Vascular endothelial dysfunction in diabetic cardiomyopathy: Pathogenesis and potential treatment targets

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Abstract

Cardiovascular complications account for significant morbidity and mortality in the diabetic population. Diabetic cardiomyopathy, a prominent cardiovascular complication, has been recognized as a microvascular disease that may lead to heart failure. Pathogenesis of diabetic cardiomyopathy involves vascular endothelial cell dysfunction, as well as myocyte necrosis. Clinical trials have identified hyperglycemia as the key determinant in the development of chronic diabetic complications. Sustained hyperglycemia induces several biochemical changes including increased non-enzymatic glycation, sorbitol–myoinositol-mediated changes, redox potential alterations, and protein kinase C (PKC) activation, all of which have been implicated in diabetic cardiomyopathy. Other contributing metabolic abnormalities may include defective glucose transport, increased myocyte fatty acid uptake, and dysmetabolism. These biochemical changes manifest as hemodynamic alterations and structural changes that include capillary basement membrane (BM) thickening, interstitial fibrosis, and myocyte hypertrophy and necrosis. Diabetes-mediated biochemical anomalies show cross-interaction and complex interplay culminating in the activation of several intracellular signaling molecules. Studies in both animal and human diabetes have shown alteration of several factors including vasoactive molecules that may be instrumental in mediating structural and functional deficits at both the early and the late stages of the disease. In this review, we will highlight some of the important vascular changes leading to diabetic cardiomyopathy and discuss the emerging potential therapeutic interventions.
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1. Introduction

The epidemic proportion of people with diabetes is alarming, and it has been estimated that by the year 2025, 300 million people will become affected by the disease (Sicree et al., 2003). Among the vast array of secondary problems associated with diabetes, cardiovascular complications significantly contribute to increasing rates of morbidity and mortality (Garcia et al., 1974; Sicree et al., 2003). Nearly 80% of the deaths associated with diabetes are due to cardiac complications (Consensus statement/American Diabetes Association, 1993; Hayat et al., 2004). Although previous studies have focused on coronary artery disease (CAD) and autonomic neuropathy as the primary cardiac complication, over the last 30 years, diabetic cardiomyopathy has been identified as a significant entity (Bell, 1995).

In 1972, Rubler et al. identified 4 diabetic patients who had heart failure without evidence of redundant cardiac abnormality such as CAD, hypertension, or other cardiovascular complication. Epidemiological, clinical, and experimental studies have subsequently identified diabetic cardiomyopathy as a distinct entity characterized by diastolic impairment (Hamby et al., 1974; Bell, 1995; Cosson & Kevorkian, 2003). Even more intriguing is the identification of diabetic cardiomyopathy as a microvascular complication (Factor et al., 1980a, 1980b; Fein & Sonnenblick, 1994; Bell, 1995). Structural and functional changes in cardiac vasculature include capillary basement membrane (BM) thickening, microaneurysms, and reduction in capillary density (Factor et al., 1980a, 1980b; Nunoda et al., 1985; Sutherland et al., 1989; Yarom et al., 1992). Cardiac microvessel endothelial cell lesions have been shown in experimental diabetes (Popov et al., 1996; Okada et al., 1998; Okruhlicova et al., 2005). Interestingly, most cardiovascular risk factors that cause alteration of other vascular endothelial cells also affect cardiac endothelium including the myocardial capillary endothelium (Henderson, 2001; Brutsaert, 2003). These studies suggest an important role of cardiac microvascular endothelial dysfunction in the initiation and perpetuation of the disease: a notion paralleled by other secondary complications such as retinopathy and nephropathy (La Selva et al., 1993; Khan & Chakrabarti, 2003a, 2003b).

The majority of diabetic cardiovascular clinical trials have been aimed at preventing CAD and autonomic neuropathy. However, experimental animal models of chronic diabetic complications have shown beneficial effects of targeting specific macromolecules in preventing the molecular changes generally associated with cardiomyopathy. Such modifications have been directly linked to high glucose-induced changes occurring in the microvascular endothelial cells (MVECs), as well as cardiomyocytes. Interestingly, hyperglycemia has been identified as a key determinant in the development and progression of diabetic complications, including endothelial cell dysfunction (Diabetes Control and Complications Trial, 1993; Feener & King, 2001; Kakizawa et al., 2004). Large scale clinical trials such as the Diabetes Control and Complications Trial and the United Kingdom Perspectives Diabetes Study have provided substantial evidence supporting the role of hyperglycemia-mediated changes in diabetic complications (Diabetes Control and Complications Trial, 1993; United Kingdom prospective diabetic study, 1996).

Diabetic biochemical modulation occurs in the endothelial cells, as well as myocytes, both of which may contribute to cell death. Long-term exposure to hyperglycemia induces many biochemical modifications, such as non-enzymatic glycation, sorbitol–myoinositol-mediated changes, redox potential alterations, protein kinase C (PKC) activation, and free fatty acid (FFA) metabolism in myocytes (Brownlee, 2001; Sheetz & King, 2002; Carvajal & Moreno-Sanchez, 2003; Khan & Chakrabarti, 2003a). It is plausible that most if not all of the biochemical abnormalities are implemented in diabetic cardiomyopathy. In addition to hyperglycemia, type 2 diabetic patients encounter the complications associated with hyperinsulinemia and dyslipidemia. These may include elaboration of vasoactive factors, defective glucose transport, increased myocyte fatty acid uptake, and altered calcium uptake (Bell, 1995; Hopfner et al., 1998; Carvajal & Moreno-Sanchez, 2003; Cosson & Kevorkian, 2003). The concert of these pathogenetic changes results in a particular sequence of events that include hemodynamic alterations and structural changes such as BM thickening, extracellular matrix (ECM) protein deposition, fibrosis, myocyte hypertrophy, and necrosis (Savage et al., 1988; Bell, 1995; Shehadeh & Regan, 1995; Lewinter, 1996; Khan & Chakrabarti, 2003a). This review will highlight the role of vascular endothelium in mediating hyperglycemia-induced structural and functional modifications, as well as the biochemical alterations, and the possible therapeutic interventions aimed at treating diabetic cardiomyopathy.

2. Functional and structural changes

2.1. Functional changes: hemodynamic alterations

Vascular endothelial cells, which utilize non-insulin-mediated mechanisms for glucose transport, are the primary targets of glucose-induced damage (Fig. 1). The early changes associated with endothelial dysfunction include hemodynamic modifications such as increased permeability and decreased blood flow (Brownlee, 2001; Sheetz & King, 2002; Khan & Chakrabarti, 2003a). These changes are associated with altered vasoactive factors, such as increased endothelin-1 (ET-1) expression and decreased nitric oxide (NO) bioavailability, leading to increased vasoconstriction and impaired vasodilatation (Khan & Chakrabarti, 2003a) (Fig. 1). We have previously shown that micro- and macrovascular endothelial cells exposed to high glucose levels increase the production of ET-1 (Chen et al., 2003b; Khan et al., 2004). Such elaboration of ET-1 is characterized as a key functional alteration in endothelial dysfunction (Sheetz & King, 2002; Khan & Chakrabarti, 2003a; Khan et al., 2004) (Fig. 2). We have further shown up-regulation of ET-1 in the target organs of diabetic complications: heart, retina, and kidney (Chen et al., 2000, 2003a; Khan et al., 2004). In normal adult heart tissue, ET has been shown to be predominantly expressed in cardiac endothelial cells (Mebazaa et al., 1993) as compared to the cardiomyocytes (Nishimura et al., 1994), further suggesting an important role of cardiac endothelial cells in diabetic cardiomyopathy. Research has also identified ET-1 up-regulation in the serum of diabetic patients (Collier et al., 1992; Haak et al., 1992; Donatelli et al., 1994; Ak et al., 2001) and vitreous of patients with proliferative diabetic retinopathy (Khan et al., 2004).

Hemodynamic studies have indicated that endothelium-dependent vasodilatory response in diabetes is impaired (McVeigh et al., 1992; Lambert et al., 1996; Dogra et al., 2001; Van de Ree et al., 2001; Johnstone et al., 1993). The exact mechanism of these changes has not been elucidated, as researchers have shown contradictory results regarding altered NO production and the expression of nitric oxide synthase (NOS). We have shown that following 1 month of diabetes endothelial NOS (eNOS) and inducible NOS (iNOS) mRNA levels are up-regulated without changes to nitrate and nitrite
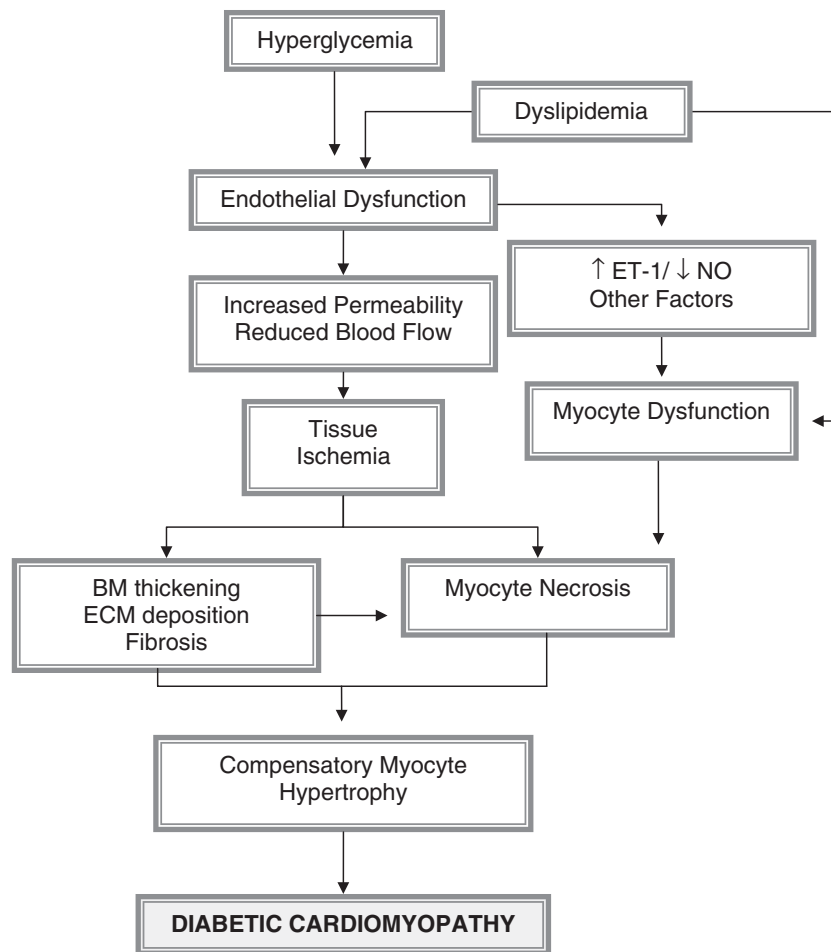
Fig. 1. Schematic illustrating the putative mechanisms leading to diabetic cardiomyopathy. Sustained hyperglycemia causes vascular endothelial cell dysfunction, resulting in increased permeability, reduced blood flow, and subsequently tissue ischemia. In response to tissue ischemia, endothelial cells release growth factors that increase basement membrane (BM) thickening and extracellular matrix (ECM) deposition. Growth factor and cytokine elaboration may also contribute to myocyte dysfunction and loss. In long-standing diabetes, this response is sustained and exacerbated, ultimately leading to diabetic cardiomyopathy and heart failure (ET-1, endothelin-1; NO, nitric oxide).
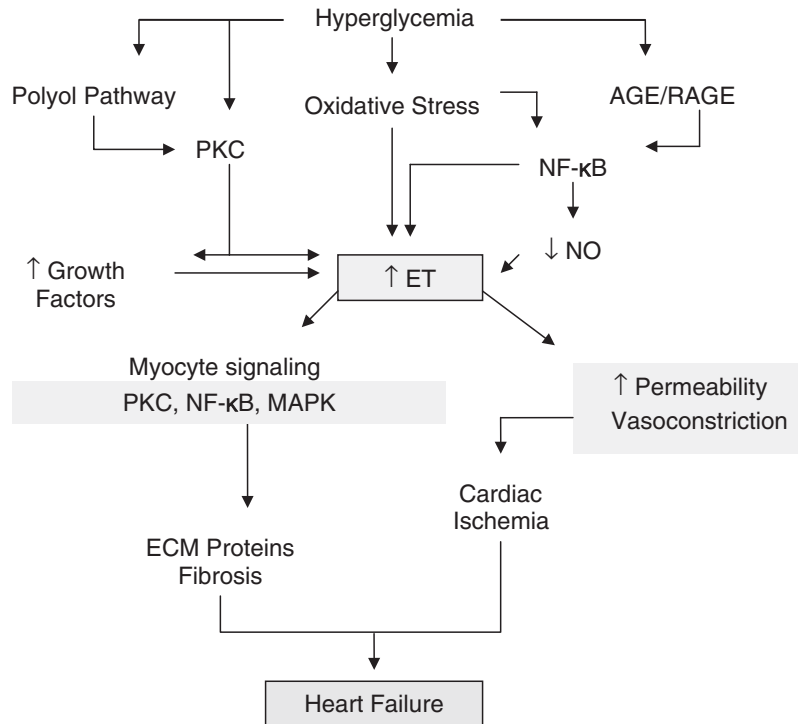
Fig. 2. Proposed mechanisms of endothelin (ET) alteration and functional consequences in cardiac endothelial cells and myocytes. High glucose-induced oxidative stress and other biochemical anomalies such as protein kinase C (PKC) activation, aldose reductase (AR) induction, and interaction of advanced glycation end-product (AGE) with their receptor (RAGE) can increase ET expression by endothelial cells. Altered ET levels may then lead to both hemodynamic (vasoconstriction/blood flow and permeability) and structural (extracellular matrix [ECM] deposition, myocyte hypertrophy) changes in the heart (NO, nitric oxide; NF-κB, nuclear factor-κB).

levels in the diabetic rat heart (Farhangkhoee et al., 2003). Increased iNOS immunoreactivity has also been demonstrated in other microvascular targets of chronic diabetes such as the retina (Abu El-Asrar et al., 2004). In addition, we have shown that cultured endothelial cells in high glucose up-regulate both eNOS and iNOS (Chen et al., 2004). These results suggest that NOS expression may be augmented in hyperglycemic conditions. Whether NO production is also increased remains to be determined with the development of more accurate techniques. In addition, the amount of NO available may be subjected to alteration by rapid sequestration. Other studies have confirmed reduced NO availability in the progression of diabetic cardiomyopathy (Joffe et al., 1999). Reports also indicate an inverse relationship between nitrite/nitrate levels and peroxynitrite levels in diabetic patients (Hoeldtke et al., 2003). Furthermore, L-arginine administration can prevent high glucose-induced Q-T interval changes in isolated rat hearts (D'Amico et al., 2001). Together, these findings suggest that NO availability and NO conversion to peroxynitrite may be physiologically significant in diabetes (Fig. 3).

The activity of all NOS enzymes, particularly eNOS, is subjected to post-transcriptional regulation by other proteins and cofactors, which may also alter the production of NO (Fleming & Busse, 1999, 2003). NOS has the capability to produce superoxide anions (O_2^-) in the absence of tetrahydrobiopterin (BH_4) or L-arginine and thus increase oxidative stress (Pou et al., 1992; Cosentino & Katusic, 1995; Pou et al., 1999). The potential of all NOS isoforms to generate O_2^- in the pathogenesis of diabetic complications is still under investiga-

tion; however, research has implicated this mechanism in the diabetic patients with CAD and in the kidneys of diabetic rats (Guzik et al., 2002; Satoh et al., 2005). Targeting vasoactive factors as a therapeutic modality has shown beneficial effects in the prevention of diabetic microvascular disease, including
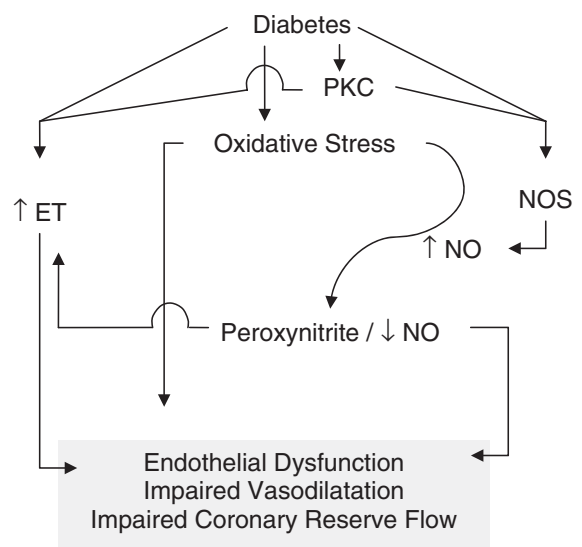
Fig. 3. Interaction between endothelin-1 (ET-1) and nitric oxide (NO) in endothelial-dependent impaired vasodilatation. Hyperglycemia-induced expression of ET and activation of protein kinase C (PKC) may regulate NO synthase (NOS) at both the transcriptional and the post-translational level. NOS-derived NO is sequestered by oxidative stress reducing NO availability and impairing vasodilatation and coronary flow reserve in diabetic cardiomyopathy.

cardiomyopathy. We have also demonstrated reduced diabetes-induced vasoconstriction in the retina following treatment with NO donor, molsidomine (Cukiernik et al., 2003). Whether targeting vasoactive factors provides an effective treatment strategy remains to be elucidated in humans with diabetes.

2.2. Structural changes: extracellular matrix protein synthesis and fibrosis

Sustained damage to endothelial cells by hyperglycemia ultimately leads to cell loss, reduced blood flow, hypoxia, and tissue ischemia (Di Mario & Pugliese, 2001; Khan & Chakrabarti, 2003b). The compensatory response to these changes may the be activation of endothelial cells and accompanying elaboration of growth factors such as vascular endothelial growth factor (VEGF) and transforming growth factor-β (Khan & Chakrabarti, 2003a, 2003b). The former influences the angiogenic response and endothelial proliferation, while the latter plays an important role in regulating the composition of the ECM. We have identified the key role of high glucose-induced transforming growth factor-β and ET-1 in the production of oncofetal fibronectin, a possible angiogenic ECM protein (Khan et al., 2004, 2005). In the rat heart, we have also shown that after 6 months of streptozotocin-induced diabetes, cardiac tissues exhibited increased fibronectin and collagen α1 (IV) expression (Chen et al., 2000). Interestingly, the changes were prevented by an ET-1 receptor antagonist bosentan (Chen et al., 2000; Khan et al., 2004, 2005). Diabetes-induced and ET-mediated increased collagen deposition may contribute to both capillary BM thickening and myocardial fibrosis. Examination of left ventricular biopsy specimens has shown increased interstitial fibrosis (Anguera et al., 1998). Furthermore, increased thickening of the BMs of heart microvessels has been demonstrated in animal models of diabetes (Saito et al., 2003). These studies suggest that diabetes-induced vasoactive/growth factors may contribute to ECM deposition and thus lead to the development of diabetic cardiomyopathy.

In response to tissue ischemia, which is precipitated by fibrosis and reduced compliance, the target organs of diabetic complications increase VEGF mRNA and protein levels (Khan & Chakrabarti, 2003b). This compensatory increase in VEGF, occurring predominantly in highly vascularized tissues such as the retina, has not been clearly established in diabetic cardiomyopathy. The expression of VEGF and its receptors has been shown to be down-regulated in the heart tissues of diabetic animals and human subjects with diabetes (Chou et al., 2002). These changes in the heart suggest that the angiogenic response is compromised, which in turn further exacerbates the hypoxic conditions and leads to severe damage to the cardiac tissues. The possibility of promoting angiogenesis in the heart may be an important therapeutic intervention, as increase in blood flow and reduced vasoconstriction may prevent tissue ischemia and myocyte necrosis. To improve the angiogenic response during cardiovascular complications, several clinical trials have implicated VEGF gene therapy as a promising therapeutic avenue (Losordo et al., 1998; Rosengart et al.,

1999; Losordo et al., 2002; Fortuin et al., 2003; Hedman et al., 2003; Kolsut et al., 2003). These studies have indicated that the administration of gene encoding VEGF can ameliorate cardiovascular complications such as myocardial ischemia and infarction and CAD. For instance, VEGF gene therapy has been shown to improve myocardial perfusion in some patients with myocardial infarction (Losordo et al., 1998). However, it should be noted that the potent vascular permeability activity of VEGF has shown undesirable outcomes. A recent report indicated lower extremity edema in patients with VEGF165 administration (Baumgartner et al., 2000). Although clinical trials targeting diabetic cardiomyopathy have not yet been conducted, animal studies have shown the benefit of VEGF gene therapy in diabetic cardiomyopathy (Yoon et al., 2005). In addition, other clinical trials aimed at diabetic neuropathy are underway and offer important therapeutic avenues (Isner et al., 2001).

The hypoxia-induced modifications to the microenvironment of the heart lead to interstitial and/or perivascular fibrosis and myocyte necrosis, which is evident in animal models of diabetes, as well as human patients who have developed secondary complications (Factor et al., 1980a, 1980b, 1981; Kawaguchi et al., 1997). The detrimental effect of cardiac fibrosis and myocyte death primarily manifest as left ventricular hypertrophy and diastolic impairment. In the majority of cases, diastolic dysfunction is subclinical, and the presentation of heart complications is not evident, as assessed by Doppler echocardiography measurements, until the disease progresses to an advanced stage (Ommen & Nishimura, 2003; Hayat et al., 2004; Zile et al., 2004). At a more pronounced stage, diastolic dysfunction includes impaired relaxation and increased susceptibility to myocardial ischemia (Kouvaras et al., 1988; Lomuscio et al., 1991; Bell, 1995; Das et al., 2004). The pathogenesis of diastolic dysfunction is directly related to ECM deposition. Our studies have indicated that increased fibronectin and collagen α1 (IV) are associated with focal scaring and myocyte death (Chen et al., 2000). Moreover, advanced glycation end-product (AGE)-induced collagen cross-linking has also been documented to induce diastolic dysfunctions (Norton et al., 1996; Aronson, 2003;). In addition to the deficiencies affecting relaxation properties, systolic dysfunction is also an important feature of diabetic cardiomyopathy (Punzengruber & Schernthaner, 1986; Yasuda et al., 1992; Mbanya et al., 2001). Contractile changes are thought to occur at a later stage in the disease process in comparison to diastolic dysfunction (Cosson & Kevorkian, 2003). These functional alterations in the heart may be directly attributable to vascular cell dysfunction, tissue fibrosis, and myocyte death. Therapeutic interventions therefore should be aimed at correcting blood flow reduction and increased matrix protein deposition. These targets may include vasoactive factor ET and fibrogenic growth factor transforming growth factor-β. Importance of early vasoactive factor alteration in cardiac hypertrophy and failure is evident in hypertensive animal models. ET is elevated in deoxycorticosterone acetate-induced hypertensive rat with cardiac hypertrophy (Lariviere et al., 1995). Right and left ventricular ET-1 levels have also been shown to be similar to

normal counterparts during the hypertrophic phase in hypertensive rat model (Iwanaga et al., 1998). However, an increase in myocardial ET-1 was observed during the transition from left ventricular hypertrophy to congestive heart failure. These studies suggest an important role of cardiac endothelial-derived ET-1 in cardiac tissue remodeling.

3. Hyperglycemic-induced biochemical alterations

3.1. Advanced glycation end-products and non-enzymatic glycation

The production of AGEs, a physiological process, has been implicated in the development of diabetic complications including cardiomyopathy (Candido et al., 2003). Glucose and other reducing sugars have the ability to react with proteins, lipids, and DNA in a process known as the Maillard reaction (John & Lamb, 1993; Raj et al., 2000). This non-enzymatic reaction yields Schiff's base (early glycation product), Amadori products (intermediate glycation products), and AGEs. During the Maillard reaction, the reactive intermediate products, methylglyoxal, 3-deoxyglucosone, and glyoxal are also produced and may participate in the development of carbonyl stress and AGE formation (Skovsted et al., 1998; Baynes & Thorpe, 1999; Miyata et al., 2000). Increased AGE formation has been reported in heart tissues of diabetic animals (Candido et al., 2003).

AGEs induce detrimental effects in both an intra- and extracellular manner. Within the cells, AGEs and their precursors modify macromolecules and nucleic acids. In the extracellular environment, AGE modification of proteins can irreversibly produce cross-links between important ECM proteins such as collagen and elastin (Brownlee et al., 1988; Airaksinen et al., 1993; Candido et al., 2003). These modifications result in impaired function of the organs, as a result of the inability tissue to be compliant and flexible (Dyer et al., 1993; McCance et al., 1993). Myocardial stiffness may result from the sum of increased deposition of collagen and cross-link formation (Norton et al., 1996; Aronson, 2003; Candido et al., 2003). These studies have also suggested that augmented production of cross-linked ECM proteins can lead to diastolic impairment (Aronson, 2003; Norton et al., 1996). AGEs may also produce adverse effects by binding to their receptors (RAGEs), which are found in several cell types, including endothelial cells, smooth muscle cells, and macrophages (Thornalley, 1998). The interaction has been reported to induce the production of cytokines and growth factors in microvascular diabetic complications (Pugliese et al., 1997; Cooper, 2004). Increased expression of RAGE has been shown in the heart tissues of diabetic rats (Candido et al., 2003). Such increased expression of RAGE was associated with connective tissue growth factor up-regulation and collagen deposition. Treatment of diabetic rats with alagebrium chloride (AGE cross-link inhibitor; ALT-711) prevented ventricular RAGE and connective tissue growth factor up-regulation. Overexpression of RAGE in the heart has also been reported to cause changes in

myocardial Ca^+ handling (Petrova et al., 2002). Furthermore, administration of AGEs in RAGE-transgenic mice prolonged the cardiomyocyte intracellular Ca^+ decay to a greater extent than myocytes from control animals, suggesting a role of AGE/RAGE interaction in cardiomyocyte dysfunction.

Potential therapeutic targets preventing the detrimental effects of AGEs include inhibitors of AGE formation and AGE cross-link breakers. Aminoguanidine, an inhibitor of AGE formation, has been shown to prevent diabetes-induced arterial wall protein cross-linking in rats (Brownlee et al., 1986). Furthermore, ALT-711 treatment of streptozotocin-induced diabetic rats causes a significant reduction in brain natriuretic peptide, left ventricular hypertrophy, and type III collagen deposition (Candido et al., 2003). Other studies have shown similar effects of ALT-711 in diabetic dogs (Liu et al., 2003). Clinical trials elucidating the implications of AGE or cross-link inhibitors on diabetic cardiomyopathy remain to be elucidated. Studies aimed at treating older individuals who have arterial stiffness with ALT-711 have shown reduced arterial compliance and thus provide a possible therapeutic intervention (Kass et al., 2001).

3.2. Aldose reductase pathway

Under physiological conditions, the majority of glucose is metabolized through the glycolytic pathway; however, during hyperglycemic conditions, the percentage of glucose oxidation through the aldose reductase (AR) pathway is increased. Such metabolic pathway switch is clearly evident in the ocular lens where the percentage of glucose metabolized by the AR pathway can increase from 3% to 30% (Gonzalez et al., 1984). Augmented glucose metabolism through the AR pathway may lead to several secondary biochemical changes in the heart (Roy et al., 1990; Brownlee, 2001; Galvez et al., 2003). The first enzymatic reaction in the AR pathway, which converts glucose to sorbitol, requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Therefore, increased AR activity yields a decrease in the NADPH/NADP$^+$ ratio. This altered redox potential results in depletion of cofactors required for antioxidant enzymes such as glutathione reductase, increasing oxidant injury (Srivastava et al., 1989). The second reaction is the oxidation of sorbitol to fructose by sorbitol dehydrogenase. Similar to the AR enzyme, sorbitol dehydrogenase requires a cofactor, nicotinamide adenine dinucleotide (NAD$^+$), which leads to the production of reduced nicotinamide adenine dinucleotide (NADH) and ultimately increases the NADH/NAD$^+$ ratio. This altered redox state resembles hypoxia and is often referred to as pseudohypoxia (Williamson et al., 1993).

Besides altered redox potential changes, the AR pathway induces several other interrelated biochemical modifications. One of these changes is the accumulation of sorbitol, which occurs as a result of slower enzyme kinetics of sorbitol dehydrogenase and the membrane impermeable nature of sorbitol (Burg, 1995; Hotta, 1997). These alterations subsequently induce osmotic stress and changes to membrane myoinositol composition, which lead to myoinositol-related

(Na$^+$/K$^+$)-ATPase defect (Greene et al., 1987). We have previously shown that supplementation with myoinositol contribute, in part, to preventing thickening of the BM in diabetic rats (Chakrabarti & Sima, 1992).

Polymorphism in AR gene has been linked to increased susceptibility of microvascular complications in diabetes (Demaine, 2003; Wang et al., 2003; Sivenius et al., 2004), implicating AR inhibition as a therapeutic modality. Several AR inhibitors including sorbinil, statil, tolrestat, and zopolrestat have been evaluated for the prevention of diabetic complications (Kador et al., 1985; Dvornik, 1992; Tsai & Burnakis, 1993; Pfeifer et al., 1997; Srivastava et al., 2005). In spite of the fact that no direct role of AR inhibition in diabetic cardiomyopathy has been established, the notion of AR inhibition in preventing diabetic cardiomyopathy is interesting. Studies have shown that administration of the AR inhibitor zopolrestat to diabetic nephropathy patients can increase their resting left ventricular ejection fraction, cardiac output, and left ventricular stroke volume (Johnson et al., 2004). Other researchers have shown that AR inhibition in patients with diabetic autonomic neuropathy showed improvements in cardiovascular performance such as enhanced resting cardiac output and maximal cardiac output (Roy et al., 1990). In animal models of diabetic complications, AR inhibition in the heart causes reduction in sorbitol accumulation, normalization of NADH/NAD$^+$ ratio, ATP preservation, and reduction in ischemic injury (Ramasamy et al., 1997; Trueblood & Ramasamy, 1998). Taken together, these studies suggest that AR inhibition may provide some beneficial effects in the prevention of diabetic cardiomyopathy; however, further studies are required to establish this therapeutic intervention.

3.3. Protein kinase C activation

Glucose-induced activation of PKC, via predominately a de novo synthesis of diacylglycerol, has been documented in both experimental animal models and human subjects with long-standing diabetes (Giles et al., 1998; Liu et al., 1999; Way et al., 2001; Way et al., 2002; Guo et al., 2003). PKC activation may lead to several important biochemical changes that have been documented in diabetic cardiomyopathy. These changes include reduced blood flow and increased vascular permeability, BM thickening, and ECM deposition (Ishii et al., 1996; Koya & King, 1998; Way et al., 2001; Hayat et al., 2004). In other target organs, PKC up-regulation has been shown to play a role in growth factor expression, activation of mitogen-activated protein kinase (MAPK), and nuclear transcription factors (Tomlinson, 1999).

There are at least 12 isoforms of PKC which exert their effects in a tissue- and isoform-dependent manner (Mellor & Parker, 1998). In the heart, diabetes-mediated PKC-β activation exhibits the greatest induction and is suggested to be the ideal candidate for therapeutic modalities (Inoguchi et al., 1992; Liu et al., 1999; Guo et al., 2003). Recent studies have implicated PKC-α, -β, and -ε in the development of diabetic cardiac hypertrophy (Way et al., 2001). These findings suggest

that PKC isoform activation may depend on the stage of the disease and the pathogenetic alteration.

Over the last few years, researchers have focused much attention on the PKC-β inhibitor, ruboxistaurin mesylate (LY333531) (Way et al., 2001). Interestingly, this specific inhibitor has been reported to reverse cardiac hypertrophy, improved fractional shortening, and cardiac injury in a transgenic mouse model with overexpression of PKC-β in the heart (Wakasaki et al., 1997). Selective inhibition of PKC-β has also been reported to correct increased albumin flux through coronary venules at the early onset of streptozotocin-induced diabetes in pigs (Yuan et al., 2000). Phase I clinical trials testing the toxicity and risks associated with PKC-β inhibition have shown no detrimental effects of ruboxistaurin mesylate (Shen, 2003; Wheeler, 2003). Although the clinical trails do not indicate the improvements specifically to diabetic cardiomyopathy, this inhibition is associated with improvements in diabetic retinopathy, nephropathy, neuropathy, and cardiac dysfunction (Way et al., 2001). Phase III clinical trials are in progress and hold some promise in terms of preventing cardiomyopathy (Shen, 2003). Recently, cardiac-specific activation of PKC-ε has been shown to prevent diabetes-induced pathogenetic changes in the heart including ventricular function and oxidative stress (Malhotra et al., 2005). Further studies investigating the differential role of various PKC isoforms may unveil which isoforms should be targeted for inhibition and which can be targeted for activation to transduce survival signals in the diabetic heart.

3.4. Oxidative stress and redox potential

The augmentation of oxidative stress has been clearly documented in the pathogenesis of diabetic complications, including cardiomyopathy (Baynes & Thorpe, 1999; Cai & Kang, 2001; Farhangkhoee et al., 2003). During long-standing diabetes, the physiological response to combat oxidative stress is overwhelmed, resulting in an imbalance between pro-oxidative and anti-oxidative compounds. The mechanism of hyperglycemia-induced oxidant injury includes glucose auto-oxidation and a number of indirect pathways (King & Loeken, 2004) (Fig. 4).

One pathway that contributes to increased oxidative stress involves the interaction of AGEs with their receptors (Wautier et al., 2001; Vlassara, 2001). The association causes intracellular changes, most notably the activation of redox transcription factor, nuclear factor-κB (NF-κB) (Brownlee, 2001). Another mechanism that contributes to glucose-induced oxidative stress is the augmented flux of substrates though the AR pathway. AR-mediated alteration of NADPH/NADP$^+$ and NADH/NAD$^+$ ratio may result in depletion of cofactors required for antioxidant enzymes and contribute to the production of AGE precursors and the PKC activator diacylglycerol (Srivastava et al., 1989; Brownlee, 2001). Activation of PKC then causes oxidative stress via activating mitochondrial NADPH oxidase, which further decreases the NADPH/NADP$^+$ ratio (Inoguchi et al., 2003).
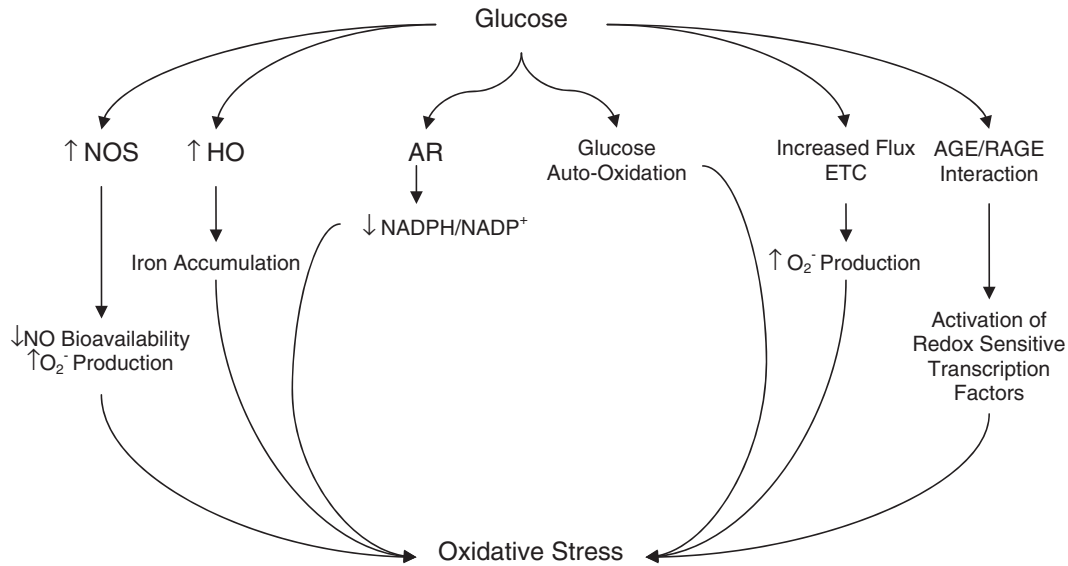
Fig. 4. Schematic representation of possible mechanisms arbitrating hyperglycemia-induced oxidative stress. Hyperglycemia may regulate reactive oxygen species (ROS) production and oxidative stress by a number of mechanisms. Increased oxidative damage may be caused by alteration of key enzymes such as nitric oxide synthase (NOS), heme oxygenase (HO), and aldose reductase (AR). For example, increased NOS activity, superoxide production, and conversion of NO to peroxynitrite can contribute to oxidative damage. Increased AR activity may lead to decreased NADPH/NADP$^+$ ratio and depletion of cofactors for anti-oxidant enzymes. Augmented HO activity may contribute to oxidative stress by increasing redox active iron accumulation in the heart tissues. Other contributing factors that increase oxidative stress include augmented glucose auto-oxidation, glucose flux through the mitochondrial electron transport chain (ETC), and interaction of advanced glycation end-products (AGE) with their receptors (RAGE) (O_2^-, superoxide).

Recently, an important pathway leading to the production of oxidative stress has been identified as increased glucose flux through the electron transport chain and subsequently augmented O_2^- production (Nishikawa et al., 2000; Brownlee, 2001). Interestingly, researchers have identified that benfotiamine, an inhibitor which prevents mitochondrial O_2^- production, can attenuate major hyperglycemia-induced biochemical pathways, including AGE formation and PKC activation (Hammes et al., 2003). Although, this compound has not been tested for the treatment of diabetic cardiomyopathy, it has been shown to be beneficial in preventing experimental diabetic retinopathy and dyslipidemia (Hammes et al., 2003; Babaei-Jadidi et al., 2004). Augmented O_2^- anion production may potentiate oxidant injury by reacting with hydroxyl radicals and hydrogen peroxide (Nishikawa et al., 2000). Superoxide may also react with NO to produce peroxynitrite, which in turn can increase lipid peroxidation, protein nitration, and oxidizes low density lipoproteins (LDLs) (Griendling & FitzGerald, 2003). The importance of NO in augmenting oxidative stress in diabetic complication is being realized. Endothelium-dependent vasodilatation has shown to be altered in patients with diabetes (McVeigh et al., 1992; Lambert et al., 1996; Dogra et al., 2001; Van de Ree et al., 2001; Johnstone et al., 1993). We have shown that following 1 month of diabetes, streptozotocin-induced diabetic heart tissues exhibit increased eNOS and iNOS mRNA levels, in association with no alteration of nitrite and nitrate production (Farhangkhoee et al., 2003). Interestingly, diabetic heart tissues also showed increased nitrotyrosine immunoreactivity (Farhangkhoee et al., 2005). These results suggest that hyperglycemia-induced NOS expression is augmented. In order to maintain vasoregulation and to counter the effects of increased vasoconstrictors (ETs), an increase in NO production would be required. Our studies indicate an increase in NOS in the heart but no significant changes in nitrite and nitrate levels as compared to controls (Farhangkhoee et al., 2003). It is plausible that NO is increased; however, the bioavailability is reduced through sequestration by reactive oxygen species (ROS). The mechanism of NO alteration and cellular dysfunction could underlie the interaction between NO and AR enzyme. Interestingly, NO donors have been reported to inhibit the activity of AR (Srivastava et al., 2003). Chemical inhibition of NOS in non-diabetic rats also increases AR activity (Chandra et al., 2002). It is plausible that basal NO regulates AR activity; however, during diabetes reduced NO availability in concert with increased oxidative stress may contribute to increased AR activity.

The alteration of the NOS, particularly eNOS, may contribute to oxidative stress and endothelial dysfunction (Ogita & Liao, 2004). In endothelial cells, eNOS is localized in the membrane invagination, known as the caveolae, by caveolin-1 (Feron et al., 1996, 1998). Caveolin-1 also co-localizes with a CD36 (Lisanti et al., 1994; Uittenbogaard et al., 2000), a scavenger receptor able to bind to modified proteins such as oxidized low density lipoproteins (ox-LDLs) (Endemann et al., 1993). We have recently shown that glucose increases CD36 mRNA and protein expression in MVECs (Farhangkhoee et al., 2005). We have further demonstrated that endothelial cells treated with high levels of glucose in the presence of ox-LDL increase uptake of the modified lipoprotein, as well as increasing oxidative stress. Specific inhibition of CD36 prevented ox-LDL uptake, oxidative stress, and endothelial dysfunction (Farhangkhoee et al., 2005). Due to the spatial arrangement of CD36 and eNOS, it is plausible that CD36-mediated increased ox-LDL uptake and ROS production

could sequester NO and further aggravate the oxidant injury. This pathway may provide a model for continuous oxidative stress production by the eNOS-caveolin-CD36 axis in diabetic microangiopathy.

Therapeutic interventions to combat oxidative stress have traditionally been aimed at using scavengers to mitigate the effects of ROS. These scavengers, however, have not been beneficial in preventing diabetes-induced damage. Compounds like benfotiamine, which prevent the formation of ROS, may provide a better avenue (Nishikawa et al., 2000; Hammes et al., 2003). Studies targeting the inhibition of proteins altered during long-standing diabetes have also shown reduction in oxidative stress (Srivastava et al., 1989; Nishikawa et al., 2000; Sonta et al., 2004). We have demonstrated a possible role of heme oxygenase (HO), a stress response protein, in mediating diabetes-induced oxidative stress in the heart (Farhangkhoee et al., 2003). HO is an enzyme that catalyzes the degradation of heme into carbon monoxide, biliverdin which is subsequently converted to bilirubin by biliverdin reductase, and free ferrous iron ($Fe2^+$) (Maines, 1997). Our results suggest that inhibition of HO system can prevent iron accumulation and diabetes-mediated oxidative stress in the heart (Farhangkhoee et al., 2003).

Downstream mediators of oxidant injury include PKC, NF-κB, and a recently identified stress-response protein, poly (ADP-ribose) polymerase (PARP). PARP is a ubiquitous enzyme that is activated by a number of factors including ionizing radiation, alkylating agents and oxidants (Decker & Muller, 2002). Activated PARP may regulate the activity of histones, transcription factors (NF-κB), and other intracellular proteins such as glyceraldehyde-3-phosphate dehydrogenase (Du et al., 2003). Researchers have shown that impaired endothelium-dependent NO release and functional changes in the heart can be prevented by PJ34, a PARP inhibitor (Pacher et al., 2002; Szabo, 2002). In addition, PARP knockout animals have been shown to be resistant against streptozotocin-induced diabetes (Masutani et al., 1999; Pieper et al., 1999). Presently, PARP inhibitors are in clinical trials to investigate the toxicity and safety of the drug.

On the basis of the aforementioned studies, inhibiting oxidative stress may provide a treatment avenue for diabetic cardiomyopathy. Studies indicate that impaired endothelium-dependent vasodilatation in both type 1 and type 2 diabetic patients may be reversed by ascorbic acid (Ting et al., 1996; Timimi et al., 1998). The mechanism of the beneficial effects of ascorbic acid on vascular responses is not yet fully elucidated. Treatment of non-diabetic hypertensive patients with ascorbic acid has resulted in a reduction of blood pressure without significant changes in systemic NO (Duffy et al., 1999). Studies with vitamin E, vitamin C, and β-carotene have, however, failed to show a significant retardation in cardiovascular disease progression (Jain et al., 1996; Marchioli, 1999; Maxwell, 1999).

3.5. Free fatty acid accumulation and oxidative metabolism

The metabolic changes associated with the diabetic myocardium have been extensively studied (Bell, 1995; Hopfner et

al., 1998; Carvajal & Moreno-Sanchez, 2003; Cosson & Kevorkian, 2003). The primary metabolic derangements include free fatty acid (FFA) accumulation, which results from both increased lipolysis of triglycerides and increased uptake, and the shift to FFA oxidation for energy requirements (Rodrigues et al., 1998). Under physiological conditions, energy from ATP is derived from the oxidation of glucose, FFAs, pyruvate, and ketone bodies. During chronic diabetes, however, ATP production is primarily derived from β-oxidation of FFAs (Cai & Kang, 2001). An increased dependence on FFA oxidation leads to aberrant metabolic changes in the myocytes which include increased oxygen consumption, formation of toxic lipid intermediates, and myocyte toxicity and loss (Rodrigues et al., 1998; Nakayama et al., 2001; Fang et al., 2004).

In addition to increased β-oxidation in cardiomyocytes, the oxidation of other compounds such as glucose and pyruvate is reduced (Cai & Kang, 2001). These changes occur due to numerous events that inhibit oxidative phosphorylation of glucose such as inhibition of pyruvate dehydrogenase (PDH) (Rodrigues et al., 1998; Cai & Kang, 2001; Hayat et al., 2004); decreased glucose phosphorylation (Cai & Kang, 2001); and decreased transcription of glucose transporters like glucose transporter 4 (Li & McNeill, 2001). Despite the evidence suggesting decreased glucose transport in cardiomyocytes, the effects of hyperglycemia-induced damage in the heart, such as increased flux of glucose through the AR pathway, still occur (Ramasamy et al., 1998; Trueblood & Ramasamy, 1998). These results suggest that glucose utilization instead of glucose transport may play a more important role in diabetic cardiomyopathy.

The role of FFA in vascular endothelial dysfunction is being realized. Increased FFA levels in diabetic patients may lead to endothelial damage by a number of mechanisms. Reports indicate increased oxidative stress in endothelial cells exposed to high levels of palmitate (Inoguchi et al., 2000). Increased FFA-induced ROS production was normalized with PKC inhibitors suggesting an important downstream role of PKC in mediating oxidative stress. Furthermore, FFA may impair endothelial NO production (Steinberg et al., 2000; Kim et al., 2005). Such a role of FFA may, in part, underlie endothelium-dependent vasodilatation in diabetics (De Kreutzenberg et al., 2000; Steinberg et al., 2000). Thus, inhibiting FFA oxidation may represent a potential therapeutic intervention. Trimetazidine (1-[2,3,4-trimethoxibenzyl]-piperazine) has been identified as the potential compound that may inhibit FFA oxidation and restore proper glucose utilization (Stanley & Marzilli, 2003). Clinical trials have shown beneficial effects of trimetazidine on left ventricular function assessed by ventricular volumes and ejection fraction (Rosano et al., 2003; Vitale et al., 2004). Other interventions have been directed at the control of lipid metabolism through the peroxisome proliferator-activated receptors (PPARs). PPARs are transcription factors that regulate multitude of genes involved in lipid metabolism (Francis et al., 2003; Gilde & Bilsen, 2003; Lee et al., 2003). Transgenic mice overexpressing PPARα show increased myocardial fatty acid oxidation rates and decreased

glucose uptake and oxidation (Finck et al., 2002). These changes were also associated with left ventricular abnormalities. Interestingly, PPARα-deficient mice are protected against the development of diabetes-induced cardiac hypertrophy (Finck et al., 2003). These studies suggest the inhibition of PPARα may be important in the prevention of diabetic dysmetabolism. Other members of the PPAR family may also be involved in diabetes-induced changes in the heart. PPARγ alteration may be the mechanism of CD36 up-regulation, which has been shown to mediate increased oxidative stress in endothelial cells (Liu et al., 2004). However, such a notion requires further experimental evidence, as many clinical studies have indicated that PPARγ agonists are important in alleviating the complications of diabetes (Bogacka et al., 2004; Hallsten et al., 2004; Wang et al., 2005). For instance, the PPARγ agonist rosiglitazone, a thiazolidinediones, was shown to enhance insulin stimulated myocardial glucose uptake (Hallsten et al., 2004). It is plausible that the beneficial effects of PPARγ agonists (normalization of energy metabolism in myocytes) supersede any detrimental effects such as CD36 alteration.

3.6. Intracellular signaling molecules

The role of intracellular kinase PKC has been extensively studied in cultured endothelial cells and target organs of chronic complications (Giles et al., 1998; Liu et al., 1999; Way et al., 2001, 2002; Guo et al., 2003). Although inhibition of PKC has shown beneficial effects in animal models, clinical trials have failed to show significant normalization of diabetes-induced changes (The PKC-DRS Study Group, 2005). These findings indicate a potential crosstalk and redundancy in signaling molecules at least in other target organs of diabetic complications. Recently, we have shown that ET-mediated up-regulation of ECM proteins in cultured endothelial cells may be arbitrated via increased activity of MAPK pathway (Xin et al., 2004). Activation of MAPK has been associated with reduced contractility of cultured cardiomyocytes (Wold & Ren, 2004). We have also demonstrated an important crosstalk between MAPK pathway, PKC, and protein kinase B (PKB) in cultured endothelial cells (Xin et al., 2004, 2005). In cultured endothelial cells, high levels of glucose increase ECM protein expression by activation of a complex cascade of protein kinases. Specific inhibition of MAPK, PKC, or PKB is able to prevent cross-activation of other kinases and normalize glucose-induced ECM protein expression.

Much of the studies aimed at elucidating the signal transducers in diabetic complications have focused on kinases which are well established in other disease models. Recently, serum- and glucocorticoid-regulated kinase has been implicated in glucose-induced ECM protein elaboration (Khan et al., 2005). We have shown that inhibition of serum- and glucocorticoid-regulated kinase-1 by small interfering RNA-mediated gene silencing leads to complete abolishment of glucose-induced fibronectin expression (Khan et al., 2005). These studies provide novel targets for preventing cardiac microvessel BM thickening and perhaps interstitial fibrosis.

4. Concluding remarks

Since the discovery of diabetic cardiomyopathy over 30 years ago, many researchers have attempted to elucidate the pathogenesis of this complication. Diabetic myocardial disease is characterized by cardiomyocyte hypertrophy, interstitial fibrosis, thickening of capillary BMs, capillary microaneurysms, and reduced capillary density. Clinical, epidemiological, and experimental data suggest that the pathogenesis of diabetic cardiomyopathy is multifactorial. The mechanisms that lead to the development and the progression of cardiomyopathy may include reduced compliance of the myocardium due to increased collagen deposition and fibrosis, microvascular dysfunction, and disturbances in cardiomyocyte energy metabolism. Reports indicate that chronic diabetes affects the MVECs in addition to the myocytes. Such a notion is paralleled in other target organs of chronic diabetic complications and represents a novel avenue for therapy. The pathogenetic role of endothelial dysfunction in diabetic cardiomyopathy is especially evident in the relationship between cardiac endothelial cells and changes in cardiac growth and performance. In addition, early structural and functional alterations in the heart may arise due to impaired blood flow and elaboration of ECM proteins, which lead to BM thickening and interstitial fibrosis. Vasoactive factors such as ET-1 and NO should therefore comprise our first line therapy to combat the adverse effects of hyperglycemia. ET-1 has been clearly established in causing vasoconstriction in target organs of diabetic complications. Such altered vasoregulation may be

Table 1
Therapeutic targets for diabetic cardiomyopathy

Therapeutic target	Potential benefits
Vasoactive factors	Normalization of blood flow
(Blockers of ET, NO enhancers)	Reduced ECM protein deposition
Aminoguanidine	Increased blood vessel compliance
(AGE formation inhibitors)	Reduced oxidative stress
	Reduced NF-κB activation
	Reduced ECM protein deposition
AR inhibitors	Normalization of pseudohypoxia
	Reduced oxidative stress
	Reduced osmotic stress
	ATP preservation
PKC inhibitors	Reduced vascular permeability
	Reduced oxidative stress
	Reduced growth factor expression
	Reduced ECM protein deposition
HO inhibitors	Reduced iron accumulation
	Reduced oxidative stress
PARP inhibitors	Normalization of endothelial function
	Reduced oxidant injury
FFA oxidation inhibitors	Normalization of FFA dysmetabolism
PPARα inhibitors	Reduced FFA oxidation
Blockers of intracellular molecules	Reduce ECM protein deposition
(PKB, MAPK, SGK, NF-κB)	Reduced oxidative stress signaling

ATP, adenosine triphosphate; AGE, advanced glycation end-products; AR, aldose reductase; ET, endothelin; ECM, extracellular matrix; FFA, free fatty acid; HO, heme oxygenase; MAPK, mitogen-activated protein kinase; NO, nitric oxide; NF-κB, nuclear factor-κB; PPAR, peroxisome proliferator-activated receptor; PARP, poly (ADP-ribose) polymerase; PKB, protein kinase B; PKC, protein kinase C; SGK, serum- and glucocorticoid-regulated kinase.

precipitated with oxidative stress-mediated NO sequestration and reduced NO availability. Elaboration of ET-1 and reduction in NO may transduce signals to vascular endothelial and contractile cells, respectively, leading to myocyte hypertrophy and increased expression of ECM proteins. BM thickening, in concert with reduced blood flow, produces ischemia in the tissue microenvironment. Such focal scarring in the heart is clearly evident in animal models of chronic diabetes.

Oxygen demand of the tissue is one of the most important signals for the elaboration of angiogenic factor, VEGF. Inability of cardiac MVECs to up-regulate VEGF or VEGF receptors could be the distinguishing factor between cardiomyopathy and highly angiogenic retinopathy. Whether such inability of the cardiac tissue to mount a proliferative/reparative response is due to the bystander cells including contractile cells remains to be determined. Gene therapy may hold great promise for increasing endothelial proliferation, restoring normal vascular function, and treating diabetic cardiomyopathy. The prospects are limited by our limited knowledge of the risks of gene therapy and by the gene transfer methods. With the current knowledge, however, it is evident that targeting one specific pathway is not practical, as a vast variety of changes are induced by chronic diabetes. The best approach may be to provide a combinatorial therapy intended to stimulate or inhibit more than one aspect of the disease process. The strategies that improve the cardiac endothelial function or re-growth of functional blood vessels to the ischemic heart are of great clinical importance. Table 1 summarizes the various molecules that may be exploited as therapeutic targets to restore vascular endothelial integrity. Inhibiting oxidative stress and restoring endothelium-dependent vasoregulation could very well represent the best strategy for therapeutic intervention.

Acknowledgments

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