Usefulness of Serum Carboxy-Terminal Propeptide of Procollagen Type I in Assessment of the Cardioreparative Ability of Antihypertensive Treatment in Hypertensive Patients

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Background—We investigated whether serum concentration of carboxy-terminal propeptide of procollagen type I (PIP), a marker of collagen type I synthesis, can be used to assess the ability of antihypertensive treatment to regress myocardial fibrosis in hypertensive patients.

Methods and Results—The study was performed in 37 patients with essential hypertension and hypertensive heart disease. After randomization, 21 patients were assigned to losartan and 16 patients to amlodipine treatment. At baseline and after 12 months, right septal endomyocardial biopsies were performed to quantify collagen volume fraction (CVF) on picrosirius red-stained sections with an automated image-analysis system. Serum PIP was measured by specific radioimmunoassay. Nineteen patients in the losartan group and 11 in the amlodipine group finished the study. Time-course changes in blood pressure during treatment were similar in the 2 groups of patients. In losartan-treated patients, CVF decreased from 5.65 ± 2.03% to 3.96 ± 1.46% (P < 0.01) and PIP from 127 ± 30 to 99 ± 26 μg/L (P < 0.01). Neither CVF or PIP changed significantly in amlodipine-treated patients. CVF was directly correlated with PIP (r = 0.44, P < 0.001) in all hypertensives before and after treatment.

Conclusions—These findings suggest that the ability of antihypertensive treatment to regress fibrosis in hypertensives with biopsy-proven myocardial fibrosis is independent of its antihypertensive efficacy. Our data also suggest that blockade of the angiotensin II type 1 receptor is associated with inhibition of collagen type I synthesis and regression of myocardial fibrosis in hypertensives. Thus, determination of serum PIP may be useful to assess the cardioreparative properties of antihypertensive treatment in hypertensives. (Circulation. 2001;104:286-291.)

Key Words: collagen ■ hypertension ■ myocardium ■ peptides ■ remodeling

A n exaggerated accumulation of fibrillar collagens type I and type III occurs throughout the free wall and interventricular septum of animals1,2 and humans3,4 with primary arterial hypertension and left ventricular hypertrophy (LVH). A rise in collagen content has been shown to raise myocardial stiffness and promote abnormalities of cardiac function and electrical activity.5,6 It has been proposed that the excess of myocardial collagen seen in hypertension is the result of both increased collagen synthesis and unchanged or decreased collagen degradation.7 A substantial body of evidence (reviewed in Weber8) suggests that angiotensin II is critically involved in fibrosis of hypertensive heart disease.

We have used serological markers of collagen turnover to address myocardial fibrosis in spontaneously hypertensive rats (SHR)9–11 and patients with essential hypertension,12–14 each of whom had LVH. Serum concentrations of the carboxy-terminal propeptide of procollagen type I (PIP), a marker of collagen type I synthesis, were higher in each setting of hypertensive heart disease, and there was a direct correlation between histological evidence of myocardial fibrosis and serum PIP in SHR and hypertensive patients. Furthermore, we have shown that the ability of the angiotensin II type 1 (AT1) receptor losartan to repair myocardial fibrosis in rats with genetic hypertension (SHR) was associated with the diminution of serum PIP.10,11

We have hypothesized that in essential hypertension, serum concentration of PIP may be used to assess the ability of antihypertensive treatment to regress myocardial fibrosis. To test this hypothesis, the present study was designed to compare changes in PIP and changes in myocardial collagen...
content in hypertensives receiving either losartan or the calcium channel blocker amlodipine as treatment.

Methods

Patients and Study Protocol
All subjects gave written informed consent to participate in the study, and the local committee on human research approved the study protocol. The study conformed with the principles of the Declaration of Helsinki. The study population consisted of 37 white patients (24 men and 13 women; mean age 58 years, range 39 to 75 years) with elevated systolic blood pressure (SBP) of >139 mm Hg and diastolic blood pressure (DBP) >89 mm Hg. All patients had appropriate clinical and laboratory evaluation to exclude secondary hypertension. Coronary artery disease was excluded as reported previously.14 Conditions associated with alterations in serum levels of PIP were excluded after complete medical examination.

No washout phase was performed to ensure continuous antihypertensive treatment (required by the ethics committee). Patients were randomized according to their previous antihypertensive treatment with diuretic agents, antiadrenergic drugs, and combined treatment. After randomization, 21 patients were assigned to losartan (losartan group) and 16 to amlodipine (amlodipine group) treatment. Diuretic medication use was reported by 2 patients in the losartan group and 5 in the amlodipine group. Antiadrenergic drugs were reported by 7 patients in the losartan group and 5 in the amlodipine group. The dosages of losartan and amlodipine were titrated to achieve the therapeutic goal of SBP and DBP <140 and 90 mm Hg, respectively. After titration, all patients in the losartan group and the amlodipine group were receiving daily dosages of 50 and 10 mg, respectively, during 12 months.

A group of 10 hearts collected from a total of 100 autopsies performed at the University Clinic of Navarra during 1998 and 1999 served as controls for myocardial fibrosis after cardiac disease was excluded. A group of 24 healthy subjects were also studied to calibrate values of PIP.

Assessment of Blood Pressure
SBP and DBP were monitored in a sitting position at each visit by use of standard cuff equipment. Mean blood pressure was calculated from the equation (SBP+2DBP)/3. Pulse pressure was the arithmetic difference between averaged SBP and DBP values.

Assessment of Left Ventricular Mass and Function
Two-dimensional targeted M-mode and Doppler ultrasound recordings were obtained in each patient as described previously.12,13 Left ventricular mass and interventricular septal thickness (IVST) were measured, and left ventricular mass index (LVMI) was calculated by dividing left ventricular mass by body surface area. The following pulsed Doppler measurements were obtained: maximal early transmitral velocity in diastole (Ve), maximal late transmitral velocity in diastole (Vl), A-wave deceleration time (DT), and isovolumic relaxation time (IVRT). The ejection fraction (EF) was calculated from measurements performed in 99m Tc ventriculography (MUGA).

All patients exhibited hypertensive heart disease as indicated by the presence of LVH (defined as LVMI >125 g/m² and/or IVST >11 mm)13 and/or diastolic dysfunction (defined as a diminished Ve :Vl ratio and/or prolonged IVRT and/or depressed DT according to age).16 None of the patients studied exhibited systolic dysfunction, as assessed by an EF <50%.

Biochemical Determinations
Serum PIP was determined by radioimmunoassay according to a method described previously.12 The interassay and intra-assay variations for determining PIP were 7% and 3%, respectively. The sensitivity (lower detection limit) was 1.20 μg of PIP per liter. Serum carboxy-terminal telopeptide of collagen type I (CITP), a marker of collagen type I degradation, was determined by radioimmunoassay according to Laviades et al.13 The interassay and intra-assay variations for determining CITP were <8%. Sensitivity was 0.50 μg of CITP per liter.

Histomorphological Study
Transvenous endomyocardial biopsy samples were taken from the mid area of the interventricular septum with a biopsyte (Cordis 96 cm, 7F) under fluoroscopic guidance after angiographic examination. Postmortem tissue studies in hypertensive human hearts have demonstrated that fibrosis present in the septum is representative of fibrosis existing in the free wall.14 The biopsy procedure was well tolerated in all cases. Histological evaluation was performed as reported previously.14 Collagen volume fraction (CVF) was determined by quantitative morphometry with an automated image-analysis system (Visillog 4.1.5., Noesis) in sections stained with collagen-specific picrosirius red (Sirius Red F3BA in aqueous picric acid).

Statistical Analysis
Differences in baseline parameters between hypertensives treated with losartan and hypertensives treated with amlodipine were tested by Student’s t test for unpaired data once normality was demonstrated (Shapiro-Wilk test); otherwise, a nonparametric test (Mann-Whitney U test) was used. Differences between hypertensives before and after treatment were tested by Student’s t test for paired data once normality was demonstrated (Shapiro-Wilk test); otherwise, a nonparametric test (Wilcoxon test) was used. Categorical variables were analyzed by χ² Fisher’s exact test when necessary. The correlation between continuously distributed variables was tested by univariate regression analysis. Values are expressed as mean±SD. A value of P<0.05 was considered statistically significant.

Results

Patient Compliance
We chose a treatment period, 1 year, just long enough to remove a significant amount of collagen from the myocardium, in keeping with the half-life of fibrillar collagen.17

During this period of time, we observed a significant dropout of patients, mainly at their own request. Two patients in the losartan group and 3 in the amlodipine group did not want to complete the study. In addition, 2 patients in the amlodipine group had to be excluded owing to nonmedical causes. No adverse effects or complications occurred.

Baseline Characteristics
Baseline clinical, hemodynamic, echocardiographic, biochemical, and histomorphological characteristics were compared for all patients in either treatment group and for patients who completed the study. No significant differences were observed between the groups in the parameters tested. Overall, the 2 groups appeared comparable.

Effects of Treatment on Blood Pressure
As shown in Figures 1 and 2, time-course changes in blood pressure were similar in the 2 groups of patients. Furthermore, final values of blood pressure were similar in the 2 groups (Tables 1 and 2). Thus, the antihypertensive efficacy of the 2 treatments was comparable.

Effects of Treatment on Cardiac Parameters
Whereas LVMI and IVST were diminished (P<0.01) after treatment in the losartan group (Table 1), these parameters were not modified by treatment in the amlodipine group (Table 2). LVH regressed after treatment in 69% of patients.
in the losartan group and 25% of patients in the amlodipine group (P<0.001).

Neither \( V_e : V_a \) ratio or IVRT changed significantly with treatment in the 2 groups of patients (Tables 1 and 2). The values of both parameters improved after treatment in 27% of patients in the losartan group and 25% of patients in the amlodipine group (Table 2). DT improved after treatment in the losartan group, the difference did not reach statistical significance (Table 1). No differences in DT were observed with treatment in the amlodipine group (Table 2). DT improved after treatment in 61% of patients in the losartan group and 46% of patients in the amlodipine group (P=NS; Figure 3).

An inverse correlation was found between CVF and DT in all hypertensives at baseline (r=0.039, P<0.05). No other correlations were found between CVF and other echocardiographic parameters measured in this study. No significant changes were observed in EF in patients receiving either losartan or amlodipine (Tables 1 and 2).

**Effects of Treatment on Myocardial and Biochemical Parameters**

Baseline CVF values were higher (P<0.001) in the 2 groups of hypertensives (Tables 1 and 2) than in control hearts (1.95±0.07%). Similarly, baseline values of PIP were increased (P<0.001) in the 2 groups of patients (Tables 1 and 2) compared with normotensive subjects (70±5 μg/L).

Whereas CVF decreased with treatment in the losartan group (5.65±2.03% versus 3.96±1.46%, P<0.01), it remained unchanged in the amlodipine group (4.93±1.08% versus 4.31±1.55%). Administration of losartan was associated with diminution in the serum concentration of PIP (127±30 versus 99±26 μg/L, P<0.01). In contrast, serum PIP did not change in the amlodipine group (104±24 versus 111±13 μg/L).

Parallel changes in CVF and PIP were observed with treatment in each patient from the 2 groups (Figures 4 and 5). Furthermore, a direct correlation was found between serum PIP and CVF (r=0.44, P<0.001) in all hypertensives before and after treatment (Figure 6). Therefore, changes in the serum marker of collagen type I synthesis reflect changes in the intensity of myocardial fibrosis in treated hypertensives.

Serum concentration of CITP did tend to increase in both groups (losartan group, 3.18±1.11 versus 3.57±1.58 μg/L; amlodipine group, 4.56±2.48 versus 4.83±3.13 μg/L). Nevertheless, the changes did not reach statistical significance.

**Discussion**

The main findings of this study are as follows: (1) a strong association exists between treatment-induced changes in collagen type I synthesis and myocardial fibrosis.

**TABLE 1. Effects of Treatment With Losartan on Hemodynamic, Hormonal, and Cardiac Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>173±30</td>
<td>137±8*</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>95±11</td>
<td>81±10*</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>118±18</td>
<td>100±9*</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>78±27</td>
<td>56±6*</td>
</tr>
<tr>
<td>Aldosterone, pg/mL</td>
<td>155±106</td>
<td>95±73</td>
</tr>
<tr>
<td>LVMi, g/m²</td>
<td>131±29</td>
<td>105±21*</td>
</tr>
<tr>
<td>IVST, mm</td>
<td>11.30±1.73</td>
<td>10.15±1.74*</td>
</tr>
<tr>
<td>V_e : V_a</td>
<td>0.92±0.31</td>
<td>0.86±0.18</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>111±16</td>
<td>105±14</td>
</tr>
<tr>
<td>DT, ms</td>
<td>201±64</td>
<td>220±30</td>
</tr>
<tr>
<td>EF, %</td>
<td>61±7</td>
<td>61±6</td>
</tr>
</tbody>
</table>

MBP indicates mean blood pressure; PP, pulse pressure. Values are expressed as mean±SD.

*P<0.01 vs values before treatment.

**TABLE 2. Effects of Treatment With Amlodipine on Hemodynamic, Hormonal, and Cardiac Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>162±43</td>
<td>137±15*</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>95±13</td>
<td>79±8†</td>
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<tr>
<td>MBP, mm Hg</td>
<td>120±14</td>
<td>98±8†</td>
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<tr>
<td>PP, mm Hg</td>
<td>76±18</td>
<td>56±7*</td>
</tr>
<tr>
<td>Aldosterone, pg/mL</td>
<td>175±153</td>
<td>99±57</td>
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<tr>
<td>LVMi, g/m²</td>
<td>134±48</td>
<td>119±45</td>
</tr>
<tr>
<td>IVST, mm</td>
<td>11.92±3.65</td>
<td>11.60±3.22</td>
</tr>
<tr>
<td>V_e : V_a</td>
<td>0.89±0.17</td>
<td>0.91±0.23</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>109±16</td>
<td>109±15</td>
</tr>
<tr>
<td>DT, ms</td>
<td>220±34</td>
<td>225±27</td>
</tr>
<tr>
<td>EF, %</td>
<td>59±10</td>
<td>63±7</td>
</tr>
</tbody>
</table>

MBP indicates mean blood pressure; PP, pulse pressure. Values are expressed as mean±SD.

*P<0.05 and †P<0.01 vs values before treatment.
lagen accumulation in myocardial tissue and treatment-induced changes in serum PIP in patients with essential hypertension; (2) the efficacy of antihypertensive treatment in reducing blood pressure does not predict its capacity to either regress myocardial fibrosis or normalize collagen type I synthesis in hypertensive patients; and (3) chronic AT₁ blockade is associated with inhibition of collagen type I synthesis and regression of myocardial fibrosis in essential hypertension.

Reparation of pathological remodeling in hypertensive heart disease is a concept focused on a regression of fibrous tissue accumulation. To date, biopsy studies to assess cardiac fibrosis were used as proof of concept. Although the biopsy procedure is a simple and safe outpatient procedure, this is an invasive methodology with obvious limitations for wide-scale application, and thus, noninvasive measures of cardiac fibrosis could prove useful.

The rate of extracellular synthesis of collagen type I can be assessed by measuring the serum concentration of PIP, which is freed during the extracellular processing of procollagen type I before collagen molecules form fibers. A number of observations have led to the proposal that increased production of PIP is a useful marker of stimulated fibrogenesis in arterial hypertension. Furthermore, we have shown recently that serum PIP is a highly sensitive and specific parameter in the identification of severe myocardial fibrosis in hypertension. In this conceptual framework, our finding that changes in serum PIP accurately reflect changes in myocardial fibrosis produced during hypertension treatment may be of interest in providing a useful and noninvasive tool to evaluate the efficacy of cardioreparative interventions.

Whereas a similar reduction in blood pressure was attained in the 2 groups of patients after treatment, only losartan-treated patients exhibited a significant reduction in CVF. This is in agreement with previous data by Brilla et al showing that myocardial fibrosis regression occurred in lisinopril-treated hypertensives but not in hydrochlorothiazide-treated patients, despite the fact that normalized blood pressure did not change significantly in either group. These findings suggest that the ability of antihypertensive drugs to decrease myocardial fibrosis in hypertensive patients is not exclusively linked to their capacity to diminish blood pressure but may also be related to their efficacy in interfering with nonhemo-dynamic fibrogenic factors (ie, angiotensin II and transforming growth factor-β [TGF-β]).

It has been shown that human cardiac fibroblasts respond to angiotensin II via AT₁-receptor-mediated collagen synthesis. Thus, it is possible that losartan blocks this effect in treated hypertensives. In support of this possibility are 2 arguments. First, we found that chronic administration of losartan was associated with reduction of serum PIP in treated hypertensives. Second, owing to its pharmacological properties, losartan does not seem to be able to influence the hepatobiliary elimination of PIP in humans.

Recently, we have shown that losartan decreases the posttranscriptional synthesis of procollagen type I molecules in the left ventricle of SHR, and this is associated with diminution of serum PIP. Thus, it can be proposed that...
losartan diminishes the synthesis of collagen type I in hypertensive patients via inhibition of the posttranscriptional processing of its precursor. Nevertheless, alternative possibilities deserve consideration. For instance, losartan has been found to suppress the expression of prolyl 4-hydroxylase in cardiac fibroblasts, a key enzyme in the posttranslational processing of procollagen α-chains that facilitates formation of the triple-stranded helical procollagen molecules and their subsequent secretion to the extracellular space. On the other hand, losartan has been shown to inhibit the activity of those procollagen type I proteinases that form collagen type I molecules and PIP by cleavage of procollagen type I molecules in the interstitial space.

It is known that angiotensin II regulates the production of TGF-β at different levels. TGF-β is a multifunctional cytokine with fibrogenic properties. Recently, an association has been found between an excess of TGF-β and increased levels of PIP in hypertensive patients. Furthermore, chronic administration of losartan was associated with inhibition of TGF-β and normalization of serum PIP in treated hypertensives. Thus, although we did not measure TGF-β in the present study, the involvement of this cytokine in the ability of losartan to diminish collagen type I synthesis and regress myocardial fibrosis cannot be excluded.

We have shown recently that chronic administration of losartan resulted in stimulation of left ventricular collagenase-mediated degradation of collagen type I fibers and increase of serum CITP in SHR. Because collagenase activity has been found to be inhibited in patients with essential hypertension, the antifibrotic effect of losartan in treated hypertensives must be due not only to its ability to diminish the synthesis of collagen type I molecules but also to its capacity to stimulate the degradation of collagen type I fibers. This is supported by the tendency for increased CITP despite the decrease in PIP observed in losartan-treated patients.

Echocardiographically determined LVH did regress with losartan treatment; in contrast, no change occurred with amlodipine treatment. Because blood pressure was controlled to a similar extent by the 2 treatments, our findings are in accordance with the perception that in addition to correction of systemic hemodynamics, modification of other factors may be determinant in the regression of hypertensive LVH induced by AT1 blockade. In this regard, we have reported that losartan corrected LVH only in those hypertensives in whom circulating levels of TGF-β were normalized during the treatment period.

Myocardial fibrosis may lead to relaxation abnormalities of the myocardium secondary to impairment of the shortening of cardiomyocytes during the relaxation phase of the cardiac cycle. A reduced Vₑ/Vₛ ratio and a prolonged IVRT reveal impaired relaxation. Because abnormalities in these 2 parameters persisted after treatment with losartan, it appears that regression of myocardial fibrosis by losartan is not associated with improvement of myocardial relaxation during diastole in hypertensives. On the other hand, accumulation of collagen fibers within the myocardium is mainly responsible for an increase in intrinsic myocardial stiffness and a decrease in ventricular distensibility. A shortened Doppler mitral A-wave DT has been suggested as a diagnostic index of left ventricular end-diastolic distensibility. We have observed that an inverse correlation exists between myocardial fibrosis and values for this parameter in all hypertensives. Furthermore, DT values increased and CVF values decreased with treatment in most patients receiving losartan. These findings are in agreement with recent data by Brilla et al showing an improvement in both DT and CVF in hypertensives treated with lisinopril. Taken together, these data suggest that regression of myocardial fibrosis may be associated with increased myocardial distensibility and improvement of this component of diastolic function in hypertensives treated with drugs that interfere with the renin-angiotensin system.

In summary, we showed for the first time that changes in serum levels of PIP may be a valuable tool with which to assess the efficacy of antihypertensive treatment to regress myocardial fibrosis in hypertensive patients. The study findings set the stage for large-scale clinical trials wherein PIP and other peptides derived from collagen turnover could prove to be definitively useful as diagnostic markers of cardioreparation in hypertensive heart disease and other cardiac diseases evolving with heart failure.

References


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