Chapter 12

Diabetic Cardiomyopathy: Focus on Oxidative Stress, Mitochondrial Dysfunction and Inflammation

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65915

Abstract

Diabetic cardiomyopathy (DCM) is an independent clinical entity defined as structural and functional changes in the myocardium because of metabolic and cellular abnormalities induced by diabetes, resulting in cardiac failure. Hyperglycemia has been seen as a major cause of DCM due to activation of different mechanisms leading to oxidative stress. Several body of evidence show that distinct pathways of oxygen and nitrogen reactive species formation contribute to myocardial impairment. Abnormal mitochondrial morphology and energetics, evoked by abnormal Ca^{2+} handling, metabolic changes and oxidative stress, are observed in DCM, suggesting a pivotal role of mitochondrial dynamics in disease pathogenesis. In addition, insulin resistance compromises myocardial glucose uptake due to cellular depletion of glucose transporter proteins, together with increased myocardial uptake of free fatty acids and augmented triglyceride levels, which cause cardiomyocyte lipotoxicity. Finally, the state of chronic low-grade inflammation, a feature of obese type 2 diabetes, seems to also play a major role in DCM progression, whose mechanisms have been progressively disclosed. In this book chapter, we review the cellular mechanism contributing to DCM development, focusing on oxidative stress, mitochondrial dysfunction and inflammation of cardiomyocytes, as well as on possible therapeutic strategies.

Keywords: diabetic cardiomyopathy, oxidative stress, mitochondrial dysfunction, inflammation, therapeutic strategies

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common endocrine deregulation worldwide, reaching pandemic proportions on a global scale [1]. In 2015, there were 415 million

people with diabetes globally and an increase to 642 million by 2040 is estimated by the International Diabetes Federation [2]. In addition, T2DM is one of the leading causes of illness and premature death, with 5 million deaths in 2015, mainly affecting developed regions ("Occidental World"), as well as many countries in development, particularly due to unhealthy lifestyle habits, such as physical inactivity and high fat and sugar diets [1].

T2DM is a major risk factor for the development of cardiovascular diseases (CVD), which are responsible for up to 65% of all deaths in diabetic patients, as well as for substantial morbidity and loss of quality of life. As T2DM progresses, the heart and blood vessels undergo changes, leading to a number of different cardiovascular complications, including coronary artery disease (CAD), stroke, peripheral arterial disease, as well as diabetic cardiomyopathy (DCM) [2].

The original finding of Rubler et al. [3] of the existence of heart failure (HF) in postmortem diabetic patients free of detectable CAD was the basis of the first use of DCM terminology. Subsequent clinical and epidemiological studies have confirmed these observations [4, 5], suggesting that diabetes can damage the cardiac tissue independently of other cardiovascular risk factors. Such associations have provided a credible existence of DCM as a unique clinical entity, independent of hypertension, CAD, left ventricular hypertrophy (LVH), atrial fibrillation, or any other known cardiac diseases, leading to HF, caused by complex relationships between metabolic abnormalities that accompany diabetes and its cellular consequences [6].

Despite the development of asymptomatic DCM for a long period of time, the metabolic anomalies at the cardiac myocyte level progresses, leading to structural and functional abnormalities. Although hyperglycemia has been classically indicated as the primary responsible, other factors seem to be involved in the evolution of the disease and several substrates have been suggested [7]. During the last years, the structural, functional, pathological and molecular aspects of the disease have been increasingly investigated, but the issue is far to be elucidated and no specific markers and therapeutics have been found so far. Unravelling the molecular mechanisms underlying DCM development and progression is crucial to identify relevant therapeutic targets and generate novel therapies tailored to reduce the risk of HF in diabetic patients.

In this book chapter, we revisit some of the main features of DCM, focusing on pathophysiological mechanisms associated with cardiomyocyte oxidative stress, mitochondrial dysfunction and inflammation. We also indicate possible therapeutic strategies targeting those important cellular events that seem to play a major role in DCM development and progression.

2. Structural and functional cardiac changes

Increasing evidences from experimental, pathologic, epidemiologic and clinical studies have been shown that diabetes results in structural and functional cardiac changes. Anatomic changes in DCM, mainly assessed by echocardiography or magnetic resonance imaging, are essentially characterized by myocardial hypertrophy and fibrosis. In addition, although many studies have shown that diabetic patients have abnormal diastolic function but preserved systolic function, which might be due to the lower sensitivity to detect systolic dysfunction by

http://dx.doi.org/10.5772/65915

some of the techniques used, the current knowledge points to the existence of a continuum of diastolic and systolic dysfunction in DCM.

2.1. Cardiac hypertrophy

One of the most important structural hallmarks of DCM is cardiac hypertrophy, which is a powerful predictor of cardiovascular events. Apoptotic and necrotic loss of cardiomyocytes causes compensatory hypertrophy of the remaining viable cardiomyocyte. Although the right ventricle can also become hypertrophic, LVH is more common and generally represents a more advanced stage of the disease. Even though the causes and mechanisms underlying LVH development in diabetic patients remain poorly understood, experimental and clinical studies have been suggesting that hyperinsulinemia, insulin resistance, hyperglycemia, and increased nonesterified fatty acids (NEFAs) may collectively play a major role. Insulin, in particular, is viewed as a growth factor in the myocardium, which is sustained by experimental findings that sustained hyperinsulinemia causes increased myocardial mass and decreased cardiac output in rats [8]. In addition, clinical and experimental data have been shown increased markers of cardiomyocyte hypertrophy, including augmented width and myofiber disarray of cardiomyocyte, as well overexpression of hypertrophic genes, namely β-myosin heavy chain, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) [9, 10].

2.2. Myocardial fibrosis, apoptosis and necrosis

Myocardial fibrosis has been indicated as another major mechanism contributing to cardiac alterations in DCM. This pathological feature of DCM that have been observed in diabetic patients without significant CAD and in animal models, results from the accumulation of interstitial glycoproteins and increased extracellular collagen matrix, which potentiates stiffening and inhibits ventricles relaxation [11, 12]. The echocardiographic features of increased left ventricular fibrosis appear in the form of impaired relaxation and diastolic dysfunction; consequently, alterations in collagen phenotype may play an important role in the impaired left ventricular diastolic filling that is typical of DCM [11]. It has been suggested that collagen is a major determinant of ventricular stiffness. In a study with rats, a correlation between increased extracellular collagen content and decrease in early mitral peak flow (decreased E/A ratio) was reported [12]. The cause for the accumulation of cardiac fibrosis in diabetes is believed to result from decreased degradation of glycosylated collagen by matrix metalloproteinases and, conversely, from excessive production of collagen by fibroblasts due to increased renin–angiotensin–aldosterone system (RAAS) activation [11]. Furthermore, increased formation in myocardial advanced glycation end-products (AGEs) has also been reported in diabetic patients, which has been attributed to hyperglycemia [13]. In fact, collagen cross-linked with AGEs causes myocardial stiffness and inhibits collagen degradation, which promotes additional collagen accumulation and fibrosis [11, 13]. This mechanism seems to be also a major contributor for the impaired left ventricular diastolic function observed in diabetic patients [13].

Finally, DCM is associated with increased myocyte cell death and apoptosis. Accelerated necrosis and apoptosis is caused by hyperglycemia, increased formation of ROS, overactivation of local RAAS system and of insulin-like growth factor-1 and transforming growth factor beta 1

(TGF-β1) [14]. While apoptosis does not cause scar formation or accumulation of interstitial collagen, because nuclear fragmentation and cell shrinkage is replaced by the surrounding cells, necrosis is able to promote the widening of extracellular compartments among myocytes and increased deposition of collagen, which causes replacement fibrosis and connective cell proliferation [15].

2.3. Diastolic dysfunction

In many cases, it has been found that abnormalities of diastolic function may advertise the subsequent progressive deterioration of cardiac function. Diastolic dysfunction is the basic hemodynamic feature and the earliest findings of DCM that can be detected using imaging techniques. The noninvasive assessment of diastolic dysfunction mainly relies on Doppler studies of diastolic transmitral inflow, flow velocities, flow patterns, isovolumic relaxation time and deceleration time, which are the most common criteria used in its evaluation. The criteria of the consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology have been used to assess left ventricular diastolic dysfunction [16].

Diastolic dysfunction is characterized by an abnormal myocardial relaxation and filling. This condition is typically manifested by reduced early diastolic filling and increased atrial filling, by augmented isovolumetric relaxation and increased number of supraventricular premature beats, as well as by amplified left ventricular end-diastolic pressure and diminished left ventricular end-diastolic volume [17–19].

Diastolic dysfunction is found in several other cardiovascular diseases, such as hypertension, hypertrophic cardiomyopathy and CAD, even with intact systolic function. However, experimental and clinical studies have shown impaired diastolic function in the absence of manifestations of congestive HF, even in prediabetes or in early stages of diabetes [19], thus suggesting that could be a useful early marker for disease prognosis. Furthermore, left ventricular diastolic dysfunction may progress to a systolic dysfunction, causing reduced left ventricular ejection fraction (LVEF) in years. Therefore, it is very important to detect left ventricular diastolic dysfunction in diabetic patients, both for early diagnosis and treatment of DCM, as well as for prevention of further systolic dysfunction.

2.4. Systolic dysfunction

Systolic dysfunction, defined as impaired ability of the heart to pump arterial blood in the periphery, is typically associated with a reduced LVEF and cardiac output. In DCM, systolic dysfunction occurs late, often when patients have already developed significant diastolic dysfunction. The presence of systolic dysfunction in the early years of diabetes has been a controversial issue, while diastolic dysfunction is more easily detected by Doppler echocardiography. The controversy relies on the fact that current techniques used to assess systolic function are less sensitive than those used for diastolic dysfunction evaluation. For this reason, more sensitive and accurately techniques for systolic assessment have been developed, such as tissue Doppler imaging (TDI) and strain rate imaging techniques, which are able to estimate left ventricular function in longitudinal, radial and circumferential ways, thus

http://dx.doi.org/10.5772/65915

allowing the detection of preclinical systolic abnormalities in diabetic patients. Currently, shortened left ventricular ejection time, decreased peak systolic velocity (S′), and smaller left ventricular fractional shortening can be the detectable parameters for identification of systolic dysfunction [20]. The prognosis in patients with depressed systolic dysfunction is poor with an annual mortality of 15–20%.

3. Overview of the molecular mechanisms involved in DCM development

The pathophysiological molecular mechanisms underlying the development and progression of DCM are multifactorial and complex and have been progressively disclosed. Some of the main features of DM are also pivotal elements in the pathogenesis of DCM, including hyperglycemia, hyperinsulinemia and insulin resistance, as well as hyperlipidemia (**Figure 1**).

Hyperglycemia has been seen as a major cause of DCM development due to activation of the classical oxidative stress pathways (polyol, hexosamine, AGEs and protein kinase C—PKC). These mechanisms cause increased production of mitochondrial reactive oxygen species (ROS), nonenzymatic glycation of proteins and glucose auto-oxidation, thus leading to cellular (cardiac) injury—glucotoxicity. Glucose and collagen interact to form Schiff bases and the fibrous network is reorganized with the so-called Amadori products, which can be transformed in AGEs. As above mentioned, the increased formation of AGEs is highly associated

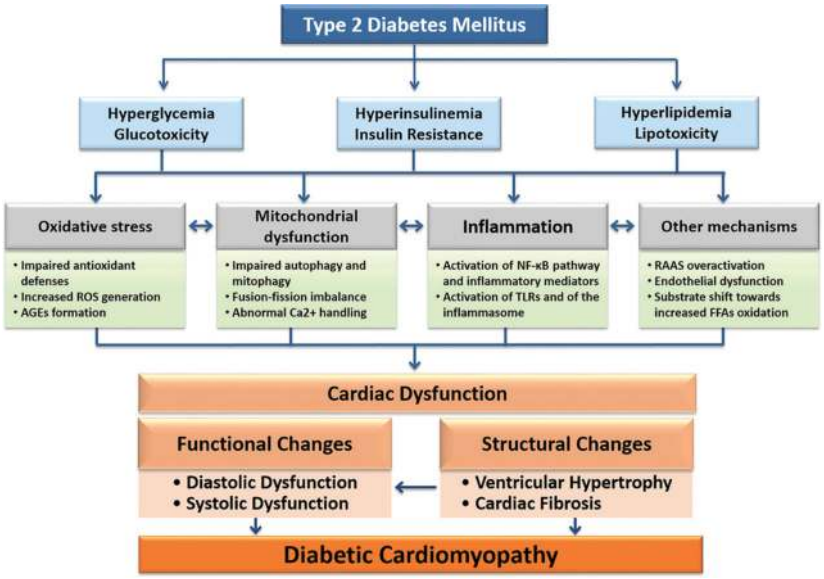

Figure 1. Metabolic abnormalities underlying T2DM that are considered the main triggers for the cellular and molecular pathways associated with structural and functional changes in DCM. Adapted with permission from Ref. [24]. *Abbreviations:* AGEs, advanced glycation end products; FFAs, free fatty acids, NF-κB, nuclear factor-κB; RAAS, renin–angiotensin–aldosterone system; ROS, reactive oxygen species; TLRs, Toll-like receptors.

with myocardial fibrosis in diabetic hearts by affecting the structural components of the extracellular matrix, such as collagen. This stable cross-linked collagen accumulate in vessel walls and in myocardial tissue, increasing diastolic stiffness of the heart and contributing to endothelial dysfunction, thus suggesting a key role of AGEs in DCM development.

Insulin resistance originates cellular depletion of glucose transporter proteins (GLUT-1 and GLUT-4) leading to reduced glucose uptake in the diabetic heart, which facilitates a substrate shift towards increased fatty acids (FAs) oxidation, resulting in reduced cardiac efficiency [21]. In brief, once inside the cardiomyocytes, the free fatty acids (FFAs) are converted into acetyl coenzyme A derivatives that will activate PKC isoforms responsible for blocking insulin cascade elevated levels of FFAs compete with glucose as energy substrate, with a shift in energy production from β-oxidation of FFAs. As a result, there is a reduced glucose utilization and oxidation, increased glucose and insulin levels, promoting insulin resistance.

Additionally, the increase concentration of FFAs and of its metabolism causes intracellular accumulation of toxic FA intermediates (such as ceramide and diacylglycerol) and formation of ROS. These mechanisms originates cardiac lipotoxicity by means of oxidative stress, cardiomyocyte apoptosis and increased myocardial consumption of oxygen, resulting in impaired contractility, mitochondrial uncoupling and decreased adenosine triphosphate (ATP) availability [22]. Intracellular deposition of FFAs is also responsible for the saturation of the mitochondrial capacity of oxidation, thus activating transcription factors, including the peroxisome proliferator-activated receptors (PPARs), which has been indicated as an inductor of cardiac lipotoxicity and dysfunction [23]. Several body of evidences show that distinct pathways of oxygen and nitrogen reactive species formation contribute to myocardial injury. Impaired mitochondrial morphology and energetics, evoked by abnormal Ca^{2+} handling, metabolic changes and oxidative stress, are observed in DCM, suggesting a pivotal role of mitochondrial dynamics in disease pathogenesis.

In addition, the state of chronic low-grade inflammation, a feature of obese T2DM, seems to also play a major role in DCM progression, whose mechanisms have been progressively disclosed [24]. The epicardial adipose tissue (EAT) that covers 80% of the heart surface and constitutes approximately 20% of the total heart weight have endocrine and paracrine properties that interfere with cardiac function, namely by the development of inflammation, insulin resistance and cardiac dysfunction [25].

As above mentioned, activation of the RAAS, locally and systemically, has been associated with the development of insulin resistance and the onset of T2DM. In addition, it has been associated with some of the hallmarks of DCM, such as increased fibrosis, oxidative damage, and cardiomyocyte and endothelial cell apoptosis and necrosis [26].

4. Oxidative stress and mitochondrial dysfunction

Mitochondria are dynamic organelles with a key role in energy transduction, signaling and cell death pathways. Consequently, mitochondrial dysfunction and oxidative stress are broadly relevant in the development of cardiovascular diseases, in both acquired and

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inherited disease [27]. Tissues with high aerobic metabolism demands, such as the heart muscle, are severely affected by a decline in mitochondrial efficiency such as in the context of diabetes, ischemia-reperfusion and aging [28, 29], associated with loss of calcium homeostasis and impaired contractile function. In fact, mitochondria comprise one-third of the volume of the heart and support the vast majority of ATP production derived from oxidation of fatty acids (FAs) and glucose, being FAs the preferred substrate in the normal adult myocardium, while failing human hearts shift to oxidizing glucose for energy production.

In the inner mitochondrial membrane, electrons deriving from the oxidation of NADH and FADH2 are funneled through the electron transport chain. This flow is coupled with the translocation of protons across the inner mitochondrial membrane to the intermembrane space, generating an electrochemical gradient. Under normal conditions, much of the energy of this gradient is used to generate ATP, as the collapse of the proton gradient through ATP synthase drives the ATP synthetic machinery. However, when the electrochemical potential difference generated by the electrochemical gradient is high (such as in high-fat or high-glucose states), or under conditions of inhibition of the ETC complexes, the life of superoxide-generating electron transport intermediates, such as ubisemiquinone radical, is prolonged [30], resulting in increased ROS generation. Although ROS are produced in multiple cell compartments, the majority of cellular ROS (approximately 90%) are mitochondrial, mainly at the level of complexes I and III of the ETC [31]. The activity of detoxifying enzymes and uncoupling proteins limits ROS generation. In the healthy myocardium, ROS concentration is tightly controlled to low steady-state level by superoxide dismutase (SOD) [32]. Superoxide anion is dismutated by mitochondrial manganese SOD into hydrogen peroxide, which is detoxified into water by the mitochondrial glutathione peroxidase (GPx), an action dependent on mitochondrial reduced glutathione (GSH) content. Mitochondrial catalase has a detoxifying effect against overproduction of hydrogen peroxide. An imbalance of antioxidant defenses that favors the accumulation of oxidants, expose mitochondria to oxidative stress, with ROS reacting with DNA, proteins and lipids, inactivating the ETC complexes and mitochondrial proteins, thus impairing both oxidative phosphorylation (OXPHOS) and inducing ROS accumulation. In the diabetic heart, increased FAs accumulation and metabolism is linked to oxidative stress [33]. Also, in the context of myocardial ischemia/reperfusion, oxidative stress is implicated in ATP depletion and cardiomyocyte death. At the onset of reperfusion, increased ROS and mitochondrial calcium influx favor induction of the mitochondrial permeability transition (MPT) and loss of mitochondrial inner membrane impermeability [34]. Recently, it has been shown that offspring of diabetic pregnancies are at risk of cardiovascular disease at birth and throughout life, with high-fat diet-exposed offspring exhibiting mitochondrial dysfunction and lipid peroxidation [35]. Each cell has normally several copies of mitochondrial DNA (mtDNA) which encodes ribosomal and transfer RNAs necessary for the synthesis of the mtDNA-encoded 13 OXPHOS polypeptides in the mitochondrial matrix [36]. The proximity to the inner membrane, the absence of protective histones, and incomplete repair mechanisms in mitochondria, renders mtDNA extremely sensitive to oxidative damage. The accumulation of mtDNA mutations due to oxidative damage results in further unbalanced ETC and increased ROS generation, perpetuating oxidative damage and enhancing inflammatory, hypertrophic, fibrotic, and cell death events in the myocardium [37]. Therefore, mechanisms

able to eliminate dysfunctional mitochondria are essential to prevent the cytotoxic impact of ROS and thus maintain cellular homeostasis.

Autophagy is a tightly regulated cellular process which promotes the turnover of dysfunctional mitochondria along with the elimination of long-lived proteins and other damaged organelles, being essential for maintaining normal cardiac function [38]. Autophagy also maintains cell viability under stress conditions by supplying amino acids for de novo protein synthesis and providing substrates for the tricarboxylic acid cycle [39], as shown by myocardial survival promoted by activation of autophagy upon starvation or ischemia [40, 41]. Autophagy is redox-dependent due to redox regulation of metabolic alterations as well as ROS-mediated modification of autophagy-regulatory proteins. Disruption of ATG5, an autophagy-related gene, results in heart failure under basal and stress conditions [42]. In turn, autophagy regulates intracellular ROS by selective elimination of dysfunctional mitochondria (mitophagy) [43] and degradation of KEAP1 and activation of the nuclear factor erythroid 2-related factor 2 (NRF2), activating the expression of antioxidant genes such as glutathione peroxidase, superoxide dismutase and thioredoxin [44]. A decline in autophagy with aging, leading to increased levels of oxidative damage and the accumulation of dysfunctional mitochondria has been proposed as an underlying cause for the pathogenesis of cardiovascular diseases prevalent in late life [45].

Besides mitophagy, mitochondrial quality control is dependent on balanced fusion and fission events that continually alter mitochondrial morphology by undergoing fission to generate discrete fragmented mitochondria or fusion to form an interconnected elongated network. This dynamic behavior shapes mitochondria to adapt metabolism to the energetic needs of the cell, allows mixing of mtDNA, lipids, proteins and metabolites, enhances communication with the endoplasmic reticulum or segregates dysfunctional or depolarized mitochondria away from the healthy network, facilitating its clearance [46]. These two processes are under the control of mitochondrial fission and fusion proteins: mitofusins (MFN1 and MFN2) and optic atrophy 1 (OPA1) mediate mitochondrial fusion while dynamin-related protein 1 (DRP1) mediates mitochondrial fission by interaction with other fission mediators such as fission protein (FIS1) [47]. Changes on mitochondrial morphology, linked to altered expression of DRP1 and MFN2, are evident during stem cell differentiation into cardiomyocytes, transitioning from fragmented rounded mitochondria into an elongated network with well-developed cristae and an efficient OXPHOS system [48]. The essential role of MFN proteins is also shown by mitochondrial fragmentation, impaired mitochondrial function and development of heart failure in models of conditional cardiac ablation of MFN 1 and 2 [49]. In post-mitotic tissues with high metabolic demands, such as the heart, abnormal mitochondrial dynamics results in the development of cardiovascular disease, due to impaired mitochondrial turnover and accumulation of fragmented and depolarized mitochondria, sources of increased ROS generation [50]. Recently, it has also been shown that DRP1 ablation results in cardiomyocyte necrosis and dilated cardiomyopathy in mice, mitophagic mitochondrial depletion and favors MPT induction, probably linked to spatiotemporal alterations in calcium signaling [51]. When exposed to calcium overload, both neonatal and adult rat cardiomyocytes exhibited increased ROS generation and mitochondrial fragmentation, which suggested that activation of the fission machinery may be an event preceding ROS generation regulated by calcium signaling [52]. Giant or mega-mitochondria have been described in a variety of

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cardiomyopathies, including those associated with mtDNA mutations [53]. The observation of increased mtDNA content, induction of genes involved in mitochondrial biogenesis, fatty acid metabolism, and glucose transport, as well as uncoupling proteins and antioxidant enzymes in mitochondrial cardiomyopathies hearts, may indicate a compensatory response, although unable to prevent energy depletion and increased ROS generation [54].

Balanced fusion–fission events are essential for normal mitochondrial biogenesis, the process by which cells increase mitochondrial mass and copy number. Among the transcription factors involved in this process, peroxisome proliferator-activated receptor-γ coactivator-1, PGC-1α, is the master regulator. This inducible coactivator acts as a coactivator of the transcription factors involved in the expression of nuclear/mitochondrial genes and bioenergetic capacity, as well as regulates cardiac fuel selection and mitochondrial ATP-producing capacity [55]. An interplay between PGC-1α and MFN-2 has been shown as follows: MFN2 is critical for the stimulatory effect of PGC-1α on mitochondrial membrane potential while PGC-1α may regulate mitochondrial fusion/fission events [56]. Besides stimulating mitochondrial biogenesis and OXPHOS, PGC-1α prevents oxidative stress by inducing ROS-detoxifying enzymes [57]. Sirtuin 3 (SIRT3) has been shown essential for the stimulatory effect of PGC-1α on both mitochondrial biogenesis and ROS-detoxifying enzymes [58]. Sirtuins (1–7) are a conserved family of NAD+-dependent lysine-modifying acylases that regulate a variety of cellular functions such as metabolic responses to diet and exercise [59]. The decline in NAD+ during aging decreases sirtuin activity thus impairing the transcription of mitochondrial OXPHOS genes which leads to cardiovascular disease, an event precipitated by SIRT1 deletion [60]. Cardiac SIRT1 is upregulated during nutrient starvation, exercise and ischemic preconditioning while downregulated during I/R [61]. SIRT3, which exhibits mitochondrial deacetylase activity, deacetylates and increases the activity of mitochondrial metabolic and antioxidant enzymes [62] as well as regulates mitochondrial fusion–fission dynamics [63]. By deacetylating and suppressing the activity of cyclophilin D, SIRT3 increases resistance to MPT induction, preventing cell death and cardiac hypertrophy [64].

5. Inflammation

Chronic low-grade inflammation is commonly associated with obesity and T2DM, and clear evidence has emerged to suggest that inflammatory process also contributes to the pathogenesis of DCM. The inflammatory signaling in cardiomyocytes usually occurs as an early response to myocardial injury and involves an increased formation of cytosolic and mainly mitochondrial ROS. Several molecular pathways have been classically associated with the inflammatory response in the cardiac tissue: increased activation of the proinflammatory nuclear transcription factor-κB (NF-κB), overexpression of cytokines [namely the tumor necrosis factor-α (TNFα), some interleukins (such as IL-1β and IL-6), chemokines (i.e., monocyte chemotactic protein-1: MCP-1), adhesion molecules (i.e., selectins and adhesion molecules (ICAM-1, VCAM-1)] and migration of leukocytes into the myocardium [24]. There is evidence that chronic progression of hypertrophy, fibrosis and ventricular dysfunction is correlated with a local increase in cytokines and activation of NF-κB [65]. Activation of NF-κB is associated with the increased release of

cytokines, such as TNF-α and IL-6, which are often involved in cardiac damage (hypertrophy and fibrosis) and left ventricular dysfunction [65, 66]. Accumulating data have been demonstrated that increased IL-1β and TNF-α are implicated in DCM, increasing epicardial thickness, promoting myocyte contractile dysfunction, thus depressing myocardial function and contributing to HF [67]. The inflammatory stimuli in the diabetic heart include hyperglycemia, hyperlipidemia, ROS, angiotensin II and the activation of Toll-like receptors (TLRs). Hyperglycemia-induced oxidative stress and inflammation seem to be deeply correlated with development of DCM. In fact, hyperglycemia activates several oxidative stress-responsive/proinflammatory transcription factors, including NF-κB, which is able to induce collagen and fibronectin synthesis, as well as to stimulate the production of inflammatory cytokines. Hyperglycemia-evoked diastolic dysfunction may be mediated partly by the macrophage migration inhibitory factor, suggesting that the NF-κB pathways may be involved in this process [68].

RAAS overactivation seems to also play an important role in the modulation of inflammation associated with DCM. In fact, Ang II not only induces vasoconstriction, cell growth and oxidative stress but also stimulates inflammation, namely by inducting cytokines release, by stimulating the production of PAI-1 and pro-inflammatory transcription factors, such as NF-κB, which in turn regulate adhesion molecules (VCAM-1 and ICAM-1) and the expression of several cytokines [69].

Activation of TLRs and the inflammasome complex has been recently proposed to play a pivotal role in cardiac inflammation and likely in the pathogenesis of DCM [70]. Accumulating evidences support the hypothesis that hyperglycemia and FFA are able to stimulate TLRs, thus inducing proinflammatory pathways in DCM. In fact, TLR-dependent NF-κB and ROS seem to be able to regulate both the priming and the posttranslational pathways required for the assembly and activation of the inflammasome, thus opening new therapeutic opportunities to DCM treatment, as further discussed.

6. Therapeutic strategies

Despite a specific therapy for the treatment or prevention of DCM is still lacking, some therapeutic strategies could present potential benefits. The advances on the knowledge of DCM pathogenesis provides us with improved management options, including lifestyle measures, strategies to improve diabetic control, lipid lowering therapy, as well as agents directed to target some of the main molecular events and mechanisms underlying DCM development and progression, including fibrosis, hypertrophy, oxidative stress and inflammation.

6.1. Physical exercise

Regular physical activity (training) has been associated with improved glycemic control and insulin sensitivity, as well as with amelioration of the metabolism of glucose and fatty acids in heart muscle, thus improving left ventricular function and attenuating diabetes induced-cardiac alterations. Physical exercise has also greater anti-inflammatory effects by decreasing the release of inflammatory cytokines from the skeletal muscles endothelial cells

http://dx.doi.org/10.5772/65915

and immune system, together with increasing the anti-inflammatory cytokines, such as adiponectin [71]. In an animal model of T2DM, the Zucker Diabetic Fatty (ZDF) rat regular aerobic exercise (training) was able to not only improve the glycemic control and attenuate dyslipidaemia, but also to promote an anti-inflammatory effect, viewed by the reduction in pro-inflammatory cytokines, such as TNF-α and CRP, and by the increment of adiponectin levels [72–74]. This effect occurred independently of weight loss and was not observed when an acute extenuating exercise was used [75].

6.2. Antidiabetic agents

Improvement of glycemic control has been shown to be associated with better outcomes in diabetic microvascular complications in many clinical trials. Even though the impact of strict glycemic control on macrovascular outcomes remains debatable, the recognized role of microvascular disease in DCM development suggests that a better glycemic control would benefit patients.

6.2.1. Insulin-sensitizing agents

Insulin resistance is a hallmark of T2DM and plays an important role in the pathogenesis of DCM. Accordingly, agents used to ameliorate insulin resistance might be useful to prevent DCM progression. The beneficial effects of insulin may rely not only on the improved glycemic control in some patients but also on cardioprotective anti-inflammatory properties. Several data show a reduction in adhesion molecules, such as ICAM-1 and E-selection, circulating CRP, IL-6 and PAI-1 due to insulin-sensitizing therapy [76]. Metformin, one of the most commonly prescribed anti-diabetic drugs and a known insulin-sensitizing agent, improves peripheral sensitivity to insulin and promotes intensive glucose control [77]. Besides, cardioprotective actions of metformin have been described, namely inhibition of hypertrophy and pro-authophagic and anti-inflammatory actions [78].

The possible beneficial effects of thiazolidinediones (TZDs) and PPAR agonists on the myocardium have been demonstrated in several studies. Pioglitazone was associated with improved diabetic cardiac function in animal models, by raising myocardial glucose uptake and improving myocardial fatty acid metabolism [79]. In addition, rosiglitazone showed an amelioration of myocardial diastolic function in T2DM patients, which was related to an antioxidant and anti-inflammatory effect [80]. Other factors involved in improved cardiac function with TZDs therapy include decreased collagen accumulation and fibrosis, as well as inhibition of cardiomyocyte hypertrophy [79, 81]. Pioglitazone and rosiglitazone stimulate the PPAR-γ, which regulates important genes for the metabolism of glucose and fat and enhance insulin sensitivity in skeletal muscle and adipose tissue [82]. Additionally, PPAR activators may have anti-inflammatory effects by inhibition of TNF-α expression at the transcriptional level due to attenuation of NF-κB activity in cardiomyocytes [83]. However, the effects of this therapy on cardiac function in patients with T2DM have not yet been fully elucidated. On the other hand, experimental studies using PPARα agonists suggested cardiac benefits related to apoptosis and hypertrophy [84]. However, further research is still required in order to fully understand the role of PPARα agonists, as well as TZDs, in DCM.

6.2.2. Incretin-based therapies

Glucagon-like peptide-1 (GLP-1), an incretin hormone rapidly released by the L-cells of the small intestine after a meal intake, presents several actions that contribute to glucose homeostasis, including stimulation of postprandial insulin secretion by pancreatic beta cells, thus improving insulin sensitivity. GLP-1 is metabolized by the enzyme dipeptidyl peptidase 4 (DPP-4), thus inactivating their insulinotropic activities. In diabetic patients, the incretin effect is partially blunted, which contributes to a poor glycemic control. The incretin-based therapies, a new class of antidiabetic drugs currently available for the treatment of diabetic patients, include DPP-4 inhibitors (such as sitagliptin) and GLP-1 receptor (GLP-1R) agonists (namely exenatide). GLP-1R and DPP-4 are expressed in several extra-pancreatic tissues, including the heart, which has encouraged studies concerning its role in cardiac physiology, as well putative cardiac and cardiovascular benefits of incretin-based therapies.

Several body of experimental and clinical data have suggested a considerable cardioprotective role of GLP-1 agonists in the myocardium. Myocardial ischemia-reperfusion injury was attenuated by GLP-1 in vitro rat hearts, showing cardioprotective and inotropic effects [85]. Furthermore, it has been shown that mice with genetic deletion of GLP-1 receptor display reduced heart rate, elevated left ventricular end-diastolic pressure and impaired left ventricular contractility and diastolic function after insulin administration. Furthermore, infusion of GLP-1 resulted in improved left ventricular function, hemodynamic status and efficiency, indicating a direct role of GLP-1 on the cardiac physiology [86]. Apart the clinical pharmacological effects on body weight reduction, amelioration of blood pressure and improvement of glycemic control and lipid profile, GLP-1R agonists have been experimentally shown to exert antioxidant, vasoprotective and anti-inflammatory properties. One of these studies showed that liraglutide exerts anti-inflammatory effect on vascular endothelial cells through increased NO production and suppressed NF-κB activation, which is at least partly mediated via AMPK activation [87]. In another study, liraglutide was able to ameliorate cardiac hypertrophy in mice [88]. However, further research is advisory to better understand the complete benefits of GLP-1R agonists in the treatment of DCM.

DPP-4 inhibitors are able to increase the endogenous contents of incretins, such as GLP-1. Besides their effect on glycemic control, beneficial actions on other tissues, including on the heart tissue, have been shown, which might be due to anti-inflammatory, antioxidant and anti-apoptotic properties [89–94]. DPP-IV inhibitors have the advantage of being available for oral administration and do not raise supra-physiological concentration of GLP-1. However, further research is needed to elucidate the effective relevance of DPP-IV inhibitors in DCM.

6.3. Other non-antidiabetic agents

6.3.1. Statins

Statins are primarily inhibitors of cholesterol biosynthesis and the control of hyperlipidemia will benefit T2DM patients. However, although the key benefits of statins were initially attributed to their lipid lowering effects, it is now known that they directly act through other cellular mechanisms, known as pleiotropic effects [95]. Statins increase the expression and

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activation of eNOS thus causing an increase in the bioavailability of nitric oxide (NO), which contribute to the reduction in blood thrombogenicity, of oxidative stress and of cell proliferation. In addition, statins may also exert anti-inflammatory effects by several pathways, including reduced activity of VCAM-1 and ICAM-1, decreased function and levels of MCP-1 and decreased CRP [96]. Atorvastatin have been associated with improved left ventricular function, reduced fibrosis and hypertrophy; the protective effects on cardiac remodeling have been attributed to its anti-inflammatory actions [97]. However, further studies should be conducted to better evaluate the possible beneficial effects in DCM.

6.3.2. RAAS inhibitors

Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are the most used drugs to block the RAAS, and there are numerous evidences suggesting that these antihypertensive agents reduce cardiovascular mortality in diabetic patients due to improvement of cardiac dysfunction [98]. ACEI and ARBs have been associated with several beneficial properties at cardiac level, including improved cardiac fibrosis, reduced collagen synthesis and deposition, amelioration of cardiomyocyte apoptosis and cardiac hypertrophy [98–100]. Experimental and clinical studies also suggest a beneficial effect on T2DM by ameliorating insulin sensitivity, enhancing glucose uptake, improving pancreatic and skeletal muscle blood flow and stimulating proliferation and differentiation of adipocytes, beyond the reduction in blood pressure [99]. Additionally, inhibition of Ang II production and/or action with ACEI and ARBs attenuate its pro-inflammatory actions. In fact, although further clinical evidences are still needed, several studies have showed beneficial effect on markers of inflammation (such as TNF-α, IL-6 and IFN-γ) in heart failure patients [100].

6.3.3. Modulators of mitochondrial function

Numerous studies have highlighted the pivotal importance of impaired mitochondrial metabolism and increased formation of ROS in the pathogenesis of cardiac dysfunction, as discussed previously. Therefore, strategies aiming at modulation of different aspects of mitochondria such as biogenesis, fusion and fission, mitophagy, MPT and ROS generation may lead to effective treatments for cardiomyopathies [101]. Modulation of sirtuins activity, exercise-induced PGC-1 activation, fission and fusion as well as autophagy/mitophagy [102–105] are examples. Interestingly, although increased ROS generation and consequent oxidative damage is associated with pathological processes, mild levels of mitochondrial-derived ROS have been proposed to induce a hormetic response. The concept of mitohormesis proposes that a mild increase in mitochondrial ROS may act as a sublethal trigger of cytoprotective long-lasting metabolic and biochemical changes against larger subsequent stresses [106]. This approach was addressed in mice as a pathway to stimulate mitochondrial energy metabolism and to induce antioxidant defenses, thus preventing cardiomyopathy induced by the cardiotoxic doxorubicin [107], an effect that was also triggered by exercise [108]. Moreover, it has been shown that the development of cardiomyopathy due to impaired mitophagy and consequent accumulation of damaged ROS-forming mitochondria can be surprisingly improved by ROS-dependent activation of compensatory autophagic pathways of mitochondrial quality control, preventing a vicious cycle of ROS formation and mitochondrial dysfunction [109].

7. Concluding remarks

Over the years, DCM has evolved from a nebulous concept to concrete reality and is now viewed as a specific clinical entity caused by the complex relationships between metabolic abnormalities that accompany diabetes, resulting in functional and structural changes in the myocardium that ultimately leads to HF. DCM involves the damage of the myocardium through several mechanisms, namely hypertrophy, fibrosis, apoptosis and necrosis of cardiomyocytes. Some of the main factors involved in diabetes pathogenesis are also pivotal in DCM development, including hyperglycemia-evoked oxidative stress and mitochondrial dysfunction (by impaired autophagy, mitophagy and fusion–fission balance), hyperlipidemia, accompanied by inflammation and a switch of substrate supply to FFAs, as well as insulin resistance. Increasing body of evidence suggests the existence of relevant links between some of these pathways, including between oxidative energy metabolism dysregulation, impaired mitochondrial morphology and energetics evoked by abnormal Ca^{2+} handling, metabolic changes and oxidative stress, as well as chronic low-grade inflammation. The improved knowledge regarding the molecular mechanisms underlying DCM development has contributed to identify novel putative targets and therapeutic opportunities for the management of DCM. Pharmacological options targeting hyperglycemia, insulin resistance and reduced sensitivity, hyperlipidemia, inflammation, oxidative stress and mitochondrial dysfunction have been increasingly investigated, and it is hoped that could significantly improve the ability to prevent and/or improve management of DCM.

Acknowledgements

Authors thank the financial support of Fundação para a Ciência e Tecnologia through (FCT) for the Sara Nunes PhD scholarship (SFRH/BD/109017/2015) and UID/NEU/04539/2013 (CNC.IBILI Consortium), as well as FEDER-COMPETE (FCOMP-01-0124-FEDER-028417 and POCI-01-0145-FEDER-007440).

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