade could have con-
downstream effector of various of these intracellular signaling mechanisms. It was reported by Takewaki et al. that the inhibition of NHE-1 activity partially decreased the activation of MAPK and protein synthesis elicited by stretch. Several still unanswered questions have arisen from the findings presented herein. How does NHE-1 inhibition reduce myocardial hypertrophy? Is the decrease in Na\(^+\) influx due to NHE-1 blockade the main mechanism? It should be kept in mind that the amount of Na\(^+\) entering through NHE-1 accounts for approximately half the total Na\(^+\) influx. A link between Na\(^+\) influx by NHE and the activation of PKC-\(\delta\) and PKC-\(\varepsilon\) isoforms in response to growth factors has been reported in isolated cardiomyocytes. The intracellular Na\(^+\) is elevated in hypertrophied myocardium, and Na\(^+\) has a direct effect on protein synthesis increase and on protein degradation decrease. Epidemiological and basic studies support the notion that Na\(^+\) is an independent factor for the development of myocardial hypertrophy. Frohlich and colleagues reported that a high Na\(^+\) diet increased not only cardiac enlargement in SHR but also cardiac mass in normotensive rats without detectable change of arterial pressure.

**Perspectives**

Clinical and epidemiological studies indicate that diastolic heart failure is mainly caused by hypertensive heart disease. It is now well recognized that it is not only the quantity but also the quality of the myocardium that is responsible for adverse cardiovascular effects in hypertensive heart disease. Increased fibrous tissue predisposes to an increased risk for diastolic and/or systolic dysfunction and, ultimately, to heart failure. Our findings that NHE-1 inhibition induces the normalization of myocardial distensibility and fibrosis—effects associated with a normalization of increased collagen synthesis—may be of particular importance for the treatment of hypertensive patients.

**References**

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