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The Role of Oxidative Stress in the Development of Diabetic Cardiomyopathy

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Abstract

Diabetes mellitus (DM) is a major global health problem, currently affecting about 460 million people while another billion have prediabetes, all costing the governments of the world over $1 trillion USAD to diagnose and treat diabetic patients, so that they can enjoy a better quality of life. DM induces hyperglycemia (HG), which in turn plays a significant role in the development of diabetic cardiomyopathy (DCM), which is responsible for over 80% of diabetic mortality. The exact mechanisms underlying DCM remain incompletely clear, although several pathological mechanisms responsible for DCM have been proposed in the literature. One such mechanism is oxidative stress (OS), which is widely considered as one of the major causes for the pathogenesis of the disease. There is a growing scientific and public interest in connecting oxidative stress with a variety of pathological conditions, including DM as well as other human diseases. HG-induced oxidative stress is a major risk factor for the development of micro-vascular pathogenesis in the diabetic myocardium, resulting in myocardial cell death, hypertrophy, fibrosis, abnormalities of calcium homeostasis and endothelial dysfunction. The aim of this review is to highlight the role of oxidative stress in the development of DCM.

Keywords: AGEs, cardiomyopathy, diabetes mellitus, heart, hyperglycemia, oxidative stress, ROS, MGO

Introduction

Diabetes mellitus (DM) has been recognized as a major cause for the development of diabetic cardiomyopathy (DCM) for decades, resulting in morbidity and mortality diabetic patients [1]. There is a large body of evidence suggesting that the body of a diabetic patient is prone to significant changes at the cellular, subcellular and molecular levels, causing structural and functional abnormalities in the myocardium and vasculature, leading to DCM [2-7]. In the development of DC, hyperglycemia (HG)

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results in tissue damage by both acute reversible changes in cellular metabolism and irreversible changes in macromolecules [8]. HG can cause long-term damage to multiple organs, resulting in sever complication [9, 10]. The micro-vasculature is a key target of HG–induced damages to small blood vessels initiating systematic complications and end organ failures [11, 12]. One major contributor to HG-induced diabetic abnormalities is increased oxidative stress (OS) [13]. The possible biochemical mechanism is increased OS [14]. Moreover, OS has been linked to the onset of DM and its complication [15, 16]. Endothelial cells dysfunction (ECD), mitochondrial dysfunction (MD), inflammation and abnormal vascular remodeling are associated with myocardial apoptosis, fibrosis and hypertrophy seen in DC [17]. ECD is a result of the activation of endothelial cells from a quiescent phenotype to vasoconstrictive, pro-inflammatory and pro-apoptotic states [18]. During DM, endothelial cells are exposed to high fluctuating blood glucose concentrations; this exposure is a known factor for ECD [19]. Furthermore, HG can stimulate the production of reactive oxygen species (ROS) as well as the production of toxic by-products of glycolysis, primarily methylglyoxal (MGO), leading to the formation of advanced glycation end-products (AGEs) [20]. In DM, the production of MGO is accelerated while its detoxification is slowed, leading to MG accumulation [21]. Elevated MGO concentrations have been shown to promote inflammatory responses that activate endothelial cells, subsequently leading to ECD and vascular damage [22]. New insights into the mechanisms that increase oxidative stress in DM might lead to novel treatment strategies [23]. This review attempts to address the role of oxidative stress in the development of DCM.

Oxidative Stress

Oxidative stress (OS) is characterized by the imbalance between free radicals and antioxidants. This imbalance could be as a result of increased free radical production and/or decreased antioxidants capacity [24, 25]. The imbalance between free radicals and antioxidant systems gives rise to free radical-mediated damage, mainly reactive oxygen species (ROS) [26, 27]. ROS can cause damage to the mitochondria together with poly (ADP-ribose) polymerase-1 (PARP) activation, leading to the inhibition of the cytosolic enzyme glyceraldehyde-3phosphate dehydrogenase (GAPDH). This inhibition initiates a series of cellular processes by activation pathways that lead to HG-associated cellular damage [28]. Inhibition of GAPDH diverts glucose from glycolytic pathways into alternative biochemical pathways, including the polyol pathway and AGE formation [29]. Under normal physiological conditions, cellular glucose is predominantly phosphorylated into glucose 6-phosphate by hexokinase, thereby entering the glycolytic pathway. Only trace amounts of non-phosphorylated glucose (about 3%) enter the polyol pathway [30]. However, during HG, there is increased flux through the polyol pathway, accounting for greater than 30% of glucose metabolism [31]. The polyol pathway converts hexose sugars such as glucose into sugar alcohols (polyols). For example, glucose can be converted into sorbitol via the action of the enzyme aldose reductase. Aldose reductase is the rate-limiting enzyme for this pathway [32]. Increased aldose reductase activity and accumulation of sorbitol have been found in diabetic animal models. As sorbitol does not easily move across cell membranes, this results in an increase in cellular osmolality, ultimately leading to cell damage. Sorbitol may also glycate nitrogen molecule on proteins, such as collagen, producing AGE products [32]. Increased polyol flux is associated with reduced concentrations of intracellular glutathione and an increase in cardiac cell apoptosis [33].

Cardiovascular Complications and AGEs

Advanced glycation end-products (AGEs), also known as glycotoxins, may play an important role in the pathobiology of DCM and heart failure [9]. AGEs are either proteins or lipids that become glycated after exposure to sugars. They are prevalent in the diabetic vasculature and contribute to the development of atherosclerosis. They have been shown to be increased in plasma by HG [9]. Currently, it is believed that AGEs are linked to many diseases [34]. In fact, AGE in the myocardium is increased in both T1DM and T2DM, and positive correlations of serum

concentration of AGEs with ventricular isovolumetric relaxation time, arterial stiffness, and carotid intimal thickness have been shown in DM [9]. Consequently, it is important to understand the pathophysiology processes of AGEs.

Formation of AGEs

AGEs are produced in our body and can also be consumed through foods. They are formed through a process called glycation. In 1912, the French Scientist, Louis-Camille Maillard investigated the non-enzymatic reaction between the free amino groups of proteins and carbonyl groups of reducing sugars or other carbonyl compounds [35]. This Maillard reaction can be divided into two parts. The first part/half proceeds until Amadori rearrangement; the second half involves AGE formation through reactions such as oxidation, dehydration, and condensation.

During the early stage, either glucose or other reducing sugars such as fructose react with a free amino group of biological amines to form an unstable compound (Figure 1). This glycation reaction results in the formation of the Schiff base, which undergoes a rearrangement to a more stable product known as Amadori product [36]. Then, the Amadori product degrades to a variety of reactive dicarbonyl compounds such as Methylglyoxal (MGO) via dehydration, oxidation and other chemical reactions. In the late stage of glycation, irreversible compounds called AGEs are formed through oxidation, dehydration and cyclization reactions. The AGEs are yellow-brown, fluorescent and insoluble adducts that accumulate on long-lived proteins, thus inducing their physiological dysfunction [37].

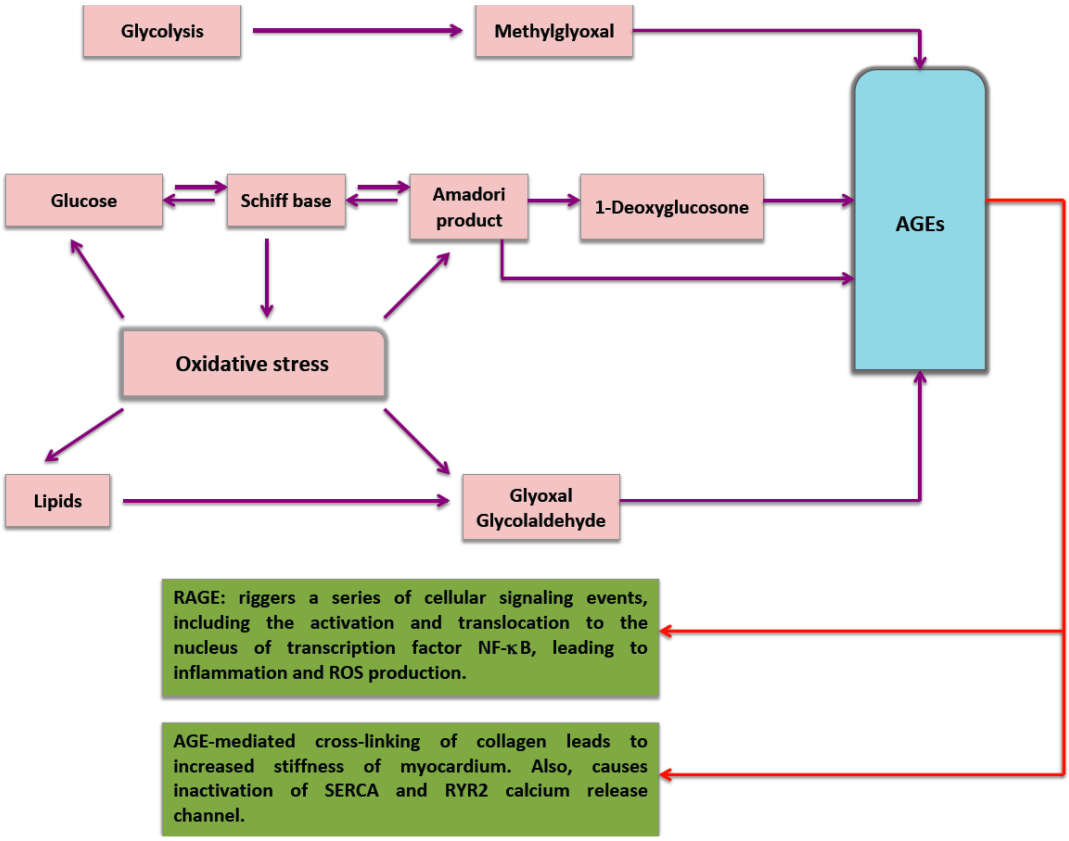

Figure 1. Flow diagram showing steps of advanced glycation end-products (AGEs) generation, leading to structural and functional changes at the cellular and subcellular level in the myocardium.

Receptors for Advanced Glycation End Products

Formation of AGEs leads to the activation of different signaling pathways mediated by a series of cell surface receptors. The most studied AGE-receptor is the multi-ligand receptor for advanced glycation end products (RAGE) [38]. RAGE is a transmembrane protein on the cellular surface that recognizes tridimensional molecules, instead of amino acid sequences, making this molecule capable of interacting with diverse ligands. RAGE represents an important factor in innate immunity against pathogens, but it also interacts with endogenous ligands, resulting in chronic inflammation. RAGE signaling has been implicated in multiple human illnesses, including diabetes, atherosclerosis, arthritis, Alzheimer's disease, and aging- associated diseases. Ligation of RAGE on the cellular surface triggers a series of cellular signaling events, including the activation and translocation to the nucleus of transcription factor NF-κB, leading to inflammation and oxidative stress [39].

Accumulation of the AGEs

Since the discovery of the Millard reaction, significant efforts have recently been made in proteomics research, which is a comprehensive study of expressed proteins in the human body. Many studies subsequently led to the identification of diseases, which cannot be cured, simply by studying gene or protein expression. The Maillard reaction has also recently attracted new attention for its role as a post-translational modification, promoted by an abnormality in the metabolism of sugars and lipids. AGEs can change the physicochemical properties of proteins, such as net negative charge degeneration and polymerization [40]. Normally, there is a balance between oxidants and antioxidant defense. During DM, the equilibrium of pro-oxidants and antioxidants shifts to the former, leading to a marked rise in reactive oxygen species (ROS). Elevated concentrations of oxidants promote the oxidation of lipids and glucose, resulting in the accelerated formation of AGEs. Alterations of extracellular proteins, such as collagen and elastin, as well as the activation of different signaling pathways after intracellular uptake, are the consequences of increasing AGE concentrations. Consequently, increasing concentrations of AGES support the formation of reactive oxygen and nitrogen species, which in turn induce further formation of AGEs [38].

Pathological Impact of AGEs

Formation of AGE can contribute to tissue damage. Its accumulation in collagen was associated with reduced collagen turnover, indicating the possibility that cross-linking of collagen makes collagen resistant to hydrolytic turnover. Such AGE-mediated cross-linking of collagen is thought to be responsible for increased stiffness of arteries and the myocardium [9]. Also, AGEs can cause inactivation of the sarcoplasmic-endoplasmic ATPASE pump (SERCA) and the Ryanodine receptor (RYR2) calcium release channel. Interestingly, DM has been closely associated with accumulation of AGEs in the myocardium and also with a positive correlation of serum concentration of AGEs with ventricular isovolumetric relaxation time, arterial stiffness, and carotid intimal thickness [9].

Production of ROS

HG can stimulate the overproduction of ROS and reactive carbonyl species (RCS). ROS and RCS are significant contributors to structural and functional abnormalities in the diabetic heart [41]. Diabetic mitochondria produce more ROS than normal mitochondria [42]. Mitochondria are the major source of ROS production. Cellular sources of ROS generation within the heart include cardiac myocytes, endothelial cells and neutrophils. Furthermore, it has been reported that the activation of the renin-angiotensin system (RAS) in DM is associated with increased oxidative damage, fibrosis and cell apoptosis [43]. Inhibition of the RAS was shown to reduce ROS production in streptozotocin-induced diabetic rats, similar to the effect observed with antioxidant treatment [44]. Angiotensin (Ang-II) given exogenously to rodents has been shown to cause cellular changes within the myocardium,

leading to hypertrophy and fibrosis [45]. ROS induces cellular damage through many mechanisms, including oxidation, interference with nitric oxide and modulation of detrimental intracellular signaling pathways. Thus, increased ROS concentration leads to cardiac dysfunction by direct damage to proteins and DNA and apoptosis [46]. RCS are diverse in chemical structures and are derived from multiple sources, including auto-oxidation of glucose and lipids, triose pathway fluxes and enzymes such as methylglyoxal synthase. RCS also have unique characteristics compared to ROS in that their half-lives are longer (minutes vs. millisecond), and they are uncharged molecules, allowing them to migrate distances far from their site of production [47].

MGO

MGO is a di-carbonyl aldehyde mainly formed as byproduct of glycolysis [48]. By its nature, MGO efficiently reacts with other molecules in the organism, causing cell and tissue dysfunction [49]. Recent studies have demonstrated that increased methylglyoxal-derived AGE concentrations in diabetic patients are associated with diabetic complications, such as DCM [50]. MGO concentrations in healthy humans have been estimated to be about 50–150 nM in plasma and 1–4 μM in tissues [22]. When the concentration of MGO exceeds these values, di-carbonyl stress occurs as a consequence of the imbalance between the generation/exposure and MGO metabolism [51]. Under physiological conditions, >99% of MGO are detoxified by the glyoxalase system [52], with minor metabolism by aldoketo-reductases (AKRs) and aldehyde dehydrogenases (ADHs), which convert MGO to hydroxyacetone and pyruvate, respectively [53].

Glyoxalase System

The glyoxalase system consists of glyoxalase 1 (Glo1), glyoxalase 2 (Glo2) and a catalytic amount of glutathione [54]. Abnormal cellular accumulation of the MGO occurs upon exposure to high glucose concentrations, oxidative stress, inflammation, and cell aging. It is associated with increased MGO-adduct content of proteins susceptible to di-carbonyl modification, collectively defined as di-carbonyl proteome [51], and with DNA instability and mutations [55]. An adequate balance between MGO concentrations and Glo1 activity is necessary to ensure detoxification of MGO from different sources and cell survival. In TIDM, MGO production increases; the increase in blood MGO may be responsible in part for the dysregulation of ECs in the vasculature of end organs. However, to the best of our knowledge, the concentration of MGO in blood of patients and animals with T1DM is typically ≤2 μM [56], which is significantly lower than the concentrations (well over 10 μM) needed to potentiate mitochondrial ROS production, and which perturbs intracellular Ca^{2+} homeostasis and impairs endothelial function *in vitro* [57]. Moreover, erythrocytes contain high concentrations of the rate-determining methylglyoxal-degrading enzyme glyoxalase-I (Glo-I), and its cofactor reduced GSH [58] that rapidly degrades this diffusible electrophile and prevents its accumulation. This discrepancy led to search for non-blood sources of methylglyoxal that could be responsible for dysregulation of ECs in T1MD. Vascular adhesion protein-1 (VAP-1) is best known as an inflammation-induced adhesin that is up-regulated in high endothelial venules to aid in the extra- vasation of leukocytes from the blood into tissues [59]. VAP-1 is also expressed on the membrane of smooth muscle cells (SMCs) [60]. In DM, endothelial cells (ECs) are directly exposed to HG, which is a known contributing factor to the loss of endothelial function (Figure 2). The ECs play an important role in cardiomyocyte viability and function and in myocardial homeostasis ECs death can lead to repeated episodes of ischemia and myocardial infarction, the death of cardiomyocytes, and the development of ventricular dysfunction, leading to heart failure [61]. Although there is now considerable scientific evidence that MGO engenders the development of vascular complications, further experimental research is needed to pinpoint the underlying causal mechanisms.

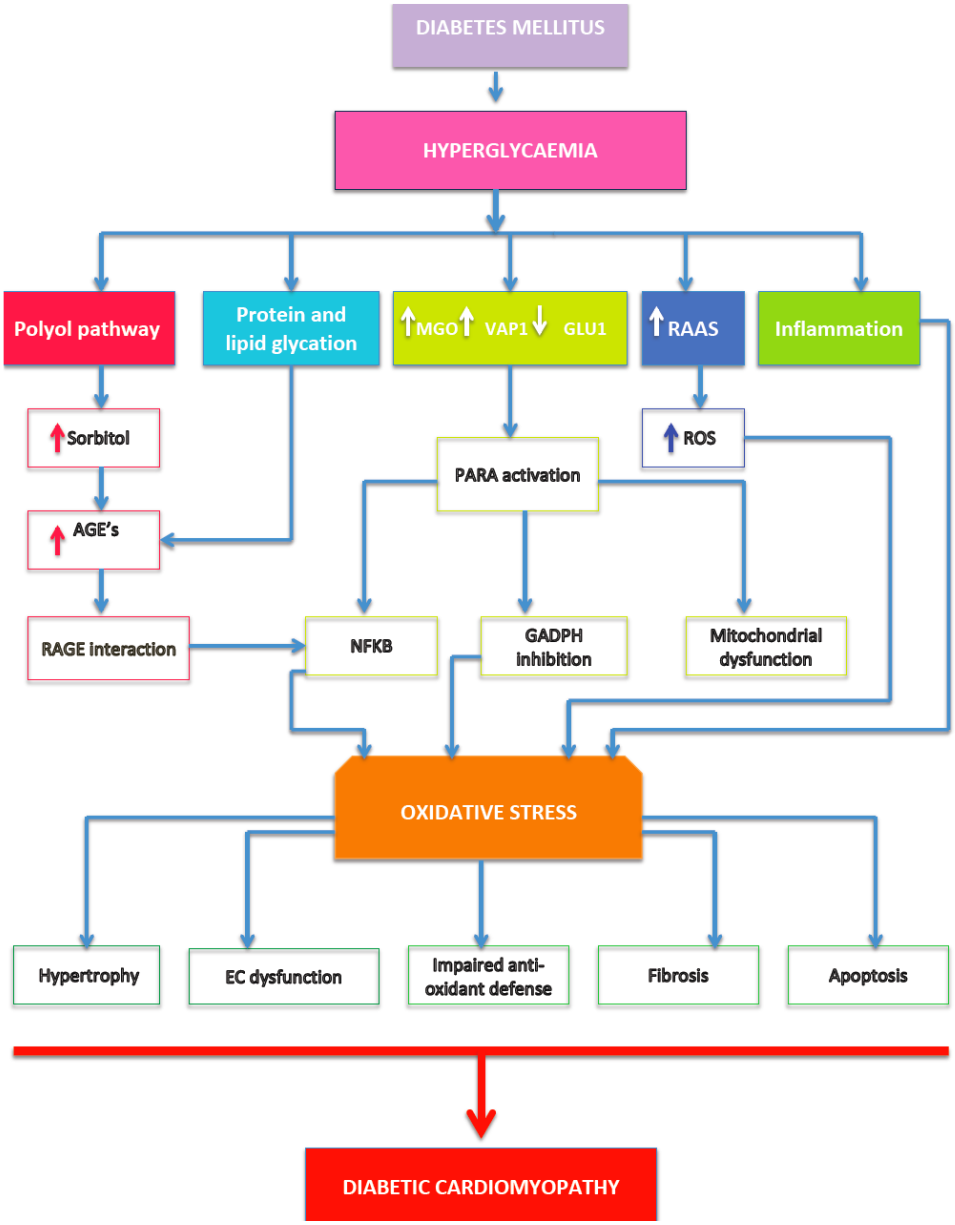

Figure 2. Flow diagram showing changes occurring during diabetes and how increased oxidative stress can lead to structural and functional changes at the cellular and subcellular level in the myocardium.

Conclusion

The flow diagram in Figure 2 is a summary of the relationship between diabetes-induced hyperglycemia and the development of diabetic cardiomyopathy, employing different signaling pathways to generate oxidative stress and subsequently cardiac dysfunction. There is considerable evidence that induction of oxidative stress is a key process in the onset of diabetic complications. The precise mechanisms by which oxidative stress may accelerate the development of complications in diabetes are only partly known. MGO accumulation has harmful effects on vascular function, by inducing insulin-resistance, hypertension, atherosclerosis, neurodegenerative disease and diabetic microvascular complications. The complete mechanism responsible for development of DCM is not fully clear, but some previous studies have identified pathways that may involve the down-expression of GLO-1 and over-

expression of VAP-1. Therefore, protecting the vasculature from MGO may be a target for future studies and therapy to postpone and reduce the development of heart failure in DM.

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