NADPH OXIDASE INHIBITORS – ARE NOVEL DRUGS IN DIABETIC NEPHROPATHY A REVIEW

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ABSTRACT
NADPH Oxidase are Novel drug in treatment of Diabetic Nephropathy. **Purpose of study**- The main purpose of the study was to find the Benefits of NADPH OXIDASE Inhibitors in Diabetic Nephropathy. **Hypothesis** – NADPH Oxidase inhibitor are novel drugs in treatment of Diabetic Nephropathy as they prevent podocyte injury, decreases extracellular matrix deposition, prevents epithelial mesenchymal transition, prevents mitochondrial injury, prevent antioxidant depletion, reduce ROS production in response to glucose and prevents signalling and activation of ERK and NF-κB activation decrease the expression of MCP-1 and prevent fibroblast proliferation and subsequently fibrosis. **Method used** –Various Quest engines and data base was used with sentence ‘NADPH Oxidase inhibitor in Diabetic Nephropathy’ and study ,report and evidences from previous articles were gathered to support the hypothesis. **Results from search Engines- as on 11/07/2017.**

1) Google: About 2,17,000 results (0.56 seconds).
2) Google Scholar: About 17,200 results (0.14 sec).
3) Pubmed: 2126.
4) Capto me: 1-10 of 8515774 records for NADPH Oxidase inhibitors in Diabetic Nephropathy - Pharmacology (ADME).

**Conclusion:** From various studies, data and report support the stated Hypothesis is factual.

**My Contribution in the review**
• In this review the above hypothesis and probable benefit NADPH Oxidase Inhibitors are well supported from the available data and study.
• All the scattered data and study about NADPH Oxidase role in pathogenesis of Diabetic Nephropathy is taken into consideration to support the hypothesis.

• All the researcher and Scholar have done the best and I give credit to them but after going through various quest engines and articles so many articles huge data is generated which is not helpful, the review article provides comprehensive and core material.

• The advantages of NADPH Oxidase Inhibitors are well elaborated in 1 to 13 points with strong study support and evidence using 25 articles to support the study.

**KEYWORD:** NADPH Oxidase, Podocyte injury, Diabetic Nephropathy.

**INTRODUCTION**

Several pathways end in ROS generation, several studies recognized the Nox family of NADPH oxidases as chief sources of ROS in many non-phagocytic cells, comprising most kidney cells. Proof suggests that amongst the seven Nox homologs, it is the Nox1 and Nox4 homologs that are essential for the destructive effects of high glucose in cultured cells and contributes to hyperglycemia-mediated microvascular and macrovascular problems of diabetes affecting retina, vasculature, heart, and kidney and organs with end artery. [Michio Hayashi 2013]

Nox1, Nox2, Nox4, and Nox5 are the NADPH oxidase homologs that are mainly expressed in the kidney, as well as glomerular and tubule-interstitial cells. [Michio Hayashi 2013]

NADPH oxidase 4 (Nox4) is a constitutively active multi-subunit enzyme, which generates superoxide from molecular oxygen using NADPH as the electron donor. It acts as an oxygen sensor, catalyzing the reduction of molecular oxygen to numerous reactive oxygen species (ROS). Nox4 is extensively expressed in the kidney (Krause KH 2004).

Nox4 is the major source of ROS in the kidneys during the early stages of diabetes mellitus. [Michio Hayashi 2013] and is up-regulated in diabetic kidney. (Rachel Yong et al 2013)

Nox4 has recently presented to be concerned in glucose induced oxidative stress in the kidney and in pro-fibrotic processes in renal cells (Sedeek M et al 2010) NADPH oxidases are proposed to play a crucial role in the development and progression of renal injury in animal models of TYPE 1 & TYPE 2 diabetic nephropathy (Jha JC et al.; 2014). Nox 4 Inhibitor and drugs are reviewed (Soni NOP 2017).
PHYSIOLOGICAL ROLE
The particular physiological function of Nox4 has not yet been clarified, but it may be an oxygen sensor that regulates erythropoietin production in the kidney. Though, it has been associated as a major source of renal ROS (Maranchie JK, Zhan Y (2005), Orient A et al 2007).

UP-REGULATORS
TGF-β up-regulate p22phox (Lee et al., 2003).
Stimulation of tubular cells by TGF-β results in an upsurge in Nox4 protein expression, an effect prevented by AMPK activation (Lee et al., 2013).

Suppression of NADPH oxidase
Endogenous adrenomedullin neutralizes the pathogenesis of diabetic nephropathy maybe due to anti-oxidative stress action through the suppression of NADPH oxidase and the renin-angiotensin system. (Michio Hayashi 2013) however, the role of adrenomedullin in diabetic nephropathy has not been clearly explained.

IDEAL NADPH OXIDASE INHIBITOR
Perfect NADPH oxidase inhibitor should neither scavenge ROS, that is, not have antioxidant actions, nor inhibit other flavoproteins or NADPH-dependent proteins. NADPH oxidase inhibitors should further not impact the expression levels of NOX or their respective binding partners. They also should not hinder with upstream signalling pathways of NOX activation but rather impede NADPH oxidase activity directly. In addition to NADPH oxidase specificity, isoform discrimination for one of the NOX isoforms is also desirable. [Sebastian Altenhöfer 2015].

CLASSIFICATION
Non-specific class: Numerous off-target effects of drugs or the inhibition of NADPH oxidases that are not unique for NOX enzymes but also occur in other (ROS generating) enzymes (Sebastian Altenhöfer 2015).


**SPECIFIC CLASS**

1. **GKT136901 and GKT137831** are the best categorised NOX inhibitors currently available. (Sebastian Altenhöfer 2015).

2. **VAS2870 and VAS3947:** Unluckily, the poor solubility and absence of pharmacokinetic and specificity data for these substances limit their in vivo use. (Sebastian Altenhöfer 2015).

3. **S17834 Polyphenol:** it activates AMPK also inhibits Nox (Sebastian Altenhöfer 2015). No pharmacokinetic or safety data have been published, mouse studies suggest sufficient oral bioavailability and safety profiles (Sebastian Altenhöfer 2015).

4. **ACD 084 (Kofler et al. 2013):** Screened a selection of active compounds from edible plants to search for NOX4 inhibitors and one such compound is ACD084 appears to have a promising ADM profile.

5. **Grindelic acid:** Inhibits Nox4 and did not inhibit NOX2 and NOX5 and it has similar Profile like ACD084 (Sebastian Altenhöfer 2015).

6. **Shionogi I and II:** The pharmaceutical company Shionogi and Co Ltd. patented pyrazolo pyrimidine but this compounds don’t fill criteria of NADPH oxidase inhibitor (Sebastian Altenhöfer 2015).

7. **EBSELEN:** Smith et al. identified ebselen and derivatives as potent NOX2 inhibitors. Ebselen is orally available and that its safety profile permitted clinical development up to phase III for the treatment of cerebral ischemia injury. Nevertheless, the drug was never brought to the market. (Sebastian Altenhöfer 2015).

8. **Celastrol:** Plant extracts from Thunder God Vine (Tripterygium wilfordii Hook F.) are used in Traditional Chinese Medicine to treat states of chronic inflammation and was identified to inhibit NOX2 in neutrophils. (Sebastian Altenhöfer 2015).

9. **Perhexiline:** is a prophylactic anti-anginal agent that is mainly prescribed in New Zealand- it inhibit carnitine palmitoyl transferase-1 and Nox 2. (Sebastian Altenhöfer 2015).

10. **Fulvene-5** -inhibits NOX2 and NOX4-mediated ROS production. (Sebastian Altenhöfer 2015).
11. Triphenylmethane derivatives-The triphenylmethane derivatives Brilliant green, Gentian violet, and Imipramin blue and all three compounds were shown to inhibit NOX4 in cellular assays in different concentrations. (Sebastian Altenhöfer 2015).

OTHER DRUGS (Soni NOP 2017)
1) Statin-Pitavastatin Inhibit Nox 4.
2) AMPK activators, e.g. AICAR (5-aminimidazole-4-carboxamide-1-riboside) or adiponectin, significantly reduced Nox4 expression, oxidative stress and podocyte injury in vitro or in vivo. Other AMPK activator is metformin used for treatment of type 2 DM.

Advantages of NADPH Oxidase INHIBITORS
1) NADPH oxidase inhibitor reduces fibronectin, type IV collagen, glomerular hypertrophy, mesangial matrix expansion, decreases urinary protein loss and prevents podocytopathy as well as fibrosis.

Evidence and supportive study
The orally obtainable small-molecule Nox1/Nox4 inhibitors from the pyrazolo pyridine chemical series have freshly drawn significant attention. Pre-clinical studies done with these inhibitors in experimental animal models point that they efficiently decrease the pathological changes detected in type 2 diabetes plus accelerated atherosclerosis as well as ischemic retinopathy, liver fibrosis, and idiopathic pulmonary fibrosis [Yves Gorin 2015].

GKT137831 suppressed NADPH oxidase activity, superoxide generation, and hydrogen peroxide production in the renal cortex of diabetic mice without affecting Nox1 or Nox4 protein manifestation. The amplified expression of fibronectin and type IV collagen was reduced in the renal cortex as well as in glomeruli, of diabetic mice treated with GKT137831. GKT137831 significantly reduced glomerular hypertrophy, mesangial matrix expansion, urinary albumin excretion, and podocyte loss. Pharmacological inhibition of Nox1/4 gives wide renoprotection in mice with pre-existing diabetes and established kidney disease. [Yves Gorin 2015].

2) NADPH oxidase inhibitor-inhibit MCP-1 and reduce macrophage infiltration.

Evidence and support from study
GKT137831 significantly reduced macrophage infiltration in glomeruli and interstitium. These results were constant with the decline of monocyte chemotactic protein-1 (MCP-1) and
macrophage infiltration witnessed in the aorta and kidneys of diabetic mice treated with GKT137831 [Yves Gorin 2015].

3) NADPH INHIBITORS reduce ROS production in response to high glucose
Evidence and support from the study
GKT137831 decreases ROS production in human podocytes exposed to high glucose. [Jha JC2014].

4) NADPH OXIDASE INHIBITOR REDUCE FIBRONECTIN IN DIABETIC RAT
Evidence and support from the study
Inhibition of Nox4 oxidase by administration of antisense oligonucleotides for Nox4 significantly reduced glomerular enlargement as well as fibronectin accumulation in glomeruli from type 1 diabetic rats (Gorin et al., 2005).

5) NADPH OXIDASE INHIBITOR REDUCE FIBROSIS
Evidence and support from the study
Nox4 is the main Nox homolog implicated in kidney myofibroblast differentiation and expression of fibronectin and other fibrotic markers in response to TGF-β (Barnes and Gorin, 2011; Bondi et al., 2010).

6) NADPH OXIDASE INHIBITOR- Reduce extracellular matrix deposition
Evidence and support from the study
In T1DM rats model the glomeruli and tubular cells showed increase in Nox4 mRNA and protein expression and down-regulation of tubular Nox4 levels after in vivo administration of antisense oligonucleotides decreases diabetes-mediated ROS production and extracellular matrix protein synthesis in the renal cortex that is mainly composed of tubular epithelial cells (Etoh et al., 2003; Gorin et al., 2005).

7) NADPH OXIDASE INHIBITOR –REDUCE TGF-β MEDIATED FIBROSIS
Evidence and support from the study
High glucose and TGF-β1 upsurges Nox4 expression and TGF-β1 decreases SOD1 expression in human proximal tubule cells. In order to decide the role of Nox4 in TGF-β1 mediated renal fibrosis, two plans to inhibit Nox4 were used, specifically the administration of a Nox4 inhibitor in in vitro and in vivo studies and Nox4 specific siRNA in in vitro studies. Both tactics consistently and considerably reduce TGF-β1 induced fibronectin and
collagen mRNA and protein expression, major extracellular matrix proteins that are upregulated in tubulointerstitial fibrosis. (Rachel Yong et al 2013).

8] NADPH OXIDASE INHIBITOR – reduce TGF-β mediated tubulointerstitial pathology

Evidence and support from the study
Elevated levels of TGFβ in diabetes, increases Nox4 expression that drives the progress of tubulointerstitial pathology. (Rachel Yong et al 2013).


Evidence and support from the study - Direct activation of Nox enzymes through the angiotensin II (AngII)/AT1 receptor (AT1R) pathway leads to oxidative stress. Ang II stimulates ROS generation in human podocytes in a Nox5-dependent manner. (Chet E. Holterman et al 2013).

Ang II-dependent rise in NADPH oxidase activity is linked with the up-regulation of Nox4, Nox2, Rac and p22phox expression in podocytes (Nistala et al., 2008; Whaley-Connell et al., 2008).

10] NADPH OXIDASE inhibitor reduce podocytopathy

Evidence and support from the study
GKT137831 significantly reduced glomerular hypertrophy, mesangial matrix expansion, urinary albumin excretion, and podocyte loss. [Yves Gorin 2015].

11] NADPH OXIDASE inhibitor reduce albuminuria

Evidence and support from the study
GKT137831 is now being assessed in a phase 2 clinical trial in patients with T2DM and albuminuria. [Yves Gorin 2015].

Direct activation of Nox enzymes through the angiotensin II (AngII)/AT1 receptor (AT1R) pathway leads to oxidative stress. Ang II stimulates ROS generation in human podocytes in a Nox5-dependent manner. (Chet E. Holterman et al 2013) Inhibiting Nox enzymes will prevent podocyte injury.
12] NADPH OXIDASE inhibitor – prevent subsequent activation of ERK PATHWAY
Evidence and support from the study
Nox4-dependent ROS production and subsequent Src/caveolin-mediated activation of epidermal growth factor receptor/ERK signalling pathway (Chen et al., 2012; Peng et al., 2009; Xu et al., 2010).

13] NADPH OXIDASE inhibitor – prevent hypertrophy by inhibiting Nox4
Evidence and support from the study
HG up-regulates Nox4 and Nox2 mRNA and protein expression in kidney Fibroblast and that is linked with an increase in ROS generation and hypertrophy (Williams and Gooch, 2014).

Current status as on 13/7/2017 checked on site ClinicalTrials.gov
Identifier: NCT02010242 Sponsor: Genkyotex Innovation SAS.

Safety and Efficacy of Oral GKT137831 in Patient With Type 2 Diabetes and Albuminuria
Clinical Trials.gov Identifier: NCT02010242.

First received: June 18, 2013.
Last updated: March 27, 2015.
Last verified: March 2015.

No Study Results Posted on ClinicalTrials.gov for this Study
About Study Results Reporting on ClinicalTrials.gov.

Study Status: This study has been completed.
Study Completion Date: March 2015.

Primary Completion Date
February 2015 (Final data collection date for primary outcome measure).

Out of so many classes of drugs only GKT137831 has reached phase 2 clinical trial and the outcome and result are still awaited. However the evidence and support from various studies as described in points 1 to 13 suggest NADPH oxidase are novel drugs in treatment of Diabetic Nephropathy.

Conflict of interest
The author declares there is no conflict of interest regarding the publication of this paper.
ACKNOWLEDGEMENT AND CREDIT
I give credit to all authors list in my work and I quote them appropriately and acknowledge them for Nobel work.

DECLARATION
The author has not conducted any animal study or Human Trial , the matter in the article is which comprises animal or human trial are the study done by researcher /scientist listed in the Reference section and cited appropriately and are taken up in the article to support the Hypothesis.

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