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Arachidonic Acid Metabolism as a Potential Mediator of Cardiac Fibrosis Associated with Inflammation

Scott P. Levick,* David C. Loch,†‡ Stephen M. Taylor,* and Joseph S. Janicki1*

An increase in left ventricular collagen (cardiac fibrosis) is a detrimental process that adversely affects heart function. Strong evidence implicates the infiltration of inflammatory cells as a critical part of the process resulting in cardiac fibrosis. Inflammatory cells are capable of releasing arachidonic acid, which may be further metabolized by cyclooxygenase, lipoxygenase, and cytochrome P450 monoxygenase enzymes to biologically active products, including PGs, leukotrienes, epoxyeicosatrienoic acids, and hydroxyeicosatetraenoic acids. Some of these products have profibrotic properties and may represent a pathway by which inflammatory cells initiate and mediate the development of cardiac fibrosis. In this study, we critically review the current literature on the potential link between this pathway and cardiac fibrosis. *The Journal of Immunology, 2007, 178: 641–646.

Cardiac fibrosis is a detrimental process whereby extracellular matrix production, in particular collagen, is enhanced in the left ventricle (LV) of the heart, leading to a decrease in compliance of the ventricle, and ultimately failure. This pathological process is intimately associated with numerous cardiovascular diseases including hypertension, myocarditis, dilated cardiomyopathy, myocardial infarction (MI), and heart failure. In many of these pathologies, inflammatory cells have been observed to be spatially and temporally associated with cardiac fibrosis (1–4).

Arachidonic acid (AA) is a free fatty acid that when liberated from cell membranes, including inflammatory cells, can be metabolized by cyclooxygenases (COXs), lipoxygenases (LOs), and cytochrome P450 monoxygenases (CYPs) to form biologically active products (5). Many of these products are known to be involved in the development of fibrosis in noncardiovascular inflammatory diseases and, therefore, may be important in cardiac fibrosis. This review will briefly present the evidence linking infiltrating inflammatory cells with the development of cardiac fibrosis and suggests the involvement of AA metabolism as a possible mediator of cardiac fibrosis.

Eicosanoids and fibrosis

The potential link between eicosanoids and fibrotic conditions such as pulmonary fibrosis is now firmly established (6). The link between fibrotic stimuli and AA metabolism was originally investigated in alveolar macrophages, which produce both COX and LO metabolites in abundance (7). Silica particles, a known fibrogenic stimulus, strongly stimulate AA metabolism in alveolar macrophages (8). The fibrogenic potential of various types of silica is related to the degree of both cytotoxicity and stimulation of 5-LO products (9). A recent study has shown that a specific leukotriene receptor antagonist reduces pulmonary fibrosis in an animal model of asthma (10), although, surprisingly, no studies with this drug class have yet been published for cardiac fibrosis. As we shall show herein, there is now ample circumstantial evidence linking eicosanoids with the development of cardiac fibrosis. This is an area that has been slow to develop compared with fibrotic diseases of other organs, and there is clearly a need to now focus on this area with available eicosanoid modulators.

Inflammatory cells and cardiac fibrosis

Schmid-Schönbein et al. (11) first suggested leukocyte involvement in the pathogenesis of hypertension-induced end-organ damage. However, the first study to indicate a possible link between inflammatory cells and LV fibrosis was conducted by Hinglais et al. (1), where young spontaneously hypertensive rats (SHRs; 8 wk old) were found to have small foci of inflammatory cells, consisting of CD4+, CD8+, OX6+ (B cell) lymphocytes, and ED1+ macrophages (resident and infiltrating), in perivascular and interstitial regions. The appearance of these inflammatory cells preceded fibrosis, however, by 12 mo of age in these hypertensive animals, fibroblasts expressing collagen I

*Cell and Developmental Biology and Anatomy, School of Medicine, University of South Carolina, Columbia, SC 29208; Department of Physiology and Pharmacology, School of Biomedical Sciences, University of Queensland, Brisbane, Australia; and Centre for Systems Biology, Faculty of Sciences, University of Southern Queensland, Toowoomba, Australia

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1 Address correspondence and reprint requests to Dr. Joseph S. Janicki, Cell and Developmental Biology and Anatomy, School of Medicine, University of South Carolina, Columbia, SC 29208. E-mail address: jjanicki@pw.med.sc.edu

2 Abbreviations used in this paper: LV, left ventricle; MI, myocardial infarction; AA, arachidonic acid; COX, cyclooxygenase; LO, lipoxygenase; CYP, cytochrome P450 monoxygenase; SHR, spontaneously hypertensive rat; PLA2, phospholipase A2; sPLA2, secretory PLA2; CysLT1, cysteinyl leukotriene; cPLA2, cytosolic PLA2; iPLA2, independent PLA2; TXA2, thromboxane A2; HETE, hydroxyeicosatetraenoic acid; LTB4, leukotriene B4; EET, epoxyeicosatrienoic acid.

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were consistently associated with inflammatory cells. In addition, all of the aforementioned inflammatory cell types were increased in areas of fibrosis. Subsequently, it has been shown that infiltrating ED1+ macrophages also localize with cardiac myofibroblasts (12).

In addition to hypertension, myocardial scarring associated with myocarditis also appears to involve the infiltration of mononuclear cells, lymphocytes, macrophages, and, to a lesser extent, neutrophils (2). Furthermore, histological analysis of myocardial biopsies from patients with dilated cardiomyopathy revealed myocardial damage, severe interstitial fibrosis (up to 41%), and the influx of lymphocytes and macrophages (3). In inflammatory cardiomyopathic patients, Pauschinger et al. (13) found an increased collagen I to III ratio, which suggested a role for inflammation in not only eliciting fibrosis but also adversely altering the ratio of collagen isofoms in the heart.

A causal relationship between inflammatory cells and fibrosis has been established using anti-MCP-1 gene therapy. Such therapy was successful in reducing overall macrophage infiltration, and subsequently interstitial fibrosis, post-MI in rats. Interestingly, the treatment did not alter the size of the infarct itself (4). This may be due to lymphocyte and neutrophil infiltration which was not reduced in the infarct zone. However, reduced macrophage numbers were associated with reduced LV total collagen, as well as interstitial collagen, in rats treated with the antifibrotic, N-acetyl-seryl-aspartyl-lysyl-proline, following MI (14). Furthermore, anti-MCP-1 was found to prevent macrophage accumulation and attenuate perivascular and interstitial fibrosis in hypertension (15). Other studies have also shown that prevention of macrophage infiltration reduces cardiac fibrosis in hypertension (16, 17). Using an alternative approach, stimulation of GM-CSF in MI rats led to dramatic increases in ED1+ macrophages at the infarct site at 7 days post-MI (18). These hearts showed significantly increased collagen by day 14 beyond that of normal MI hearts.

As mentioned earlier, lymphocytes are also spatially located with macrophages in fibrotic areas of the LV. This may be because Th1 cells release cytokines (e.g., IFN-γ, TNF, and TGF-β) that activate macrophages, whereas Th2 cells release IL-4 and IL-13, which “deactivate” macrophages (19). Therefore, T cells may exert effects on fibrosis through the regulation of macrophage activity. The potential importance of T cells was clearly demonstrated by Yu et al. (20) who recently presented compelling evidence that modulating T cell phenotype has a dramatic impact on cardiac fibrosis. They demonstrated that a shift toward the Th1 phenotype was associated with increased total collagen, increased collagen cross-linking, and an increased LV stiffness. Conversely, a shift to the Th2 phenotype caused a decrease in total collagen, decreased collagen cross-linking, and decreased LV stiffness. Unfortunately, this study did not investigate macrophage infiltration; therefore, no conclusions can be drawn as to whether or not these effects were achieved by manipulating macrophage activity.

Mast cells are also known to play roles in cardiovascular pathologies since cardiac mast cell numbers are increased in dilated cardiomyopathy (21) and in the infarct zone post-MI (22). Hara et al. (23) further reported that aortic banding in mast cell-deficient mice resulted in reduced perivascular fibrosis in comparison with wild-type mice that had also undergone aortic banding, suggesting a role in hypertension-induced LV fibrosis. Alternatively, Brower et al. (24, 25) have demonstrated that mast cells are involved in the matrix metalloproteinase-regulated breakdown of the extracellular matrix in heart failure. Taken together, these findings indicate that mast cells may be capable of playing dual roles in the regulation of fibrosis in the heart.

The evidence presented herein clearly links inflammatory cells, including macrophages, T cells, and mast cells, with cardiac fibrosis from varying pathologies. These infiltrating inflammatory cells are capable of releasing numerous cytokines, which may regulate the further infiltration of inflammatory cells as well as cardiac fibroblasts. However, another mechanism by which these infiltrating inflammatory cells may contribute to cardiac fibrosis may be through metabolites of AA.

AA metabolism and cardiac fibrosis

Phospholipase A2 (PLA2) enzymes. While not products of AA, PLA2 enzymes are critical for AA metabolism since they liberate AA from the cell membrane by hydrolyzing glycosphospholipids. Secretory PLA2 (sPLA2) enzymes are of particular interest since group IIA mRNA is expressed in cardiac myocytes (26), and numerous types have been detected in the myocardium, including groups IIA, IIE, IIF, V, and X (27). Following MI, group IID is also detectable in damaged cardiomyocytes. The fact that these enzymes are differentially located in the LV suggests that they may have somewhat differing roles. However, the similarity in localization to the area of damaged cardiomyocytes by sPLA2-IIA and sPLA2-V suggests that these two enzymes may act synergistically, with sPLA2-V being identified at the acute stage in ischemic areas of the myocardium staining positive for fibronecin (28). In fact, group V sPLA2 is important in AA release and subsequent production of cysletinyl leukotrienes (CysLTs) and PGE2 by macrophages (29). At present, the precise roles of sPLA2 enzymes in cardiovascular disease are unknown, and evidence is lacking describing the involvement, if any, of many of these enzymes in the development of cardiac fibrosis. However, inflammatory cells, including macrophages (30, 31), T cells, and mast cells (32–35), also produce sPLA2-IIA, and levels of this enzyme are increased in MI, correlating with disease severity (36, 37) and TNF-α concentration (38). Furthermore, sPLA2-IIA has been shown to localize to the border of the infarcted area, binding to the plasma membrane of ischemic as well as normal cardiac myocytes (37). Interestingly, Nijmeijer et al. (38) induced sPLA2-IIA binding to myocytes under conditions mimicking ischemia and concluded that sPLA2-IIA may influence the size of the damaged area by progressing nonlethally affected cardiac myocytes to late-stage apoptotic/necrotic cells (38), thus potentially laying the foundation of the scarring process.

Furthermore, there is evidence for a role for sPLA2-IIA in the development of hypertension-induced cardiac fibrosis. Inhibition of sPLA2-IIA in young SHR during the development of hypertension completely prevented perivascular and interstitial fibrosis, independent of changes in systolic blood pressure and LV hypertrophy (39). This was achieved without reducing the level of infiltrating macrophages, which is consistent with the concept that macrophages may provide a source of sPLA2-IIA and presumably AA (and associated metabolites). It should be noted that, although the sPLA2-IIA inhibitor used in this study was shown to be highly selective for sPLA2-IIA (40), several PLA2 enzymes were not known to exist at the time that these studies were conducted. Therefore, the selectivity against these enzymes...
enzymes is unknown. However, it was shown not to have inhibitory effects on the closely related sPLA2-V (39). Future advances in this field require the development of even more highly selective PLA2 inhibitors to pinpoint their various roles in pathophysiology.

PLA2 enzymes may be important by eliciting AA release from inflammatory cells so that it may then be metabolized by other cells, such as cardiac fibroblasts, to produce profibrotic products. To this end, Ghesquiere et al. (41) demonstrated that macrophage sPLA2-IIA was critical to the development of fibrotic lesions. Furthermore, macrophage-like P388D1 cells appear to release sPLA2-IIA extracellularly, which then reassociates with the membrane to cause the extracellular release of AA (30, 31). Therefore, sPLA2-IIA from inflammatory cells could act to release AA from neighboring cells (42). In support of this, Reddy and Herschman (33) described a situation where sPLA2 can be released by nearby cells and stimulate 3T3 fibroblasts to produce PGs. In addition to macrophages, mast cells are also capable of releasing AA (34, 43) by an sPLA2-IIA-mediated mechanism (34). In fact, mast cells, when incubated with sPLA2-IIA, release AA, which is preferentially used to generate COX products over LO metabolites (35). It is also well established that cytosolic PLA2-a (cPLA2-a) is critical for the production of eicosanoids from the intracellular release of AA in inflammatory cells. sPLA2 enzymes appear to be important in amplifying this release of AA (29, 31). If cPLA2-a is essential to initiate sPLA2-induced AA release, then this enzyme may represent a better target than sPLA2-IIA since cPLA2-a has been shown to be responsible for almost all the AA released by neutrophils (44).

Most PLA2 within the heart does not require calcium for activity (45). Ventricular myocytes have been shown to contain calcium-independent PLA2 (iPLA2), which can be stimulated by TNF-α to induce AA release (46). iPLA2 is involved in cardiac myocyte contractility (47) and contributes to tachyarrhythmias following ischemia (48). McHowat et al. (49) detected cPLA2 and sPLA2 isoenzymes in the cytosol of normal hearts, while cPLA2 and iPLA2 were localized to the sarcolemma. In failing hearts, there was a shift in sPLA2-induced AA release, then this enzyme may be calcium independent. Presently, however, it is not known if iPLA2 plays a role in cardiac fibrosis.

**COX metabolism**

Metabolism of AA by COX-1 or COX-2 (Fig. 1) is an essential step in the synthesis of prostanoids (e.g., PG12, PGD2, PGE2, PGF2α, and thromboxane A2 (TXA2)) and is probably the most researched pathway of AA metabolism. While early studies using nonselective inhibitors of the COX enzymes found no effect on scar size following MI (50), there is now enough evidence to suggest that COX metabolism may be important. Mice devoid of the PG12 (IP) receptor develop hypertension, LV hypertrophy, and fibrosis (51). This LV fibrosis was subsequently shown to be prevented by deletion of the receptor for TXA2 (TP), in addition to deletion of the PG12 receptor, and was independent of hypertension and hypertrophy. Such findings emphasize the importance of the cardioprotective vs pathological relationship that exists between PG12 and TXA2.

In ischemic-reperfusion studies, aspirin administered at a dose (25 mg/kg/day) that inhibits TXA2, but not PG12, led to reduced collagen in areas of the LV remote to the infarct zone, without altering the size of the infarct itself (52). This suggests that COX metabolism, or more specifically, TXA2, may play a role in the development of interstitial fibrosis but is less important for the formation of the scar itself. Furthermore, the addition of the PG12 analog, beraprost, has been shown to reduce the rate of growth, DNA synthesis, and collagen I and III mRNA in cardiac fibroblasts (53). The stable metabolite of TXA2, TXB2, is known to be elevated in the acute period following MI (54), whereas COX-2 inhibition significantly reduced plasma TXB2 levels and improved myocardial function in acute MI (55). Furthermore, COX-2-generated PGE2 is also elevated following MI (56), and while antagonism of the PGE2 receptor (EP1) reduced renal fibrosis in the SHR (57), the specific role of this receptor in cardiac fibrosis has not been evaluated. However, mice lacking the COX-2 gene develop significant cardiac fibrosis (58). Several selective COX-2 inhibitors have been shown to attenuate fibrosis in areas of the LV remote to the infarct itself, concomitant with a decrease in TGF-β1 (56). Furthermore, cardiac fibrosis induced by the chemotherapeutic agent, doxorubicin, is prevented in COX-2-knockout mice (59). However, the use of selective COX-2 inhibitors as a treatment for cardiac fibrosis seems impractical given the association of these drugs with adverse cardiovascular events (60). A better strategy may be the use of antagonists of the specific receptors.

To date, there is no evidence implicating PGD2 or PGF2α in the development of cardiac fibrosis. However, PGD2 is metabolized to PGJ2, which is further metabolized to 15-deoxy-D12,14-PGJ2, a natural ligand for the peroxisome proliferator-activated receptor γ (61), and known to have antifibrotic effects in bleomycin-induced lung injury (62). While this pathway is yet to be investigated with regard to cardiac fibrosis, there is potential for activation of this pathway to be of therapeutic value.
LO metabolism

The LO pathways (Fig. 1) involve the conversion of AA to 5-, 12-, or 15-hydroperoxyeicosatetraenoic acids by 5-, 12-, or 15-LO, respectively. Hydroperoxyeicosatetraenoic acids are rapidly metabolized to 5-, 12-, or 15-hydroxyeicosatetraenoic acid (HETE), respectively (63–67). 5-LO is the key enzyme in the synthesis of leukotriene B₄ (LTB₄) and CysLTs (LTC₄, LTD₄, and LTE₄) (66).

To date, no direct evidence exists for a role for 5- or 15-LO metabolism in cardiac fibrosis; however, there is some circumstantial evidence implicating the 5-LO pathway. LTB₄/LTE₄ receptor antagonism reduced the extent of myocardial necrosis to determine their antifibrotic potential in the heart.

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