Increased Collagen Type I Synthesis in Patients With Heart Failure of Hypertensive Origin
Relation to Myocardial Fibrosis

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Background—We investigated whether increased collagen type I synthesis and deposition contribute to enhancement of myocardial fibrosis and deterioration of cardiac function in patients with hypertensive heart disease (HHD).

Methods and Results—We studied 65 hypertensives with left ventricular hypertrophy subdivided into 2 groups: 34 patients without heart failure (HF) and 31 patients with HF. Transvenous endomyocardial biopsies of the interventricular septum were performed to quantify the amount of fibrotic tissue and the extent of collagen type I deposition. The carboxy-terminal propeptide of procollagen type I (PIP), an index of collagen type I synthesis, was measured by radioimmunoassay in serum samples from the coronary sinus and the antecubital vein. Compared with normotensives, the amount of collagen tissue, the extent of collagen type I deposition, and coronary and peripheral PIP were increased (P<0.01) in the 2 groups of hypertensives. These parameters were also increased (P<0.01) in HF hypertensives compared with non-HF hypertensives. Coronary PIP was higher (P<0.01) than peripheral PIP in hypertensives but not in normotensives. The amount of collagen tissue was inversely correlated with the ejection fraction and directly correlated with both coronary and peripheral PIP in all hypertensives.

Conclusions—These findings suggest that an excess of cardiac collagen type I synthesis and deposition may be involved in the enhancement of myocardial fibrosis that accompanies the development of HF in HHD. In addition, our data show that the heart secretes PIP via the coronary sinus into the peripheral circulation in patients with HHD. Thus, PIP determined in peripheral blood can be a useful marker of myocardial fibrosis in these patients. (Circulation. 2004;110:1263-1268.)

Key Words: collagen ■ heart failure ■ hypertension ■ myocardium ■ peptides

Hypertension is the major risk factor for heart failure (HF).1 The failure of the hypertensive myocardium involves a complex of events at the molecular and cellular levels, including alterations in the metabolism of extracellular matrix.2 A number of studies performed in postmortem human hearts3-5 and endomyocardial human biopsies6-9 have shown that the amount of fibrillar collagen is abnormally increased in the myocardium of hypertensive patients with left ventricular hypertrophy (LVH). It has been proposed that the excess of myocardial collagen seen in hypertensive heart disease (HHD) is primarily a result of the uncoupling between increased synthesis and unchanged or decreased degradation of collagen type I fibers (reviewed by Weber10). Hemodynamic loading, ischemia, hormones, and growth factors may be involved in such an uncoupling (reviewed by Weber10). Myocardial fibrosis is now considered a major determinant of altered diastolic filling and compromised systolic pump function in HHD (reviewed by Burlew and Weber11 and Díez et al12).

We have hypothesized that enhancement of myocardial fibrosis secondary to increased collagen type I synthesis and deposition contributes to the development of HF in patients with HHD. To test this hypothesis, we studied the serum concentrations of the carboxy-terminal propeptide of procollagen type I (PIP), an index of collagen type I synthesis (reviewed by López et al13), and the extent of collagen type I deposition in the myocardium of patients with HHD and either no manifestations of HF or clinically overt HF. In addition, the relation between the amount of myocardial fibrillar content and cardiac function was analyzed in the same patients.

Methods

Patients

All subjects gave written informed consent to participate in the study, and the institutional review committee approved the study...
protocol. The study conformed to the principles of the Helsinki Declaration.

The hypertensive population consisted of 65 white patients with systolic blood pressure and diastolic blood pressure of >139 and 89 mm Hg, respectively. All patients had appropriate clinical and laboratory evaluation to exclude secondary hypertension. All patients exhibited HHD as indicated by the presence of LVH in the echocardiogram (see below). Other cardiac diseases associated with myocardial fibrosis (eg, coronary artery disease, hypertrophic cardiomyopathy, and aortic stenosis) were excluded after complete medical examination, which included a diagnostic cardiac catheterization. None of the patients presented any condition associated with alterations in the serum levels of PIP (alcoholic liver disease, metabolic bone disease, hyperthyroidism, renal insufficiency).

Whereas 34 patients did not present a medical history or current manifestations of HF (non-HF hypertensives), 31 patients had a previous diagnosis of HF (HF hypertensives). The diagnosis of HF syndrome was made on a clinical basis by the presence of at least 1 major and 2 minor Framingham criteria. In addition, HF diagnosis was reinforced by the presence of a depressed ejection fraction (EF) and/or Doppler signs of diastolic dysfunction in all patients. Furthermore, hemodynamic evidence of myocardial failure was obtained in each patient by measuring elevated left ventricular end-diastolic pressure and pulmonary capillary wedge pressure (>12 mm Hg in both cases). After the diagnosis of HF had been established, the patient’s clinical status was assessed according to his extent of disability via the New York Heart Association (NYHA) functional classification. Three HF hypertensives presented NYHA class II, 19 presented class III, and 9 presented class IV.

A group of 12 normotensive subjects (7 men and 5 women; mean age, 56 years; range, 39 to 72 years) were used as control subjects for histomorphological and biochemical studies. They were subjects without LVH and with clinically presumed coronary artery disease who were found to lack the disease at a coronary angiography.

### Assessment of Left Ventricular Dimensions, Mass, and Function

2D echocardiographic imaging, targeted M-mode recordings, and Doppler ultrasound measurements were obtained in each patient. Left ventricular mass was measured and left ventricular mass index (LVMI) was calculated as previously described. The presence of LVH was established when LVMI was >111 g/m² for men and >106 g/m² in women. Following pulsed-Doppler measurements were obtained: maximum early transmural velocity in diastole; maximum late transmural velocity in diastole; the deceleration time of the early mitral filling wave; and isovolumic relaxation time. EF was calculated according to Quinones et al.

### Histomorphological and Immunohistochemical Studies

Three transvenous endomyocardial biopsies were taken from the middle area of the interventricular septum with a bioprobe, Cordis 96 cm (7F), under fluoroscopic guidance after angiographic examination, as previously reported. The fraction of myocardial volume occupied by fibrillar collagen (CVF) was determined by quantitative morphometry with an automated image analysis system (AnalySYS, Soft Imaging System GmbH, Hamm). In sections stained with collagen-specific picrosirius red (Sirius red F3BA in aqueous picric acid).

For immunohistochemical study, the peroxidase-labeled dextran polymer method was used. The primary antibody used was against collagen type I (Biogenesis) at a dilution of 1:10. The myocardial surface area with positive staining for collagen type I was analyzed by quantitative morphometry with the automated image analysis system mentioned above.

The histomorphological study was performed by a pathologist blinded to the other characteristics of the studied patients.

### Determination of PIP

PIP was determined in serum samples from the coronary sinus and the left antecubital vein by radioimmunoassay according to a method previously described. The interassay and intra-assay variations for determining PIP were 7%. The sensitivity was 5 μg of PIP/L.

### Statistical Analysis

To analyze the differences between the normotensive group and the 2 groups of hypertensive patients, a 1-way ANOVA followed by a Student-Newman-Keuls test was used. Differences between 2 groups of patients were tested by a Student’s t test for paired data once normality was demonstrated; otherwise, a nonparametric test (Wilcoxon test) was used. Differences between 2 groups of patients were tested by a Student’s t test for unpaired data once normality was demonstrated; otherwise, a nonparametric test (Mann-Whitney U test) was used. The correlation between continuously distributed variables was tested by univariate regression analysis. Values are expressed as mean±SEM. A value of P<0.05 was considered statistically significant.

### Results

#### Clinical Characteristics of the Hypertensives

Baseline clinical characteristics of the 2 groups of patients are presented in the Table. Twenty-one non-HF hypertensives

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-HF Hypertensives</th>
<th>HF Hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58±2</td>
<td>64±2*</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>22/12</td>
<td>24/7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28±1</td>
<td>29±1</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>8</td>
<td>...</td>
</tr>
<tr>
<td>Loop diuretic</td>
<td>...</td>
<td>31</td>
</tr>
<tr>
<td>Digitalis</td>
<td>...</td>
<td>9</td>
</tr>
<tr>
<td>ACEI or ARA</td>
<td>...</td>
<td>31</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>α-Blockers</td>
<td>2</td>
<td>...</td>
</tr>
<tr>
<td>Ca²⁺ antagonists</td>
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<td>...</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>174±5</td>
<td>142±3†</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>95±2</td>
<td>87±2*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>78±5</td>
<td>73±3</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>128±7</td>
<td>170±10†</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>10.70±0.30</td>
<td>9.70±0.20*</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>109±3</td>
<td>107±3</td>
</tr>
<tr>
<td>Vₑ/Vₛ</td>
<td>0.89±0.04</td>
<td>0.35±0.16*</td>
</tr>
<tr>
<td>Tₑₑ, ms</td>
<td>211±11</td>
<td>199±11</td>
</tr>
<tr>
<td>EF, %</td>
<td>0.61±0.01</td>
<td>0.40±0.02†</td>
</tr>
</tbody>
</table>

HF indicates heart failure; BMI, body mass index; ACEI, ACE inhibitor; ARA, angiotensin II type 1 receptor antagonist; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; LVMI, left ventricular mass index; LWT, left ventricular wall thickness; LVEDD, left ventricular end-diastolic diameter; IVRT, isovolumic relaxation time; Vₑ, maximal early transmural velocity in diastole; Vₛ, maximal late transmural velocity in diastole; Tₑₑ, deceleration time; and EF, ejection fraction. Values are expressed as mean±SEM and No. of patients.

*P<0.05, †P<0.01 vs non-HF patients.
were receiving antihypertensive medication in monotherapy or combination at the time of enrollment. No significant differences in blood pressure were found between treated and untreated non-HF hypertensives (data not shown). Most HF hypertensives were treated with the combination of a loop diuretic, a β-blocker, and either an ACE inhibitor or an angiotensin II type 1 receptor antagonist. None of the patients in this group were being treated with aldosterone antagonists. A depressed EF (<0.50) was found in 61% of HF hypertensives.

Assessment of Myocardial Fibrosis

Values of CVF measured in the 2 groups of patients were higher (P<0.01) than the values measured in control subjects (1.95±0.07%). In addition, CVF was increased (P<0.001) in HF hypertensives (7.91±0.55%) compared with non-HF hypertensives (5.38±0.31%). Figure 1 (top) shows a representative picture of collagen deposition in the myocardium of the 3 groups of subjects. Whereas the abnormal accumulation of fibrous tissue was seen as a scattered deposition in non-HF hypertensives, a more diffuse interstitial deposition leading to a marked disarray of myocardial architecture was observed in HF hypertensives.

According to our previous classification of myocardial fibrosis in hypertension,16 we classified patients with CVF <6% as patients with nonsevere fibrosis and patients with CVF >6% as patients with severe fibrosis. Thus, severe fibrosis was present in 23% and 71% of non-HF and HF hypertensives, respectively.

CVF did tend to be increased in HF hypertensives with depressed EF compared with HF hypertensives with preserved EF (8.13±0.66% versus 7.15±1.01%), but the difference did not reach statistical significance. Values of CVF measured in HF hypertensives with NYHA class IV (9.48±2.12%) did tend to be higher than values measured in HF hypertensives with NYHA classes II and III (6.84±0.55% and 7.29±0.69%, respectively), but the differences were not statistically significant.

The extent of collagen type I deposition was higher (P<0.001) in the 2 groups of patients than in control subjects (2.03±0.55%). This parameter was increased (P<0.05) in HF hypertensives (7.88±1.35%) compared with non-HF hypertensives (4.97±0.49%). In addition, a strong direct correlation was found between the extent of collagen type I deposition and CVF in all patients (r=0.952, P<0.001).

A representative picture of collagen type I deposition in the myocardium of the 3 groups of subjects is shown in Figure 1 (bottom). Collagen type I stained large strands and a reticular network of fibrotic tissue through the myocardium of HF hypertensives. In contrast, collagen type I reacted moderately around groups of cardiomyocytes in non-HF hypertensives.

Assessment of Collagen Type I Synthesis

As shown in Figure 2, both coronary and peripheral PIP were increased (P<0.01) in the 2 groups of patients compared with control subjects (84±11 and 82±8 μg/L, respectively). Peripheral PIP was higher (P<0.05) in HF hypertensives (139±6 μg/L) than in non-HF hypertensives (122±6 μg/L) (Figure 2). Similarly, the values of coronary PIP were higher (P<0.01) in HF hypertensives than in non-HF hypertensives (166±7 versus 142±5 μg/L) (Figure 2).

Coronary PIP was higher (P<0.001) than peripheral PIP in the 2 groups of patients, no significant differences between these 2 parameters were observed in control subjects (Figure 2). In addition, a direct correlation was found between coronary and peripheral PIP (r=0.776, P<0.001) in all patients (Figure 3).

Analysis of Associations

The EF was inversely correlated with CVF (r=-0.393, P<0.001) (Figure 4) and coronary (r=-0.387, P<0.05) and peripheral (r=-0.256, P<0.05) PIP in all patients. Figure 5 shows that CVF was directly correlated with both coronary (r=0.759, P<0.001) and peripheral (r=0.867, P<0.001) PIP in all patients. Direct correlations were found between the extent of collagen type I deposition and coronary (r=0.751,
and peripheral ($r=0.757, P<0.01$) PIP in all patients.

**Discussion**

The main findings of this study are as follows: (1) an association exists between histologically assessed fibrillar collagen accumulation in the myocardium and deterioration of cardiac function in patients with HHD, (2) myocardial accumulation of collagen type I is strongly associated with biochemically assessed increased collagen type I synthesis in patients with HHD, and (3) serum PIP may be a useful marker of myocardial fibrosis in these patients.

Initially, fibrosis of the myocardial interstitium compromises the rate of relaxation, diastolic suction, and passive stiffness, contributing to impaired diastolic function. In accordance with this, we have recently shown that an association exists between myocardial collagen content and left ventricular chamber stiffness in patients with HHD. A continued accumulation of fibrous tissue further impairs diastolic filling and now compromises transduction of cardiomyocyte contraction into myocardial force development, thus impairing systolic performance. In support of this possibility, we found that an association exists between increase of myocardial accumulation of collagen type I fibers and deterioration of EF in hypertensives. Furthermore, we found that the values of CVF were higher in HF hypertensives with the most severe functional impairment (ie, depressed EF) and with the most severe NYHA class (ie, class IV). Although these differences were not statistically significant, overall, they are consistent with the conclusion that myocardial fibrosis participates in the deterioration of systolic function in HF hypertensives.

Fibrillar collagen type I is synthesized in the fibroblasts as a procollagen precursor containing an amino-terminal and a carboxy-terminal propeptide of PIP in 3 groups of subjects. Bars represent mean±SEM.

**Figure 2.** Serum concentration of carboxy-terminal propeptide of PIP in 3 groups of subjects.

Figure 3. Direct correlation ($y=0.79x+55.56$) between serum concentration of carboxy-terminal propeptide of procollagen type I measured in coronary blood (PIP cb) and serum concentration of PIP measured in peripheral blood (PIP pb) in all patients. Non-HF hypertensives are depicted with open circles and HF hypertensives with solid circles.

Figure 4. Inverse correlation ($y=-2.42x+73.30$) between CVF and EF in all patients. Non-HF hypertensives are depicted with open circles and HF hypertensives with solid circles.
propose that the exaggerated synthesis of collagen type I extent of collagen type I deposition and CVF allow us to HF. Furthermore, the associations found between PIP and the mally stimulated in patients with HHD, namely, in those with coronary PIP suggests that the heart is largely the source of significant correlation observed between peripheral PIP and the coronary sinus in these patients. Furthermore, the highly highly suggests that this peptide is released from the heart through organs such as the liver and the lung.26 Furthermore, we coronary blood to the peripheral blood in patients with HHD suggests that this peptide is released from the heart through the coronary sinus in these patients.25 Given the earlier evidence showing that PIP is produced in various tissues, including the heart, lung, liver, muscle, and bone.21,22 all of these organs are possible sources of circulating PIP. Our finding that there is a gradient of PIP from the coronary blood to the peripheral blood in patients with HHD suggests that this peptide is released from the heart through the coronary sinus in these patients. Furthermore, the highly significant correlation observed between peripheral PIP and coronary PIP suggests that the heart is largely the source of circulating PIP in HHD. These findings would suggest that the cardiac synthesis of collagen type I molecules is abnormally stimulated in patients with HHD, namely, in those with HF. Furthermore, the associations found between PIP and the extent of collagen type I deposition and CVF allow us to propose that the exaggerated synthesis of collagen type I molecules is critically involved in the development of collagen type I-dependent myocardial fibrosis in patients with HHD.

A role for angiotensin II–induced fibrosis has been proposed in the development of HF in various cardiomyopathies.23 Furthermore, a number of studies (reviewed by Weber24) suggest that angiotensin II may stimulate the synthesis of collagen type I, thus leading to enhanced deposition of collagen fibers in the hypertensive heart. Nevertheless, although all HF hypertensives included in this study were under treatment with either ACE inhibitor or angiotensin II type 1 receptor antagonist, severe myocardial fibrosis was present in most. Thus, the participation of other profibrotic factors in HF-associated myocardial fibrosis must also be taken into account (eg, aldosterone). Interestingly, a reduction in serum PIP has been reported in HF patients receiving conventional therapy when spironolactone was added to the treatment, thus suggesting a role for aldosterone in collagen type I synthesis in these patients.25

Various experimental and clinical observations have demonstrated that high serum concentrations of peripheral PIP are associated with histomorphologically proven ongoing fibrosis in organs such as the liver and the lung.26 Furthermore, we have shown previously that an association exists between the serum concentration of peripheral PIP and myocardial fibrosis in non-HF hypertensives.16,20,27 In the present study, we expand this association to HF hypertensives. The clinical usefulness of peripheral PIP in the management of HHD, namely, when HF develops, is supported by 3 kinds of arguments. First, the measurement of serum PIP is easy to perform and analyze, and it has good performance characteristics and low cost. On the other hand, serum PIP makes physiological sense; that is, its changes reflect well the variations in collagen type I deposition in the myocardium. Finally, as demonstrated previously, pharmacologically induced changes in the serum concentration of PIP are significantly associated with changes in the outcome of HF patients.25

Limitations of the Study
This was a study involving a relatively small number of patients, but because of the nature of the goals under investigation, this design is appropriate. In addition, it must be recognized that therapy with different types of antihypertensive drugs in the 2 groups of patients may have confounded the findings and their interpretation. Nevertheless, one must consider that, because they are standard therapies for hypertension and HF, it is unreasonable to withdraw them for purposes of this investigation.

We performed biopsies of the right side of the interventricular septum to assess the structural effects of left ventricular pressure loading. However, as shown by Pearlman et al.,28 fibrosis present in the septum in postmortem tissue from hypertensive human hearts is representative of fibrosis existing in the free wall.

Exaggerated myocardial accumulation of collagen type I fibers in the hypertensive heart is the result not only of stimulated synthesis but also of uncharged or inhibited degradation of collagen type I molecules.29 Thus, myocardial...
fibrosis observed in hypertensives from this study may also be the consequence of an insufficient cleavage of these molecules by matrix metalloproteinases.

Because an excess of collagen type III deposition occurs in the myocardium of patients with HHD, and picrosirius red binds to collagen molecules other than type I, such as type III, we cannot exclude the possibility that the changes in myocardial fibrosis found in our patients may be also caused by changes in the deposition of fibril-forming collagen type III molecules. In addition, other components of the extracellular matrix (e.g., glycoproteins and proteoglycans) that have not been studied here may also be altered in the hypertensive heart.

In conclusion, we report that an excess of cardiac collagen type I synthesis and deposition is associated with the enhancement of myocardial fibrosis that accompanies the development of HF in HHD. In addition, we show for the first time that the heart secretes PIP into the systemic circulation. The available data set the stage for large long-term clinical studies intended to definitively test this hypothesis.

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References
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