Fibrosis in hypertensive heart disease: role of the renin-angiotensin-aldosterone system

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Organs are composed of highly differentiated, very specialized parenchymal cells surrounded by stroma consisting of extracellular matrix, tissue fluid, and undifferentiated pluripotent mesenchymal cells. Parenchymal cells distinguish one organ from another based on their unique morphologic features and their highly specialized functions. Disproportionate stromal growth, relative to parenchymal growth, termed "fibrosis" (sclerosis or cirrhosis), represents a pathologic structural remodeling of an organ [1].

Fibrosis presents in several different morphologic patterns. A reparative (replacement) fibrosis appears at sites of parenchymal cell loss. For example, microscopic scarring of ventricular myocardium follows cardiac myocyte necrosis [2–4], whereas a macroscopic scar follows segmental infarction [5,6]. A perivascular fibrosis of intramural vessels with extensions into the contiguous interstitial space has been referred to as a "reactive fibrosis" because loss of parenchyma is not a requisite [7]. A perivascular-interstitial fibrosis, for example, accompanies chronic elevations in the effector hormones of the renin-angiotensin-aldosterone system [8–11].

Absolute fibrosis is defined as increased collagen concentration of an organ and consists predominantly of fibrillar collagen types I and III that appear secondary to altered collagen turnover (synthesis greater than degradation). Collectively, fibrosis is a common pathway to organ failure. This holds true for such diverse organs as the heart [12,13], kidneys [14,15], lungs [16,17], and liver [18].

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Under certain circumstances, a regression of fibrous tissue occurs after the repair process has been completed. This is related to fibrillar collagen degradation by matrix metalloproteinases [19,20]. In other cases, there is a persistent fibrosis (eg, infarct scar of the myocardium) that is essential for organ integrity. Lastly, a progressive fibrosis can occur (eg, experimental glomerulonephritis, chronic elevation of circulating angiotensin II [ANGII] or aldosterone) and destroys organ architecture and accounts for its failure [20–23]. The need to prevent fibrosis or to regress it, once formed, represents a major challenge. Various strategies can be taken [14].

Myocardial fibrosis in hypertensive heart disease

Clinical evidence

The essential criterion in defining hypertensive heart disease (HHD) is a greater than normal heart mass in the absence of a cause other than arterial hypertension. It is now accepted, however, that besides left ventricular hypertrophy (LVH), alterations in myocardial structure as listed below account for loss of tissue homogeneity and pathologic remodeling that appears in HHD:

Cellular alterations
  Cardiomyocytes
    Hypertrophy, atrophy, apoptosis, necrosis
  Noncardiomyocytes
    Hyperplasia and apoptosis of fibroblasts
    Hypertrophy or hyperplasia of vascular smooth muscle cells

Noncellular alterations
  Interstitial and perivascular fibrosis
  Microscopic scarring
  Medial thickening of coronary arterioles
  Diminished number of capillaries

A growing body of evidence indicates that myocardial fibrosis is one of the key pathologic features of myocardial remodeling in HHD. In fact, a number of studies performed in postmortem human hearts [24–26] and endomyocardial human biopsies [27–30] have shown that myocardial collagen volume fraction, a morphometric measure of the amount of tissue collagen, is constantly increased in patients with HHD compared with normotensive controls (Fig. 1). Furthermore, an exaggerate accumulation of fibrillar collagen types I and III within the myocardial interstitium and surrounding intramural coronary arteries and arterioles has been evidenced immunohistochemically in patients with HHD [31].

Potential mechanisms

Collagen types I and III are the major fibrillar collagens produced by fibroblasts in the adult heart. They exhibit the characteristic triple helical
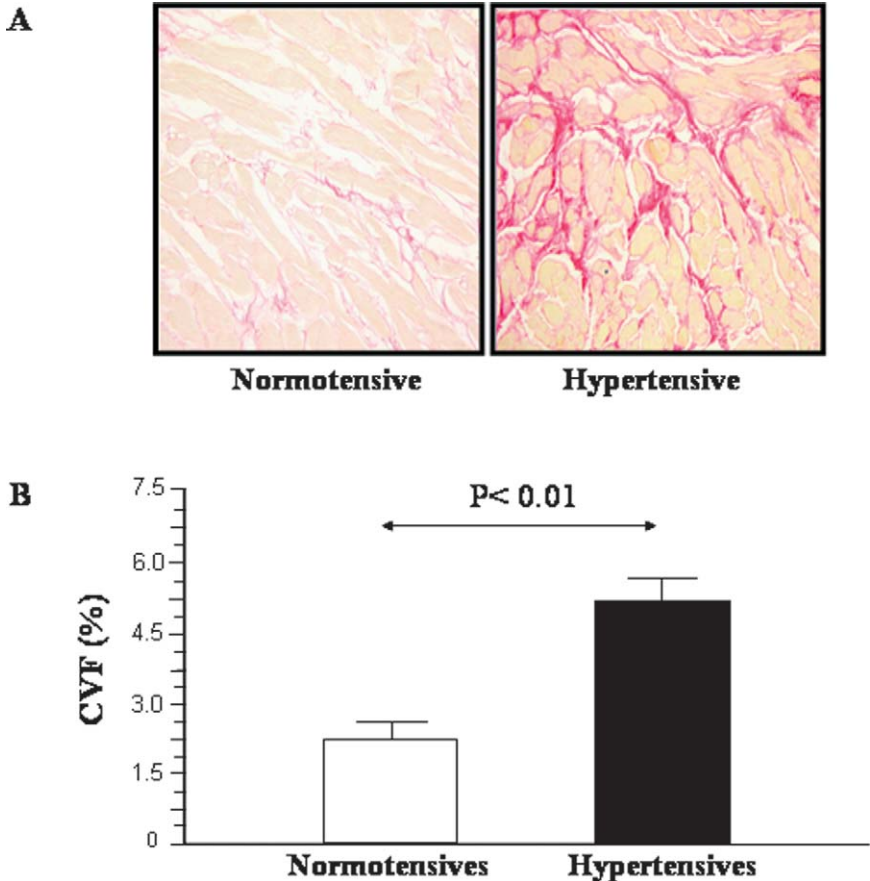
A

B

Fig. 1. (*A*) Histologic sections of interventricular septal specimen biopsies from humans. Sections were stained with picrosirius red, and the interstitial collagen was identified in red (original magnification ×20). (*B*) Collagen volume fraction (CVF) determined in interventricular septal specimen biopsies from humans. (*Adapted from* Querejeta R, Varo N, López B, Larman M, Artiñano E, Etayo JC, et al. Serum carboxy-terminal propeptide of procollagen type I is a marker of myocardial fibrosis in hypertensive heart disease. Circulation 2000;101:1729–35; with permission.)

conformation formed by three polypeptide chains (α chains). Fibrillar collagen of the heart provides the structural scaffolding for cardiomyocytes and coronary vessels and imparts cardiac tissue with physical properties that include stiffness and resistance to deformation [12]. In addition, fibrillar collagen may also act as a link between contractile element of adjacent cardiomyocytes and as a conduit of information that is necessary for cell function.

As in other organs, collagen turnover of normal adult heart results from the equilibrium between the synthesis and degradation of collagen types I and III molecules [32]. The synthesis of collagen molecules follows the

normal pattern of protein synthesis, but it differs from the synthesis of many proteins in that the newly formed α chains undergo a number of post-translational modifications. Extracellular degradation of collagen fibers is mediated by collagenase and other members of the matrix metalloproteinase family of zinc-containing endoproteinases. The active form of collagenase can be inhibited by interaction with naturally occurring specific tissue inhibitors of matrix metalloproteinases (TIMPs).

As shown by in vivo experiments, chronic pressure overload stimulates both procollagen gene expression and collagen protein synthesis leading to excessive collagen deposition and fibrosis [33]. In addition, in vitro studies have shown that procollagen type I synthesis is stimulated in cardiac fibroblasts submitted to cyclic mechanical load [33]. Hemodynamic overload of the left ventricle caused by systemic hypertension may play a role in myocardial fibrosis.

Nevertheless, two types of findings suggest that besides the mechanic factor, nonhemodynamic factors may also contribute to myocardial fibrosis in human hypertension. First, myocardial fibrosis has been found not only in the left ventricle but also in the right ventricle [25,34] and the interventricular septum [35] in postmortem studies of human hearts with HHD. Second, recent studies have shown that the ability of antihypertensive treatment to regress fibrosis in hypertensives with biopsy-proved myocardial fibrosis is independent of its antihypertensive efficacy [29,36]. The current view is that myocardial fibrosis may be the consequence of the loss of reciprocal regulation that normally exists between molecules that stimulate (eg, ANGII) and molecules that inhibit fibrillar collagen turnover [37]. Factors modulating collagen types I and III turnover in the myocardium include the following:

Factors that facilitate the synthesis of collagen
    Vasoactive substances
        Angiotensin II, endothelin-1, catecholamines
    Growth factors
        Transforming growth factor-β_1, platelet-derived growth factor, basic fibroblast growth factor, insulin-like growth factor-1
    Hormones
        Aldosterone, deoxycorticosterone
    Cytokines
        Interleukin-1
    Adhesion molecules
        Ostepontin

Factors that facilitate the degradation of collagen
    Vasoactive substances
        Bradykinin, prostaglandins, nitric oxide, natriuretic peptides
    Growth factors
        Hepatocyte growth factor

Hormones
  Glucocorticoids
Cytokines
  Tumoral necrosis factor-α, interferon-γ
Endogenous peptides
  N-acetyl-seryl-aspartyl-lysyl-proline

An excess of stimulators, caused by either absolute stimulator over-production or by their relative overabundance because of a deficit in inhib-itor formation can promote fibrosis.

Clinical impact

Several arguments support the concept that myocardial fibrosis has a particularly important influence in the process of heart failure associated with cardiac remodeling [38]. First, interstitial fibrosis contributes to ven-tricular wall stiffness and consequently impairs cardiac compliance, con-tributing to impaired diastolic function. Second, because neither the collagen network nor the fibroblasts contribute to systolic contraction, increased collagen deposition and fibroblast volume means that systolic work is being performed by a smaller proportion of the cardiac mass, contributing to systolic dysfunction. Third, perivascular fibrosis leads to increased distance that oxygen must diffuse and potentially lowers Pa_{O_2} for the working car-diomyocytes. Finally, electrical coupling on the cardiomyocytes may be im-paired by the accumulation of collagen proteins and fibroblasts because such accumulation causes morphologic separation of cardiomyocytes.

Role of angiotensin II in myocardial fibrosis

Various lines of evidence support a role for ANGII as a critical candidate factor to induce myocardial fibrosis in HHD.

In vivo evidence

Animal models

Endogenous elevations in circulating ANGII that accompany unilateral renal artery stenosis [39] or the infusion of exogenous ANGII [40] are associated with increased blood pressure and fibrosis. The appearance of such fibrous tissue formation is preceded by increased expression of ANGII type 1 (AT_1) receptors, transforming growth factor-β_1 (TGF-β_1), and mRNA for collagen types I and III [41]. In addition, development of fibrosis involves proliferating fibroblasts and cell differentiation into myofibroblasts [42]. Two observations suggest that the ability of ANGII to induce cardiac fibrosis in these models is independent of its hypertensive action. First, fibrosis in the renal artery stenosis model develops in both low-pressure right and left atria and right ventricle and high-pressure left ventricle [43].

Second, cardiac fibrosis in the ANGII infusion model can be prevented by either angiotensin converting enzyme (ACE) inhibitors or AT_1 receptor antagonists, but not hydralazine or prazosin, despite a similar antihypertensive efficacy of these compounds [44,45]. The critical role of ANGII in hypertension-associated cardiac fibrosis is further supported by the observation that experimental infrarenal aortic binding, which does not induce ANGII, causes blood pressure elevation and cardiomyocyte hypertrophy but not cardiac fibrosis [43].

The hypertensive Ren2 rat provides a well-established model of ANGII-dependent cardiac hypertrophy [46]. Several studies have revealed that interstitial and perivascular fibrosis, along with extensive collagen types I and III deposition are present in Ren2 [47–49]. Increased cardiac renin and ANGII levels have been described in this transgenic rat model [46]. In addition, cardiac lesions are very sensitive to ACE inhibition and AT_1 receptor antagonism in Ren2 rats [50]. As a result, the development of hypertrophy and fibrosis in the heart of these animals has been attributed, at least partially, to a local activation of the cardiac renin-angiotensin system.

Pharmacologic studies

Experimental findings. Pharmacologic interventions with ACE inhibitors or AT_1 receptor antagonists have underscored the potential importance of ANGII in the mediation of cardiac fibrosis in pathologic conditions, such as primary hypertension. In rats with spontaneous hypertension and LVH, myocardial fibrosis has been shown to regress by treatment with the ACE inhibitor lisinopril [51]. This effect occurred independently of the drug's antihypertensive effect [51]. It has been found that chronic AT_1 receptor antagonism with losartan resulted in reversal of fibrosis, inhibition of the posttranscriptional synthesis of procollagen type I, inhibition of TIMP-1 expression, and stimulation of collagenase activity in the left ventricle of adult rats with spontaneous hypertension [52,53]. Analysis of the individual data showed that the intensity of these myocardial changes was independent of the antihypertensive efficacy of the drug [52,53].

Clinical findings. The fibrogenic role of ANGII in humans has been investigated in three recent prospective trials of limited size using biopsy-proved myocardial fibrosis in patients with essential hypertension and LVH. Schwartzkopff et al [28] studied 14 patients before and after 1 year of treatment with the ACE inhibitor perindopril. Structural analysis revealed diminution of perivascular and interstitial fibrosis with treatment. The observed regression of fibrosis on ACE inhibitor treatment was observed in the non–pressure-overloaded right ventricle, indicating that the antifibrotic effect was not accounted for by left ventricular pressure reduction alone. Brilla et al [29] randomized 35 previously treated patients with controlled blood pressure to receive either the ACE inhibitor lisinopril or the diuretic hydrochlorothiazide for 6 months. Only patients randomized to lisinopril

had a significant reduction in myocardial fibrosis. Blood pressure reduction was similar in patients treated with either lisinopril or hydrochlorothiazide. Finally, López et al [36] studied 37 treated patients with uncontrolled blood pressure. After randomization, 21 patients were assigned to the AT_1 receptor antagonist losartan and 16 to the calcium channel blocker amlodipine for 12 months. Whereas myocardial fibrosis decreased significantly in losartan-treated patients, this parameter remained unchanged in amlodipine-treated patients. A similar reduction of blood pressure in losartan-treated patients than in amlodipine-treated patients was reported in this study. Collectively, these observations support the concept that in addition to pressure overload, ANGII induces myocardial fibrosis in essential hypertension.

Cellular and molecular mechanisms

Increasing evidence strongly indicates that ANGII exerts multiple pro-fibrotic effects within the heart including induction of fibroblast hyperplasia, activation of collagen biosynthetic pathways, and inhibition of collagen degradative pathways (Fig. 2). In addition, available data indicate that these effects may result from either the direct action of ANGII or a synergistic cooperation between this peptide and other profibrotic factors.

Stimulation of fibroblast proliferation

In vitro studies of rat and human cardiac fibroblasts have shown that ANGII stimulates cell proliferation by the AT_1 receptor [54]. Results in the literature indicate that the proliferative response of fibroblasts to ANGII might well be mediated by stimulation of the synthesis of growth or in-flammatory substances like platelet-derived growth factor and cytokines, by integrin activation caused by adhesion proteins, or by a combination of these mechanisms [55,56].

For instance, ANGII strongly up-regulates the expression of osteopontin and its ligand αVβ3 integrin in rat and human cardiac cells [57,58]. Interestingly, elevated left ventricular osteopontin expression has been reported in the Ren2 rat model characterized by high myocardial ANGII concentrations [49]. Monoclonal antibodies directed against either osteopontin or αVβ3 completely blocked the mitogenic effect of ANGII on cultured rat
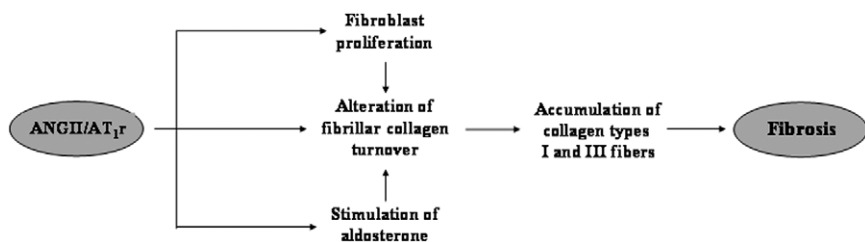

Fig. 2. Potential pathways involved in angiotensin II (ANG II)-mediated fibrosis.

cardiac fibroblasts [58], suggesting that osteopontin mediates ANGII-induced fibroblast proliferation acting by an integrin-dependent pathway.

Stimulation of collagen synthesis

Although different signaling pathways of the AT_1 receptor may subserve direct ANGII-induced collagen synthesis in cardiac fibroblasts [59], recent data suggest that the MAP/ER kinase pathway seems to play a major role [60]. The end result of signaling mechanisms is activation of transcription factors, which bind to various cis-acting elements in the regulatory sequences of α1 and α2 collagen type I and α1 collagen type III genes [60,61]. This, in turn, couples with gene expression and the synthesis of collagen types I and III precursor molecules [62,63].

A number of studies, however, provide strong evidence that ANGII indirectly regulates collagen synthesis by cardiac fibroblasts by specific growth factors [64]. The principal candidates include $TGF-\beta_1$ and endothelin-1.

In fact, ANGII has been shown to induce collagen type I gene expression by activation of $TGF-\beta_1$ signaling pathways (eg, connective tissue growth factor and Smad proteins) and these effects were blocked by the AT_1 receptor antagonist losartan [65]. ANGII has been also shown to increase the expression of $TGF-\beta_1$ in cultured cardiac fibroblasts by stimulation of the AT_1 [54]. Recent data suggest that a Krüppel-like zinc-finger transcription factor 5 (also known as BTEB2 and IKLF) is critically involved in ANGII-induced $TGF-\beta_1$ expression, collagen synthesis, and development of cardiac fibrosis [66]. Besides up-regulation of cardiac gene $TGF-\beta_1$ expression, ANGII has been reported to convert latent $TGF-\beta_1$ to the active protein in vivo in the heart [67].

Endothelin-1 is synthesized and released by cardiac fibroblasts in response to the interaction of ANGII with the AT_1 receptor [68] and has been shown to stimulate the synthesis of collagen types I and III in these cells [69]. In several rat models of arterial hypertension, blockade of endothelin receptors is associated with decrease in left ventricular collagen accumulation [70,71].

There is some in vivo evidence that ANGII also influences post-translational processing of cardiac fibrillar collagen. It has been shown that ANGII infusion is associated with stimulation of prolyl 4-hydroxylase (an enzyme that mediates hydroxylation of procollagen α chains in the endoplasmic reticulum of cardiac fibroblasts) in the rat left ventricle [72]. In addition, it has been reported that immunoreactive prolyl 4-hydroxylase concentration decreases significantly in the ventricle of post–myocardial infarction rats treated with the AT_1 receptor antagonist losartan [73].

Inhibition of collagen degradation

In addition to collagen synthesis, ANGII stimulation of the AT_1 receptor has been shown to regulate collagen degradation by attenuating interstitial collagenase activity in adult rat [54] and human [74] cardiac fibroblasts and by enhancing TIMP-1 production in rat heart endothelial cells [75].

A number of factors may mediate the inhibitory effect of ANGII on cardiac collagen degradation (eg, TGF-β_1 and plasminogen activator inhibitor-1). Cell culture studies on human fibroblasts show that exposure of these cells to TGF-β_1 in the presence of other growth factors (eg, epidermal growth factor and basic fibroblastic growth factor) resulted in down-regulation of collagenase and up-regulation of TIMP-1 [76,77]. Similar findings have been reported in the fibrotic myocardium of TGF-β_1 transgenic mice [78].

Activation of the AT$_1$ receptor in human cardiac fibroblasts has been shown to promote stimulation of plasminogen activator inhibitor-1 expression [79]. This stimulatory effect has been confirmed in the left ventricle of ANGII-induced hypertensive rats [80]. Plasminogen activator inhibitor-1 inhibits the activation of collagenase and other matrix metalloproteinases and thereby collagen degradation [81,82].

Interaction with aldosterone

Experimental studies have demonstrated the central role of aldosterone in promoting cardiac fibrosis, probably through a direct action on the heart mediated by cardiac mineralocorticoid receptors [83–85]. In fact, aldosterone has been shown to stimulate collagen synthesis through the mineralocorticoid receptor in isolated cardiac fibroblasts [86]. In experimental studies on rats with renovascular hypertension, hyperaldosteronism, or spontaneous hypertension, however, the aldosterone antagonist spironolactone was able to prevent or reverse the development of myocardial fibrosis even though the drug did not normalize blood pressure and did not prevent LVH [8,43,87–89]. An increase in this mineralocorticoid may be a mechanism for ANGII-induced cardiac fibrosis in some forms of arterial hypertension.

Interestingly, an increase in the density of AT$_1$ receptors has been observed in the heart of aldosterone-salt–treated rats [90]. In addition, the AT$_1$ receptor antagonist losartan prevents fibrosis and up-regulation of collagen types I and III mRNAs in the heart of aldosterone-salt–treated rats [91]. Taken together, these findings support the hypothesis that one mechanism by which aldosterone induces cardiac fibrosis involves ANGII acting through AT$_1$ receptors. Because the production of aldosterone is activated in the hypertrophied left ventricle of rats with spontaneous hypertension [92] and hypertensive patients [93], it is possible that aldosterone contributes to ANGII-mediated myocardial fibrosis in primary hypertension.

The potential clinical relevance of these interactions is given by several observations. In essential hypertension, a low dose of the aldosterone antagonist canrenone added to antihypertensive treatment has been shown significantly to improve left ventricular diastolic function [94]. This improvement, not accounted for by changes in blood pressure and left ventricular mass, can be ascribed to a direct action of the drug on the myocardium. This is further supported by recent studies showing that chronic administration

of either spironolactone [95–99] or potassium canrenoate [100] is associated with a reduction in the circulating levels of markers of collagen turnover in patients with different cardiac diseases that evolve with myocardial fibrosis.

Summary

Structural homogeneity of cardiac tissue is governed by mechanical and humoral factors that regulate cell growth, apoptosis, phenotype, and extracellular matrix turnover. ANGII has endocrine, autocrine, and paracrine properties that influence the behavior of cardiac cells and matrix by AT_1 receptor binding. Various paradigms have been suggested, including ANGII-mediated up-regulation of collagen types I and III formation and deposition in cardiac conditions, such as HHD. A growing body of evidence, however, deals with the potential role of aldosterone, either local or systemic, in inducing cardiac fibrosis. Aldosterone might also mediate the profibrotic actions of ANGII. To reduce the risk of heart failure that accompanies HHD, its adverse structural remodeling (eg, myocardial hypertrophy and fibrosis) must be targeted for pharmacologic intervention. Cardioprotective agents must reverse not only the exaggerated growth of cardiac cells, but also regress existing abnormalities in fibrillar collagen. Available experimental and clinical data suggest that agents interfering with ACE, the AT_1 receptor, or the mineralocorticoid receptor may provide such a cardioprotective effect.

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