Biochemical Assessment of Myocardial Fibrosis in Hypertensive Heart Disease

Begoña López, Arantxa González, Nerea Varo, Concepción Laviades, Ramón Querejeta, Javier Díez

Abstract—Fibrous tissue accumulation is an integral feature of the adverse structural remodeling of cardiac tissue seen with hypertensive heart disease. Given the importance of fibrous tissue in leading to myocardial dysfunction and failure, noninvasive monitoring of myocardial fibrosis by use of serological markers of collagen turnover could prove a clinically useful tool, particularly given the potential for cardioprotective and cardioreparative pharmacological strategies. An emerging experimental and clinical experience holds promise for the use of radioimmunoassays of various serological markers of fibrillar collagen type I and type III turnover in arterial hypertension. More specifically, the measurement of serum concentrations of procollagen type I C-terminal propeptide (a peptide that is cleaved from procollagen type I during the synthesis of fibril-forming collagen type I) may provide indirect diagnostic information on both the extent of myocardial fibrosis and the ability of antihypertensive treatment to diminish collagen type I synthesis and reduce myocardial fibrosis. This approach represents an exciting and innovative strategy, and available data set the stage for larger trials, in which noninvasive measures of fibrosis in hypertensive heart disease could prove useful. (Hypertension. 2001;38:1222-1226.)

Key Words: collagen ■ hypertension, essential ■ myocardium ■ remodeling ■ renin-angiotensin system

A substantial increase in fibrillar collagen deposition, which leads to increased interstitial and perivascular fibrosis (Figure 1), has been observed in the cardiac ventricles of animals and humans with arterial hypertension (for review, see Weber1). Reactive or reparative hypertensive myocardial fibrosis is the result of both increased collagen type I and III synthesis by fibroblasts and unchanged or decreased extracellular collagen degradation.2 Hemodynamic and nonhemodynamic factors may be involved in the disequilibrium between myocyte growth and collagen turnover that occurs in hypertension.3 A number of experimental studies provide evidence that angiotensin II can mediate myocardial fibrosis independently of mechanical load either directly or via specific growth factors (for review, see Weber4 and Dostal5).

As shown experimentally6 and clinically,7 a rise in collagen content increases myocardial stiffness and promotes abnormalities of cardiac function, whereas its regression normalizes stiffness and function. In addition, the perivascular accumulation of collagen fibers may impair the vasodilator capacity of intramyocardial coronary arteries and contribute to the decrease in coronary reserve that is commonly seen in the hypertensive heart.8 On the other hand, alterations in the electrical activity of the left ventricle in hypertensive patients have been shown to be related to the degree of myocardial fibrosis.9

Although microscopic examination of cardiac biopsies is the most reliable method for documenting and measuring myocardial fibrosis, the development of noninvasive methods to indicate the presence of myocardial fibrosis in hypertensive patients would have broader application. In this regard, a correlation between echoreflectivity and histologically assessed collagen was recently shown in the heart of hypertensive patients,10 suggesting the possibility of noninvasive ultrasonic characterization of myocardial texture in hypertensive heart disease.

We have applied a biochemical method based on the measurement of serum peptides derived from the tissue synthesis and degradation of fibrillar collagens to monitor the turnover of these molecules in rats with spontaneous hypertension (SHR) and in humans with essential hypertension.11 Because collagen type I is much more abundant in cardiac fibrosis than is collagen type III,12,13 this brief review will focus on biochemical monitoring of collagen type I metabolism.

Biochemical Assessment of Collagen Type I Synthesis and Degradation

Fibrillar collagen is synthesized in the fibroblasts as procollagen containing an N-terminal and a C-terminal propeptide (Figure 2).14 After procollagen has been secreted into the

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extracellular space, the propeptides are removed by specific proteinases, allowing integration of the rigid collagen triple helix into the growing fibril (Figure 2).14

The 100-kDa procollagen type I C-terminal propeptide (PIP) is cleaved from procollagen type I during the synthesis of fibril-forming collagen type I and is released into the blood stream (Figure 2). A stoichiometric ratio of 1:1 exists between the number of collagen type I molecules produced and that of PIP released. Circulating PIP is cleared from the blood by the liver (Figure 2).15 Several clinical observations have demonstrated that high serum levels of the propeptide, measured by specific radioimmunoassay, reflect ongoing fibrosis in organs such as the liver and the lung.16 Therefore, the serum concentration of PIP can be considered as a useful marker of collagen type I synthesis in conditions of preserved liver function.

The rate-limiting step in the degradation of collagen type I fibrils is catalytic cleavage by interstitial collagenase (Figure 2).17 This enzyme cleaves all 3 α-chains of collagen at a single, specific locus located at a distance three fourths of the way from the N-terminal. The resulting 36-kDa and 12-kDa telopeptides maintain their helical structure and are resistant to further proteolytic degradation. The big telopeptide spontaneously denatures into nonhelical gelatin derivatives, which in turn are completely degraded by interstitial gelatinases (Figure 2).

The small 12-kDa pyridinoline cross-linked C-terminal telopeptide resulting from the cleavage of collagen type I (CITP) is found in an immunochemically intact form in blood, where it appears to be derived from tissues with a stoichiometric ratio of 1:1 between the number of collagen type I molecules degraded and that of CITP released (Figure 2).18 CITP appears to be cleared from the circulation via glomerular filtration (Figure 2). In recent clinical studies, serum concentrations of the telopeptide measured by radioimmunoassay were found to be related to the intensity of the degradation of collagen type I fibrils.16 Therefore, the serum concentration of CITP can be considered as a useful marker of collagen type I degradation in conditions of normal renal function.

Figure 1. Histological section of myocardial biopsy specimen from a patient with essential hypertension, showing interstitial (A) and perivascular (B) fibrosis. (picrosirius red stain, ×20)

Figure 2. Diagrammatic depiction of the different compartments of fibrillar collagen turnover. The origin and destination of the serum markers of collagen type I synthesis (procollagen type I C-terminal propeptide or PIP) and collagen type I degradation (collagen type I pyridinoline cross-linked C-terminal telopeptide or CITP) are indicated.
PIP and CITP in Arterial Hypertension

Experimental Studies
In recent studies,19–21 we measured serum PIP and CITP concentrations by specific radioimmunoassays in normotensive Wistar-Kyoto rats (WKY), SHR, SHR chronically treated with the ACE inhibitor, quinapril, SHR chronically treated with the angiotensin type I receptor antagonist (ARA) losartan. All animals were male adults (30 to 36 weeks old), and SHR exhibited left ventricular hypertrophy and fibrosis at the onset of the studies. The presence and intensity of interstitial and perivascular fibrosis of the left ventricle was evaluated using quantitative morphometry in sections in which collagen was specifically stained and using immunohistochemical techniques.

In untreated SHR, compared with WKY, we found more extensive interstitial and perivascular fibrosis, increased collagen volume fractions, more marked accumulation of collagen type I, increased serum PIP concentrations, and similar serum CITP concentrations.19–21 Interestingly, a direct correlation was found between the collagen volume fraction and serum PIP in untreated SHR.19

In quinapril-treated SHR19 and losartan-treated SHR,20,21 compared with untreated SHR, we found a marked reduction in fibrosis, lower collagen volume fractions, diminished accumulation of collagen type I, decreased PIP concentrations, and a tendency to increased CITP concentrations. In losartan-treated SHR, these effects were observed at doses that did not normalize blood pressure.21

The ratio between PIP and CITP, an index of the degree of coupling between the synthesis and degradation of collagen type I,22 was abnormally increased in untreated SHR and became normalized after treatment in SHR with both agents (Figure 3A).19–21

Clinical Studies
We measured serum PIP and CITP concentrations by specific radioimmunoassays in patients with essential hypertension who had never been treated and in normotensive individuals who acted as controls.23–25 Measurements were repeated in hypertensive patients after chronic treatment with either the ACE inhibitor lisinopril23,24 or the ARA losartan.25

Baseline serum PIP concentrations were increased in hypertensive patients compared with normotensive individuals.23–25 No significant differences were found between baseline serum CITP levels in hypertensive patients compared with normotensive individuals.24 The ratio between PIP and CITP was higher in hypertensive patients than in normotensive individuals (Figure 3B).25 Serum PIP concentrations correlated directly with the left ventricular mass index in the hypertensive group.23 In addition, serum PIP related directly to the severity of ventricular arrhythmias in the hypertensive group.23

Patients treated with lisinopril23,24 or losartan25 had normalization in blood pressure, regression of left ventricular mass index, improvement of diastolic dysfunction, and a diminution in the number of ventricular extrasystoles. Serum PIP concentration decreased to normal values, and CITP concentration tended to be increased in these patients.23,24,27

The ratio between PIP and CITP was normalized in losartan-treated patients (Figure 3B).27

In 2 recent studies,26,27 transvenous endomyocardial biopsies were performed, and collagen volume fraction was determined on picrosirius red-stained septal tissue sections with an automated image analysis system. Hearts from autopsies performed in normotensive subjects were used as controls in these studies.26,27

In one of these studies, we found that serum PIP concentrations correlated directly with collagen volume fraction in hypertensive patients (Figure 4).26 Furthermore, using receiver operating characteristic curves, we observed that a cutoff of 127 μg/L for PIP provided 78% specificity and 75% sensitivity for predicting severe myocardial fibrosis, with a relative risk of 4.80 (95% confidence interval, 1.19 to 10.30).26

In the other study, we observed a strong association between treatment-induced changes in tissue collagen content and treatment-induced changes in serum PIP in hypertensive patients.27 Furthermore, the ability of antihypertensive treatment to reduce blood pressure did not predict its capacity to either regress myocardial fibrosis or normalize collagen type I synthesis in these patients.27 Thus, chronic angiotensin type 1 blockade with losartan, but not chronic calcium channel...
blockade with amlodipine, resulted in a decrease of both collagen volume fraction and PIP in hypertensive patients.27

**Discussion**

Collectively, these data suggest the following: (1) tissue metabolism of collagen type I is abnormal in rats and humans with primary hypertension and myocardial fibrosis; (2) pharmacological interference of the renin-angiotensin system normalizes collagen type I metabolism and regresses myocardial fibrosis in SHR and essential hypertensive patients; (3) serum concentrations of PIP and CTPP may be of interest for assessing the synthesis and degradation, respectively, of collagen type I fibers in patients with essential hypertension; and (4) serum PIP may be a useful marker of collagen type I–dependent myocardial fibrosis in hypertensive patients.

If an equilibrium is to exist between collagen synthesis and degradation, as proposed by Laurent,28 our findings of increased serum PIP concentrations and normal serum CTPP concentrations in SHR and in hypertensive patients suggest that the intensity of the extracellular degradation of collagen type I is not enough to equilibrate the increased extracellular synthesis of collagen type I. This, in turn, can result in organ fibrosis (i.e., heart, vessels, and kidney) in primary hypertension.

The effects of ACE inhibitors and ARAs on serum PIP suggest that these agents inhibit the synthesis of collagen type I in hypertension. ACE inhibitors and ARAs can inhibit the synthesis of fibrillar collagen via suppression of the direct stimulatory action exerted on fibroblasts by angiotensin II.29

Our data in SHR treated with doses of losartan that do not normalize blood pressure21 and the dissociation of the effects on PIP and blood pressure in patients treated with either losartan or amlodipine27 support a predominant direct role for angiotensin II in fibroblast-mediated overproduction of collagen type I in hypertension.

The tendency to increased CTPP despite the decrease in PIP observed in treated SHR19–21 and treated hypertensive patients23–25 suggest that the pharmacological interference with the renin-angiotensin system results in degradation of collagen type I fibers. This is supported by the recent observation that chronic blockade of angiotensin type 1 receptors is associated with stimulation of left ventricular collagenase in SHR.21

It is clear that PIP detectable in serum is not exclusively heart-specific. Nevertheless, we have calculated that in the SHR,19,22 changes in the cardiac compartment of collagen type I can alter concentrations of PIP in the circulation and that other extracardiac sources able to elevate the serum concentrations of PIP can be excluded. Whether this is also the case in hypertensive patients, we can propose that measurement of serum PIP concentrations may provide indirect diagnostic information on the development of collagen type I–dependent myocardial fibrosis in arterial hypertension in the absence of liver or renal disease. This is further supported by the relations here reported between serum PIP and collagen volume fraction in the free left ventricular wall of SHR and the interventricular septum of hypertensive patients.

On the other hand, our findings that pharmacological interventions that regress myocardial fibrosis also decrease serum PIP suggest that determination of this peptide may be useful to assess the cardio reparative properties of antihypertensive treatment in hypertensives.

**Perspectives**

The available experimental and clinical data suggest that the biochemical monitoring of fibrillar collagen type I turnover may provide a potential non-invasive method of documenting both the extent and mechanisms of hypertensive myocardial fibrosis and in assessing pharmacological measures designed to prevent its appearance or even to cause its regression.

Recent clinical trials27,28 performed with endomyocardial biopsies underscore the potential for targeting myocardial fibrosis in hypertensive patients using a cardio reparative strategy. Since the use of cardiac biopsies is an invasive methodology not useful for wide-scale application, and beyond that may be subject to sampling error, serum tests can be performed on a frequent basis. The studies here reviewed have set the stage for larger on-going trials wherein serological markers of fibrillar collagen turnover would be of great interest to assess the antifibrotic ability of pharmacological interventions aimed to repair the myocardium in cardiac diseases.30

**References**


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