Arterial hypertension induces numerous alterations in the composition of cardiac tissue, which, in turn, result in structural remodeling of the myocardium. This remodeling is due to a range of pathologic mechanisms associated with mechanical, neurohormonal and cytokine processes that affect both cardiomyocyte and non-cardiomyocyte compartments of the myocardium. One of these processes involves disruption of the equilibrium between the synthesis and degradation of type-I and type-III collagen molecules. The result is excess accumulation of type-I and type-III collagen fibers in interstitial and perivascular spaces in the myocardium. The clinical significance of myocardial fibrosis lies in its contribution to the development of cardiac complications in hypertensive patients. This brief review focuses on the mechanisms of myocardial fibrosis and their clinical consequences. In addition, the techniques used for diagnosing myocardial fibrosis and the main therapeutic strategies for reducing fibrosis are also discussed.

Key words: Collagen. Fibrosis. Systemic arterial hypertension. Peptides.

INTRODUCTION

Sustained elevation of blood pressure (BP) is associated with a significant increase in cardiovascular morbidity and mortality in hypertensive patients. This is because arterial hypertension (AHT) can damage the structure and alter the function of the heart, brain, and kidney. Specifically, patients with AHT are at threat of developing a series of structural and functional alterations of the heart that constitute so-called hypertensive heart disease (HHD). Left ventricular hypertrophy (LVH) forms the macroscopic injury characteristic of HHD, but a series of microscopic changes underlie this giving rise to an entity known as myocardial remodeling. Cardiomyocyte hypertrophy and apoptosis, myocardial fibrosis, and intramyocardial artery and arteriole wall hypertrophy are the definitive structural elements of myocardial remodeling present in HHD.
MOLECULAR AND CELLULAR BASES

Like other organs, the heart consists of highly differentiated parenchymal cells, cardiomyocytes, and stroma formed by the extracellular matrix, tissue fluid, and undifferentiated multipotent mesenchymal cells. The cardiac extracellular matrix is mainly made up of fibrillar and non-fibrillar collagen, laminin and elastin fibers, proteoglycans and integrins. Fibrillar type I and III collagen molecules are the most abundant in adult heart and exhibit their typical triple helical shape due to the spatial orientation of their α-polypeptide chains. Fibrillar collagen serves as a structural framework for cardiomyocytes and the intramyocardial vasculature, which makes it resistant to distortion during the cardiac cycle. Furthermore, fibrillar collagen connects the contractile elements of adjacent cardiomyocytes, as well as acting as a transducer of cardiac muscle contraction toward the ventricular chamber. Although there is a shortage of fibrillar collagen in specific cardiac chambers; the amount of fiber accumulation is inversely related to the number of cardiomyocytes and directly related to their degree of hypertrophy.

CAUSAL MECHANISMS

The excess of myocardial collagen fibers in HHD is the result of a combination of an increase in collagen synthesis, by fibroblasts and myofibroblasts, and a decrease or absence of change in its degradation by matrix metalloproteinases. This hypothesis is supported by experimental findings that show overexpression of procollagen type I genes (collagen type I precursor) and reduced collagenase (enzyme governing collagen type I degradation) activity in hypertrophied left ventricle of spontaneous hypertensive rats (SHR). The combination of different factors (hemodynamic, hormonal, genetic and environmental) can give rise to this imbalance.

Hemodynamic Factors

In vivo experiments have shown that chronic pressure overload stimulates both gene expression and collagen protein synthesis in the myocardium, which favors excess deposition of collagen fibers and the resulting fibrosis. Furthermore, in vitro studies have shown that procollagen type I synthesis is stimulated in cardiac fibroblasts under mechanical cyclic overload, like that produced under AHT conditions. Thus, hemodynamic left ventricular overload due to AHT can favor myocardial fibrosis.

Several clinical observations support this possibility. Tanaka et al. found that CVF increased from the exterior to the interior of the left ventricular free wall in hypertensive human hearts, which is probably a reflection of the transmural gradient of parietal stress. Rossi reported that when hypertensive patients’ hearts were grouped according to their hypertensive myocardial fibrosis presents the following defining characteristics: there is an initial excessive deposition of type III collagen fibers, followed by type I as the process progresses; the fibers are arranged as bundles lining the interstices and around the intramyocardial vessels; fiber accumulation is not limited to the left ventricle and is also present in the other cardiac chambers; the amount of fiber accumulation is inversely related to the number of cardiomyocytes and directly related to their degree of hypertrophy.

There are few studies on the prevalence of myocardial fibrosis in HHD. A study which established degrees of fibrosis based on comparing CVF values between normotensive subjects without LVH and hypertensive patients with LVH, verified that 11% of the patients presented null-minimum fibrosis, 58% mild-moderate fibrosis, and 31% severe fibrosis (Figure 1). Thus, fibrosis is a practically constant lesion in the myocardium of HHD patients.

ABBREVIATIONS

ANG II: angiotensin II.
HHD: hypertensive heart disease.
ACE: angiotensin-converting enzyme.
ELISA: enzyme-linked immunosorbent assay.
EF: ejection fraction.
CVF: collagen volume fraction.
AHT: arterial hypertension.
LVH: left ventricular hypertrophy.
BP: blood pressure.
PICP: carboxy-terminal propeptide of procollagen type I.
PPAR-γ: peroxisome proliferator-activated receptor-γ.
r-AT1: angiotensin II type 1 receptor.
RIA: radioimmunoassay.
SHR: spontaneously hypertensive rats.
CVF: collagen volume fraction.
EF: ejection fraction.
ELISA: enzyme-linked immunosorbent assay.
ACE: angiotensin-converting enzyme.
HHD: hypertensive heart disease.
ANG II: angiotensin II.
In addition to hemodynamic factors, non-hemodynamic factors can also contribute to the development of myocardial fibrosis in AHT. As mentioned, the first refers to the presence of myocardial fibrosis not only in the left ventricle but also in the right, as reported in post-mortem studies of hearts from HDD patients. Second, recent studies have shown that the ability of antihypertensive treatment to regress myocardial fibrosis in hypertensive patients is independent of its antihypertensive efficacy. Thus, the current view is that the development of myocardial fibrosis can also be a consequence of the hormonal factors that stimulate fibrillar collagen metabolism predominating over those which inhibit it (Table 1). Among these, the renin-angiotensin-aldosterone system (RAAS) agonists are especially relevant. Aldosterone is another hormonal factor that can be relevant in the development of myocardial fibrosis. Chronic aldosterone infusion in uninephrectomized animals induces fibrosis in the myocardium.

TABLE 1. Hormonal Factors Stimulating and Inhibiting Myocardial Fibrillar Collagen Metabolism

<table>
<thead>
<tr>
<th>Stimulating factors</th>
<th>Inhibiting factors</th>
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<tbody>
<tr>
<td>Angiotensin II</td>
<td>Bradykinin</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>Other growth factors (PDGF, TGF-β1)</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Desoxycorticosterone</td>
<td>Natriuretic peptides</td>
</tr>
<tr>
<td>Endothelin</td>
<td>Glucocorticoids</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP)</td>
</tr>
<tr>
<td>Adhesion molecules (ICAM-1, VCAM-1)</td>
<td></td>
</tr>
<tr>
<td>Osteopontin</td>
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</table>

In addition, various findings indicate that interactions between factors produced by cardiomyocytes (e.g., osteopontin), macrophages (e.g., plasminogen activator inhibitor 1), and fibroblasts (e.g., transforming growth factor-beta) would mediate the profibrotic effects of ANG II. Furthermore, fibrosis could be part of a reparative response to the inflammation and oxidative stress induced by ANG II through interacting with r-AT1 located in cardial microvasculature cells.21
ratts fed with a high-salt diet is associated with marked accumulation of collagen fibers in both heart ventricles. In this model, cardiac fibrosis is prevented by spironolactone, a mineralocorticoid receptor blocker, and so the fibrotic mechanism of aldosterone would involve interacting with this receptor present in cardiac fibroblasts and myofibroblasts. In addition, activation of the mineralocorticoid receptor can facilitate the profibrotic action of ANG II via upregulation of r-AT1 expression. It is relevant to note that the profibrotic action of aldosterone seems to be independent of BP, given that mineralocorticoid receptor inhibition with eplerenone reduces the myocardial fibrosis produced in mice with chronic pressure overload in the absence of significant changes in systemic BP.

**Genetic and Environmental Factors**

Some findings indicate that genetic factors play a role in modulating hypertensive myocardial fibrosis. A microsatellite marker for rat ACE gene has been identified, making it possible to differentiate the alleles of this gene and its association with different degrees of enzyme activity in plasma. Higher degrees of ACE activity in the left ventricle and a more extensive development of ventricular fibrosis in response to isoproterenol have been found in rats carrying allele B than in rats carrying allele L treated with the same compound. On the other hand, in a recent study, our group analyzed the influence of A1166C polymorphism of the r-AT1 gene on the ability of losartan to inhibit collagen type I synthesis and regress myocardial fibrosis in HDD patients. The patients were genotyped for this polymorphism and divided into 2 subgroups: AA and AC/CC. Collagen synthesis was significantly greater in the AA patients and decreased with losartan treatment more than in the AC/CC patients. Although the molecular bases for this association are not very clear, they may be related to changes in RAAS cardiac activity.

In the same way that it is known that excess salt intake facilitates the development of LVH in animals and humans with AHT, regardless of its effects on BP, recent experimental findings indicate that this could also be applicable to myocardial fibrosis. Thus, a recent study found that increased salt intake was associated with the development of biventricular myocardial fibrosis in SHR rats, but not in normotensive Wistar-Kyoto rats; furthermore, there was no association between elevated BP and increased CVF in SHR. These findings indicate the potential for myocardial fibrosis of the hypertensive genetic substrate interacting with the exogenous factors commonly linked to AHT.

**CLINICAL CONSEQUENCES**

As shown in Figure 2, myocardial fibrosis can contribute to ventricular dysfunction, reduced coronary flow reserve and ventricular arrhythmias adversely affecting cardiovascular outcomes in HDD patients.
CVF adversely affects diastolic stiffness (promoting diastolic dysfunction), whereas a fourfold increase in CVF or more is associated with an additional increase in diastolic stiffness and a reduction in systolic elasticity (promoting systolic dysfunction).

Several clinical findings support this. Recently, our group has shown that there is a direct association between myocardial collagen content and the stiffness of the left ventricular chamber in HHD patients (Figure 3A), and that regression of the severe fibrosis induced by losartan in these patients is accompanied by reduced myocardial stiffness. Sugihara et al. found that CVF was the most relevant factor associated with diastolic dysfunction in hypertensive patients. Brilla et al. found that the reduction in CVF after chronic treatment with the ACE inhibitor lisinopril was associated with improved left ventricular diastolic function in AHT patients. Our group and McLenachan and Dargie have reported an inverse association between CVF and ejection fraction (EF) in HDD patients. Finally, studies done in patients with HDD-associated heart failure have found an association between reduced myocardial fibrosis and improved cardiac function in such patients.

Reduced Coronary Flow Reserve

HHD patients can present symptoms and signs of myocardial ischemia, although the coronary arteries may appear normal under angiography. Reduced coronary flow reserve is probably the cause of myocardial ischemia in these cases.

Functional and structural alterations in the coronary microcirculation have been reported in HHD that can be associated with reduced coronary flow reserve, including endothelial dysfunction, media layer thickening with reduced lumen and collagen fiber accumulation in the perivascular area. It has been reported that chronic treatment with the ACE inhibitor perindopril induces an increase in coronary flow reserve in hypertensive patients associated with significant perivascular fibrosis regression and a slight, although nonsignificant, reduction in hypertrophy of the arteriolar media layer. Isoyama et al. have shown the relevance of perivascular collagen in impaired coronary flow reserve in experimental studies. It was found that, after declamping the aorta, normalized BP induced regression in the hypertrophied arteriolar media layer; however, coronary flow reserve was only normalized after collagen accumulation in the adventitia was inhibited with α-aminopropionitril. Thus, it can be assumed that perivascular fibrosis is a limiting factor regarding intramyocardial vessel distensibility in HDD patients.

Ventricular Arrhythmias

Epidemiological studies, such as the Framingham study, have shown a high incidence of ventricular arrhythmias in HHD patients. Arrhythmias are associated with greater mortality and sudden death in these patients. McLenachan and Dargie analyzed the possible correlates of ventricular arrhythmias in HHD patients and found that patients with arrhythmias had greater left ventricular mass and CVF values than patients without arrhythmias. Ejection fraction and the number of coronary vessels with significant stenosis (>50%) were similar in the 2 groups of patients. Thus, the high incidence of arrhythmias in HHD patients cannot be attributed exclusively to the occurrence of coronary artery disease or left ventricular dysfunction, but can be related to fibrosis and the adaptive phenotypic changes in LVH-associated cardiomyocytes.

Fibrosis could cause arrhythmias via anatomical uncoupling due to myocardial heterogeneity and via a reentry mechanism generated by the zigzag propagation of the transverse wavefront.
patients.46,47 However, reproducing the information and the increase in CVF in the hearts of HDD reduction in cyclic variation in the backscatter signal echoreflectivity has recently been shown as a context, an association between alterations in abnormal tissue, such as fibrotic tissue.45 In this those observed when ultrasounds interact with interaction of the ultrasound waves with normal tissue enables the identification and characterization of its widespread application.

Histological Diagnosis

Given that myocardial fibrosis is a histopathological lesion, the most reliable diagnostic method would be endomyocardial biopsy. In general, the endomyocardial biopsy procedure is not technically complex and is clinically safe for the patient.44 This assertion is especially relevant taking into account that fibrosis existing in the interventricular septum has been shown to be representative of that on the left ventricular free wall,13 which means that the risk of complications is further reduced by biopsy of the septum via the right ventricle using a venous approach. Nevertheless, it should be recognized that endomyocardial biopsy is an invasive technique that, due to its technical requirements, presents obvious limitations regarding its widespread application.

Diagnosis Via Imaging Methods

Ultrasonic characterization of myocardial tissue can be useful when evaluating the effects of antihypertensive treatment on remodeling. Furthermore, being able to assess fibrosis can be useful when evaluating the effects of antihypertensive treatment on remodeling.

Biochemical Diagnosis

In recent years, alternative methods to previous ones have been developed based on the immunochromatographic determination of peptides derived from the metabolism of collagen type I and III present in the blood (Table 2). Of all the peptides studied, only one, the carboxy-terminal propeptide of procollagen type I (PICP), meets the requirements to consider it both a circulating marker of cardiac collagen type I synthesis and a myocardial fibrosis biomarker.35

The method is based on the following (Figure 4): cardiac fibroblasts and myofibroblasts secrete the procollagen type I precursor molecule into the interstitial space. This is converted into the final collagen type I fiber-forming molecule due to the action of specific proteinases that hydrolyze the terminal peptides of the precursor. Specifically, a specific carboxypeptidase hydrolyzes PICP that, via the cardiac venous and lymphatic systems, flows into the systemic circulation. One molecule of PICP appears in the blood for each procollagen type I molecule that is transformed into one collagen type I molecule, where it can be detected via a specific radio-immunossay (RIA) or enzyme-linked immunosorbent assay (ELISA).

Pilot studies conducted by our group have shown that PICP serum concentrations are abnormally higher in HDD patients1 and SHR,35 and that in both cases serum concentrations of this peptide are directly correlated with CVF (Figure 5A). We have recently shown that PICP concentrations increase progressively as HDD evolves, reaching their highest concentrations in heart failure (CHF) patients, and have a direct

### TABLE 2. Peptides Derived From Collagen Type I and Type III Metabolism That Can Be Determined in Blood

<table>
<thead>
<tr>
<th>Peptides Produced During</th>
<th>Synthesis</th>
<th>Degradation</th>
</tr>
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<tbody>
<tr>
<td>Reference molecule</td>
<td>PICP, PINP</td>
<td>ICTP</td>
</tr>
<tr>
<td>Collagen type I</td>
<td>PICP, PINP</td>
<td>PIIINP</td>
</tr>
<tr>
<td>Collagen type III</td>
<td>PHIIIP</td>
<td>PIIINP</td>
</tr>
</tbody>
</table>

ICTP indicates carboxy-terminal telopeptide of collagen type I; PICP, carboxy-terminal propeptide of procollagen type I; PINP, N-terminal peptide of procollagen type I; PIIINP, N-terminal peptide of procollagen type III.
correlation with the myocardial content of collagen type I. Furthermore, in the same study, we proved the cardiac origin of PICP, given that in hypertensive patients there is a gradient between PICP values measured in coronary blood and those in peripheral blood (Figure 5B), as well as a direct and highly significant correlation between the two. Finally, in other studies we have reported that PICP values and the amount of myocardial fibrosis are modified in parallel under antihypertensive treatment both in SHR and in patients with CHF and normal heart function and in patients with CHF and HHD. Although preliminary, these data indicate that the PICP present in the peripheral blood of HHD patients is essentially of cardiac origin and provides a reliable index of the amount of collagen type I fibers present in the myocardium, as well as changes in the amount of fibrosis induced by the treatment.

In the same way that cerebral and atrial natriuretic peptides are considered biomarkers of systolic dysfunction in CHF patients, it would be interesting to explore whether PICP can be of value as a biomarker of structural myocardial damage in these patients. In fact, we could infer from this observation that serum concentrations of PICP are significantly higher in HHD and CHF patients with depressed EF than in patients with preserved EF. However, such differentiation would not be valid for patients with heart failure deriving from other etiologies, above all those with ischemic heart disease.

THERAPEUTIC ASPECTS

The time may have come to propose that the treatment of hypertensive patients should not focus exclusively on normalizing BP, but should also have as an objective the prevention or correction of structural and functional alterations in AHT target organs. With regard to HHD, the European Society of Hypertension and the European Society of Cardiology state in their guidelines on treating hypertension that “Future studies should investigate treatment-induced effects on indices of collagen content or fibrosis of the ventricular wall, rather than on its mass only.” From this perspective, drugs with the ability to repair myocardial fibrosis will be, basically, those which reestablish equilibrium between the factors that stimulate and those that inhibit the metabolism of collagen type I and type III molecules.

Antihypertensive Drug Findings

This cardioreparative concept has been proven clinically in several prospective studies of limited size where biopsies were used to quantify myocardial
fibrosis. Brilla et al\textsuperscript{15} showed that treating HHD patients with lisinopril reduces myocardial fibrosis independently of BP monitoring and LVH regression, whereas treatment with hydrochlorothiazide does not have such an effect. Fibrosis reduction is associated with improved left ventricular diastolic function. Our group has shown that 1 year of losartan treatment is associated with reduced PICP serum concentrations and reduced CVF in HDD patients.\textsuperscript{18} However, patients treated with amlodipine do not show significant changes in either parameter, despite having similar antihypertensive efficacy.\textsuperscript{16} In a later study, we found that the ability of losartan to induce regression of severe fibrosis in HHD patients is independent of its ability to reduce BP or left ventricular mass and is associated with reduced ventricular chamber stiffness.\textsuperscript{18} Taken together, these data confirm what is found in SHR, where it has been verified that the pharmacological interference on the action and production of ANG II in experimental hypertension is effective in the regression of cardiac fibrosis, independent of its antihypertensive effect.\textsuperscript{8,56}

Recently, we reported that HDD/CHF patients undergoing chronic treatment with torasemide present greater reductions in serum PICP concentrations and reduced CVF in patients treated with furosemide (Figure 6).\textsuperscript{37} Unlike furosemide, it is noteworthy that torasemide is able to inhibit the adrenal secretion of aldosterone\textsuperscript{57} and its bonding to mineralocorticoid receptor,\textsuperscript{58} as well as decreasing transcardiac extraction of aldosterone in CHF patients.\textsuperscript{59} Based on this, it could be hypothesized that its effects on cardiac fibrosis occur by inhibiting the profibrotic actions of aldosterone.

Findings With Other Compounds

Several experimental works have explored alternative therapeutic strategies to reduce myocardial fibrosis. Thus, it has been shown that tranilast [N(3,4-dimethoxycinnamoyl) anthranilic acid]\textsuperscript{60} and AcSDKP (N-acetyl-seryl-aspartyl-lysyl-proline)\textsuperscript{61} reduce inflammation and cardiac fibrosis in rats with experimental hypertension via mechanisms probably related to TGF-β inhibition. It has also been found that fenofibrates-peroxisome proliferators-activated receptor-α (PPAR) activators-reduce myocardial fibrosis in mineralocorticoid-induced hypertensive rats, probably by preventing inflammatory mediator release associated with the NF-κB pathway.\textsuperscript{62} Finally, the proteasome inhibitor MG132 has been shown to eliminate expression of collagen molecules in isolated fibroblasts and reduce myocardial fibrosis in SHR.\textsuperscript{63} Although this group of findings opens new perspectives on the treatment of myocardial fibrosis, additional studies are needed before implementing their therapeutic use in HHD.

Findings Via Other Procedures

Recently, it has been reported that cardiac resynchronization therapy reduces CVF in patients with CHF of varying etiology.\textsuperscript{64} A recently published study reported that the beneficial effects of cardiac resynchronization therapy on cardiac morphology, function and performance are associated with reduced PICP serum concentrations in CHF patients.\textsuperscript{65} The specific mechanisms by which resynchronization reduces collagen type I synthesis and deposition in the myocardium of these patients remains to be clarified.

On the other hand, experimental studies have shown that intracardiac injection of human mesenchymal stem cells reduces fibrosis in animals with myocardial infarction,\textsuperscript{66} probably by releasing factors that inhibit the metabolism of fibrilar collagen.\textsuperscript{67}

CONCLUSIONS

Myocardial fibrosis forms the histomorphological substrate of HDD. Available data indicate that the
RAAS is determinant in myocardial fibrosis developing in HHD. Fibrosis can contribute to the transition of LVH to congestive heart failure in hypertensive patients, as well as to developing other complications specific to HHD. From this point of view, the clinical treatment of these patients should involve something more than the diagnosis and normalization of AHT and LVH. A more thorough approach would include measures also aimed at detecting and treating myocardial fibrosis. Some preliminary evidence indicates that measuring serum PICP can prove useful in diagnosing myocardial fibrosis in HHD patients. On the other hand, evidence already exists showing that the aim of reducing myocardial fibrosis is achievable in HHD patients, mainly via the use of drugs that interfere with the RAAS. Taken together, this information forms the basis for undertaking large, long-term clinical trials to clarify whether the diagnosis and regression of myocardial fibrosis helps to improve the prognosis and evolution of HHD patients.

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53:1445-51.

52:3439-44.

51:1420-6.

50:1263-8.

49:2028-35.

48:1164-5.


45:1164-5.

44:385-93.


42:286-93.

41:401-93.

40:1445-51.


38:439-44.

37:114-126.

36:220-6.

35:111-114.

34:439-44.


32:865-79.

31:27:14-163.

30:12-6.

29:3439-44.

28:1164-5.


26:359-66.

25:1445-51.

24:1420-6.


22:60-1.


20:1011-53.


18:353-61.

17:1445-51.

16:286-35.

15:18:36:35.

14:359-66.

13:1455-41.


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10:1047-57.