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Monitoring fibrillar collagen turnover in hypertensive heart disease

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1. Hypertensive myocardial fibrosis

A substantial increase in fibrillar collagen has been observed in the cardiac ventricles of animals [1] and humans [2] with arterial hypertension. Either reactive or reparative hypertensive myocardial fibrosis is the result of both increased collagen types I and III synthesis by fibroblasts and unchanged or decreased extracellular collagen degradation [3]. Hemodynamic and non-hemodynamic factors may be involved in the disequilibrium between collagen synthesis and degradation that occurs in hypertension [4].

As shown experimentally [1] and clinically [5,6], a rise in collagen content increases myocardial stiffness and promotes abnormalities of cardiac 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, which is commonly seen in the hypertensive heart [7]. 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 [6].

Although microscopic examination of cardiac biopsies is the most reliable method for documenting and measuring myocardial fibrosis, the development of non-invasive methods to indicate the presence of myocardial fibrosis in hypertensive patients would be useful. We have therefore applied a biochemical method based on the measurement of serum peptides derived from the tissue formation and degradation of fibrillar collagens to monitor the turnover of these molecules in rats with spontaneous hypertension (SHR) and in patients with essential hypertension.

2. Biochemical assessment of fibrillar collagen synthesis and degradation

2.1. Fibrillar collagen synthesis

Fibrillar collagen is synthesized in the fibroblasts as procollagen containing an amino-terminal and a carboxy-terminal propeptide (Fig. 1) [8]. After procollagen has been secreted into the extracellular space, the propeptides are removed by specific proteinases, allowing integration of the rigid collagen triple helix into the growing fibril (Fig. 1) [8].

The 100-kDa procollagen type I carboxy-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 (Fig. 1). A stoichiometric ratio of 1:1 exists between the number of collagen type I molecules produced and that of PIP released [8]. Circulating PIP is cleared from the blood by the liver (Fig. 1) [9]. Therefore, the serum concentration of PIP has been proposed as a useful marker of collagen type I synthesis in conditions of preserved liver function [10]. This has been confirmed by several clinical observations demonstrating that high serum levels of the propeptide measured by specific radioimmunoassay reflect continuous tissue fibrosis [11–13].

The 42-kDa procollagen type III amino-terminal propeptide (PIIIP) is formed during the conversion of procollagen type III to collagen type III and is released into the blood in a stoichiometric fashion (Fig. 1) [8]. As PIIIP appears to be cleared from the blood via hepatobiliary elimination (Fig. 1) [14], the serum levels of this propeptide have been proposed as a useful marker of collagen type III synthesis in conditions of preserved bile excretion [10]. This is sustained by a diversity of clinical

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[15–17] observations demonstrating that high serum levels of the propeptide reflect ongoing tissue fibrosis.

2.2. Fibrillar collagen degradation

The rate-limiting step in the degradation of collagen type I fibrils is catalytic cleavage by interstitial collagenase (Fig. 1) [18]. This enzyme cleaves all three alpha chains of collagen at a single, specific locus located at a distance of three-fourths from the amino-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 non-helical gelatin derivatives which, in turn, are completely degraded by interstitial gelatinases (Fig. 1).

The small 12-kDa 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 (Fig. 1) [19]. CITP appears to be cleared from the circulation via glomerular filtration (Fig. 1) [19]. In recent studies [19–21], serum concentrations of the telopeptide were found to be related to the intensity of the degradation of collagen type I fibrils in patients with normal renal function.

3. Fibrillar collagen synthesis and degradation-derived peptides in arterial hypertension

3.1. Animal studies

In a recent study [22], we measured serum PIP and CITP concentrations by specific radioimmunoassays in normotensive Wistar-Kyoto rats (WKY), SHR and SHR treated with the angiotensin converting enzyme (ACE) inhibitor, quinapril, for 20 weeks. The Masson trichrome stain was used to evaluate the presence and intensity of interstitial and perivascular fibrosis of the left ventricle, and the deposition of collagen type I was assessed by immunohistochemistry using a specific monoclonal antibody.

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 (Table 1) [22]. In quinapril-treated SHR, compared with untreated SHR, we found marked decreases of fibrosis, lower collagen volume fractions, diminished accumulation of collagen type I, decreased PIP concentrations and similar CITP concentrations (Table 1) [22]. A direct correlation was found between the collagen volume fraction and serum PIP in

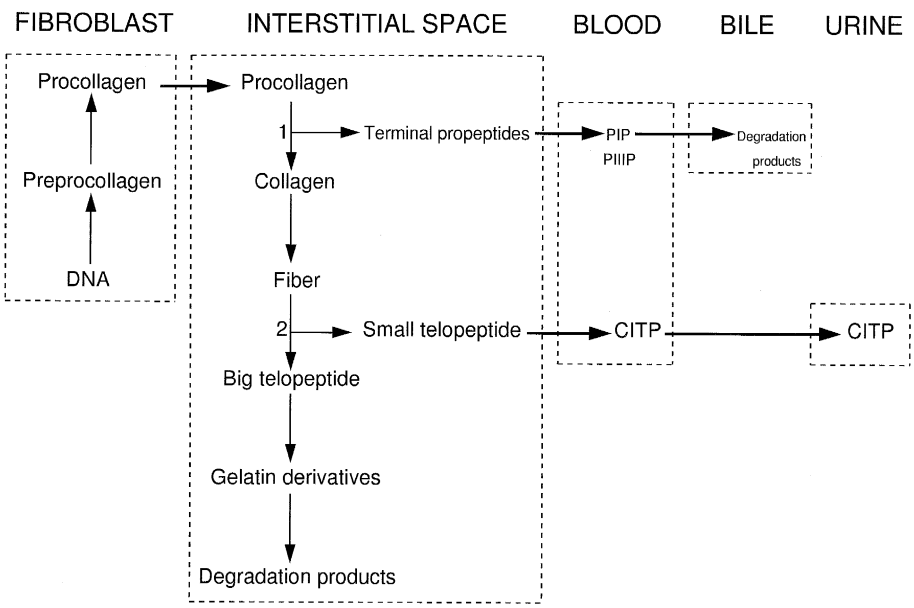

Fig. 1. Diagrammatic depiction of the different compartments of fibrillar collagen turnover. The origin and destination of the serum markers of collagen type I synthesis (PIP), collagen type III synthesis (PIIIP) and collagen type I degradation (CITP) are indicated. 1 = procollagen-specific amino- and carboxy-terminal proteinases; 2 = collagenase.

Table 1
Serum markers of fibrillar collagen turnover in arterial hypertension

Experimental condition	PIP (μg/l)	CITP (μg/l)	PIP/CITP	PIIP (ng/ml)
Rats				
Normotensive Wistar-Kyoto (WKY)	8.56 ± 0.56	5.24 ± 0.17	1.60 ± 0.27	–
Spontaneously hypertensive (SHR)	13.13 ± 0.94 *	5.86 ± 0.19	2.20 ± 0.39 **	–
Quinapril-treated SHR (Q-SHR)	8.88 ± 0.62	5.79 ± 0.19	1.53 ± 0.22	–
Humans				
Normotensive individuals (NI)	108 ± 6	–	–	8.47 ± 0.77
Essential hypertensive (EH)	139 ± 6 ***	–	–	10.08 ± 0.39 ***
Lisinopril-treated EH (L-EH)	111 ± 5	–	–	8.42 ± 0.56

Adapted from Refs. [22–24]. Values are expressed as mean ± s.e.m. PIP = procollagen type I carboxy-terminal propeptide; CITP = collagen type I carboxy-terminal telopeptide; PIIP = procollagen type III amino-terminal propeptide.
* $P < 0.01$ compared to WKY and Q-SHR; ** $P < 0.05$ compared to WKY and Q-SHR; *** $P < 0.001$ compared to NI and L-EH.

untreated SHR [22]. The ratio between PIP and CITP was abnormally increased in untreated SHR and became normalized after treatment in quinapril-treated SHR (Table 1) [22].

3.2. Human studies

We recently measured serum PIP [23] and PIIIP [23,24] concentrations by specific radioimmunoassays in patients with essential hypertension who had never been treated and in normotensive individuals who acted as controls. Patients with conditions associated with elevated serum PIP or PIIIP concentrations were excluded. Measurements were repeated in hypertensive patients after treatment with the ACE inhibitor lisinopril for 6 months.

Baseline serum PIP [23] and PIIIP [23,24] concentrations were increased in hypertensive patients, compared with normotensive individuals (Table 1). Serum PIP concentrations correlated directly with the left ventricular mass index in the hypertensive group [23]. In addition, serum PIP concentrations related directly to the Lown-Wolf grade of ventricular arrhythmias in the hypertensive group [23]. On the other hand, serum PIIIP concentrations correlated inversely with maximal early transmitral flow velocity measured during diastole [24] and with the ratio between maximal early transmitral flow velocity and maximal late transmitral flow velocity measured during diastole [23] in the hypertensive group.

Patients treated with lisinopril had normalization in blood pressure, regression of left ventricular mass index, amelioration of diastolic filling and a diminution in the number of ventricular extrasystoles [23,24]. Serum PIP and PIIIP concentrations decreased to normal values in these patients (Table 1) [23,24].

4. Discussion

The findings of the above studies [22–24] show an increase in serum concentrations of PIP and PIIIP in essential hypertensive patients and in SHR. The relations observed between serum PIP and PIIIP and parameters of myocardial mass and composition, function and electrical activity of the left ventricle suggest that these circulating procollagen-derived propeptides may reflect continuing myocardial accumulation of fibrillar collagen molecules in arterial hypertension.

On the other hand, if an equilibrium is to exist between collagen synthesis and degradation, as proposed by Laurent [25] our findings of normal serum concentrations of CITP and increased PIP/CITP ratio in SHR [22] 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 in this model of genetic hypertension.

Interestingly, we have calculated that in the SHR [22,26] changes in the cardiac compartment of collagen type I can alter concentrations of PIP and CITP in the circulation and that other extracardiac sources able to elevate the serum concentrations of PIP and CITP can be excluded. We therefore propose that measurement of serum PIP and CITP concentrations may provide indirect diagnostic information on the development of myocardial fibrosis in arterial hypertension.

Due to their pharmacological properties neither quinapril [27] nor lisinopril [28] seems to stimulate either the hepatic or the renal elimination of the above three peptides. Accordingly, the effects of ACE inhibitors on serum PIP and PIIIP concentrations and the ratio PIP/CITP suggest that these agents influence the synthesis and/or the degradation of fibrillar collagen. ACE inhibitors can inhibit the synthesis of fibrillar collagen via suppression of the direct stimulatory action exerted on fibroblasts by mechanical strain, angiotensin II and aldosterone [29]. On the other hand, it has been shown that active collagenase is selectively inhibited by the plasminogen activator inhibitors (PAIs) [30]. Since angiotensin II has the capacity to induce PAIs [31], it can be proposed that ACE inhibition may stimulate collagen degradation via the suppression of angiotensin-II-dependent PAI synthesis.

5. Conclusions and perspectives

The preliminary experimental and clinical data reviewed here suggest that the biochemical monitoring of

fibrillar collagen turnover may provide a potential non-invasive method of assessing both the presence and mechanisms of myocardial fibrosis and the ability for cardiac repair of antihypertensive drugs in hypertensive patients.

It is clear that none of the collagen tissue peptides detectable in serum is exclusively heart-specific or unambiguously reflects either fibrogenesis, fibrolysis or fibrosis in hypertensive heart disease. However, if the diagnosis is not established, which is still based primarily on histological assessment, measurement of these peptides or a combination of them should provide information not available by any other known parameter. Nevertheless, we are aware that further clinical and experimental studies are necessary to definitively validate this approach.

While a cardiac-based biopsy protocol is not manageable and beyond that may be subject to sampling error, serum tests can be performed on a frequent basis. This kind of patient control will definitely be necessary, once the availability of potent antifibrotic drugs triggers clinical trials or even leads to novel standard treatment for fibrogenic cardiac diseases, a development which can be anticipated in the immediate future.

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