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REVIEW

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Hyperglycemia-induced oxidative stress in diabetic complications

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Abstract Reactive oxygen species are increased by hyperglycemia. Hyperglycemia, which occurs during diabetes (both type 1 and type 2) and, to a lesser extent, during insulin resistance, causes oxidative stress. Free fatty acids, which may be elevated during inadequate glycemic control, may also be contributory. In this review, we will discuss the role of oxidative stress in diabetic complications. Oxidative stress may be important in diabetes, not just because of its role in the development of complications, but because persistent hyperglycemia, secondary to insulin resistance, may induce oxidative stress and contribute to beta cell destruction in type 2 diabetes. The focus of this review will be on the role of oxidative stress in the etiology of diabetic complications.

Keywords Oxidative stress · Free radicals · Diabetic complications

Introduction

There is much evidence that oxidative stress is involved in the etiology of several diabetic complications (Giugliano and Ceriello 1996; Feldman et al. 1997; Ruggiero et al. 1997; McDonagh and Hokama 2000; Vinik et al. 2000; Kowluru and Kennedy 2001; Chang et al. 2003). Oxidative stress results when the rate of oxidant production exceeds the rate of oxidant scavenging. During diabetes or insulin resistance, failure of insulin-stimulated glucose uptake by fat and muscle causes glucose concentrations in blood to remain high. Consequently, glucose uptake by insulin-independent tissues increases. Increased glucose flux both enhances oxidant production and impairs antioxidant defenses by multiple interacting pathways which are described here and in Fig. 1. Glucose can cause nonenzymatic glycation of proteins, which can be oxidants (Baynes and Thorpe 1999). Free glucose activates aldose reductase activity and the polyol pathway, which decreases NADPH/$NADP^+$ ratios (Dunlop 2000). Excess glucose may activate protein kinase C (PKC) by several mech-anisms, including through de novo synthesis of diacylglycerol (DAG), by activation of phospholipase C, and by inhibition of DAG kinase (Xia et al. 1994, 1996; King et al. 1996). PKC increases oxidative stress by activating mitochondrial NADPH oxidase (Inoguchi et al. 2000). Increased oxidative glucose metabolism itself increases mitochondrial production of the superoxide anion ($O_2^{\cdot -}$), which will then be converted to the hydroxyl radical ($OH^{\cdot -}$), and hydrogen peroxide (H_2O_2) (Nishikawa et al. 2000). In addition, mitochondrial $O_2^{\cdot -}$ production may inhibit activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity (Nishikawa et al. 2000). As a result, glycolytic intermediates upstream of GAPDH accumulate, leading to increased substrate-directed activity of the de novo DAG synthetic pathway, which further activates PKC and NADPH oxidase, as well as the hexosamine biosynthetic pathway (Nishikawa et al. 2000). Glucosamine-6-phosphate, produced by the hexosamine biosynthetic pathway, inhibits activity of glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme in the pentose shunt pathway (Kanji et al. 1976). Since G6PD activity is coupled to reduction of $NADP^+$ to NADPH, activation of the hexosamine biosynthetic pathway would further decrease NADPH/$NADP^+$ ratios. Decreased NADPH/$NADP^+$ ratios, resulting from inhibition of G6PD or stimulation of NADPH oxidase, can increase oxidative stress by two mechanisms, first, by decreasing the regeneration of the important cellular antioxidant,

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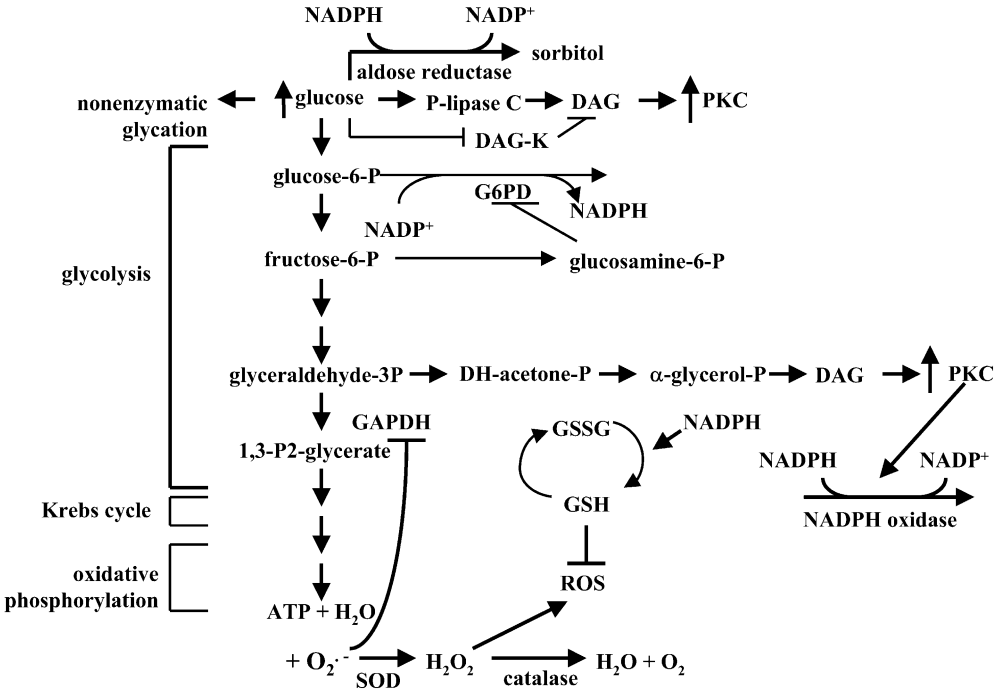

Fig. 1 Schematic diagram of pathways that contribute to oxidative stress in response to increased glucose flux

reduced glutathione (GSH) from oxidized glutathione (GSSG), and second, by decreasing availability of NADPH, thereby decreasing activity of catalase, the enzyme responsible for converting the reactive oxygen species (ROS), H_2O_2, to H_2O. Indeed, glutathione scavenging activity and NADPH content are decreased in vascular endothelial tissues by high glucose conditions (Kashiwagi et al. 1996). In addition, in vascular tissue, inflammation can stimulate inducible nitric oxide synthase (iNOS) expression by macrophages and smooth muscle cells, leading to the free radical, nitric oxide ($NO^{\cdot}$). $NO^{\cdot}$ can react with $O_2^{\cdot -}$ to produce the highly reactive peroxynitrite ($ONOO^{\cdot -}$), which can increase lipid peroxidation, protein nitration, and oxidize LDL (Griendling and FitzGerald 2003). In addition to oxidative stress resulting from glucose flux and metabolism, metabolism of free fatty acids (FFA), which are elevated in diabetes and insulin-resistance, requires β-hydroxylation and utilizes acetyl-CoA. FFA metabolism can increase oxidant status, as demonstrated by activation of PKC activity and a decrease in the NF-κB inhibitor, IκB-α (Itani et al. 2002). Thus, there is no single pathway by which oxidative stress is increased by diabetes-induced hyperglycemia. This being said, if oxidative stress is ultimately responsible for the pathogenesis of diabetic complications, it may be particularly difficult to prevent or attenuate the adverse effects of hyperglycemia, owing to the multiple pathways by which hyperglycemia causes oxidative stress.

Mechanisms by which oxidative stress may be responsible for the pathology of diabetic complications

There is increasing evidence that acute changes in redox status of enzymes and transcription factors, as well as changing ratios of NAD^+ cofactors, participates in signaling during normal cellular physiology. For example, H_2O_2, resulting from mitochondrial metabolism, can activate the c-Jun N-terminal kinase (JNK) and inhibit glycogen synthase activity (Nemoto et al. 2000), and production of H_2O_2 is necessary to activate DNA synthesis in response to growth factors such as PDGF (Sundaresan et al. 1995). Expression of circadian rhythm-responsive genes has recently been shown to be regulated by fluctuations of $NADP^+$ and NADPH (Rutter et al. 2001), and NAD^+ increases the association of GAPDH with a transcriptional complex that activates expression of the gene encoding histone H2B during S phase (Zheng et al. 2003).

However, chronic or excessive increases in oxidant production can adversely affect cellular physiology and function. For example, if PKC or NOS activities are necessary for normal tissue function, excessive PKC and NOS activities may disturb the normal function of affected tissues. Furthermore, while physiological production of H_2O_2 promotes cell cycle progression in response to growth factors, excessive production of H_2O_2 does not accelerate DNA synthesis, but, paradoxically, may induce cell cycle arrest by oxidation-induced degradation of Cdc25C (Savitsky and Finkel 2002). Chronic or excessive oxidative stress may interfere with the normal function of tissues affected by diabetic hyperglycemia, by increasing blood flow and disturbing hemodynamics in the retina

(Kunisaki et al. 1995; Kowluru and Kennedy 2001), contractility of vascular smooth muscle cells (Sharpe et al. 1998), and decreasing neural conductivity in peripheral nerves (Hounsom et al. 2001). These defects may, in part, be due to altered activities of signaling pathways that regulate differentiated function of these tissues. In addition, differentiation of progenitor cells that serve to repair and regenerate differentiated tissues may be impaired (Stepanovic et al. 2003). In diabetic complications, the effects of oxidative stress may not be sufficient to elicit massive tissue destruction, nevertheless, oxidant damage in individual cells may reach sufficient threshold to cause DNA strand breaks and induce cell death (Du et al. 2003), and this may impair the integrity and function of the entire tissue.

In addition to affecting the static function of signaling pathways, hyperglycemia-induced oxidative stress can affect gene expression. Oxidative stress induces expression of genes which attempt to protect cells from oxidant-induced damage of proteins, DNA, and lipids. For example, genes encoding free radical scavenging enzymes, and enzymes that regulate DNA repair or cell cycle arrest, including genes which stabilize p53, are induced by oxidative stress (Hancock et al. 2001; Liu et al. 2001; Napoli et al. 2001; Owuor and Kong 2002; Nicoletti and Stella 2003). Many of these genes are regulated by NF-κB. Normally, NF-κB is maintained in an inactive state in the cytoplasm due to association with IκB, however, in response to cellular disturbances, such as inflammation or oxidative stress, IκB is phosphorylated by Iκ-kinase, and NF-κB is released to activate genes to restore appropriate redox homeostasis (Garcia-Ruiz et al. 1995; Ozes et al. 1999; Yamamoto and Gaynor 2001). If restoration of homeostasis is unsuccessful, or of sufficient magnitude, oxidative stress will induce cell death through activation of signal transduction pathways, including the p38/mitogen-activated protein kinase (MAPK) pathway, and caspases, poly-ADP-ribosyltransferase (PARP), and p53-dependent activation of proapoptotic genes and repression of antiapoptotic genes (Owuor and Kong 2002; Nicoletti and Stella 2003; Oren 2003). Oxidation can also alter DNA binding of transcription factors such as NF-κB and p53 by oxidizing or nitrosylating free thiols in the DNA binding regions (Marshall et al. 2000). There are likely to be other mechanisms by which oxidative stress can regulate gene expression that do not involve NF-κB. For example, oxidative stress inhibits expression of the developmental control gene, *Pax3* (Chang et al. 2003), and the segment of DNA which is required for oxidative stress inhibition does not contain a binding element for NF-κB or any other redox-recognized transcription factor (Wang and Loeken, unpublished results).

In addition to altering expression of genes that are involved in cell survival or death, oxidative stress can interfere with the expression of genes that are necessary for differentiated cell function. For example, hyperglycemia-induced pathways activate expression of genes encoding the IGF-1 receptor, TGF-βI, plasminogen activator I, and endothelin 1 in renal glomeruli (Sugimoto et al. 1996; Hoffman et al. 1998; Benigni et al. 2000; Goldberg et al. 2002) (although inhibition of endothelin 1 has also been reported; Shin et al. 1995), and activate expression of vascular endothelial growth factor in vascular smooth muscle cells (Natarajan et al. 1997). Yet, whether altered expression of these genes is precipitated by oxidative stress, and whether altered gene expression is primarily responsible for the pathogenesis of the diabetic complication can be difficult to demonstrate. However, in diabetic embryopathy, a diabetic complication in which the early embryo of a mother with diabetes develops congenital malformations, there is evidence that oxidative stress-induced alteration of gene expression is primarily responsible. Many laboratories have shown that oxidative stress is increased in rodent embryos in experimental models of diabetic embryopathy (Hagay et al. 1995; Trocino et al. 1995; Eriksson and Siman 1996; Sivan et al. 1996; Viana et al. 1996; Siman and Eriksson 1997a, b; Yang et al. 1997; Wentzel and Eriksson 1998; Wentzel et al. 1999). Hyperglycemia-induced oxidative stress is associated with altered expression of genes that regulate redox status in embryos (Forsberg et al. 1996; Wentzel et al. 1999; Cederberg et al. 2000). Maternal hyperglycemia alters expression of genes that control essential developmental processes, such as *Pax3*, which controls neural tube and neural crest development (Phelan et al. 1997; Fine et al. 1999), and oxidative stress mediates the adverse effects of hyperglycemia to inhibit *Pax3* expression (Chang et al. 2003). As a consequence of insufficient *Pax3* expression, differentiation of Pax3-dependent tissues fails, and malformations such as neural tube and cardiac outflow tract defects occur. There are likely to be other developmental control genes whose expression is impaired by oxidative stress and lead to maldevelopment of other organs. Thus, determining how oxidative stress disturbs expression of genes which control differentiation or differentiated function will reveal essential processes in the progression of diabetic complications.

Is oxidative stress responsible for pathology?

The previous discussion cites evidence that diabetes- or insulin resistance-induced hyperglycemia causes oxidative stress, and that if oxidant induction of homeostatic mechanisms are insufficient to restore physiologic redox status, normal cellular function could be impaired, and tissue integrity could be affected. However, demonstration that hyperglycemia-induced oxidative stress is primarily responsible for the pathology of diabetic complications has been difficult, and may be tissue-specific. For example, certain microvascular complications, such as retinopathy and nephropathy, fail to occur in nondiabetic insulin-resistant patients, even though these tissues generate increased $O_2^{\cdot -}$ production in response to excess glucose and FFA. This could indicate that other metabolic disturbances subsequent to the onset of diabetes contribute to these complications, or else that there is a threshold effect required to elicit tissue damage. On the

other hand, there is evidence, both from in vivo and in vitro studies with animal models, that ameliorating oxidative stress can prevent or attenuate several diabetic complications, including nephropathy, retinopathy, macrovasculopathy, and embryopathy. Specifically, vitamin E normalizes retinal blood flow and PKC activity in vascular tissue of diabetic rats (Kunisaki et al. 1995, 1996). Diabetic embryopathy of rat or mouse embryos is prevented by vitamin C, vitamin E, superoxide dismutase, *N*-acetyl-cysteine, or glutathione ethyl ester (Sivan et al. 1996; Viana et al. 1996; Siman and Eriksson 1997b; Wentzel et al. 1997; Chang et al. 2003). In addition, inhibition of the polyol pathway with aldose reductase inhibitors may reduce the effects of hyperglycemia on diabetic nephropathy, and this appears to be due to decreased oxidative stress (Dunlop 2000). Moreover, preventing mitochondrial $O_2^{\cdot -}$ production by an inhibitor of electron transport or an uncoupler of oxidative phosphorylation, or increased expression of superoxide dismutase, prevent the increase in PKC activity, formation of advanced glycation end-products, activity of the polyol pathway, and NF-κB activation in cultured bovine endothelial cells (Nishikawa et al. 2000).

Despite this experimental evidence, results of clinical studies have not provided compelling evidence that antioxidants prevent the progression of diabetic complications. In support of positive benefit from antioxidant treatment, α-lipoic acid has provided relief from diabetic neuropathy, but this could, in part, be due to increased glucose transport into fat and muscle, thereby decreasing oxidant production (Greene et al. 2001; Konrad et al. 2001; Packer et al. 2001). Tests of vitamin C, alone or in combination with vitamin E, have been shown to improve microalbuminuria and endothelium-dependent vasodilation (Beckman et al. 2001; Gaede et al. 2001). In an 8-month-long study of 36 patients with type 1 diabetes for less than 10 years duration, high doses (1,800 IU/day) of vitamin E restored normal retinal blood flow and renal filtration (Bursell et al. 1999). Similarly, in two other studies (Secondary Prevention with Antioxidants of Cardiovascular Disease in End-Stage Renal Disease, or SPACE, and Cambridge Heart Antioxidant Study, or CHAOS) of patients (not necessarily diabetic) who either had preexisting ischemic heart disease or were on kidney dialysis, 400 or 800 IU vitamin E prevented myocardial infarction (Stephens et al. 1996; Boaz et al. 2000). However, these studies were of relatively short duration and small sample size. Notably, in the 4-year-long Heart Outcomes Prevention Evaluation (HOPE) study of more than 3,600 diabetic patients, some of whom already displayed microalbuminuria, 400 IU/day of vitamin E in combination with an ACE inhibitor did not provide microvascular or cardiovascular protection (Lonn et al. 2002). There are several differences in design of each of these studies which may reconcile these seemingly contradictory observations. First, antioxidants may provide short-term relief of oxidative stress and restore normal function, but it may be difficult to provide sufficient antioxidants like vitamin E or vitamin C that are not enzymatically regenerated (unlike GSH) at sufficient concentrations to scavenge free radicals on a long-term basis. Second, in tissues of patients with long-standing diabetes and preexisting structural and functional pathology, it may be impossible to eliminate or reverse tissue damage. Third, if patterns of gene expression have been altered by oxidative stress, it may not be possible to reverse this process and restore normal patterns of gene expression.

Therapeutic potential to prevent diabetic complications by blocking oxidant effects

As indicated above, it may not be possible to completely reverse diabetic complications with antioxidants once they have been established, but further investigation is needed to determine whether it may be possible to slow down progression, or prevent the onset of complications by preemptive therapy prior to the development of tissue damage. Certainly, use of uncouplers to prevent the increase in mitochondrial $O_2^{\cdot -}$ production would not be of therapeutic value. However, inhibition of specific pathways that are activated as a consequence of increased oxidative stress and glucose flux may be sufficient to prevent certain complications. For example, use of specific PKC inhibitors slows the progression of diabetic vascular dysfunction in rats (Ishii et al. 1996) and may be useful to treat diabetic retinopathy and nephropathy in patients. There may be novel agents, such as the thiamine derivative, benfotiamine, which can prevent the stimulation of the hexosamine pathway, PKC activity, and production of advanced glycation end-products, through activation of the pentose shunt pathway (Hammes et al. 2003). While use of high doses of a natural antioxidant such as vitamin E may seem preferable to pharmacologic intervention, whether accumulation of the lipid-soluble vitamin following long-term administration could have adverse consequences should be investigated. It should also be remembered that oxidative stress may not be the precipitating event leading to all diabetic complications. For example, individuals with insulin resistance are exposed to hyperglycemia and FFA-induced oxidative stress, and yet they do not develop the pathology of the retina and kidney that occur in diabetic patients. Similarly, oxidative damage accumulates during natural aging, and yet retinopathy and nephropathy do not occur in the elderly who do not have diabetes. Therefore, there may be specific effects of diabetes on some tissues that leads to pathology at the tissue level, in which oxidative stress is not the primary factor.

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