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Role of peroxynitrite in the pathogenesis of cardiovascular complications of diabetes

Pál Pacher¹ and Csaba Szabó²

¹ National Institutes of Health, NIAAA, Laboratory of Physiologic Studies, 5625 Fishers Lane MSC 9413, Bethesda, Maryland 20852, USA

² Department of Surgery, UMD NJ-New Jersey Medical School, Newark, New Jersey 07103, USA

Abstract

Hyperglycemic episodes, which complicate even well-controlled cases of diabetes, lead to increased polyol pathway flux, activation of protein kinase C and accelerated non-enzymatic formation of advanced glycation end products. Many of these pathways become activated in response to the production of superoxide anion. Superoxide can interact with nitric oxide, forming the potent cytotoxin peroxynitrite. Peroxynitrite attacks various biomolecules in the vascular endothelium, vascular smooth muscle and myocardium, eventually leading to cardiovascular dysfunction via multiple mechanisms. This review focuses on emerging evidence suggesting that peroxynitrite plays a key role in the pathogenesis of the cardiovascular complications of diabetes, which underlie the development and progression of diabetic retinopathy, neuropathy and nephropathy.

Introduction

Diabetes mellitus is one of the most prevalent (the estimated lifetime risk of developing diabetes for individuals born in 2000 is 32.8% for males and 38.5% for females) and costly chronic diseases, which significantly reduces life expectancy [1]. The majority of diabetic complications are related to pathophysiological alterations in the vasculature, with macro- and microvascular disease being the most common cause of morbidity and mortality in patients with diabetes mellitus. Atherosclerosis is the most common macrovascular complication of diabetes, and increases the risk for stroke, myocardial infarction and peripheral artery disease, the latter being the leading cause of limb amputation in civilized countries. Microvascular complications comprise retinopathy and nephropathy — the leading causes of blindness and renal failure [1].

Hyperglycemic episodes, which complicate even well-controlled cases of diabetes, are closely associated with increased oxidative and nitrosative stress, which can trigger the development of diabetic cardiovascular complications (Figure 1). This review focuses on the role of nitrosative stress, particularly that of the reactive oxidant peroxynitrite (formed from the reaction of superoxide with nitric oxide), in the pathogenesis of diabetic complications.

Pathogenesis of diabetic endothelial dysfunction: role of oxidative stress

Endothelial dysfunction in many diseases precedes and predicts, as well as predisposes, for subsequent, more severe vascular alterations. As such, endothelial dysfunction has been documented in various forms of diabetes, and even in pre-diabetic individuals [2–7].

Corresponding author: Pacher, Pál (pacher@mail.nih.gov).

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The pathogenesis of endothelial dysfunction in diabetes is complex. The diabetic state is associated with increased oxidative stress, which plays an important role in the development of diabetic complications. Hyperglycemia triggers increased polyol pathway flux, altered cellular redox state, increased formation of diacylglycerol and the subsequent activation of specific protein kinase C isoforms, and accelerated non-enzymatic formation of advanced glycation end products [8–10]. Activation of many of these pathways results from the production of oxygen-derived oxidants, such as superoxide anion (Figure 1). Superoxide production, therefore, plays a significant role in the pathogenesis of the diabetes-associated endothelial dysfunction [9–11]. The cellular sources of superoxide anion are multiple and include NADH/NADPH and xanthine oxidases, the mitochondrial respiratory chain, the arachidonic acid cascade (including lipoxygenase and cyclooxygenase), and microsomal enzymes [8]. Hyperglycemia-induced superoxide generation contributes to the increased expression of NAD(P)H oxidase which, in turn, generates more super-oxide anion. Among these pathways, mitochondrial generation of superoxide appears to play the most crucial role in diabetic complications. Hyperglycemia also favors increased expression of inducible nitric oxide synthase (iNOS) through the activation of nuclear factor- κ B (NF- κ B), which can increase the generation of nitric oxide (NO) [7,12]. Superoxide anion can quench NO, thereby reducing the efficacy of the potent endothelium-derived vasodilator system that participates in the homeostatic regulation of the vasculature [9].

Pathogenesis of diabetic endothelial dysfunction: role of nitrosative stress and peroxynitrite

Superoxide anion is able to interact with nitric oxide, which is produced physiologically by constitutive sources such as the endothelial isoform of nitric oxide synthase. This process results in the formation of the strong oxidant peroxynitrite, which attacks various biomolecules leading (among other processes) to the production of a modified amino acid, nitrotyrosine [13]. Nitrotyrosine was initially considered a specific marker of peroxynitrite generation, but now is generally regarded an index of reactive nitrogen species, rather than a specific indicator of peroxynitrite formation [14].

Peroxynitrite attacks various biomolecules, leading to cardiovascular dysfunction via multiple mechanisms [15^{*}]. One of these pathways involves DNA strand breakage and consequent activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP; for reviews, see [15^{*},16]). PARP-1 activation emerges as a key process in the development of diabetic cardiovascular dysfunction both in diabetic animals and in humans [3,17,18] and might also contribute to the development of other diabetic complications such as nephropathy, neuropathy and retinopathy [19^{*}]. Recent work on endothelial cells in conditions of elevated extracellular glucose has identified a novel mechanism by which PARP activation regulates the development of various pathways related to diabetic complications. In this scheme, elevated superoxide generation from the mitochondria (directly, or indirectly via the generation of peroxynitrite) induces DNA strand breaks and activates PARP which, in turn, induces the poly(ADP-ribosyl)ation of glyceraldehyde-3-phosphate dehydrogenase. The resulting metabolic alterations activate NF- κ B, aldose reductase and the polyol pathways [20,21].

Accumulating evidence supports the hypothesis that diabetes is associated with increased nitrosative stress and peroxynitrite formation in numerous tissues, both in experimental animals and in humans [22]. For example, increased nitrotyrosine plasma levels are detectable in type 2 diabetic patients [23] and iNOS-dependent peroxynitrite production is increased in platelets from diabetic individuals [24]. Hyperglycemia induces increased nitrotyrosine formation in the artery wall of monkeys [25] and in diabetic patients during a period of postprandial hyperglycemia [26,27]. Increased nitrotyrosine immunoreactivity has also been demonstrated in the microvasculature of type 2 diabetic patients, which correlates with fasting blood glucose,

endothelial dysfunction, and increased levels of HbA1c, intracellular adhesion molecule and vascular cellular adhesion molecule [3]. Toxic actions of peroxynitrite and/or nitrotyrosine in the cardiovascular system are also supported by evidence showing that the degree of cell death and/or dysfunction correlates with levels of nitrotyrosine in endothelial cells, myocytes and fibroblasts from heart biopsies of diabetic patients [28], hearts of streptozotocin-induced diabetic rats [29], and working hearts of rats during hyperglycemia [30]. Nitrotyrosine is directly harmful to endothelial cells [31], and high glucose-induced oxidative and nitrosative stress pathologically alters the prostanoid profile of human endothelial cells [4,32]. It has recently been suggested that there may be several phases in the pathogenesis of high glucose-induced endothelial injury: the short-term effect appears to depend upon combined oxidative and nitrosative stress with peroxynitrite formation, whereas the long-term effect is related to the generation of reactive oxygen species. In both cases, protein kinase C ultimately mediates the changes in vascular permeability [33].

Angiotensin II — a known factor in the pathogenesis of diabetic cardiovascular complications — can induce superoxide formation, which is mediated (at least in part) by vascular NAD(P)H oxidases. Reactive oxidant species, in turn, can exert direct oxidative effects, but can also signal through pathways involving mitogen-activated protein kinases, tyrosine kinases and transcription factors, leading to events such as inflammation, hypertrophy, remodeling and angiogenesis [34]. Recent work demonstrates that angiotensin II can also induce intra-endothelial peroxynitrite formation [35,36], as well as PARP activation [36].

Consistent with the role of peroxynitrite/nitrotyrosine in the pathogenesis of diabetic vascular dysfunction, neutralization of peroxynitrite with the metalloporphyrin peroxynitrite decomposition catalyst FP15 ameliorated the endothelial and cardiac dysfunction in a streptozotocin-induced murine model of diabetes [37].

Diabetic cardiomyopathy: role of nitrosative stress and peroxynitrite

Diabetic cardiomyopathy has been recognized for many decades and is characterized by complex changes in the biochemical, mechanical, structural and electrical properties of the heart, which can underlie the development of early diastolic or late systolic dysfunction (or both), and the increased incidence of cardiac arrhythmias in diabetic patients. Diabetes is now considered a potent independent risk factor for mortality in patients hospitalized with heart failure, particularly in females [38].

Accumulating pre-clinical and clinical evidence suggests that increased sympathetic activity, an activated cardiac reninangiotensin system, myocardial ischemia or functional hypoxia, and elevated circulating levels of glucose can result in oxidative and nitrosative stress in the cardiovascular system of diabetic animals and humans. As such, oxidative and nitrosative damage might be critical in the early onset of diabetic cardiomyopathy [28–30,37,39]. In cardiomyocytes grown in culture medium containing elevated glucose, pathophysiological alterations can be attenuated by antioxidants, NOS inhibitors and peroxynitrite-neutralizing agents, suggesting that these alterations are induced by oxidative and nitrosative stress [40]. Nitrotyrosine was also detected in cardiac myocytes from myocardial biopsy samples obtained from diabetic and diabetic-hypertensive patients [28] and in a mouse model of streptozotocin-induced diabetes [29]. Perfusion of isolated hearts with high glucose led to upregulation of iNOS, an increase in coronary perfusion pressure and an increase in NO and superoxide generation, conditions favoring the production of peroxynitrite; this was accompanied by the formation of nitrotyrosine and cardiac cell apoptosis [30].

As mentioned above, experimental and clinical evidence suggest that nitrosative stress and peroxynitrite play an important role in the pathogenesis of diabetic cardiomyopathy. It appears that pharmacological neutralization of peroxynitrite improves vascular and/or cardiac function,

not only in experimental models of diabetes but also in other pathophysiological conditions of the heart that are associated with increased peroxynitrite formation. These include acute myocardial infarction, chronic ischemic heart failure, doxorubicin-induced cardiomyopathy and diabetic cardiomyopathy [41–43,15*]. The mechanism by which neutralization of peroxynitrite protects against cardiovascular dysfunction involves protection against vascular and myocardial tyrosine nitration, PARP activation and lipid peroxidation, as all of these have previously been linked to diabetic cardiomyopathy as well as to peroxynitrite-induced cardiac injury [15*]. Additional mechanisms of peroxynitrite-mediated diabetic cardiac dysfunction include inhibition of myofibrillar creatine kinase [41] and succinyl-CoA:3-oxoacid CoA-transferase [44] and activation of metalloproteinases [15*].

The observations described above support the concept that peroxynitrite is a major mediator of myocardial injury in various pathophysiological conditions, and its effective neutralization can be of significant therapeutic benefit [15*]. Accumulating experimental evidence suggests that peroxynitrite might also be involved in the pathogenesis of primary diabetes [37], diabetic microvascular injury in retinopathy [45–47], nephropathy [48,49] and neuropathy [50*,51,52*], but the detailed description of these studies is beyond the scope of the present review [22].

Conclusions and implications

Several lines of evidence reviewed herein support the view that nitrosative stress and peroxynitrite-induced damage play a pivotal role in the pathogenesis of diabetic cardiovascular complications (see Figure 1). Neutralization of reactive nitrogen species and inhibition of downstream effector pathways, including the inhibition of PARP activation, emerge as novel approaches for the prevention or reversal of diabetic cardiovascular complications, as well as of neuropathy, nephropathy and retinopathy. Of note, hyperglycemia is a strong trigger of reactive oxygen and nitrogen species formation in the cardiovascular system; therefore, tight glycemic control in diabetics could be one of the best ways to prevent reactive species production and initiation of downstream pathways of myocardial injury. It remains to be seen whether various therapeutic drugs with well-known cardiovascular protective effects (e.g. antioxidants, statins, β -blockers, angiotensin-converting enzyme inhibitors) are able to suppress peroxynitrite formation and PARP activation in the cardiovascular system.

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Figure 1. Mechanisms of cardiovascular dysfunction in diabetes: role of superoxide and peroxynitrite. Hyperglycemia induces increased superoxide anion ($\text{O}_2^{\bullet -}$) production via activation of multiple pathways including xanthine and NAD(P)H oxidases, cyclooxygenase, uncoupled NOS, glucose autooxidation, the mitochondrial respiratory chain, polyol, and advanced glycation end products (AGEs). Superoxide activates AGEs, PKC, polyol (sorbitol), hexosamine and stress-signaling pathways, leading to increased expression of inflammatory cytokines, angiotensin II (Ang II), endothelin-1 (ET-1) and NAD(P)H oxidases, which in turn generate more superoxide via multiple mechanisms. Hyperglycemia-induced superoxide generation might also favour increased expression of NOSs through the activation of NF- κ B, which increases the generation of NO. Superoxide anion quenches NO, thereby reducing the efficacy of a potent endothelium-derived vasodilator system. Superoxide can also be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) and interact with NO to form a reactive oxidant, peroxynitrite (ONOO^-), which induces cell damage via lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration, and activation of matrix metalloproteinases (MMPs). Peroxynitrite also acts on mitochondria to decrease the membrane potential (Ψ) and trigger the release of proapoptotic factors such as cytochrome c (Cyt c) and apoptosis-inducing factor (AIF). These factors mediate caspase-dependent and -independent apoptotic death pathways. Peroxynitrite, in concert with other oxidants (e.g. H_2O_2), causes strand breaks in DNA, activating the nuclear enzyme PARP-1. Mild damage to DNA activates

the DNA repair machinery. By contrast, once excessive oxidative- and nitrosative stress-induced DNA damage occurs, overactivated PARP-1 initiates an energy-consuming cycle by transferring ADP-ribose units (small red spheres) from NAD^+ to nuclear proteins, resulting in rapid depletion of the intracellular NAD^+ and ATP pools, slowing the rate of glycolysis and mitochondrial respiration, and eventually leading to cellular dysfunction and death. Poly(ADP-ribose) glycohydrolase (PARG) degrades poly(ADP-ribose) (PAR) polymers, generating free PAR polymer and ADP-ribose, which signals to the mitochondria to induce AIF release. PARP-1 activation also leads to the inhibition of cellular glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) activity which, in turn, favours the activation of PKC, AGEs and the hexosamine pathway, leading to increased superoxide generation. PARP-1 also regulates the expression of a variety of inflammatory mediators, which might facilitate the progression of diabetic cardiovascular complications.