An Insight in to the Pathogenesis of Diabetic Vascular Diseases: Role of Oxidative Stress and Antioxidants

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Abstract

The profound effects of hyperglycaemia on the vascular tree are the major causes of morbidity and mortality among patients suffering from diabetes. Diabetic Vascular Diseases (DVD) includes accelerated forms of atherosclerosis due to endothelial dysfunction and microangiopathy of retinal vessels. A host of several studies indicate that increased oxidative stress play a pivotal role in the development and progression of diabetic vascular diseases. The metabolic abnormalities due to oxidative stress are linked to the structural and functional changes in the vasculature, consequently resulting in atherosclerosis and diabetic retinopathy. Oxidative stress brings alterations in downstream transcription factors which result in changes in gene expression, myocardial substrate utilization, myocyte growth, endothelial function and myocardial compliance. Based on this, an approach towards investigating new and effective antioxidant therapies could serve as potential therapeutic implications in preventing the deleterious effects of oxidative stress on vasculature.

This review aims to understand the underlying mechanisms involved in the pathogenesis of vascular complications in diabetes with special emphasis on the role of oxidative stress towards development of these complications and also describe the role of antioxidants as therapeutic interventions for DVD.

Keywords: Hyperglycaemia; Oxidative stress; Endothelial dysfunction; Atherosclerosis; Diabetic retinopathy

Introduction

Diabetes mellitus is a metabolic disorder, characterized by defective or deficient insulin secretory process, glucose underutilization and increased blood sugar. It is congenital or acquired inability to transport sugar from blood stream into the cells. Multiple etiologies have been found which segregates diabetes into two major forms: Type I or insulin dependent diabetes mellitus (IDDM) and Type II or non-insulin dependent diabetes mellitus (NIDDM) [1]. Type I diabetes is an autoimmune disease in which patient’s immune system react against islet antigens and destroy the beta cell. Type II diabetes is a polygenic syndrome characterized by insulin resistance with more severe β-cell deficiency [1]. Both the types are associated with characteristic long term complications. The number of people with diabetes is increasing rapidly due to aging population, growth of population size, urbanization and high prevalence of obesity and sedentary lifestyle [2]. As per International Diabetes Federation, about 365 million people suffered from diabetes in 2011 and this number is expected to rise up to 552 million by 2030 [3].

Diabetic vascular diseases (DVD) are the most serious microvascular and macrovascular complications of diabetes. Vascular complications in diabetes include accelerated forms of atherosclerosis due to endothelial dysfunction and microangiopathy of retinal vessels. The underlying molecular mechanisms for DVD are still debatable but hyperglycaemia-induced oxidative stress has been proposed as the major precipitating factor in various studies [4-6].

This review article highlights the molecular mechanisms behind the development of vascular complications in diabetes with main focus on the role of oxidative stress as well as the implication of antioxidants for the prevention of DVD.

Metabolic Status in Diabetes

The metabolic effects of insulin is closely related to its effect on the vascular system, such that the effect of insulin on the endothelium augments its metabolic effect [7]. The major role of insulin in the vascular system is to stimulate the production of endothelial derived Nitric Oxide (NO) which mediates vasodilation [8]. In normal circumstances, insulin mediated activation of insulin receptors in the endothelium and glucose processing organs such as adipose tissue and skeletal muscle, leads to a activation of downstream signaling pathways.

Initially phosphorylation of Insulin Receptor Substrate (IRS) activates phosphotyrosilninostol-3-kinase (PI3K) [9], which then generates phosphotyrosilninostol-3,4,5-triphosphate (PIP3). PIP3 becomes phosphorylated to activate a serine kinase phosphoinositide-dependent kinase (PDK) which ultimately activates Akt, a serine/threonine protein kinase. Akt directly phosphorylate to activate a serine kinase phosphoinositide-dependent kinase (PDK) which ultimately activates Akt, a serine/threonine protein kinase. Akt directly phosphorylate endothelial NO synthase (eNOS) in the endothelium and GLUT-4 translocation in glucose processing organs [10], leading to vasodilation which facilitates the glucose uptake. Alteration in insulin signaling along with other metabolic disturbances such as increase in the levels of free fatty acids (FFA) causes inhibition of IRS phosphorylation, thereby inhibiting the downstream signaling and ultimately causing endothelial dysfunction and insulin resistance, as shown in Figure 1 [11].

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Complications in Diabetes

In chronic hyperglycaemia, the sugar that normally serves as substrate, fuel and signal takes on the darker role of toxin [12] and is the major cause of the harmful effects of diabetes on various tissues of body [13]. The deleterious effects include macrovascular complications such as coronary artery disease, stroke and peripheral artery disease and microvascular complications such as retinopathy, nephropathy and neuropathy [14,15].

Vascular Complications in Diabetes

Among the several listed complications, effects of diabetes on the vascular tree are the major causes of mortality and morbidity in both types of diabetes. In light of this fact, it becomes more important to understand the relation between diabetes and vascular diseases. The vascular complications of diabetes include accelerated forms of atherosclerosis, increased risk of myocardial infarction, stroke and microangiopathy of renal and retinal vessels [16].

The central pathogenic mechanism for the vascular complications is the process of atherosclerosis which serves as a major risk factor for myocardial infarction, ischemic heart disease and stroke. Diabetes, as a metabolic syndrome, leads to abdominal obesity and hyperlipidemia which in turn promote vascular complications independent of atherosclerosis [17]. Finally, high glucose in diabetes can itself directly affect the myocardium and hence an independent cause of heart failure [18,19]. Recent clinical studies demonstrated that the risk of myocardial infarction (MI) in people with diabetes is equivalent to risk in non-diabetics with a previous history of MI [20]. In view of such facts American Heart Association and American Diabetes Association has recommended that diabetes should be considered as coronary artery disease risk equivalent rather than a risk factor [21].

Risk of stroke related dementia and stroke related mortality is also elevated in diabetic patients [22]. The DCCT trial demonstrated 42% risk reduction in all cardiovascular events and 57% reduction in stroke and death from CVD following intensive treatment of Type I diabetic patients [23].

Besides these macrovascular complications, microvascular complications involving smaller blood vessels like renal and retinal vasculature result in equally devastating consequences. Retinal complications termed as retinopathy, eventually leading to blindness, and begins to develop as early as seven years before the clinical diagnosis of diabetes and thus is the earliest complication of diabetes [24]. Evidences suggest that incidence of diabetic retinopathy can be reduced in 90% of the cases with early treatment [25].

Cardiovascular Complications in Diabetes

Cardiovascular diseases accounts for upto 80% of premature mortality in diabetic patients [26]. The major cardiovascular diseases related to diabetes include atherosclerosis which is a major risk factor for Coronary Artery Disease (CAD) [27]. Enhanced myocardial dysfunction in diabetes leads to accelerated heart failure independent of CAD and is more specifically termed as diabetic cardiomyopathy [28]. In addition prolonged hypertension, chronic uncontrolled hyperglycemia, microvascular diseases, glycosylation of myocardial protein and autonomic neuropathy aggravate the development of congestive heart failure in diabetic heart [29].

The cardiovascular complications of diabetes were earlier considered to be caused by slowly progressing structural changes. But with the emergence of modern techniques, it is now clear that cardiac dysfunction occurs soon after the development of metabolic abnormalities and much before the development of any structural changes. This suggest that complex events occurring at cellular and molecular level plays a major role in the development of cardiovascular dysfunction in diabetes [30,31].

Retinal Complications in Diabetes

Diabetic retinopathy is the most common and tissue specific...
microvascular complication of diabetes. Its effect as impairment of vision is well established but its importance beyond visual impairment remains to be explored. Studies reveal that people with diabetic retinopathy have high risk of developing systemic vascular complications like CAD, heart failure and nephropathy which is attributed to a common genetic link between retinopathy and other systemic vascular complications [32-34].

Vascular and neuronal degeneration are the prime changes that manifest themselves as diabetic retinopathy [35,36]. The features of diabetic retinopathy include increase in vascular permeability, neovascularisation, retinal cell apoptosis and leukocyte adhesion [37,38].

Several factors like accumulation of advanced glycation end products (AGE), polyol pathway, Protein kinase C activation, increased expression of growth factors and oxidative stress have been implicated in the pathogenesis of diabetic retinopathy [24]. Out of these, oxidative stress plays a crucial role in mediating the vascular dysfunction associated with diabetic retinal changes [39,40].

9. Role of Oxidative Stress in Diabetic Vascular Complications

Oxidative stress is defined as excess formation or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) which includes free radicals such as superoxide (O$_2^-$), hydroxyl (OH$^-$), peroxyl (•RO$_2^-$), hydroperoxyl (•HRO$_2^-$) as well as non-radical species such as hydrogen peroxide (H$_2$O$_2$) and hypochlorous acid (HOCl) [41]. ROS generated under physiological conditions is required for cell signalling and defence mechanisms but excess generation of ROS play an important role in the pathological event. In diabetes, ROS is the major determinant for the poor prognosis of vascular complications [42]. The molecular alterations leading to increased ROS/RNS production include increased expression of NADPH oxidase, formation of advanced glycation end products (AGEs) and activation of protein kinase-C, endothelial nitric oxide synthase uncoupling (eNOS) and polyol pathway [42,43].

Overproduction of ROS/RNS cause damage to cell structures, nucleic acid, lipids and proteins [44,45]. Oxidative stress can also result in damage to protein transporters, increase the concentration of intracellular calcium and lipid peroxidation [46]. These events lead to activation of stress sensitive pathways like NF-κB, p38-MAPK, JNK/ SAPK that are ultimately responsible for insulin resistance, β-cell activation of stress sensitive pathways like NF-κB, p38-MAPK, JNK/intracellular calcium and lipid peroxidation [46]. These events lead to activation of stress sensitive pathways like NF-κB, p38-MAPK, JNK/ SAPK that are ultimately responsible for insulin resistance, β-cell activation of stress sensitive pathways like NF-κB, p38-MAPK, JNK/INOS and polyol pathway [42,43].

Oxidative stress mediated endothelial dysfunction

Endothelial dysfunction is the prime manifestation of vascular complications in diabetes [50]. It involves the loss of activity of NO, leading to increased in the activity of Nf-kB, that causes increased production of chemokines and cytokines which are ultimately responsible for atherosclerotic changes [51]. Besides mediating vasodilation, NO also cause inhibition of monocyte adhesion, inhibition of vascular smooth muscle cell proliferation, inhibition of platelet adhesion and activation of intrinsic coagulation pathway [52]. All these factors have an important role in the pathogenesis of atherosclerosis [53]. Therefore, decreased production of NO in diabetes is one of the major molecular factor leading to vascular complications.

Diabetes induced oxidative stress further deteriorates the situation as excess superoxide radical react with available NO, thereby generating cytotoxic peroxynitrite (ONOO$^-$) [54]. Formation of peroxynitrite inturn inactivates NO leading to decreased NO as explained above [52]. In addition to the inactivation of NO, ONOO-also alters the function of other biomolecules by mediating protein nitration and lipid peroxidation. Protein nitration inhibits potassium channels that normally mediate vascular relaxation [55]. ONOO-further causes oxidation of tetrahydrobiopterin, which is a cofactor for nitric oxide synthase (NOS). This leads to uncoupling of NOS which then produces superoxide radical instead of NO, thereby leading to increased risk of vascular complications [56]. In addition to superoxide radical, increased production of hydrogen peroxide by high glucose leads to apoptosis and pathologic angiogenesis in endothelial cells [57].

Pro-atherosclerotic Effects of Oxidative Stress

Besides oxidative stress, dyslipidemia is also a common metabolic disturbance associated with diabetes, as 97% of diabetics are dyslipidemic [58-60]. Atherogenic dyslipidemia, is characterised by increased levels of very low density lipoprotein (VLDL), low density lipoprotein (LDL) and decreased high density lipoprotein (HDL) [61,62].

Oxidative stress due to diabetes leads to oxidation of the LDL particles, producing oxidized LDL which are pro-atherogenic, and behave as foreign particle to the immune system. It thus, produces abnormal biological response leading to plaque formation (Figure 2), in atherosclerosis and other associated vascular dysfunctions in diabetes [63,64].

Oxidative stress in diabetes increases the formation of AGE (vide infra) [67], which in turn leads to glycation of LDL and HDL particles that eventually changes the half-life of LDL and HDL. Increase in half-life of LDL increases its pro-atherogenic potential and decrease in half-life of HDL decreases its protective effect against atherosclerosis [68-70] (Figure 3).

Oxidative Stress in the Development of Microangiopathy

Retinopathy is the common clinical implication due to diabetes induced microangiopathy. Increased production of AGE and its expression on its receptor RAGE in retinal microvasculature in people with diabetes leads to increased protein kinase C (PKC) [71,72]. This PKC increase the oxidative stress in retina which in turn increases the accumulation of AGE in retinal microvasculature [67]. This positive feedback system leads to consequences such as retinal cell apoptosis, neovascularisation, activation of NF-κB, microaneurysm and loss of function of other biomolecules by mediating protein nitration and oxidation of tetrahydrobiopterin, which is a cofactor for nitric oxide synthase (NOS). This leads to uncoupling of NOS which then produces superoxide radical instead of NO, thereby leading to increased risk of vascular complications [56]. In addition to superoxide radical, increased production of hydrogen peroxide by high glucose leads to apoptosis and pathologic angiogenesis in endothelial cells [57].
Figure 2: Atherogenic dyslipidemia in diabetes leading to vascular complications and involvement of oxidative stress.

Figure 3: Role of glycation of lipoproteins in the etiology of atherosclerosis and the involvement of oxidative stress.
PKC, among which PKC-β is the major one activated in vasculature and retina [76]. PKC isoform regulates vascular permeability, blood flow and neovascularisation and increased PKC activation can therefore lead to changes such as increase in permeability of retinal vessel, neovascularisation and endothelial proliferation [79,80].

Oxidative stress also induces microangiopathy through indirect mechanisms like increased expression of vascular endothelial growth factor (VEGF), a proangiogenic factor as observed in diabetic retinopathy [81]. Physiologically, VEGF has a key role in embryonic and early postnatal vasculogenesis and angiogenesis. In adults, it occurs at several sites in the vascular bed and is a potent vasodilator. It has the capacity to promote formation of collateral vessels that has role in ischemic injury and wound healing [82]. In the pathological states such as oxidative stress, the physiological roles of VEGF take the darker side and its excess activation leads to consequences like hyperpermeability of retinal membrane, angiogenesis [83]. These changes are responsible for visual loss in case of diabetic retinopathy (Figure 4).

In addition to retinopathy, we recently demonstrated diabetes induced microangiopathy in the bone marrow due to increased intracellular oxidative stress [84]. Bone marrow microangiopathy lead to the endothelial cell dysfunction, leaky endothelial cell barrier, thereby affecting the survival of bone marrow stem cells. Treating the diabetic animals with anti-oxidant Benfotiamine markedly reduced the microangiopathy, thereby confirming the role of oxidative stress in the development of microangiopathy [84]. Further, we also demonstrated that diabetes induces bone marrow endothelial cells barrier dysfunction through activation of RhoA-Rho-associated kinase pathway, a potent activator of oxidative stress [85].

These evidences provide a clear role for oxidative stress in the development of diabetes induced microangiopathy, thereby suggesting the potential therapeutic role for anti-oxidants in people with diabetes to prevent or delay the development of vascular complications.

Role of Antioxidants in Preventing DVD

Diabetes has reached epidemic proportion fuelled by an aging population and rapidly increasing obesity. Among the varieties of complications associated with diabetes, vascular complications are the leading cause of death and disability among the people with diabetes [26]. While, by convention, the focus of the drug therapy in diabetes mellitus is on the glycaemic control, there is an urgent need towards addressing the associated complications due to diabetes. Hyperglycaemia-generated ROS appears to be an important element in the genesis of severe diabetic complications including atherosclerosis and microangiopathy. Antioxidants have emerged as an effective therapy in the prevention of DVD. Besides their role in inhibiting the activity of ROS, antioxidants have been reported to exhibit protective effects through various mechanisms [86]. A well-established role of oxidative stress is also supported by the various epidemiological, experimental as well as clinical studies, showing protective effects following long term administration of antioxidants by several mechanisms [79,87-91].

Among various antioxidants reported to exert beneficial effects against diabetic vascular damage, antioxidants such as vitamin C, vitamin E, β-carotene, α-lipoic acid, bioflavonoids, as well as phenolic constituents present in various medicinal plants have been well recognized. Lynch et al. [92] demonstrated the protective role of Vitamin C in the development of atherosclerosis via inhibition of oxidation of LDL, in addition to its well-recognized role in neutralizing the ROS. Vitamin E supplementation has also been reported to possess beneficial role in preventing and delaying the onset of cardiovascular events in diabetic patients by reducing lipoproteins and reducing the activity of oxidative stress enzymes such as glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) [93,94]. Another natural antioxidant, Rosmarinic acid, has been found to protect aortic endothelial function and structural alterations in diabetic rats by through its anti-inflammatory and antioxidant properties [95].

The antioxidant and anti-atherogenic properties of turaine have been known from past two decades [96,97]. A study by Wang et al. [98] demonstrated the protective effects of Tauarine against diabetes-mediated vascular endothelial dysfunction by downregulating the expression of lectin-like oxidLDL receptor-1 (LOX-1) a well-known receptor for intercellular adhesion molecule-1 (ICAM-1) in the aorta. In line with these findings, the beneficial role of turaine on diabetic vasculature was extensively evaluated by other experimental and clinical studies [99,100]. Zhu et al. [101] investigated the rescue potential of lycopene, a potent antioxidant compound, in endothelial dysfunction by reducing the oxidative stress, hence indicating its use in preventing DVD associated with endothelial dysfunction.

Ginkgo biloba, known to be rich in antioxidant constituents is recently reported to exhibit beneficial effects in diabetic retinopathy by improving retinal capillary blood flow rate in type II diabetic individuals [102,103]. Recently, Kumar et al. [104,105] investigated the retinoprotective properties of Moringa oleifera in STZ-diabetic rats. M. oleifera exhibit its retinoprotective effect via antioxidant, anti-inflammatory, and anti-angiogenic mechanisms, which is attributed to its antioxidant constituents. Similarly, another naturally occurring phenolic antioxidant, Resveratrol, found in wine has been demonstrated to decrease vascular lesions and VEGF induction in retinas of diabetic mice, suggesting its mitigating role in diabetic retinopathy [106]. Citrus fruits are rich in flavonoids that have high antioxidant potential. Hesperetin, a dietary flavanoid, found in many citrus fruits is reported to possess ameliorative effect in diabetes induced vasculopathy via anti-angiogenic mechanism by inhibition in the expression of VEGF and PKC-β [105]. In another study 106 these authors also reported the ameliorative effect of hesperetin in diabetic retinopathy via inhibiting retinal oxidative stress, neovascularisation as well as apoptosis in STZ-induced diabetic rats.

In addition to the pharmacological management of oxidative stress to combat the vascular complications of diabetes, recent advances in the understanding of underlying molecular mechanisms has resulted in the development of novel therapies for the treatment of DVD. This includes gene therapy for modulation of pro-survival genes [107] and most recently the therapeutic modulation of microRNAs for the post-
transcriptional regulation of genes involved in the development of DVD [108]. While these studies have provided promising results in the pre-clinical setting, a translation research in larger diabetic population will be required to investigate the therapeutic outcome of these novel therapeutic modalities.

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