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Review Article

SIGNALLING PATHWAY OF NADPH MEDIATED ROS-RNS IN DIABETIC NEPHROPATHY

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ABSTRACT
Disorder of physiological signaling functions of reactive oxygen species (ROS) superoxide (ROS are chemically reactive molecules containing oxygen), hydrogen peroxide, reactive nitrogen species (RNS) nitric oxide and peroxynitrite are an important feature of diabetes mellitus. The balance between the production of ROS, notably superoxide anion (O^{-2}) and hydrogen peroxide (H_2O_2), and the antioxidant defense system that includes superoxide dismutase (SOD) and peroxidases, determines the degree of oxidative stress. An increased production and/or decreased metabolism of ROS have been implicated in the pathogenesis of renal injury in diabetes mellitus (DM). Thus the regulation of ROS generation in diabetes by antioxidants seems to be a promising target for management of Diabetic nephropathy.
Keywords: NO, eNOS, ROS, PKC, NOX4

INTRODUCTION

Diabetes mellitus, a metabolic disorder associated with various macrovascular and microvascular complications including nephropathy. Nephropathy is one of major microvasular complication associated with the chronic diabetes mellitus. The uncontrolled glucose level results in structural as well as functional changes in the kidney. The common structural changes such as thickening of glomerular basement membrane, glomerulosclerosis, glomerular hypertrophy, podocyte loss, mesangial cell expansion, and tubulointerstitial fibrosis. After numerous afford it has been noted that the multiple signaling pathways are involved in the pathogenesis of diabetic nephropathy which include angiotensin-II (ANG-II), endothelin-1 (ET-1), advanced glycation end products (AGEs), endothelial nitric oxide synthase (eNOS), ROS, transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), nuclear factor kappa B (NF-κB), lipid peroxidation, and collagen-4 but it is worthwhile to note that ROS-RNS mediated oxidative stress through Nicotinamide adenine dinucleotide phosphate (NADPH) overproduction seems to be a centrally involved in the pathogenesis of diabetes induced nephropathy. Major ROS & RNS consist of paramagnetic free radicals (superoxide O_2^{-}, hydroxyl OH^{-}, peroxy radical ROO, nitric oxide free radical, diamagnetic molecules (H_2O_2 and peroxynitrite $ONOO^{-}$) which are products of the reactions of these free radicals. Thereby, the objective of this review is the suppression of damaging ROS-RNS signalling in biological processes whereby ROS-RNS induced by NADPH play a major role in pathogenesis and progression of diabetes induced nephropathy.

NADPH OXIDASE

NADPH oxidase is a critical component for ROS generation in macrophages and neutrophils and many other cell including mesangial cells. NADPH oxidase, a major source of superoxide generation, is composed of six subunits including two membrane associated components (p22 phagocytic oxidase (phox) and gp91phox) and four cytosolic components (P47phox, p40phox, p67phox, and small GTPase Rac). NADPH oxidase is activated by membrane translocation of three cytosolic proteins (p47phox, p67phox, and small GTPase Rac). At the membrane, these proteins assemble with gp91phox-p22phox heterodimer and induce a conformational change of gp91phox, which results in superoxide production. The activated NADPH oxidase generates superoxides by transferring an electron inside the cells across the membrane, and the electrons thus transferred are coupling with molecular oxygen to generate superoxides, known as reactive free radicals[1].

NADPH is formed during glycolysis or oxidative phosphorylation and exerts antioxidant activity by regeneration of reduced glutathione. Glutathione act as important intracellular antioxidant by reacting with ROS and organic peroxides[2-3]. Thus, antioxidant defense system will reduce the level of NADPH by coversion of NADPH to $NADP^{+}$. In renal vessels, macula densa, thick ascending limb of loop of henle, distal tubules, collecting ducts, interstitial fibroblasts, glomerular podocyte and messengial cells are documented to consist of enzyme NADPH oxidase which is responsible for significant production of superoxide radical[4]. Activation of NADPH oxidase may be due to assembly of the subunits and translocation of p47phox to the membrane is necessary.

NADPH OXIDASE INDUCE NOS

Nitric oxide (NO) production plays a central role in modulating endothelial function[5]. NO is generated from the metabolism of L-arginine by the enzyme NOS, of which there are three isoforms: the constitutive types, brain (bNOS) and endothelial (eNOS), and the inducible type (iNOS)[6]. iNOS is induced de novo by various stimuli, including hyperglycemia[7], while the mitochondrial-generated superoxide can inhibit eNOS, although enough NO is still produced[8]. NADPH oxidase generated superoxide radicals can react with NO forming peroxynitrite, which is a potent oxidant and nitrosylating agent. Furthermore, this reaction can cause NO deficiency, NO normally regulates tubuloglomerular feed back and renal blood flow, and is involved in regulation of natriuresis. The NO deficiency can be worsened by the fact that oxidative stress promotes activation of vasoconstrictors[9]. Moreover, NO deficient animal models have shown to develop glomerulosclerosis and proteinuria, as well as hypertension and renal failure[9]. Expression of p47phox is increased in podocytes, glomeruli, loop of henle, cortical distal tubules, and medullary collecting ducts in diabetic rats[10-11]. Further NADPH oxidase inhibitor, apocyanin decreases the expression of gp91phox and

activation of p47phox in diabetic rats[11]. Furthermore, increased NADPH oxidase activity will decrease NADPH/$NADP^+$ ratio, causing oxidative stress by TCA cycle enzyme complex α-ketoglutarate dehydrogenase[12]. In addition to high glucose, free fatty acid[13], oxidized LDL, hyperlipidemia[14-15], ANG-II in mesangial cell[16], and endothelial cell[17] are reported to activate NADPH oxidase. Furthermore, increased superoxide produced within the glomerular microcirculation decrease NO bioactivity on mesangial contraction and arterioal tone and may contribute to many of the renal hemodynamic and vascular abnormalities in diabetic nephropathy[18].

On the other hand a major defense of endothelial cells against vascular injury is eNOS, which generates NO in the presence of the substrate L-arginine, and the cofactor (6R)-5-6-7-8-tetrahydro-Lbiopterin (BH4). NADPH oxidases are major sources of ROS in endothelium and are activated in animal models of hypertension and diabetes. Superoxide ($O_2^{\cdot -}$) reacts avidly with vascular NO to form peroxynitrite ($ONOO^-$), leading to BH4 oxidation and subsequent promotion of $O_2^{\cdot -}$ production by eNOS itself, so-called "eNOS uncoupling"[19-20]. Uncoupled eNOS is detected in conditions associated with oxidant stress, hypertension and diabetes[19].

Recently, it has been suggested that eNOS-derived NO is a critical regulator of endothelial junctional integrity and serves to maintain the low basal permeability of endothelium[21]. Importantly, a recent study has demonstrated that NO prevents high glucose-induced endothelial apoptosis by suppressing NF-κB activity[22]. In addition, it has been shown that NO prevents aldose reductase activation, sorbitol accumulation and potentially suppresses vascular complications during diabetes[23].

NADPH OXIDASE INDUCE ROS

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[24]. The ROS are generated via NADPH-oxidase system by the interaction of membrane-bound flavocytochrome b_{558} (heterodimer of gp91phox and p22phox) with various cytosolic proteins (p47phox, p67phos, p40phox, and a GTP-binding protein, p21rac)[25-26] as a result $O_2^{\cdot -}$ is generated, which gets dismutated to H_2O_2. The relevance of this system in renal pathobiology lies in the fact that Nox 4, a homologue of neutrophil gp91phox, is expressed in the kidney[25]. Besides AGEs, PKC, DAG, IP_3 (inositol 1, 4, 5-trophosphate), and TGF-β the metabolites of cyclo-oxygenase (COX) pathway and can also activate NADPH oxidase under high glucose ambience[25-26]. The high glucose, as compared to normal glucose, induced a three-fold increase in ROS production by bovine aortic endothelial cells and that overexpression of uncoupling protein (UCP)-1 or manganese superoxide dysmutase (MnSOD) effectively prevented ROS overproduction by high glucose suggesting that the mitochondria is the source of high glucose-induced superoxide generation[28]. Moreover, decreased fibronectin expression by the inhibitors of oxidase, apocynin, and diphenylene iodonium (DPI) suggests a potential role of NADPH oxidase in hyperglycemic injury and relevance in redox-sensitive processes, i.e., cell growth, apoptosis, migration, and extracellular matrix (ECM) modeling that are modulated by various signaling pathways and transcription and growth factors such as TGF-β.

On the other hand the angiotensin converting enzyme inhibitor (ACEi) and ANG-II receptor blocker (ARB) inhibited p47phox and nitrotyrosine expression in diabetic kidney suggesting that inhibition of ANG-II may confer renoprotection in diabetes, in part, through prevention of ROS overproduction[19, 27].

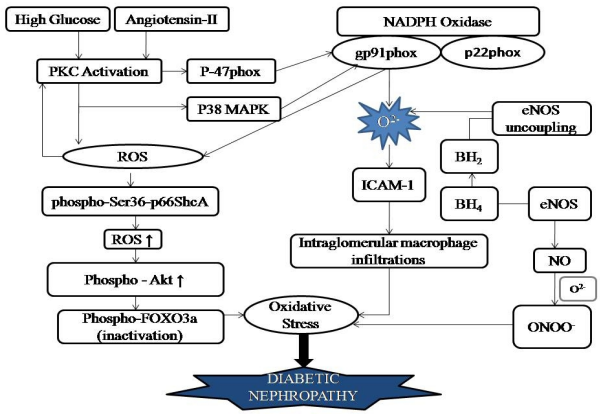

Fig-1. The NADPH mediated ROS-RNS signalling involve in Diabetic Nephropathy.

PKC-Protein Kinase C, ROS-Reactive oxygen species, e NOS-endothelial nitric oxide synthase, ICAM-1 (Intercellular Adhesion Molecule 1), Akt also known as Protein Kinase B (PKB), Tetrahydrobiopterin deficiency (THBD, BH4D) also called THB or BH4 deficiency. High glucose (HG)-induced ROS formation resulted in the phosphorylation of Ser36-p66shcA protein that promoted further ROS formation. ROS overproduction activated Akt/PKB kinase, which phosphorylated and inactivated FOXO3a protein, enhanced oxidative stress. Diabetes increased the levels of transforming growth factor TGFβ that led to the inactivation of phospho-FOXO3a protein and the enhanced oxidative stress.

CONCLUSION AND DISCUSSION

Present findings demonstrate importance of NADPH mediated ROS and RNS signaling in many enzymatic cascades regulating the development of diabetic nephropathy. It might also be suggested that the deregulation of ROS signaling is probably the beginning of pathological changes in diabetes.

Investigation of ROS and RNS signaling in enzymatic cascades which are responsible for developing diabetic nephropathy could be a fascinating task, promising the discovery of new pharmaceutical agents and methods for the treatment of these pathologies. Aforementioned findings suggest the potential usefulness of the inhibitors of ROS & RNS signaling in gene/enzymatic processes for the treatment of diabetic nephropathy.

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