Diabetic nephropathy: Aggressive involvement of oxidative stress

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ABSTRACT

Diabetic nephropathy is a major microvascular complication of diabetes mellitus and the most common cause of end-stage renal disease worldwide. Hyperglycemia, a well-recognized pathogenetic factor of long-term complications in diabetes mellitus, generates reactive oxygen species (ROS) mainly through NADPH oxidase, polyol pathway and advanced glycation products. Further, diabetes activates protein kinase C which promotes excessive formation of oxidative stress in the renal cellular milieu. Additionally, ROS mediate hyperglycemia-induced activation of signal transduction cascades and transcription factors leading to transcriptional activation of profibrotic genes in the kidney. However, maintaining intensive glycemic control and timely use of current therapies may help to reduce oxidative stress mediated progression of diabetic nephropathy.

Keywords: Diabetic nephropathy, reactive oxygen species, glycation, polyol pathway, NADPH oxidase pathway

Introduction

Diabetes is a metabolic disorder characterized by hyperglycemia and often leads to numerous microvascular complications, including nephropathy. Diabetic nephropathy is a major risk factor for cardiovascular disease, and the majority of individuals with diabetic nephropathy die of cardiovascular disease. The increased oxidative stress was noted in the poor glycemic control patients with chronic diabetes mellitus. High intracellular glucose concentration in chronic diabetes mellitus causes activation of polyol pathway (also known as the sorbitol–aldose reductase pathway), hexosamine biosynthetic pathway and PKC are major sources of reactive oxygen species (ROS), which are collectively involved in the pathogenesis of diabetic nephropathy. ROS are believed to play a key role in the pathogenesis of diabetic complications because of their reactive chemical property to directly oxidize and damage DNA, protein, lipid, and carbohydrate. ROS activates signal transduction cascades and transcription factors leading to transcriptional activation of profibrotic genes. Further, PKC, transforming growth factor-α1 (TGF-α1) and angiotensin II (Ang II) stimulated by hyperglycemia-induced ROS, in turn, generate and signal through ROS and thus involve in glomerular mesangial expansion and tubulointerstitial fibrosis. It is now clear that the overproduction of ROS in diabetes is a direct consequence of hyperglycemia and various types of cells including endothelial, vascular smooth muscle, mesangial, and tubular epithelial cells, are capable of producing ROS under hyperglycemic condition. Interestingly, in early diabetic nephropathy, dysfunction of vascular endothelial cells is a major consequence in the pathogenesis of diabetic nephropathy.

These observations indicated that hyperglycemia is the major risk factor for microvascular complications of diabetes. In addition to maintaining glycemic control, strategies to reduce oxidative stress during diabetes mellitus may brandish beneficial effects on progression of diabetic nephropathy. In this review include evidence that ROS play an important pathogenic role in the development of diabetic nephropathy, and will explore major therapeutic interventions.

Reactive oxygen species (ROS)

ROS are continuously generated in physiological condition and are effectively eliminated by several antioxidative systems. ROS includes mainly superoxide or hydroxyl radicals and other are alkoxyl, peroxyl, plus non-radical derivatives of oxygen, specifically hydrogen peroxide and ozone, which plays a major part in cell signaling, ageing and degenerative disease. The amount of ROS produced is finely balanced with the antioxidant...
activity and when it exceeds cellular defence power or diminishes the production of antioxidants can lead to increased oxidant-derived tissue injury or oxidative stress.  

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**Sources of ROS in diabetes mellitus**

The high glucose concentration in chronic diabetes mellitus induces oxidative stress by generating ROS through an activation of number of enzymatic and non-enzymatic sources in the body. The major sources of ROS in diabetes include polyol pathway, advanced glycation and uncoupling of NADPH oxidases (Fig 1).

**NADPH oxidase pathway**

In diabetes, NADPH oxidase is a major source of generation of ROS. NADPH oxidase is located in plasma membrane of various renal cell types, including mesangial and proximal tubular cells, vascular smooth muscle cells, endothelial cells and fibroblasts.  

The NADPH oxidase complex comprises several isoforms, now designated as the nox family, particularly nox4 isoform, a 578-amino acid protein and a major source of ROS in the renal milieu and thus NADPH oxidase dependent overproduction of ROS play a key role in promoting hyperglycemia-induced oxidative stress. The NADPH oxidase increase oxidative stress and finally results in development of diabetic nephropathy in rats. In addition to this, by increasing oxidative stress in renal milieu, it results in increased expression of fibronectin and collagen-1, mesangial expansion and albuminuria, which

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**Figure 1.** Figure depicting the major sources of ROS pertaining in the glucotoxicity pathways in diabetic nephropathy. AGE, advanced glycation end products; RAGE, receptors for advanced glycation end products; ROS, reactive oxygen species; NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase; PKC, protein kinase C; NFκB, nuclear factor κB; TGF-β1, transforming growth factor-β1; AR, aldose reductase; SDH, sorbitol dehydrogenase; CTGF, connective tissue growth factors.
were markedly attenuated by the treatment with apocynin, a specific inhibitor of NADPH oxidases. Thus, NADPH oxidase may be one of potential target deserving further investigation in the development of drug in the treatment of diabetic nephropathy.

**Polyol pathway**

In cell, unused glucose in the cytosol is diverted to the polyol pathway, which involves two enzymatic reactions: the first is the reduction of glucose to sorbitol by the action of aldose reductase, and the second oxidation of sorbitol to fructose by the action of sorbitol dehydrogenase. Reduction of glucose to sorbitol uses NADPH and oxidation of sorbitol increases NADH with a resultant rapid change in the cytoplasmic redox state and enhanced production of ROS. Decreased NADPH may compromise reduction of glutathione in oxidatively stressed cells. However, chronic hyperglycemia increases ROS generation and causes excessive consumption of NADPH in the polyol pathway, which inhibits reduced glutathione, the vital substrate for glutathione-peroxidase-mediated cellular antioxidant activity, which ultimately results in disturbance in antioxidant property. Hence, polyol pathway is considered as a major source of ROS generation in the pathogenesis of diabetic nephropathy.

**Advance glycation**

Advanced glycation end products (AGEs) are diverse group of molecules and are well known heterogenous compounds formed nonenzymatically through an interaction of reducing sugar with free amino group of proteins, lipids and nucleic acids. Numerous studies reported that the increased production of AGEs has been implicated in the pathogenesis in diabetic nephropathy. AGEs exert their effects through receptors and binding proteins, such receptors are RAGE and AGE. Further, binding of AGEs with receptor for AGE (RAGE) potentially activates NADPH, which contributes to excessive production of renal ROS and its binding on the tubular surface has been implicated in the pathogenesis of tubular cell injury, potentially via NADPH oxidase. Binding to RAGE activates PKC-α-mediated activation of NADPH oxidase and NFκB, which in turn induces production of various inflammatory cytokines and generation of mitochondrial ROS, thereby, further aggravating oxidative stress in the kidney. In chronic hyperglycemia, excessive ROS production in mitochondria may result in ROS dependent damage to mitochondria and considered as a major event in the pathogenesis of tissue damage in diabetes mellitus. In addition, AGEs stimulate collagen mRNA expression by activating PKC and upregulating TGF-β1 in cultured mesangial cells. The detrimental role of AGEs is further conformed by the fact that AGEs can induce podocyte damage and hypertrophy since these events occurs in the diabetic nephropathy. Growing evidence suggests that AGE-RAGE interaction and resultant production of ROS in the kidney play a pivotal role in the induction and progression of diabetic nephropathy.

**Oxidative stress and kidney damage**

The relation between ROS and DNA is of critical concern as DNA damage may endorse the development and progression of microvascular complications. Diabetic kidney may be mainly vulnerable to structural and functional damage caused by chronic hyperglycemia-induced ROS generation. Various oxidative stress parameters viz. thiobarbituric acid reactive substances (TBARS), reactive carbonyl derivate (RCDs), and total sulfhydryl groups (TSHGs) - in serum and urine of patients with DN have been assessed. Urine TBARS, RCDs, and TSHGs were proposed as possible markers for oxidative damage of kidney in DN. A biomarker of DNA oxidation of guanine residues, 8-hydroxydeoxyguanosine, has been found to be significantly higher in mononuclear cells of subjects with diabetes than in non-diabetic subjects. Chronic hyperglycemia essentially facilitates a state of chronic oxidative stress in the renal milieu, the long-term consequence of which is progressive loss of glomerular and tubular cells. In diabetes, chronic hyperglycemia sustains the oxidative stress by excessive generation of ROS in glomerular and tubular cells, via overexpression of NADPH oxidase and contributes to renal tissue injury. The activation of glomerular sterol regulating element binding protein-1c (SREBP-1c) plays an important role in the progression of diabetic nephropathy by inducing NADPH oxidase-mediated oxidative stress in glomeruli. Diabetes-induced PKC-α activation significantly contributes to renal accumulation of type IV collagen, laminin, and fibronectin by increasing the expression of TGF-β in the glomeruli of diabetic rats. Further, PKC is involved in hyperglycemia-induced...
overexpression of vascular endothelial growth factors (VEGF) in podocytes. Furthermore, supportive study reported that PKC is involved in ROS production in cultured mouse podocytes exposed to high concentration of glucose. Additionally, it has further confirmed that the use of different antioxidants significantly inhibit high glucose-induced activation of PKC in mesangial cells, suggesting that ROS mediate high glucose-induced activation of PKC. Thus, the aggressive involvement of hyperglycemia mediated ROS formation via activation of PKC may exacerbate the diabetic nephropathy, and it further confirms the detrimental role of oxidative stress in diabetic kidney.

Persistent hyperglycemia-induced ROS production has been implicated in early podocyte damage and apoptosis. Recent evidence suggests that glomerular podocytes have a key role in early proteinuria, and reduced podocyte number was reported in patients with diabetic nephropathy. In next step of disease progression, enhanced deposition of the extracellular matrix occurs, owing to increased production and/or decreased degradation of its components such as type IV collagen, laminin and fibronectin. Further, morphological change in diabetic nephropathy such as thickening of the glomerular basement membrane, with expansion of the mesangium, results in diabetic glomerulosclerosis. Experimental studies suggest that amelioration of excessive oxidative stress by antioxidant therapies may potentially prevent glomerular and podocyte damage in diabetic nephropathy.

Enhanced oxidative stress in conjunction with increased angiotensin II (Ang-II) levels activates TGF-β, which stimulates the mesangial matrix synthesis. Activation of renin-angiotensin system (RAS) induced by ROS worsens the renal damage in diabetic nephropathy. Buffering the production of ROS appears to be a promising therapeutic option to ameliorate renal damage from diabetic nephropathy, however, various studies have exhibited a minimal reno-protection by these agents. Interruption of the RAS has yielded much better outcome in terms of reno-protection and progression of diabetic nephropathy. Various aspects of oxidative stress coupled together with the damage induced by RAS have been reported with the anticipation to yield an impetus for designing new generation of specific antioxidants that are potentially more effective to reduce renovascular complications of diabetes. Further, increased Ang-II increases the renal production of ROS by activating NADPH oxidase. Furthermore, TGF-β has been shown to involve in NADPH oxidase mediated generation of ROS in mesangial cells exposed to high glucose. Enhanced and sustained activation of TGF-β by increased ROS production in diabetes mellitus ultimately result in excessive extracellular matrix remodeling in the mesangium and promotion of fibrotic processes in the tubulointerstitium.

**Treatment of diabetic nephropathy: Radical approach**

In the physiological state, some of the major endogenous antioxidant molecules such as superoxide dismutase (SOD), glutathione peroxidase, catalase, heme oxygenase and biliverdin reductase prevents oxidative stress mediated renal cell injury. Phospholipid hydroperoxide glutathione peroxidase, an intrinsic renal cytoprotective antioxidant enzyme mainly expressed in glomerular podocytes, parietal epithelial cells, as well as tubular epithelial cells plays a prominent role in preventing lipid peroxidation. SOD is the first-line physiological defense against oxidative stress and this notation further supported by the fact that diabetic mice transgenic for Cu/Zn SOD had significantly lower urinary albumin excretion, glomerular hypertrophy, and glomerular expression of TGF-β I and collagen IV protein compared to non-transgenic mice. Further, it has been noted that overexpression of MnSOD in bovine aortic endothelial cells prevents high glucose-induced activation of PKC, NK-kβ, hexosamine, and AGE pathways. Hyperglycemia is a major controller of oxidative stress and thus strict glycemia control remains the cornerstone of the current standard therapeutic approaches and may help to ameliorate oxidative stress. On other hand, different therapies such as ACE inhibitors (such as captopril, lisinopril, and imidapril) and AT1 receptor blockers (such as losartan, irbesartan, and olmesartan) have been observed in numerous experimental and clinical studies to have therapeutic potential in the treatment of diabetic nephropathy. In fact, generation of ROS as a result of excessive advanced glycation in diabetes mellitus and thus, the inhibition of renal formation of AGEs was suggested to provide a unique prospect in the treatment of diabetic nephropathy. Administration of OPB-9195, a
novel AGE inhibitor has been demonstrated to possess therapeutic potential in prevention and progression of diabetic nephropathy by suppressing the expression of TGF-â, VEGF and type IV collagen in the kidney of OLETF rats. 

Chronic hyperglycemia results in overexpression of PKC and TGF-β, which causes increased generation of ROS. Thus, inhibition of PKC was understood to represent a novel strategy in the management of diabetic nephropathy. Administration of ruboxistaurin (LY333531), an inhibitor of PKC has been noted to afford renoprotection by reducing albuminuria, inhibiting the accumulation of extra cellular matrix protein and decreasing the expression of glomerular TGF-β in experimental diabetic nephropathy. Resveratrol (RSV) a stilbenoid is reported to ameliorate renal injury and increased mitochondrial biogenesis with Mn-SOD dysfunction in the kidney of db/db mice, through improvement of oxidative stress via normalization of Mn-SOD function and glucose-lipid metabolism. RSV exerts antioxidative activities via AMPK/SIRT1-independent pathway. Another natural resinous substance Propolis and its extract have antioxidant properties and a strong antioxidant effect of propolis have been reported to ameliorate oxidative stress and delay the occurrence of diabetic nephropathy in diabetes mellitus. The pharmacological agents inhibiting these target sites have been reported to halt the progression of diabetic nephropathy. However, further clinical studies are needed to illuminate their therapeutic potential in treating diabetic patients with nephropathy.

Conclusion

Diabetic nephropathy is the most common cause of progressive renal damage and end stage renal failure in patients with diabetes mellitus. While the exact cause of diabetic nephropathy remains unknown, oxidative stress coupled with chronic hyperglycemia may have an important role in the pathogenesis of glomerular and tubular functional and structural abnormalities. Numerous novel target sites such as TGF-β, AGEs, PKC, and NADPH oxidase, have been identified to play a pathogenic role in the progression of diabetic nephropathy. There has been an increase in the knowledge of the role of oxidative stress in diabetic nephropathy which has directed to the investigation of a number of therapeutic strategies, the success of which has been so far limited. However, timely and judicious use of recent therapies to maintain good glycemic control, lipid levels adequate and blood pressure, along with lifestyle measures such as regular exercise, optimization of diet and smoking cessation, may help to reduce oxidative stress and endothelial cell dysfunction and slow the progression of diabetic nephropathy until more effective therapies become available. Further, antioxidant therapy seems to be promising in preventing the induction and progression of diabetic nephropathy along with ACE inhibitor and AT1 receptor blockers, which provide direct renoprotective effects.

References


