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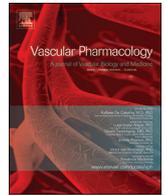
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Review

Perivascular fibrosis and the microvasculature of the heart. Still hidden secrets of pathophysiology?

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ABSTRACT

Perivascular fibrosis, the deposition of connective tissue around the vessels, has been demonstrated crucially involved in the development of cardiac dysfunction. Although cardiac fibrosis has been shown to be reversible under certain experimental conditions, effective anti-fibrotic therapies remain largely elusive. Therefore, perivascular fibrosis currently represents a major therapeutic target for cardiovascular diseases. The main topic of this review will be to address the mechanisms underlying perivascular fibrosis of the vasculature within the myocardium, with a special focus on perivascular fibrosis of small vessels, microvascular dysfunction and disease.

1. Introduction

Regulation of blood flow or vessel resistance and capacitance in the cardiac wall as well as in other organs is based on several mechanisms. Among these, the most important are autoregulation, metabolic regulation and mechanisms dependent by endothelium-derived factors, hormones and innervation [1,2]. The balance between each of them depends on the organ supplied by these vessels and is strongly connected to the organ physiology. The presence of perivascular fibrosis might limit the ability of the regulatory mechanisms to work properly, and thereby represent a limitation in oxygen and nutrient supply and washout of metabolites.

Perivascular fibrosis, characterized by an increased amount of connective tissue around the vessels, especially collagen, has been demonstrated in brain, kidney, heart, liver, lung, skin and skeletal muscle [3–7]. Since oxygen extraction is already high at rest in the heart, increase in oxygen delivery under constant perfusion pressure is dependent on the ability to increase coronary flow through dilatation of prearterioles and arterioles. Malfunction or fibrotic remodeling of the microcirculation are therefore major threats to cardiac health. Macroscopic remodeling of the heart implies changes in shape and size of the heart, whereas at the microscopic level remodeling involves both the

intra- and extracellular compartments and substantial rearrangements at a molecular level. Multiple conditions, including those associated with endothelial dysfunction and coronary microvascular dysfunction, might trigger perivascular fibrosis [3]. This review manuscript aims to describe the major cellular and molecular mechanisms involved in perivascular fibrosis of the vessels within the myocardium, with a special focus on perivascular fibrosis of small vessels, microvascular dysfunction and disease [8].

2. The anatomy and histology of the microvasculature in the heart

The microvasculature consists of capillaries together with vessels in close proximity to the capillaries: prearterioles with a diameter of 100–150 μm , and arterioles with a diameter < 100 μm , precapillary sinuses and corresponding venules. Capillaries are found close to the cardiomyocyte, which is surrounded by several capillaries securing a short oxygen diffusion distance [9]. The distance between endothelial cells in the capillaries and the cardiomyocyte membrane might be as low as 1 μm , and depending of the thickness of myocytes the diffusion radius will be in the range 3–12 μm . This assures that different forms of paracrine communication can take place, and that diffusion of oxygen (O_2), carbon dioxide (CO_2), nitric oxide (NO) and other lipid soluble

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and low molecular weight compounds is facilitated. The precapillary sinuses are thin walled short vessels with a diameter of 30–50 μm , approximately 3 times the capillaries, and several capillaries spread out from the sinuses. Kaneko et al. proposed a role of the sinuses in storing blood during diastole that can be spread to the capillaries during systole [9].

The distance from arterioles to the corresponding vein, i.e. the length of a capillary in the heart, has been estimated to be 350 μm . The degree of overlap between capillary beds in the human heart is not large but connections have been observed. However, above a certain level, each capillary bed will be independent of the neighboring capillary bed. Interestingly, Kaneko et al. report results consistent with the presence of a micro-circulatory unit based on their observation of micro-necrosis [9]. Regions of myocardial micro-necrosis and micro-fibrosis were observed in the autopsied heart without coronary artery disease, and were similar in size to the distances from the central arteriole to both adjacent venules.

3. Coronary microvascular function

A method to examine coronary microvascular function is by measuring maximum hyperemic blood flow and baseline coronary flow, using an ultrasound probe positioned to the left coronary artery or with myocardial contrast echocardiography or *positron emission tomography* (PET) [10]. Coronary flow reserve (CFR) is calculated as the ratio of hyperemic peak diastolic flow velocity to baseline peak diastolic flow velocity [8]. This relative coronary flow is particularly useful for the characterization of a distinct stenosis, whereas absolute coronary flow with maximal dilatation is often more informative for the description of coronary pathologies with global involvement, such as coronary microvascular fibrosis [11]. In an experimental setting the difference between baseline and hyperemic conditions induced by inhalation of 1% and 2.5% isoflurane respectively, are often used [12]. Examination of coronary microvascular function by CFR does not distinguish between reduced reserve due to reduced density of micro-vessels and reduced ability to dilate [8]. Therefore, histology is always an important supplement to clarify what structural alterations are behind change in function. The normal value of CFR greatly depends on the technique used for quantification, but the most recent literature considers CFR < 2.0 sufficiently abnormal to result in ischemia [13]. Any rise in baseline myocardial blood flow can lead to underestimation of true CFR. It also has to be taken into consideration that the level of maximum coronary blood flow is in linear relationship with coronary perfusion pressure. Hence, any concomitant peripheral vasodilation leading to hypotension can underestimate CFR. Microvascular dysfunction and diminished coronary flow reserve result in hypoxia upon increased oxygen demand [14].

4. Role of different cell types of the vessel wall in perivascular fibrosis

Several different cell types within the vascular wall can contribute to perivascular fibrosis development and progression, promoting pathological consequences on cardiac function and remodeling. Undoubtedly, *fibroblasts* are crucially involved in the development of perivascular fibrosis (Fig. 1). The volume fraction of heart tissue occupied by fibroblasts is small, whereas the relative number of cells is much higher [15,16]. A recent report showed that cardiac fibroblasts normally account for around 13% of the cell number in the heart, a number that is lower than reported by earlier studies [16]. In this study based on flow-cytometry assays, pericytes were not mentioned. Whether pericytes are included or not may account for some of the variability between studies. In addition, multiple new studies established cardiac fibroblasts as a plastic population of cells that can adopt the expression of cardiogenic gene programs [17] and rapidly change their phenotype in response to local microenvironment [18]. The ability of

fibroblasts to produce excessive fibrotic extracellular matrix upon activation by stress stimuli and inflammation results in remodeling of the vessel wall and extracellular matrix [19,20]. Epigenetic changes in fibroblasts are expected to take place in a lifetime perspective and add further to the complexity [21]. Interestingly, reprogramming fibroblasts into myocytes like cells has been repeatedly examined, and these cells seem not doomed to collagen production [22–24].

With respect to non-myocyte cells in the heart, it now seems clear that *endothelial cells* outnumber fibroblasts [15]. Endothelial cells of the coronary vessels might have different phenotypes, depending on their localization in the vessel tree. The possibility of endothelial to fibroblast transformation has also been suggested [25,26]. Awareness of the importance of coronary endothelial-myocardial signaling for normal heart function raises the question of how fibrotic events close to the micro-circulation might disturb heart function under pathological conditions [27]. Deposition of extracellular matrix between endothelial cells of the capillaries and cardiomyocytes might hamper contractile function. In this context, development of in vitro experimental preparations combining myocytes - endothelial cells to study coupling between these two cells during normal and pathophysiological conditions, are valuable [27–29].

Pericytes are periendothelial mesenchymal cells residing close to endothelial cells and their basement membrane [30,31]. These cells are gaining increasing interest and are now acknowledged as important constituents in the normal wall of micro-vessels, with important functions for growth, regeneration and endothelial and smooth muscle function. Cardiac pre-capillary arteriole, capillary, and post-capillary venule, constituting the core microcirculatory unit, are richly covered by pericytes, with < 1% of the length of this microcirculatory unit being free of pericytes [32]. There are approximately 3.6×10^7 pericytes/cm³ of left ventricular tissue and the number of pericytes exceeds the number of myocytes per unit volume of left ventricular tissue [32]. The detailed knowledge regarding their role in perivascular fibrosis is uncertain. One important functional characteristic of these cells is the formation of gap junctions with other pericytes and with endothelial cells [32]. In a recent study, it was shown that a network of perivascular mesenchymal-stem-cell-like cells (identified by the expression of the marker Gli1) can generate myofibroblasts and therefore participate to the development of fibrosis in multiple organs including the heart [33]. These cells were localized to the pericyte niche in the microvasculature, adjacent to endothelial cells. Gli1⁺ cells did not exhibit a typical pericyte signature and appeared as more immature cells, that can however gain markers of mature pericytes (such as NG2) once activated.

Smooth muscle cells are responsible for regulating vascular resistance and have a clear role in the sequence of events leading to the development of perivascular fibrosis [34–36]. They are sensitive to pressure stress and respond to a variety of humoral factors including factors released from nerve endings, angiotensin II, endothelin and other compounds, which are known to promote fibrosis [37,38]. Recently, it was demonstrated in mice and in cell cultures that loss of caveolin-1 from the cell membrane attenuated perivascular fibrosis and collagen accumulation due to angiotensin II [37]. Under the influence of cytokines and growth factors, smooth muscle cells undergo phenotype alteration from contractile to a proliferative and migrating phenotype, which leads to structural changes in the vessel wall [35].

Finally, fibrosis is also usually associated with inflammation and activation of *immune cells* [39]. In particular, monocytes as well as infiltrating macrophages also play a pivotal role in secretion of extracellular matrix (ECM) components and in ECM remodeling. In addition, extensive crosstalk between inflammatory cells, fibroblasts and ECM actively modulates the fibrotic response [39].

5. Collagen and ECM

Interstitial matrix in the heart consists of a macromolecular network

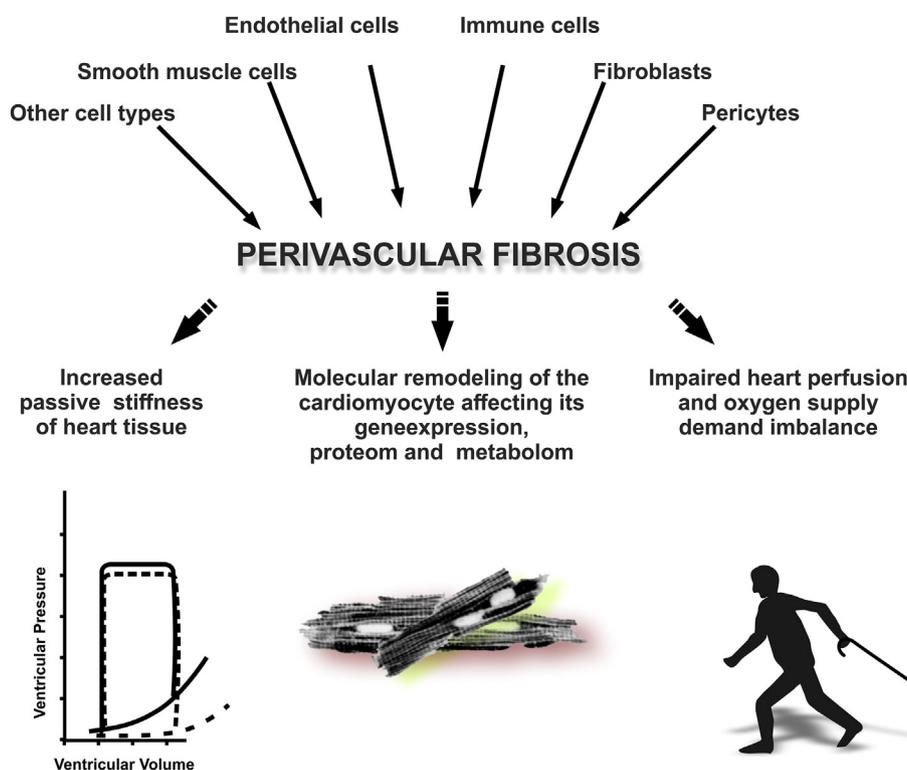


Fig. 1. Perivascular fibrosis: Multiple cells are involved in addition to fibroblasts, and the consequences of perivascular fibrosis affect cell, organ and integrated physiology.

of fibers, with collagen and glycosaminoglycan as main parts [4]. Collagen is the principal structural protein of the matrix and is mainly synthesized by fibroblasts. Elastin, fibronectin and laminin are additional protein components of the matrix. Collagen fibrils in bundles can easily be visualized by light microscopy of histological slices using different staining protocols like picrosirius red, Masson's trichrome and immunohistochemistry which enables semiquantification of amounts relative to other structures. Collagen types I and III are the most abundant in the myocardium, and collagen type I alone represents the majority of cardiac collagen (85%), controlling stiffness by providing resistance to stretch and deformation. Type III collagen is less abundant, but more specific to the heart compared to collagen I and provides elasticity [40,41].

ECM is not a static compartment, but a result of dynamic equilibrium between synthesis and degradation. Degradation is regulated mainly by matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) [41–44]. TIMPs and MMPs play crucial roles in matrix remodeling in the heart including the perivascular space. Extracellular matrix remodeling is seen as both a reparative and reactive process. Interestingly, circulating collagen turnover products have been proposed as biomarkers of fibrosis [45,46], even if such findings might be restricted to generalized vascular fibrosis. Carboxy-terminal propeptide of procollagen type I (PIP) in serum has been used as a marker of collagen I metabolism. The MMPs and TIMPs are synthesized and secreted by a number of cell types, especially fibroblasts but also smooth muscle cells and endothelial cells, and importantly, mammalian myocytes also synthesize and release MMPs and TIMPs. Under conditions that change the activity of these enzymes, the composition, the amount and anatomical locations of ECM can be altered. The general classification of MMPs is based on substrate specificity, but several of the MMPs can degrade a number of different matrix components [43,44,47,48]. After synthesis, the MMPs are secreted into the extracellular space as a proenzyme, or zymogen. A large number of MMPs have been characterized, but the classes of MMPs that may have particular relevance in the heart are the collagenases, which include MMP-1; the stromelysins,

which include MMP-3; the gelatinases, which include MMP-9 and MMP-2; and the membrane-type MMPs. Subtypes MMP1 and TIMP1 are well expressed in the heart, and changes in their expression have been particularly useful in experimental studies of heart diseases. Both elastin and collagen are degraded by MMP-9 during remodeling, but the turnover of elastin is much slower than collagen. Thus with MMP-9 activation, degraded matrix is replaced by stiffer collagen, promoting dysfunction and probably endothelial-myocyte (E-M) uncoupling [49].

6. Hormones, paracrine factors and inflammatory mediators

Inhibitors and antagonists of the renin-angiotensin system and adrenergic stimulation are standard treatment in heart failure. Angiotensin II is a main mediator of cardiac and perivascular fibrosis. Fibroblast angiotensin 1 (AT1) receptor activation leads to transforming growth factor beta (TGF β) activation and reactive oxygen species production by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX). TGF β activation, through SMAD-dependent or -independent pathways, activates the fibrotic genetic program, which comprises fibroblast proliferation, change in gene expression and protein synthesis including collagen synthesis [24]. This in turn can promote leukocyte infiltration, matrix degradation, collagen deposition, and importantly the myofibroblastic transdifferentiation [24,39]. Angiotensin II also cooperates with aldosterone to trigger perivascular and interstitial fibrosis. Doubling of intracardiac aldosterone concentration in genetically modified mice led to local activation of pro-fibrotic factors combined with an inhibition of anti-fibrotic factors [50].

Adrenergic agonists are on a regular basis used experimentally to produce collagen remodeling and perivascular and interstitial fibrosis [51]. However, it has been recently shown that cardiac β 3-adrenergic receptors protect from fibrosis in response to haemodynamic stress by modulating NO and oxidant stress-dependent paracrine signaling to fibroblasts [52].

Cytokines and chemokines like TGF β and monocyte chemoattractant protein 1 (MCP-1) are expressed in increased amounts concomitant with

development of perivascular fibrosis. Multiple experimental studies interfering with the cytokine TGF β and TGF β signaling pathways have acknowledged the significant importance of this pathway in fibrosis [53]. However, so far no treatment directed against cytokines and chemokines has been proven useful in terms of perivascular fibrosis [54]. Endothelins, potent vasoconstrictors with proliferative and mitogenic properties, are mainly paracrine factors. With respect to perivascular fibrosis and microvascular dysfunction a significant role of the endothelin-1 isoform has been confirmed as a key driver of angiotensin II-induced cardiac fibrosis [55]. It has been proposed that transformation of vascular endothelial cells into myofibroblasts, known as endothelial-mesenchymal transition, is a crucial event in the development of perivascular fibrosis. Interestingly, hepatocyte growth factor (HGF) was able to suppress TGF β or angiotensin II induced transformation of human coronary endothelial cells to myofibroblasts as well as transformation of resting fibroblasts to myofibroblasts [25]. Upon pressure overload stress HGF-transgenic mice exhibited a reduced amount of cardiac fibrosis compared to pressure-overloaded nontransgenic controls, particularly in the perivascular region [25].

7. Pathophysiology and disease

Increase in perivascular fibrosis in the heart is not specific for one disorder, but is seen in a variety of pathological conditions [8]. In most studies, due to technical issues, perivascular fibrosis is not distinguished from interstitial fibrosis in general in the heart. Fibrosis in the form of excess connective tissue at these two locations is often expected to develop in parallel although this is not always the case. For example, the marked changes observed with aging affect both the identified perivascular compartment and the extracellular space [56,57]. This reflects the situation that commonly occurs during age-dependent conditions like hypertension and diabetes, which are both associated with ECM remodeling. Hence, elderly patients with hypertension and left ventricular hypertrophy (LVH) typically exhibit extracellular fibrosis associated with impaired diastolic function and often develop signs of ventricular ischemia. Another important pathophysiological aspect is the presence of multiple comorbidities with disorders like autoimmune rheumatic diseases (ARDs) and kidney disease, which also affect the vessel wall and promote fibrosis [56–58].

7.1. Cardiac hypertrophy and related conditions

LVH combined with fibrosis, is a typical consequence of prolonged untreated arterial hypertension [59]. During hypertension fibrosis starts in the perivascular space and expands beyond that space with time if the condition is left untreated. Seminal works done in late 90's, studied structural alterations at the arteriolar level in two different groups of patients with cardiac hypertrophy, signs of myocardial ischemia but no coronary artery disease. Findings were compared with control heart samples. One group suffered from hypertensive heart disease, and peri-arteriolar collagen accumulation and increase in the media of the arteriolar wall characterizing this group. The other group had been diagnosed with hypertrophic cardiomyopathy, and morphological examination of subendocardial biopsies demonstrated reduced microvessel density but no perivascular fibrosis [60,61]. Arterial collateral vessels are present in the heart in variable degree, and in humans arterial collaterals develop as a response to progressive atherosclerosis and regional ischemia in the heart [62]. Interestingly, studies indicate that development of arterial collaterals in the adult heart is correlated with bradycardia and absence of hypertension [63]. Dysfunction or perfusion defects at the microcirculatory level is not compensated by collateral growth. Micro-vascular dysfunction is masked if macro-vascular disease develops. Female gender, pregnancy and exercise harbor anti-fibrotic factors protecting the microvasculature of the heart. LVH is a characteristic of the athlete heart and also to some degree of the pregnant heart, but fibrosis including perivascular fibrosis is absent

[64]. When pregnant female rats were exposed to angiotensin II for two weeks and compared to exposed non-pregnant females, the angiotensin II-induced fibrosis was significantly reduced by pregnancy state [65]. More detailed knowledge of the molecular processes behind the beneficial role of exercise and female sex (including the pregnancy state) is warranted. The molecular mechanisms linking this to inhibition of perivascular fibrosis are only partly described. Laminar shear stress along the endothelial luminal surface maintains the endothelium healthy. Change of endothelial cells to more pro-inflammatory phenotype might occur when blood flow velocity is not sufficient [66].

Although microvascular fibrosis is part of heart failure pathophysiology, whether it is a cause or consequence of cardiac pathological remodeling, and temporal relations is difficult to determine [67]. Microvascular fibrosis has especially been put forward in relation to heart failure with preserved ejection fraction [26,68]. A large fraction of heart failure patients does not have reduced left ventricular ejection fraction indicating that stroke volume is reduced due to constrictive remodeling and/or diastolic dysfunction resulting in reduced filling of the ventricle in diastole. The active part of relaxation is dependent on cellular events like the cellular energy situation and calcium handling, the passive part on structural factors including degree of fibrosis. Microvascular fibrosis might impair both phases.

7.2. Post-infarction remodeling of the heart

A reparative process follows myocardial infarction with scar tissue gradually replacing necrotic and apoptotic cardiomyocytes and fragmented extracellular matrix, thus securing against rupture of the wall [43,44,49]. Persistent myofibroblast activation distinguishes pathological fibrosis from the physiological wound healing described above. Inflammatory activity and increase in stress upon tissue adjacent to the infarcted area might induce and maintain myofibroblast activation leading to perivascular fibrosis as part of expansion of remodeling, causing dysfunction and deterioration of global heart function [69]. Post infarct remodeling is age and sex dependent, partly due to the impact of age and sex on fibroblast function [56,70].

7.3. Obesity, insulin resistance and diabetes

Vascular dysfunction is observed in obesity, insulin resistance and type II diabetes. Fibrotic remodeling of the vessel wall is present at systemic level involving several organs. Remodeling of cardiac vessels with diabetes and obesity seem to affect both larger arteries and the microcirculation. The hypertrophy and fibrosis often observed in animal models of diabetes and obesity are considered responsible for the development of diastolic dysfunction in obesity [71–73]. There is a growing understanding of the mechanistic role of microvascular fibrosis in this condition. This includes a strong local influence of angiotensin II as well as endothelial-myocyte uncoupling among other factors [74,75]. Obesity and diabetes often exist with other common comorbidities affecting the heart (such as hypertension and coronary artery disease) and dissection of the contribution of metabolic disease in remodeling of the heart is challenging. Obstructive sleep apnea is often observed in these patients and is associated with intermittent hypoxia, which is thought to trigger the development of perivascular fibrosis. Comorbidities characterize the patient population with diastolic dysfunction and heart failure with preserved ejection fraction (HFpEF), and microvascular fibrosis could be the common denominator [26]. The need to understand the functional coupling between myocytes and capillary endothelium and the consequence of perivascular fibrosis affecting the microcirculation, calls for development of appropriate experimental models of this syndrome.

8. Conclusions

To investigate perivascular fibrosis and microvascular dysfunction

in the heart has been a challenge in both experimental and clinical cardiovascular research. This is in spite of - or maybe caused by - the findings that in most common cardiovascular diseases cardiac microcirculation is affected and fibrotic remodeling takes place. The reasons for the complexity are the temporal nature, the spatial diversity and the multiple different cells involved. However, renewed interest in the topic provides hope for a better understanding of the pathophysiological events leading to clinical manifestations.

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