AMERICAN JOURNAL OF PHYSIOLOGY
HEART AND CIRCULATORY PHYSIOLOGY

Am J Physiol Heart Circ Physiol 326: H655–H669, 2024.
First published January 19, 2024; doi:10.1152/ajpheart.00559.2023

REVIEW

Cardiac Fibroblasts

Fibroblasts and platelets: a face-to-face dialogue at the heart of cardiac fibrosis

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Abstract

Myocardial fibrosis is a feature found in most cardiac diseases and a key element contributing to heart failure and its progression. It has therefore become a subject of particular interest in cardiac research. Mechanisms leading to pathological cardiac remodeling and heart failure are diverse, including effects on cardiac fibroblasts, the main players in cardiac extracellular matrix synthesis, but also on cardiomyocytes, immune cells, endothelial cells, and more recently, platelets. Although transforming growth factor-β (TGF-β) is a primary regulator of fibrosis development, the cellular and molecular mechanisms that trigger its activation after cardiac injury remain poorly understood. Different types of anti-TGF-β drugs have been tested for the treatment of cardiac fibrosis and have been associated with side effects. Therefore, a better understanding of these mechanisms is of great clinical relevance and could allow us to identify new therapeutic targets. Interestingly, it has been shown that platelets infiltrate the myocardium at an early stage after cardiac injury, producing large amounts of cytokines and growth factors. These molecules can directly or indirectly regulate cells involved in the fibrotic response, including cardiac fibroblasts and immune cells. In particular, platelets are known to be a major source of TGF-β1. In this review, we have provided an overview of the classical cellular effectors involved in the pathogenesis of cardiac fibrosis, focusing on the emergent role of platelets, while discussing opportunities for novel therapeutic interventions.

antifibrotic drugs; antiplatelet drugs; cardiac fibroblast; platelets; TGF-β

CARDIAC FIBROSIS

Fibrosis is characterized primarily by excessive deposition of collagen and other extracellular matrix (ECM) proteins, leading to their accumulation in a tissue. This phenomenon is typically induced by an acute or chronic tissue injury and constitutes a major cause of organ dysfunction (1–3). Although cardiac dysfunction results from many etiologies including myocardial infarction, valvular diseases, hypertension, or myocarditis, they are almost all characterized by fibrous tissue formation. Indeed, as the heart has no regenerative capacity, the loss or damage of cardiomyocytes induces an inflammatory phase, which involves immune cell infiltration and upregulation of inflammatory genes leading to fibrosis (2–6). The type and the extent of fibrosis are closely related to the etiology of cardiac conditions (2, 7, 8). After a myocardial infarction, cardiac fibrosis is initially reparative, reflecting the replacement of dead cardiomyocytes by a collagen-based scar, called cicatricial or replacement fibrosis (2, 3, 6, 8, 9). The latter is therefore essential for survival in the early post-myocardial infarction stages. However, fibrosis extends into the healthy noninfarcted tissue, known as interstitial fibrosis, inducing deleterious effects on cardiac structure and function (2, 7). Thus, fibrosis drastically alters myocardial compliance, increasing passive stiffness, and contributing to diastolic dysfunction (10). Small changes in collagen content have been shown to exert significant effects on the passive mechanical properties of the heart (11, 12). In addition, fibrosis disturbs cardiac electrical conduction, leading to a predisposition to arrhythmia (13–15). Finally, collagen accumulation around the small coronary arteries and arterioles can lead to microvascular dysfunction and impair the diffusion of oxygen from the vessels to the cardiomyocytes (16, 17). In other cardiac diseases, such as left ventricular pressure overload caused by aortic stenosis or hypertension, metabolic syndrome, and myocarditis, fibrosis is mainly interstitial as it develops in the absence of significant cardiomyocyte loss, increasing myocardial stiffness and causing diastolic dysfunction (2, 7–9). Ultimately, fibrosis accelerates the progression to heart failure and is strongly correlated with adverse outcome (11, 18–21). Therefore, myocardial fibrosis is a complex process that has become a prime focus of cardiac research.

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Submitted 8 September 2023 / Revised 2 January 2024 / Accepted 10 January 2024

CLASSICAL CELLULAR EFFECTORS OF CARDIAC FIBROSIS

Cardiac fibroblasts are described as the main players in the synthesis of cardiac ECM proteins and the development of fibrosis in response to heart injury. However, there is increasing evidence that other cell types present in the injured heart, including immune cells, endothelial cells, and cardiomyocytes, also contribute significantly to fibrosis (6, 7). In recent years, several studies have suggested that blood platelets, by infiltrating the infarcted (22–27) or hypertensive myocardium (28–31), and by recognizing pathogens responsible for myocarditis (32, 33) are involved in the pathogenesis of cardiac fibrosis. The present review highlights this newly recognized role of platelets.

Cardiac Fibroblasts and Myofibroblasts

Cardiac fibroblasts are found within the cardiac tissue, surrounding cardiomyocytes, and are one of the largest cell populations in the mammalian heart (34–36). In a healthy heart, cardiac fibroblasts remain quiescent and maintain the cardiac ECM homeostasis (34, 37). By secreting structural ECM proteins such as collagen and elastin, cardiac fibroblasts provide a scaffold for cardiomyocytes and other cardiac cells, but also a storage network for latent growth factors, such as transforming growth factor-β (TGF-β) (8, 37). In addition, cardiac fibroblasts secrete enzymes involved in ECM turnover, such as matrix metalloproteinases (MMPs) and their inhibitors (TIMPs), which regulate the balance between ECM synthesis and degradation (7, 38). Following cardiac injury, cardiac fibroblasts are activated and their proliferation and differentiation into myofibroblasts are promoted. Myofibroblasts have a cellular phenotype characterized by the expression of α-smooth muscle actin (αSMA) stress fibers (1, 3, 8, 34, 37). Interestingly, recent studies have pointed toward a spectrum of cardiac fibroblast phenotypes, which are probably more complex than a simple binary state between activated and quiescent fibroblasts (34). Multiple cell types, such as endothelial cells (39), pericytes (40), and bone marrow-derived myeloid cells, (41) have been suggested as potential sources of activated cardiac fibroblasts (1, 34). However, recent studies using lineage tracing strategies have revealed that most activated cardiac fibroblasts within the injured heart are derived from resident cardiac fibroblasts (42, 43). Activated cardiac fibroblasts display enhanced synthetic properties, and are the main source of structural ECM proteins, matricellular proteins, and enzymes involved in the ECM processing. They therefore contribute primarily to the development of fibrosis (3, 44–46). Cardiac fibroblast activation is triggered by a large variety of molecules, such as growth factors [TGF-β, platelet-derived growth factor (PDGF)], cytokines [interleukine-6 (IL-6), IL-1β, tumor necrosis factor-α (TNF-α)], hormones (angiotensin II, aldosterone, epinephrine, norepinephrine), and neuropeptides (endothelin-1). These molecules are highly present in the pathological heart due to inflammation and adaptive responses (1, 2, 4, 8). The increased mechanical stress observed in the pathological heart also activates cardiac fibroblasts through mechanosensitive pathways, involving focal adhesions, integrins, stretch-activated channels, and transient receptor potential channels (1, 7, 47–49).

Immune Cells

Immune cells are key regulators of cardiac fibrosis as they orchestrate the inflammatory phase that precedes and triggers the fibrotic process. Neutrophils are recruited from the circulating blood to the site of injury by danger-associated molecular patterns (DAMPs) released from necrotic cardiomyocytes (38, 50, 51) and inflammatory mediators secreted by resident cardiac cells (52). Neutrophils are the first immune cells to invade the injured heart after myocardial infarction (38, 50, 51) or pressure overload (53). Infiltrated neutrophils secrete large amounts of ROS, myeloperoxidase, MMPs, cytokines, and chemokines that are involved in ECM degradation and monocytes recruitment to propagate the inflammatory response (38, 51, 54, 55). Interestingly, neutrophils play a critical role in the polarization of macrophages toward an anti-inflammatory and reparative phenotype. Therefore, neutrophil depletion in the infarcted mouse heart has been shown to enhance cardiac fibroblast activation and fibrosis (56).

Macrophages are the predominant immune cell type in the infarcted (50, 51, 57) and hypertensive heart (53). They are derived from circulating monocytes (58) and resident macrophages (38, 50, 51, 53). Their main role is to phagocyte dead cells and ECM debris. Macrophages also secrete cytokines, growth factors, proteases, and matricellular proteins participating in cardiac ECM remodeling (7, 51, 54, 55, 59, 60). Macrophages have been shown to regulate the activity of neutrophils by secreting paracrine factors (61). Moreover, macrophages and monocytes were found to directly regulate cardiac fibroblast activation by secreting fibrotic mediators (62–64). Finally, their phenotypic change during the process is a key step in the transition from inflammation to fibrosis (50, 55, 65).

The heart contains a number of resident mast cells located close to the vessels and in the epicardium (53). These cells store a wide range of molecules, such as TNF-α, histamine, and proteases, that are rapidly secreted upon degranulation and act as paracrine factors (38, 50, 53, 66, 67). Studies have shown an increase in the number of mast cells in the heart following myocardial infarction (67, 68) or pressure overload (53). Interestingly, resident cardiac mast cells have been described as important initiators of the postinfarct inflammatory response (67) and a major source of IL-6 in the heart following transaortic constriction (TAC) (53).

There is strong evidence to suggest that subpopulations of lymphocytes T and B infiltrate the heart after myocardial infarction (9, 50, 57, 69) or pressure overload (53) and may play a critical role in regulating the inflammatory and fibrotic responses. It has been reported that different lymphocyte subpopulations exert distinct roles in the fibrotic response, by both secreting various inflammatory mediators and by direct adhesion and actions on cardiac fibroblasts (7, 9, 50, 54, 57, 69).

Finally, dendritic cells have also been shown to infiltrate the infarcted (50, 70, 71) and hypertensive (53) heart where they regulate the inflammation and the angiogenesis.

The resolution of inflammation is a complex process crucial for fibrosis development, involving several mechanisms: the release of proresolving factors that promote inflammation resolution, the apoptosis of neutrophils, and their phagocytosis

by macrophages, as well as the transition of macrophages from a proinflammatory to an anti-inflammatory phenotype (38). Insufficient inflammatory response, as well as persistent inflammation, can promote further tissue damage (38, 56, 72).

Endothelial Cells

Endothelial cells are the most abundant nonmuscular cell type in the adult mammal heart and have been shown to contribute to cardiac fibrosis in several ways (36). First, endothelial cells are activated after cardiac injury, which induces upregulation of adhesion molecules on their surface, while promoting leukocyte recruitment to the site of injury (7, 52). In addition, endothelial cells are an essential source of cytokines and chemokines (57). Finally, endothelial cells can undergo endothelial-to-mesenchymal transition, thus contributing to the pool of activated cardiac fibroblasts (73). Although previous results reported that approximately 30% of activated cardiac fibroblasts are derived from endothelial-to-mesenchymal transition (39), a recent study using robust lineage tracing demonstrated that there are in fact very few endothelium-derived cardiac fibroblasts (42), indicating that the endothelial-to-mesenchymal transition plays only a limited role in cardiac fibrosis.

Cardiomyocytes

Following cardiac injury, necrotic cardiomyocytes release a wide variety of molecules (DAMPs) which are the main stimulus for the induction of the inflammatory response preceding fibrosis development (7, 8). Therefore, massive cardiomyocyte death after infarction promotes a significant inflammatory reaction followed by a considerable fibrotic response, whereas this phenomenon is less evident in other cardiac fibrosis models. Moreover, stressed cardiomyocytes have been reported to secrete cytokines, such as IL-6 and IL-11, influencing inflammatory cells (53) and cardiac fibroblasts (74, 75).

TRANSFORMING GROWTH FACTOR-β

TGF-β Expression, Activation, and Signaling

There are three TGF-β isoforms, including TGF-β1, -β2, and -β3, which are encoded by distinct genes. While all three isoforms are expressed in the mammalian heart, their specific actions have not yet been studied in vivo, and the majority of studies have focused on the role of TGF-β1. While cardiac fibroblasts, cardiomyocytes, leukocytes, and platelets actively produce TGF-β de novo, the relative contribution of each cardiac cell type to TGF-β secretion in the pathological heart remains unclear and therefore requires further investigation. TGF-β is initially secreted as a dimer, forming a complex with the latency-associated peptide (LAP), which maintains TGF-β molecules into a latent nonactive form. TGF-β/LAP complexes also bind to latent TGF-β binding protein (LTBP), keeping the secreted TGF-β within the ECM (76). Therefore, the cardiac ECM contains a large amount of latent TGF-β/LAP/LTBP that can be rapidly activated following injury. The latent complexes can be activated by several factors including proteases (MMPs, cathepsins, serine proteases, and calpain), integrins, reactive oxygen species (ROS), thrombospondin-1, and acidic environment (76–79). Once activated, TGF-β binds to its constitutively active type II receptor (TβRII) on the surface of various target cells. It triggers the dimerization and phosphorylation of TGF-β type I receptor (TβRI), along with the activation of downstream intracellular signaling pathways. The canonical TGF-β-meditated pathway involves the transcriptional cofactors Smads. TGF-β can also activate noncanonical cascades, including the mitogen-activated protein kinases (MAPK), NADPH oxidase (Nox), phosphatidylinositol 3-kinase (PI3K), and small GTPases (47, 80).

TGF-β in the Injured Heart

TGF-β expression is significantly upregulated in animal models of myocardial infarction or pressure overload, as well as in patients with heart failure (76). The action of TGF-β depends on its concentration, specific characteristics of the cellular environment, and the type of injury (76). Consequently, it exerts pleiotropic effects in the injured heart, influencing immune-cell response, angiogenesis, cardiomyocyte survival, and cardiac fibroblast activation (4, 76, 80). Primarily, TGF-β serves as a key regulator of the immune response. Various lines of evidence suggest an anti-inflammatory role of TGF-β following myocardial infarction. Indeed, TGF-β has been shown to reduce neutrophil infiltration into the ischemia-reperfusion-subjected heart by decreasing endothelial cell-leukocyte interactions (81). In addition, systemic inhibition of TGF-β has been demonstrated to promote neutrophil recruitment and the expression of proinflammatory cytokines in a mouse model of ischemia-reperfusion (82). Finally, the myeloid cell-specific invalidation of Smad3 results in a defective transition of macrophages toward an anti-inflammatory phenotype after left anterior descending (LAD) coronary artery permanent ligation (83). Contrastingly, TGF-β has been reported to contribute to monocyte invasiveness into the infarcted heart (84). The specific deletion of TGF-β receptors in cardiomyocytes was found to attenuate neutrophil infiltration and MMP-9 activation after LAD permanent ligation, ultimately leading to reduced mortality due to wall rupture (85). Although TGF-β is well described in regulating T cell functions (86), its relative contribution to the regulation of these cells in the injured heart remains unknown despite evidence of inducing an anti-inflammatory environment in atherosclerotic plaques by modulating regulatory T cell activation (87). In vitro evidence suggests that TGF-β reduces endothelial expression of proinflammatory chemokines and adhesion molecules, thereby inhibiting leukocyte recruitment (88). However, TGF-β has been demonstrated to induce proinflammatory signaling in endothelial cells in a mouse model of atherosclerosis (89). The extent to which TGF-β contributes to angiogenesis and neovascularization in vivo, after myocardial infarction or pressure overload, remains unknown. The effect of TGF-β on cardiomyocytes is also controversial. It has been described to exert an antiapoptotic effect on cardiomyocytes through p42/p44 MAPK activation (90). Conversely, the cardiomyocyte-specific invalidation of Smad3 was shown to reduce cardiomyocyte apoptosis in rat and mouse models of ischemia-reperfusion (91). Adding to the complexity, mouse models of pressure overload have produced conflicting results, with reports of both prohypertrophic (92, 93) and antihypertrophic effects of TGF-β on cardiomyocytes (94). On the

contrary, the critical role of TGF-β in cardiac fibroblast activation and differentiation remains strongly established, irrespective of the specific pathological injury (1, 3, 8, 76).

PLATELETS

Platelets are small anucleate blood cells of 2- to 4-μm diameter, which are derived from the hematopoietic lineage by fragmentation of megakaryocytes. This process, called thrombopoiesis, occurs continuously in the bone marrow or, as shown more recently, in the spleen and lungs (81). Thrombopoiesis results in the release into the circulation of 5,000 to 10,000 platelets per megakaryocyte. Platelets survive between 8 and 12 days in human blood, compared with 3–4 days in mice, before being removed from vessels by neutrophils and macrophages, and transported to the spleen and liver for elimination. They are the most prevalent blood components after red blood cells and are typically known to be central players in hemostasis and thrombosis. However, platelet functions extend far beyond hemostasis and are currently involved in the regulation of many physiological and pathological processes (81–83).

Platelets in Hemostasis and Thrombosis

The primary physiological function of platelets is hemostasis, where they contribute to the formation of a thrombus at a damaged vascular site to limit blood loss. Under physiological conditions, platelets circulate in the bloodstream in a resting state, without interacting with the subendothelium. However, in response to vascular injury caused by erosion or atherosclerotic plaque rupture, the integrity of the endothelial layer is disrupted, exposing the subendothelial extracellular matrix (ECM) to blood components. This event triggers platelet adhesion to the injured endothelium, followed by platelet activation, aggregation, and ultimately, the formation of a thrombus. It is important to note that while hemostasis is a normal physiological process, thrombosis represents a pathological condition. In thrombosis, a hemostatic clot forms within the lumen of a blood vessel, obstructing blood flow and leading to ischemia (95, 96).

Platelet adhesion to endothelium.

The initial anchoring results from the interaction between the endothelial von Willebrand factor (vWF) binding protein, attached to soluble vWF, and the platelet membrane glycoprotein (GP)Ib-IX-V, which leads to platelet rolling. This process enables a direct association between exposed collagen fibers and the platelet collagen receptors, GPVI and α2β1 integrin, which stabilize platelet adhesion. Other adhesive proteins, including fibronectin, laminin, and thrombospondin, are also involved in the adhesion process by binding to their specific receptors at the platelet surface (83–85).

Platelet activation.

Platelet adhesion to the injured endothelium induces the activation of various platelet intracellular signaling pathways leading to an increase in cytosolic calcium (Ca^{2+}), with subsequent platelet activation (86). It results in the secretion of granules and the release of secondary agonists which further potentiates thrombus growth by recruiting and activating additional platelets at the site of injury.

Platelets contain two major types of secretory granules: α-granules and dense granules. The α-granules, ranging in diameter from 200 to 500 μM and numbering between 50 and 80 per platelet (97), constitute the most abundant secretory organelles in platelets. Upon platelet activation, they release a diverse array of soluble proteins into the circulation. This includes chemokines, growth factors, proangiogenic and antiangiogenic factors, coagulation factors, and immune mediators, as detailed in Table 1 (95, 97, 98). In addition, α-granules house adhesive proteins crucial for platelet-platelet and platelet-endothelial cell interactions. Among these granules are membrane-bound proteins, such as P-selectin and CD40L, which become expressed on the platelet surface, contributing to interactions with immune cells (95, 97, 99). Proteins within α-granules play essential roles in various physiological functions, encompassing hemostasis, thrombosis, inflammation, angiogenesis, wound repair, and antimicrobial host defense. Dense granules, approximately 150 nm in diameter with four to six per platelet, contain over 200 nonprotein molecules, including cations, nucleotides, polyphosphates, and bioactive amines (Table 1) (95, 97). These molecules predominantly participate in the secondary aggregation response, crucial for amplifying and sustaining platelet activation through positive feedback mechanisms (95, 97, 99–101). In addition to granules, activated platelets release lysosomes containing enzymes, such as acid hydrolases, involved in the degradation of proteins, lipids, and carbohydrates (102). The exact role of platelet lysosomes remains not fully understood. Furthermore, platelets secrete microvesicles (100–1,000 nm) that carry growth and angiogenic factors, coagulation factors, enzymes, adhesion molecules, chemokines, and cytokines, complement proteins, apoptosis regulators, bioactive lipids, and microRNAs (Table 1) (103, 104). Platelet-derived microvesicles are abundant in blood and are now recognized as important mediators of intercellular communication (104).

Platelets activation also results in the exposure of negatively charged phospholipids (phosphatidylserine) on platelet surface, supporting the binding and activation of coagulation factors (81). Activated platelets secrete thromboxane A_2, which is synthesized from platelet membrane phospholipids, and acts as a secondary agonist by stimulating and recruiting additional platelets. Finally, platelet activation leads to a conformational change of the α2bβ3 membrane receptor, from a low- to a high-affinity state for fibrinogen, resulting in aggregation through the formation of interplatelet bridges and the formation of platelet plug at the site of injury (87, 88).

Platelet aggregation and thrombus formation.

The thrombus formation is the result of a well-coordinated series of events. It is initiated by the activation of the coagulation cascade which is a series of enzymatic reactions, involving serine proteases called coagulation factors, and results in the conversion of prothrombin to thrombin. Thrombin ultimately converts fibrinogen to fibrin, which polymerizes into an insoluble fibrin network creating a fibrous blood clot to seal the injured area. Thrombin is also the most potent platelet agonist that strongly contributes to platelet activation and amplifies the coagulation cascade (88, 89). The coagulation cascade is a powerful response which is

Table 1. *Examples of platelet α-granule and dense granule content*

	Chemokines	Growth and Angiogenic Factors	Pro-/Anticoagulant Factors and Fibrinolytic Proteins	Immune Mediators	Membrane-Bound and Adhesive Proteins
α-Granules	I-309 (CCL1) MCP-1 (CCL2) MIP-1α (CCL3) RANTES (CCL5) MCP-3 (CCL7) TARC (CCL17) GRO-α (CXCL1) PF-4 (CXCL4) (CXCL4L1) ENA78 (CXCL5) NAP-2, PBP, CTAPIII, β-thromboglobulin (CXCL7 and precursors) IL-8 (CXCL8) SDF-1α (CXCL12)	TGF-β1 VEGF bFGF HGF IGF-1 PDGF BDNF EGF CTGF Angiopoietin-1 MMP-1, -2, -9 TIMP-1, -4 BMP-2, -4, -6 Angiostatin Thrombospondin Endostatin	Factor V Factor XI Factor XIII Prothrombin α_2-macroglobulin α_2-antiplasmin Plasmin PAI-1 TFPI Antithrombin Protein S Plasminogen	Complement C3 precursor Complement C4 precursor β1H globulin Factor D Factor H C1 inhibitor IgG Thymosin-β4 Thrombocidins	P-selectin CD40L GPIb-IX-V GARP GPVI Fc receptors PECAM vWF Vitronectin Fibronectin Fibrinogen Thrombospondin Fibrocystin Integrins Tetraspanins Glut-3 CD36 Scamp2 CD109
	Cations	Bioactive Amines	Nucleotides		
Dense granules	Ca^{2+} Mg^{2+} K^{+} Polyphosphate Pyrophosphate	Serotonin Histamine	ATP ADP		

MCP-1 and -3, monocyte chemoattractant protein-1 and -3; MIP-1α, macrophage inflammatory protein-1α; TARC, thymus- and activation-regulated chemokine; PF-4, platelet factor-4; ENA-78, epithelial neutrophil-activating protein-78; NAP-2, neutrophil-activating peptide-2; PBP, platelet basic protein; CTAPIII, connective tissue-activating protein III; SDF-1α, stromal cell-derived factor-1; TGF-β1, transforming growth factor- β; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; HGF, hepatic growth factor; IGF-1, insulin-like growth factor-1; PDGF, platelet-derived growth factor; BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor; CTGF, connective tissue growth factor; MMP, matrix metalloproteinase; BMP, bone morphogenetic protein; PAI-1, plasminogen activator inhibitor-1; TFPI, tissue factor pathway inhibitor; GARP, glycoprotein a repetitions predominant protein; PECAM, platelet endothelial cell adhesion molecule; vWF, von Willebrand factor; Scamp2, secretory carrier membrane protein 2.

finely regulated by a complex inhibitory system called the fibrinolysis and mediated by the plasmin (90).

Platelets Beyond Hemostasis and Thrombosis: At the Core of Cardiac Fibrosis and Inflammation

A growing body of evidence supports that platelet functions extend far beyond hemostasis and thrombosis. Indeed, as previously explained, they exhibit the capability to release a wide variety of biomolecules that are functionally involved in a large number of physiological or pathological responses, such as angiogenesis (91), wound healing (92–94), inflammation, and fibrosis (Fig. 1) (29, 95, 99, 103).

In addition to endothelial injury, various other stimuli can prompt platelet activation and recruitment into the heart (Fig. 2). Platelets express pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and nucleotide-binding oligomerization domain-like receptors (NLRs), enabling them to recognize damage-associated molecular patterns (DAMPs) released by necrotic cardiomyocytes or pathogen-associated molecular patterns (PAMPs) expressed by pathogens (98, 105). Furthermore, in instances of vascular inflammation (such as systemic inflammation or atherosclerosis), activated endothelial cells express various factors responsible for platelet activation, including von Willebrand factor (vWF), tissue factor, reactive oxygen species (ROS), and others (95, 98). Lastly, angiotensin II and oxidative stress observed in conditions like hypertension and/or metabolic syndrome may also activate platelets (29, 106–108).

Hence, platelets have been demonstrated to infiltrate both the vasculature and the interstitial myocardium following ischemia (22–27) or pressure overload (28–31)-induced injuries. Notably, in the ischemic mouse myocardium, platelet recruitment begins as early as 2 min after reperfusion, progressively increasing up to 60 min (25). This rapid recruitment underscores platelets as among the first blood cells to respond to ischemia-reperfusion injury, as the appearance of neutrophils occurs more slowly, starting from 30 min of reperfusion (25). Magnetic resonance and PET-scan imaging have confirmed the very early recruitment of platelets after ischemia-reperfusion, showing their presence in the ischemic myocardium reaching peak accumulation at 2 h after reperfusion, followed by a decline by 4 h (26, 27). During this period, platelets predominantly colocalize with leukocytes (27). In addition, Xu et al. (25) demonstrated that the activation and recruitment of platelets into the ischemic heart are dependent on the duration of ischemia. In a mouse model of LAD coronary artery permanent ligation, platelets were observed to accumulate in the heart as early as 6 h after myocardial infarction, becoming more prominent by 72 h. This study revealed that platelets constitute one of the initial waves of inflammatory cells accumulating within the

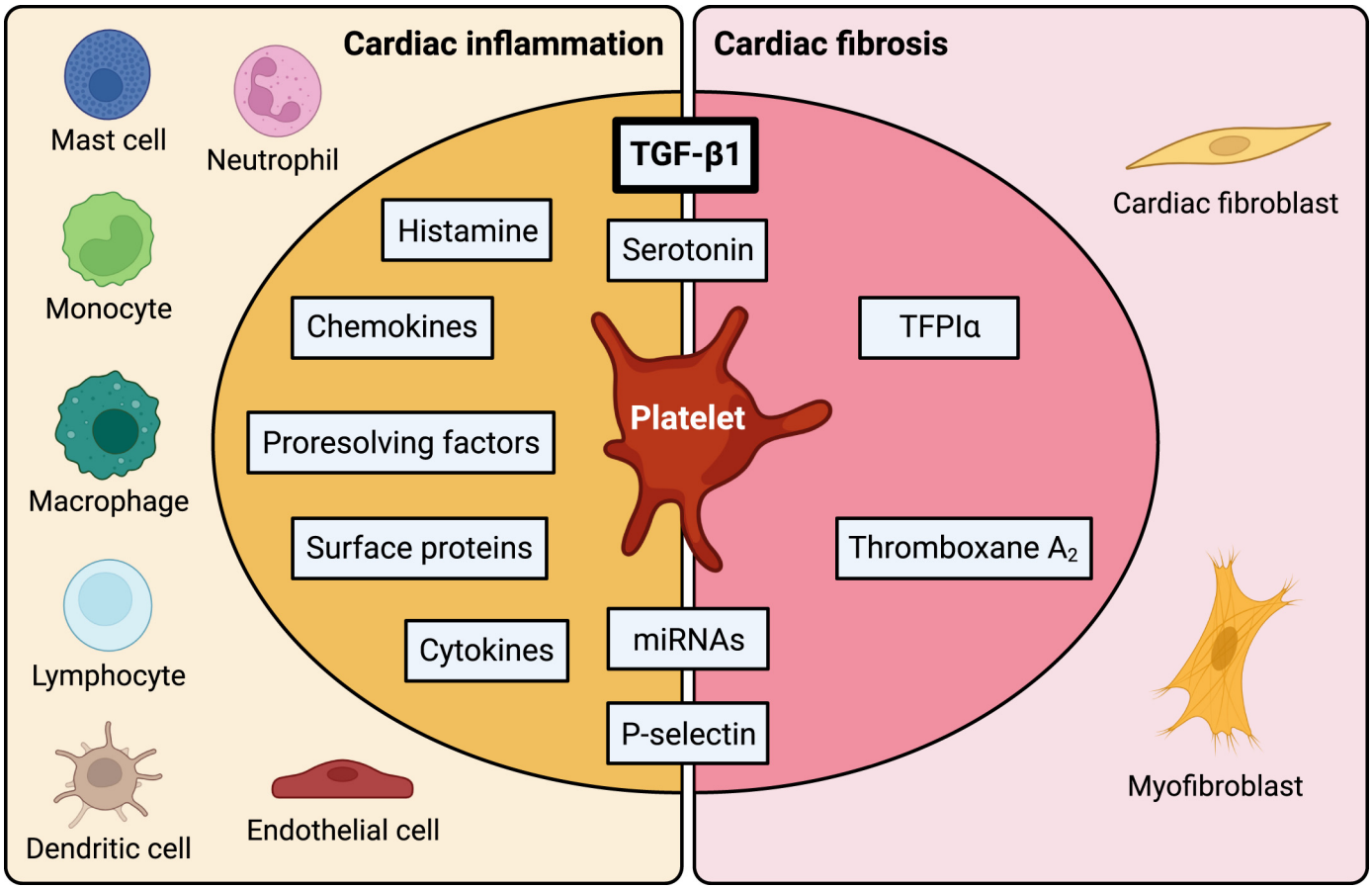

Figure 1. Contribution of platelet-derived mediators to myocardial inflammation and/or fibrosis. TFPIα, tissue factor pathway inhibitor-α; TGF-β1, transforming growth factor-β1. Created with a licensed version of BioRender.com.

infarcted myocardium, colocalizing with leukocytes at 6 h post-MI (24). Although platelets were still present within the ischemic myocardium at 1 day and 7 days after ischemia-reperfusion (22), the question of whether platelets are cleared from the site of injury or persist in the late stages of cardiac fibrosis development after myocardial infarction remains to be investigated.

In the hypertensive heart, platelets have been observed after 7 days of angiotensin II infusion (28). However, in response to transverse aortic constriction (TAC), platelet recruitment was initiated as early as 3 days postsurgery. In this model, platelet accumulation reached its peak at 7 days after TAC, with a subsequent decline observed by 1 mo (30, 31). Furthermore, an elevation in circulating platelet activation was noted in mice at 3 days after TAC compared with the sham group, gradually decreasing at 1 wk and 4 wk postsurgery (30). The persistence of platelets within the heart at later stages of pressure overload-induced injury remains unknown. Interestingly, platelets were identified in the atrium of patients with hypertension suffering from atrial fibrillation. In addition, these patients with hypertension exhibited increased circulating platelet activity compared with normotensive individuals (29).

More recently, platelets have been demonstrated to accumulate and play a pivotal role in cardiac infectious diseases. Notably, they were implicated in a mouse model of autoimmune myocarditis (32) as well as in human endomyocardial specimens with inflammatory myocarditis (33). In these investigations, platelets were observed to infiltrate the myocardium at 2 days following the induction of autoimmune myocarditis in mice, with their numbers significantly reduced after 2 wk and 1 mo (32, 33). Interestingly, it was revealed that the peak number of platelets occurred before immune cell infiltration, suggesting that platelets serve as a first-line defense against infection (32).

Platelets in cardiac fibrosis.

Platelets as a source of TGF-β. As previously explained, TGF-β is the most important factor in cardiac fibroblast activation and fibrosis development (1, 8, 76). Platelets constitute a major source of TGF-β1, as they contain 40–100 times more TGF-β1 than other cell types (95,96, 99, 103). Therefore, platelets are the leading regulator of TGF-β1 concentrations in plasma. Indeed, it has been demonstrated that total TGF-β1 levels are reduced by approximately 45% in the plasma of mice with a platelet-specific deletion of TGF-β1. Furthermore, profound thrombocytopenia induced by injecting monoclonal anti-αIIbβ3 antibodies in mice, reduces plasma levels of total TGF-β1 (103). Platelet TGF-β1 is contained in α-granules and rapidly released upon

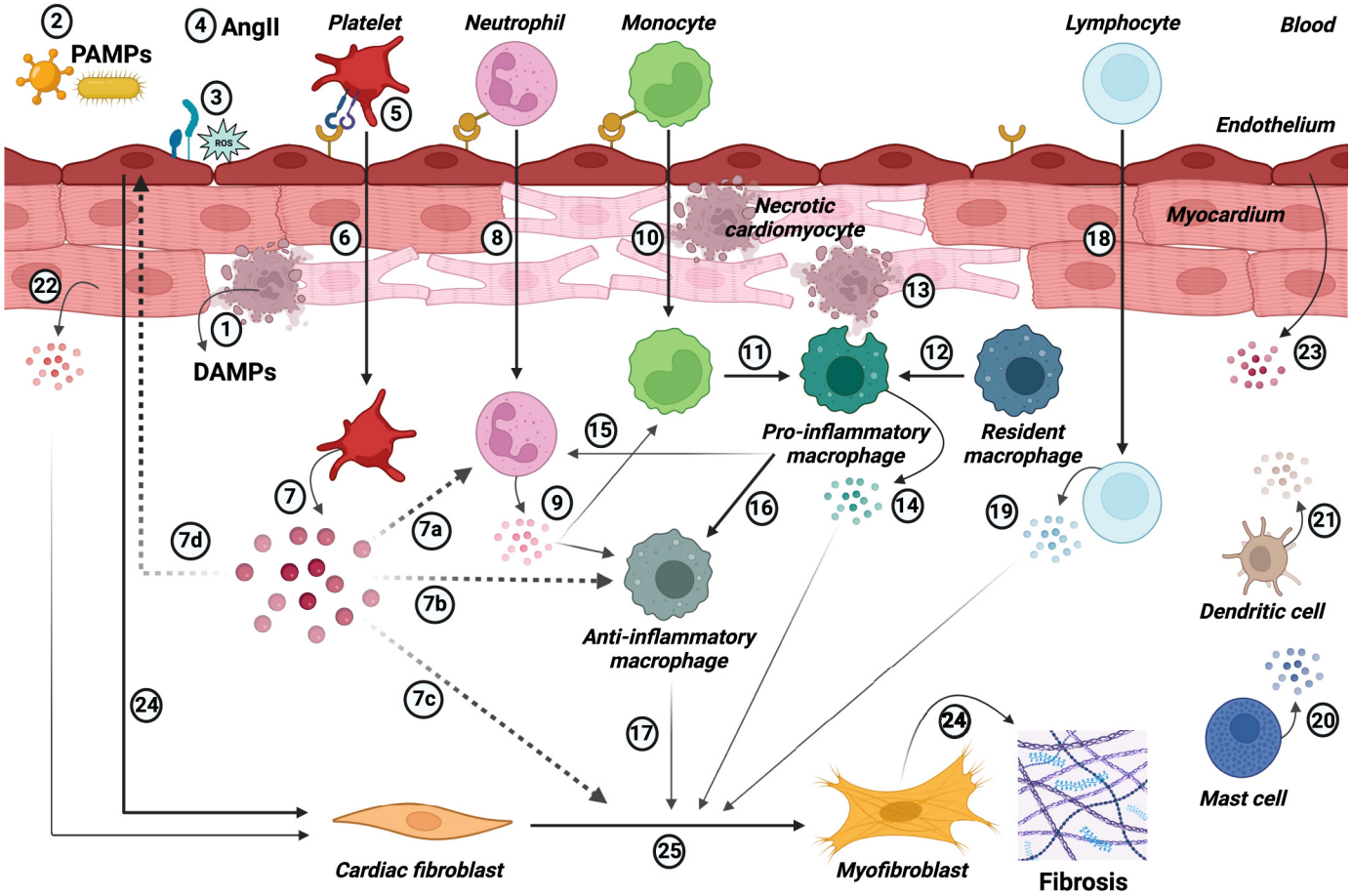

Figure 2. Platelets are at the core of cardiac inflammation and fibrosis. *1*: necrotic cardiomyocytes release danger-associated molecular patterns (DAMPs), which are recognized by platelet-surface receptors, inducing platelet activation. *2*: pathogens express pathogen-associated molecular patterns (PAMPs), which are recognized by platelet-surface receptors, inducing platelet activation. *3*: activated endothelial cells express von Willebrand factor, tissue factor and reactive oxygen species (ROS) responsible for platelet activation. *4*: hypertension induces angiotensin II (ANG II) expression responsible for platelet activation. *5*: upregulation of adhesion molecules on endothelial cells surface promoting platelet and leukocyte recruitment to the site of injury. *6*: infiltration of platelets into the injured myocardium. *7*: platelets release factors influencing the inflammatory and fibrotic responses. *7a*: platelets influence neutrophil recruitment. *7b*: platelets influence differentiation of proinflammatory macrophages toward an anti-inflammatory phenotype. *7c*: platelets influence cardiac fibroblast activation. *7d*: platelets influence angiogenesis. *8*: Infiltration of neutrophils into the injured myocardium. *9*: infiltrated neutrophils secrete factors, which are involved in extracellular matrix degradation, monocyte recruitment and polarization of macrophages toward an anti-inflammatory phenotype. *10*: infiltration of monocytes into the injured myocardium. *11*: infiltrated monocytes differentiate into proinflammatory macrophages. *12*: activation of resident macrophages. *13*: proinflammatory macrophages phagocyte dead cardiomyocytes and extracellular matrix debris. *14*: proinflammatory macrophages secrete factors influencing neutrophil activity, extracellular matrix remodeling and cardiac fibroblast activation. *15*: differentiation of macrophages from a proinflammatory toward an anti-inflammatory phenotype. *16*: anti-inflammatory macrophages influence cardiac fibroblast activation. *17*: infiltration of different subpopulations of lymphocytes into the injured myocardium. *18*: infiltrated lymphocytes secrete factors influencing the inflammatory and fibrotic responses. *19*: degranulation of resident mast cells influencing the inflammatory response. *20*: dendritic cells secrete factors influencing inflammation and angiogenesis. *21*: surviving cardiomyocytes secrete factors influencing immune cells and cardiac fibroblasts. *22*: endothelial cells secrete factors influencing inflammatory and fibrotic responses. *23*: endothelial-to-mesenchymal transition. *24*: activation of cardiac fibroblasts and differentiation into myofibroblasts. *25*: myofibroblasts secrete large amounts of extracellular matrix proteins inducing fibrosis. Created with a licensed version of BioRender.com.

platelet activation. Latent TGF-β1 is present in two different pools in platelets. The first pool, representing 95% of total platelet TGF-β1, corresponds to the TGF- β1 associated with LTBP and LAP. The second pool corresponds to the TGF-β1 complexed with LAP, without LTBP. Recent results suggest that the TGF-β1/LAP/LTBP pool is released into plasma in a single step, whereas the TGF-β1/LAP-containing complex is trapped on platelet surface before being released into plasma upon activation (109). Indeed, it has been found that activated platelets express TGF-β1 on their extracellular surface through its binding to the glycoprotein A repetitions predominant protein (GARP) (100, 110). However, the mechanism by which active TGF-β1 is generated from latent TGF-β1/GARP complexes on platelets remains poorly understood. Platelet TGF-β1 was shown to modulate a number of biological processes including cardiac fibrosis. In this context, Meyer et al. provided the first evidence that TGF-β1 originating from platelets contributes to cardiac fibrosis. Using a platelet specific TGF-β1 deletion transgenic mouse model, these authors demonstrated that mice lacking platelet TGF-β1 developed less interstitial and perivascular fibrosis following pressure overload generated by transverse aorta constriction. These mice were partially protected from cardiac hypertrophy and systolic dysfunction (103). A

few years later, Varshney et al. demonstrated that aortic stenosis progression could be delayed in the absence of platelet TGF-β1. This was associated with a significant decrease in fibroblast myodifferentiation and fibrosis in aortic valve tissue (95). More recently, platelet-derived TGF-β1 was found to promote angiotensin II-induced atrial fibrosis and atrial fibrillation in mice. In this study, TGF-β1 from platelets was shown to directly promote myodifferentiation of cardiac fibroblasts (29).

Platelets as a source of other fibrotic molecules. In addition to TGF-β1, numerous mediators of fibroblast activation are contained in platelet granules and microvesicles, and some of them have been shown to be involved in the regulation of cardiac fibrosis. Indeed, serotonin released by platelets has been described to directly affect neonatal rat cardiac fibroblasts by promoting their migration and myodifferentiation and by increasing the secretion of TGF-β1 and MMPs (97). Furthermore, activated platelets have been found to stimulate cardiac fibrosis in a mouse model of angiotensin-II hypertension by secreting P-selectin (98). Then, platelet thromboxane A_2 was shown to stimulate cardiac fibroblast myodifferentiation, profibrotic gene expression, and consequently cardiac fibrosis (101). Platelet-derived tissue factor pathway inhibitor-α (TFPIα) was reported to prevent cardiac fibrosis by reducing thrombin generation and thrombin-mediated platelet activation in mice (102). Recently, platelets have emerged as a rich source of microRNAs (miRNAs), acting as paracrine mediators that tightly regulate surrounding cells, including cardiac fibroblasts (22, 111–114). Notably, despite lacking nuclei, platelets contain mRNAs, miRNAs, and the necessary enzymes for the conversion of precursor miRNAs (pre-miRNA) into mature miRNAs (115). Intriguingly, studies have revealed that platelet-derived miRNAs play a crucial role in modulating cardiac fibrosis following ischemia-reperfusion. In a genetic mouse model featuring megakaryocyte/platelet-specific invalidation of the pre-miRNA processing ribonuclease Dicer, inducing a disruption in miRNA processing in platelets, researchers observed worsened myocardial remodeling and exacerbated cardiac fibrosis in platelet-specific Dicer knockout mice subjected to transient left coronary artery ligation (22). Other molecules influencing cardiac fibroblasts such as thrombospondin-1 and PDGF are highly enriched in platelet granules (2, 104, 116). However, it is not clear whether these platelet-derived molecules directly contribute to cardiac fibrosis.

Although the correlation between platelet count in the bloodstream and liver fibrosis is well established (117, 118), there is currently a lack of studies investigating a similar correlation in the context of the heart. Interestingly, the mean platelet volume (MPV) has been identified as a prognostic marker in heart failure, predicting adverse cardiovascular events and associated with high mortality rates (119–123). However, the connection between MPV and cardiac fibrosis remains unexplored.

Platelets in cardiac inflammation.

Given the high interconnectedness between the inflammatory and fibrotic processes, platelets may regulate cardiac fibrosis indirectly by modulating inflammation. Indeed, in recent years, the role of platelets in inflammatory reactions and immune response modulation has emerged and they are now considered as full-blown immune cells (81, 82, 124, 125). Platelets secrete a wide range of immunomodulatory factors (chemokines, cytokines, adhesive proteins, miRNA, etc.) containing in their α-granules and microvesicles. They also directly interact with leukocytes through their surface proteins (P-selectin, α2bβ3 integrin, GpIbα), contributing to leukocyte recruitment, trans-endothelial migration, and activation (24, 25, 125). Therefore, circulating platelets have been shown to interact with monocytes, thereby promoting P-selectin-mediated delivery of platelet-derived proinflammatory factors, and ultimately the formation of atherosclerotic lesions in apolipoprotein-E-deficient mice (126). Moreover, platelets have been described to induce a proinflammatory phenotype in monocytes (127). Recently, platelets were reported to induce upregulation of osteopontin in macrophages, inducing inflammation into the abdominal aortic wall (128). In addition, platelet microvesicles-derived microRNAs were demonstrated to play an important role in cardiac inflammation following ischemia-reperfusion injury (22). Finally, platelet-derived serotonin was shown to induce neutrophil degranulation, enhancing post-myocardial infarction inflammation (129).

Nonetheless, platelets have also been described to exert anti-inflammatory effects. Indeed, they release an abundance of anti-inflammatory cytokines, such as TGF-β1 and proresolving mediators including lipoxin A4, maresin 1, and resolvin E1, which attenuate neutrophil trafficking and promote their apoptosis (125, 130, 131). Moreover, platelets were implicated in the modulation of macrophage phenotype toward their reparative function (93, 125, 132). Interestingly, platelets were shown to drive the differentiation of profibrotic macrophages by secreting CXCL4 (105). Altogether, these data reveal the complexity of platelets' contribution to inflammation and its resolution. Additional studies are needed to further clarify this process, which is challenging but might lead to attractive new therapeutic targets for the management of cardiac fibrosis.

ANTIFIBROTIC THERAPIES

Although cardiac fibrosis represents a major medical issue, current antifibrotic drugs have limited efficiency. Moreover, they are insufficient to inhibit fibrotic remodeling and avoid its progression to heart failure (4, 5, 80, 106). Therefore, the development of novel and effective antifibrotic therapeutic strategies for heart diseases is crucial. A wide range of studies have targeted classical signaling pathways involved in fibroblast activation and inflammation following injury (5, 7, 80, 106, 107). Numerous compounds are currently in clinical trials for the treatment of fibrosis from different etiologies. However, few have so far proved sufficient beneficial effects in the context of cardiac fibrosis (108). Indeed, while some of these led to promising results in reducing cardiac fibrosis in animal models, several major challenges for translating them into human clinical practice still remain.

Targeting TGF-β Signaling

Because of its critical involvement in the cardiac fibrotic response, TGF-β signaling remains an attractive therapeutic

target. To date, various types of TGF-β inhibitors have been tested in preclinical studies for the treatment of cardiac fibrosis. Neutralizing anti-TGF-β antibodies were used in rodent models of myocardial infarction and hypertrophic cardiomyopathy. These studies have been inconclusive, with conflicting results depending on the timing and dose of administration (133–136). In addition, cardiovascular toxicities of anti-TGF-β antibodies have been demonstrated (137). The soluble TβRII, which acts as a competitive inhibitor of TGF-β, has shown promising effects when injected on *days 3–4* after myocardial infarction, whereas it has been ineffective when injected a few weeks later (138, 139). On the contrary, Ikeuchi et al. have demonstrated that soluble TβRII overexpression during the early stage after myocardial infarction exacerbates ventricular dilatation and increases mice mortality, whereas the inhibition of TGF-β signaling during the later stage after myocardial infarction alleviates cardiac remodeling (140). Interestingly, adenoviral overexpression of decorin, a TGF-β antagonist, has been shown to inhibit hypertension-induced cardiac fibrosis in rats (111). GW788388 is a TGF-β receptor I (ALK5) inhibitor that has been demonstrated to reduce fibrosis after myocardial infarction in rats (112). It has also been found to inhibit cardiac fibrosis in an experimental mouse model of Chagas' heart disease (113, 114). Although GW788388 appears to have beneficial antifibrotic effects, these preclinical data need to be confirmed. Indeed, SM16, another ALK5 inhibitor, has been shown to induce beneficial effects on pressure overload-induced cardiac fibrosis while increasing left ventricular dilatation and mortality in mice (115). In addition, ALK5 inhibitors have been found to induce heart valve damage in rats (141). Pirfenidone is an antifibrotic drug currently approved for the treatment of idiopathic pulmonary fibrosis. Although its exact mechanism of action is not fully understood, it is thought to exert its effects by inhibiting TGF-β expression. The beneficial effects of Pirfenidone on cardiac fibrosis have been demonstrated in several animal models (117–121, 142). The PIROUETTE clinical trial demonstrated that administration of pirfenidone is associated with a significant reduction in cardiac fibrosis whereas it unfortunately does not improve diastolic function (122). Finally, tranilast is used for the treatment of allergic disorders. Although its mechanism of action is not fully understood, tranilast inhibits the expression and activity of TGF-β. It has been shown to

Table 2. *Preclinical in vivo studies using antiplatelet drugs and their effects on cardiac fibrosis*

Dose	Administration	Treatment Duration	Animal	Model	Major Findings	Reference
Clopidogrel 50 mg/kg/day	ig	For 7 days	Mouse	ANG II	↓Inflammation ↓Fibrosis	(28)
Clopidogrel Loading: 15 mg/kg/day Maintenance: 5 mg/kg/day	ig	For 3 wk	Mouse	ANG II	↓inflammation ↓fibrosis ↓atrial fibrillation	(29)
Ticagrelor Acute: 10 or 30 mg/kg Chronic: 300 mg/kg/day Clopidogrel Acute: 12.5 mg/kg Chronic: 62.5 mg/kg/day	Acute: ip Chronic: in diet	Acute: 5 min before reperfusion Chronic: Starting 1 day after I/R, for 4 wk	Rat	I/R	↓infarct size ↓apoptosis ↓fibrosis Improve cardiac function No effect	(146)
Aspirin 25 mg/kg/day	ip	From 2 days before to 21 days after LAD	Rat	LAD	↓fibrosis	(147)
Aspirin 10 mg/kg/day	ig	From 4 days before to 4 wk after TAC	Mouse	TAC	↓fibrosis	(148)
Ticagrelor 300 mg/kg/day Aspirin 20 mg/kg/day Prasugrel 15 mg/kg/day Ticagrelor + Aspirin 300 and 20 mg/kg/day	Chow	From 1 wk after I/R, for 1 wk or 3 wk	Rat	I/R	Improve cardiac function ↓fibrosis ↑regenerative cells No effect	(149)
Aspirin 15 mg/kg Clopidogrel 11 mg/kg Ticagrelor 27 mg/kg Prasugrel 1.5 mg/kg	ig	1 wk before LAD or From 1 wk before to 1 wk after LAD or 1 wk after LAD	Mouse	LAD	↓inflammation ↓fibrosis Improve cardiac function when treating before surgery	(150)
Cilostazol 0.1% wt/wt	Chow	From 7 days before to 28 days after ANG II	Mouse	ANG II	↓hypertrophy ↓fibrosis	(151)

ig, intragastrically; ip, intraperitoneal; ANG II, angiotensin II; TAC, pressure overload generated by transaortic constriction; LAD, myocardial infarction induced by permanent ligation of left anterior descending coronary artery; I/R, ischemia-reperfusion.

reduce cardiac fibrosis in rodents. In summary, although some anti-TGF-β therapies have shown encouraging results, the pleiotropic effects as well as the context and time-dependent actions of TGF-β after cardiac injury make anti-TGF-β therapy challenging. Therefore, more specific agents should be developed to safely and robustly inhibit TGF-β signaling.

Platelets as a New Potential Therapeutic Tool for Cardiac Fibrosis

Given the key role of platelets in inflammatory and fibrotic processes, platelet-targeting may be a promising therapeutic strategy (Table 2). The use of antiplatelet drugs has already shown encouraging beneficial effects in the treatment of liver and pulmonary fibrosis (123, 143, 144). In the cardiovascular field, platelet depletion with an anti-GPIbα antibody strongly reduces thrombus fibrosis and adverse vein wall remodeling in a mouse model of inferior vena cava stenosis, indicating that platelets are a major contributor to thrombus fibrosis (145). Several studies have found that treatment with antiplatelet drugs significantly reduces fibrosis in different models of cardiac injury. First, the inhibition of platelet activation by the irreversible ADP receptor blocker clopidogrel has been shown to prevent cardiac inflammation and fibrosis in a mouse model of angiotensin II-induced hypertension (28). More recently, Liu et al. (29) demonstrated that clopidogrel reduces angiotensin II-induced platelet accumulation, inflammation, and fibrosis in the atrium. Second, ticagrelor, a reversible ADP receptor blocker, has been described to decrease collagen expression and fibrosis after ischemia-reperfusion injury. Interestingly, despite a similar degree of platelet inhibition, clopidogrel had no effect on cardiac fibrosis in this study (146). Inhibition of cyclooxygenase-1 by aspirin has been shown to decrease interstitial fibrosis in infarcted rat hearts (147) and attenuate pressure overload-induced cardiac fibrosis by inhibiting ERK1/2 and Akt/β-catenin signaling pathways in mice (148). Moreover, ticagrelor and aspirin have been found to reduce collagen expression in a rat model of ischemia-reperfusion (149). A recent study showed that different anti-platelet drugs, namely aspirin, clopidogrel, ticagrelor and prasugrel, significantly reduce inflammatory cell infiltration and cardiac fibrosis in a mouse model of myocardial infarction (150). Finally, Cilostazol has been found to reduce angiotensin II-induced perivascular and interstitial fibrosis in mice through the activation of the cyclic AMP-protein kinase A pathway (151).

CONCLUSIONS

Although current antifibrotic therapies have proven to slow the progression of heart failure, they remain insufficient. It is therefore essential to find new therapeutic strategies targeting cardiac fibrosis. Ongoing research is focusing on novel therapeutic strategies based on cell transplantation or reprogramming, biomaterials engineering, noncoding RNAs, and epigenetic modifiers (4, 106, 107). However, the complexity of the cell types and signaling pathways involved in the fibrotic process makes these approaches rather difficult. Platelets are classically known for their role in hemostasis and thrombosis, but it is now well described that their function extends far beyond these processes. They have been shown to infiltrate the myocardium in the very acute phase after myocardial infarction (22–27), pressure overload (28–31), and myocarditis (32, 33), where they secrete large amounts of molecules, therefore directly or indirectly regulating cells involved in the fibrotic response, including cardiac fibroblasts and immune cells. Therefore, platelets represent an interesting candidate for the management of cardiac fibrosis. Indeed, several studies have demonstrated the beneficial effects of antiplatelet therapies on the cardiac fibrotic response (28, 29, 145–151). However, the exact molecular and cellular mechanisms involved are still poorly understood, requiring further research. As platelets store a large number of molecules, it would be interesting to study the relative contribution of platelet-derived profibrotic factors to myocardial fibrosis. In this regard, the use of transgenic mice with invalidation of megakaryocyte-specific genes would provide additional knowledge.

Nonetheless, TGF-β is well established as a key regulator of cardiac fibrosis, and platelets have been identified as a significant source of TGF-β1 (130–133). The specific targeting of platelet-derived TGF-β could prove to be a promising therapeutic avenue. In this regard, the GARP protein emerges as a potential candidate. GARP associates with latent TGF-β1 on the platelet surface, triggering its activation (152, 153). While GARP expression has been identified in T-regulatory lymphocytes, platelets, and some endothelial and fibroblast cell lines, only T-regulatory lymphocytes and platelets have been demonstrated to generate active TGF-β1 from GARP/latent TGF-β1 complexes (135, 152, 153). Therefore, investigating the role of platelet GARP in active TGF-β1 production following heart injury holds significant interest. GARP represents a promising target that could play a crucial role in TGF-β1 activation in the injured heart.

GRANTS

C.D. and J.B. are supported by a fellowship from the Fonds National de la Recherche Scientifique et Médicale (FNRS, Belgium) and the Fonds pour la formation à la Recherche dans l'Industrie et dans l'Agriculture (FRIA, FNRS, Belgium), respectively. L.B. is supported by a grant from Walloon Excellence Research Institute WELRI-WELBIO (Belgium). S.H. works as a senior research associate at FNRS, Belgium.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.D. prepared figures; C.D., J.B., and S.H. drafted manuscript; C.D., J.B., L.B., C.B., and S.H. edited and revised manuscript; C.D., J.B., L.B., C.B., and S.H. approved final version of manuscript.

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