

Diabetic Nephropathy: Review of Novel Experimental and Clinical Strategies

Original Article

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ABSTRACT

Background: Diabetic nephropathy (DN) is considered a severe disorder which affects health worldwide. New more active treatment options are required. Despite a large number of drugs already being available for treatment of hyperglycemia in diabetes, current oral antidiabetic agents often do not provide adequate glycemic control. Renin-angiotensin-aldosterone system (RAAS) blockers are novel agents to slow the progression of DN.

Method: The present review provides an overview of recent studies on DN management, especially the use RAAS blockers and their antidiabetic effects. References were mainly identified through PubMed search until December 2016 and backtracking of references in pertinent studies.

Results: This review reporting the novel mechanisms and benefits of targeting the RAAS by the use of ACEIs; ARB or direct renin inhibitors in glucose lowering, antioxidant and anti-inflammatory effects and kidney protection.

Conclusion: These drugs targeting the RAAS can be used as monotherapy or in combination therapy in hypertensive-diabetic patients to protect them from DN.

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Key Words: ACEIs; aliskiren; diabetic nephropathy; RAAS.

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BACKGROUND

Diabetic nephropathy (DN) is considered the major dangerous complication of both type I and II diabetes. Its complications severely influence the patient life and it is considered the main cause of renal failure, even in developed or non-developed countries. Early treatment delays or prevents these severe complications. Preclinical studies on experimental animals are important for discovery of new molecules or treatment strategies.

The pathophysiology of DN is involved high blood pressure (BP), fluid retention, continuous albuminuria, decreased glomerular filtration rate (GFR), and glomerulosclerosis because of end stage renal disease (ESRD). Elevated oxidative stress and inflammatory mediators are involved in the underlined mechanism of DN^[1-4]. In addition to decrease antioxidant defense mechanisms; kidney nitric oxide synthase (NOS) reported to be decreased in experimental DN^[5]. Numerous endogenous modulators as endothelin-I^[6], angiotensin II^[7], arginine vasopressin^[8], urotensin-II^[9], asymmetric dimethylarginine^[10], caveolin^[11], C-reactive protein^[12], and leptin^[13] have been reported with DN. In addition to many other factors intermediate the evolution of nephropathy in

diabetic patients includes hyperglycemia, atherosclerosis and hypertension^[14] (Figure 1).

EXPERIMENTAL MODELS OF NEPHROPATHY

Increasing number of patients with ESRD who need dialysis lead to the evolution of many animal models to study the different mechanisms underlying nephropathy and to develop new drugs for treatment^[15].

Induction of diabetes nephropathy

Streptozotocin (STZ)

Streptozotocin is an alkylating agent; nitrosourea class; used in treatment of Islets of Langerhans cancer. The mechanism of action of STZ is by damage of pancreatic β -cells of the Islets of Langerhans by damages the DNA, so it is used in induction of diabetes in animals. Single dose of STZ in rats (40, 50, 55, 60 or 65 mg/kg i.p. or i.v.) leads to hyperglycemia in 72 hours^[15, 16]. Nephropathy observed in rats in 1–2 months after induction. DN is showed by critical rise in serum creatinine level, blood urea nitrogen (BUN), and glomerulosclerosis^[15]. STZ-induced DN leads to decrease eNOS activity and decrease NO, which triggers nephropathy. Szabo *et al.*,^[17] noted that STZ administration

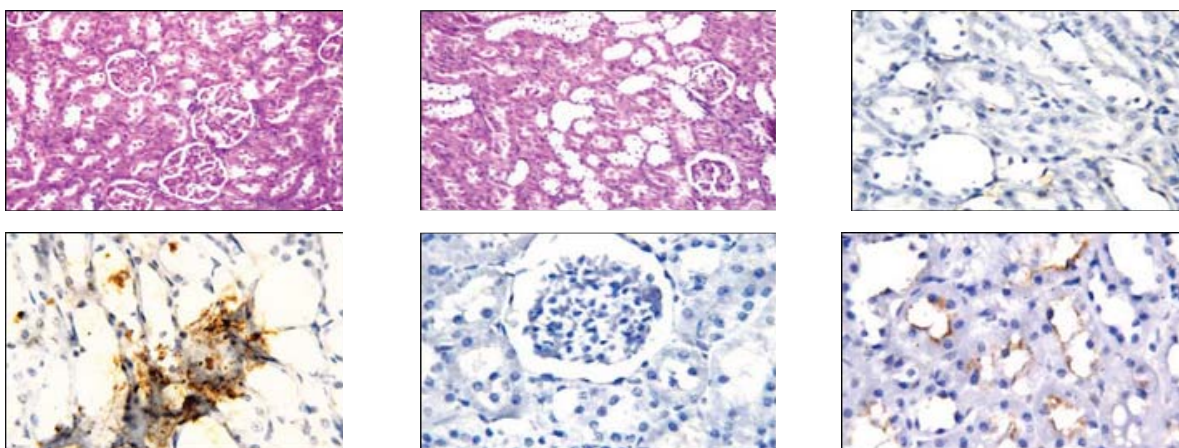


Fig. 1 (a-f): Photomicrographs for kidney sections from normal rats and rats with diabetic nephrotoxicity induced by streptozotocin (fig. 1a, 1b: H&E-stained; fig. 1c, 1d: immunohistochemistry staining for determination of Caspase3; fig. 1e, 1f: immunohistochemistry staining for determination of TGF- β) ($\times 200$) (Mahfoz *et al.*, 2016).

1a: Kidney of rat in normal control group: showing normal histological structures of the glomeruli and tubules of the cortex. 1b: Kidney of rat in diabetic control group: showing degeneration and coagulative necrosis in lining epithelial cells of some individual tubules at the cortex. Fig. 1c: kidney of rat in normal control group showing negative immunoreaction using caspase 3. Fig. 1d: Kidney of rat in diabetic control group showing strong positive immunoreaction using caspase 3. Fig. 1e: Kidney of rat in normal control group showing negative immunoreaction using TGF- β . Fig. 1f: Kidney of rat in diabetic control group showing mild immunoreaction using TGF- β .

(35 mg/kg i.p., once) in rats in which $\frac{1}{2}$ kidney is resected and the other one removed, lead to macro albuminuria in 80 days, with significant increase in triglycerides, serum creatinine and glomerulosclerosis^[18]. In mice; STZ (125 mg/kg i.p.) administration for 2 days induced DN^[19]. STZ-induced DN is a common used model in experimental studies; DN appeared within 4–6 weeks after a single dose^[17].

Genetic model

Important, non-obese model for studying molecular basis of DN using: Goto-Kakisaki (GK) rat. It is characterized by hyperglycemia; at age 24 months nephropathy appeared^[20].

Cyclosporine A-induced nephropathy

Cyclosporine is an immunosuppressant and inhibitor of calcineurin. It is not used clinically because of its nephropathy side effect^[21]. Administration of 7.5 mg/kg/day or 15 mg/kg/day s.c. of cyclosporine in rats lead to nephropathy^[7, 22].

Anthracycline-induced nephropathy

Doxorubicin is an anthracycline antibiotic. Due to its toxicity on heart and kidney, it is used now as anticancer drug for breast, bladder, thyroid and stomach cancer. Doxorubicin used in dose of 2 mg/kg i.v., two times per day for 5 months to induce rats' nephropathy. While 20 mg/kg i.v. once in mice developed serious nephropathy^[23, 24].

Electrolyte nephropathy

Abnormalities of electrolytes level in kidney was found to induced nephropathy due to dysfunction of nephron

which caused mainly by decrease Mg^{2+} and K^{+} content. Disodium hydrogen phosphate diet for rats, for 12 days induces nephropathy^[25].

Ethylene glycol model of nephropathy

Ethylene glycol was used as antifreeze material in cooling systems. 50–400 mg/kg/day ethylene glycol p.o. for 12 months induced nephropathy. This is due to oxalic acid; which is the metabolic product of ethylene glycol. Oxalic acid precipitated as crystals in kidney. This resulted in increased ROS and renal degeneration^[26].

Aminoglycoside-induced nephropathy

Nephrotoxicity is a severe side effect of gentamicin which limits its use. Kumar *et al.*, (2000)^[27] reported that (80 mg/kg, i.p.; gentamicin) for one week in rats induced nephropathy associated with high ROS.

Cadmium model of nephropathy

Cadmium is used in metal coatings but chronic exposure lead to nephrotoxicity^[28]. Uriu *et al.*, (1998)^[29] reported that 0.18 mg/kg i.p., of cadmium 3 times per week for 3 months in rats induced nephropathy.

Carbon tetrachloride-induced nephropathy

Chronic exposure to carbon tetrachloride (CCl₄) is hepatotoxic and nephrotoxic due to its metabolic end products^[30]. Administration of 0.5 ml/kg s.c., of CCl₄, 3 times per week for 7 weeks induced nephropathy in rats^[31].

Germanium dioxide (GeO₂) model of nephropathy

GeO₂ is used as additive in foods, medicines, and cosmetics. For its anti-microbial, anti-tumor and immunomodulatory properties. Yanagisawa *et al.*, (2000)

reported that 5.16 g/day of GeO₂ in diet for rats for 5 months developed nephropathy^[32].

Mercury chloride (HgCl₂) induced nephropathy

HgCl₂ (1 mg/ kg s.c.) resulted in DN in rats when used on days 0, 2, 4, 7, 9 and 11^[33].

Cisplatin model of nephropathy

It is an anticancer agent. 7 mg/kg iv, once was reported to induce nephropathy in rats within one week^[34].

Vomitoxin model of nephropathy

Vomitoxin is got from the fungus *Fusarium graminearum*. This fungus is grown on wheat and maize. It causes renal failure among kids and young. 25 mg/kg of vomitoxin, PO, one time per day was found to induce nephropathy in mice^[35].

NEW BIOMARKERS FOR DIABETIC NEPHROPATHY

Albuminuria is the gold standard biomarker of DN. However albuminuria has some limitations as large variability and low sensitivity^[36]. So novel biomarkers for DN are very important for early diagnosis or treatment evaluation, are the following:

Cystatin C

Cystatin C, a protease inhibitor, is a low molecular weight protein, freely filtered by glomeruli. Urinary cystatin C level early increased in Zucker diabetic fatty rats before severe renal damage^[37].

Immunoglobulins

Urinary IgM or IgG excretion marks that a damage or large pore present in the glomerular capillary wall. It considered an early predictor of progression of type 1 DN^[38, 39].

Type IV collagen

Increased collagen excretion in urine is observed to be increased in type 2 DN^[40].

Podocytes and podocyte-associated molecules

Podocytes are structural components of glomerular barrier. Urinary excretion of podocytes is considered an early potential marker of DN^[41, 42].

Transferrin

Transferrin is a plasma protein, more filtered through glomerular filtration barrier than albumin^[38]. Urinary transferrin excretion is considered an early potential marker for early detection of DN.

Ceruloplasmin

Ceruloplasmin is plasma copper-carrying protein. Urine excretion of ceruloplasmin reported even in normo-albumin-urea diabetic patients^[43].

Transforming Growth Factor - β (TGF- β)

TGF- β is considered an important growth factor^[44]. It is observed to be increased in type 1 and 2 DN^[45, 46]. TGF- β is even elevated in normo-albumin-uric diabetic patients; it is considered an important biomarker for the pathogenesis of DN^[47].

Oxidative stress biomarkers

Elevated serum Malondialdehyde (MDA) and Nitric oxide (NO) and decreased serum superoxide dismutase activity (SOD) are reported in DN^[48-50].

Inflammation biomarkers: TNF- α , MCP-1, IL-6, VDBP

Previous studies showed that pro-inflammatory cytokines like TNF- α ^[48], Vitamin D-Binding Protein (VDBP)^[51] and IL-6^[52] were reported in DN.

MANAGEMENT OF DIABETIC NEPHROPATHY

There are several lines in treatment of patients with DN. Hyperglycemia control, lowering of elevated blood pressure, and decreasing salt in diet slow the progression and complications of DN.

Role of renin angiotensin aldosterone system (RAAS) in DN

RAAS is known by its significant role in controlling cardiovascular homeostasis. RAAS rely on the biotransformation of angiotensinogen (Ang) to angiotensin I (Ang I) and Ang II by the effect of angiotensin converting enzyme (ACE)^[53]. Ang II function is mediated through Angiotensin 1 receptor (AT1R) and Angiotensin 2 receptor (AT2R). Functions of Ang II include: proinflammatory, pro-oxidative, vasoconstriction, hypertrophic and proliferative effects^[53] (Figure 2).

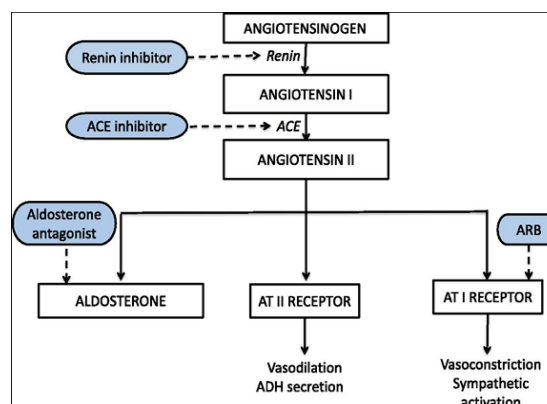


Fig. 2: Flowchart showing the clinical effects of RAAS and the sites of action of ACEIs, Renin inhibitors and ARBs.

RAAS is found in all tissue, including the blood vessels, heart, kidney, brain, and skeletal muscles pancreas, adipose tissue^[54]. Insulin resistance and pancreatic β cell

dysfunction are correlated to RAAS function^[55]. The negative feedback effect of Ang II on AT1R is the main mechanism responsible for RAAS harmful effects^[56].

Insulin resistance is reported to decrease by increase ACE2/Ang/Mas receptor axis. ACE2 gene therapy also enhances hyperglycemia control in diabetic mice due to its observed ability to elevate the action of bradykinin^[54, 57] (Figures 2, 3). Furthermore, bradykinin influences insulin production and effect. It is resulted in β cell injury and affects the transcriptional expression of glucose transporter 2 (GLUT2)^[58-60].

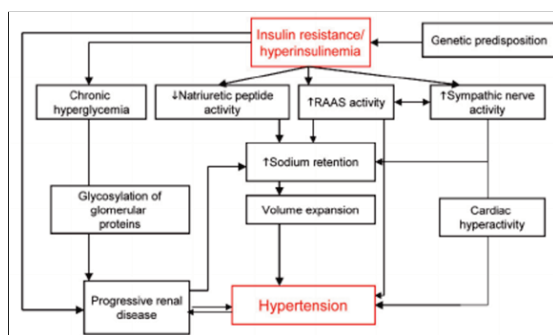


Fig. 3: Relation between insulin resistance, RAAS and hypertension.

Antidiabetic mechanisms of ACEIs and ARBs:

Increasing spread of metabolic syndrome and type 2 diabetes has generated interest in metabolic effects of antihypertensive drugs. The main focus was on the effects of diuretics and beta-blockers on carbohydrate and lipid metabolism^[61, 62].

Previous recent studies have reported the ability of some new antihypertensive agents to lead to less metabolic side-effects than diuretics, and also decrease the risk for type 2 diabetes. Drugs with antidiabetic and antihypertensive effects are considered more important clinically^[63, 64].

Previous clinical, preclinical, in-vitro and in-vivo studies which involved the inhibition RAAS have reported a potential relation between RAAS and insulin resistance. AngII may enhance glucose digestion by its consequences for insulin flagging pathways, tissue blood stream, sympathetic effects and oxidative pressure^[65-67].

ACEIs or ARBs may enhance glucose digestion by interference with AngII age or AngII receptor initiation. To be sure, given a portion of the confirmation collected to date, it is conceivable that pharmacologic of the meddling RAAS may some time or another demonstrate fit for enhancing insulin affectability and diminishing the hazard for diabetes. Late preclinical and clinical investigations have announced that ACEIs may build insulin affectability^[65, 67].

The consequences of these investigations are fascinating to propel trials to ponder the capacity of ACEIs to diminish the occurrence of new-beginning type 2 diabetes^[62, 68]. Past

examinations recommended that the antidiabetic properties of ACEIs might be through increments in nitric oxide, bradykinin level and GLUT4 glucose transporter^[65, 69, 70].

Beyond examinations in rats missing bradykinin B2 receptors and in rats dealt with an ACEI and a bradykinin antagonist proposed that the insulin-sharpening impacts of ACEIs encompass something other than decreases in Ang II tiers^[65, 70]. Increments in bradykinin level from changing over compound hindrance may enhance glucose digestion by influencing insulin flagging pathways, nitric oxide creation and translocation of GLUT4^[69, 71].

To the degree that the antidiabetic impacts of ACEIs are auxiliary to impedance with Ang II-subordinate systems that advance insulin protection, one may anticipate that ARBs will be more successful than ACEIs in enhancing insulin protection and avoiding type 2 diabetes. Paolisso *et al.*, (1997)^[72] noticed that losartan-initiated increments in entire body glucose transfer were associated with losartan-prompted increments in femoral supply route bloodstream. Nonetheless, few examinations have been manufactured from the insulin-polishing influences of ACEIs as opposed to ARBs and, to this point, no sizeable scale clinical trials have checked out the ability of ACEIs and ARBs to diminish the danger for diabetes^[73-75].

Some examiners have encouraged that the inhibitory impacts of AngII on insulin-flagging pathways won't be intervened via both kind 1 or kind 2 Ang II receptors and that some other sort of Ang receptor is probably covered^[76].

Clinical trials utilizing ARBs have given some aberrant help to the likelihood that Ang II receptor barricade may enhance insulin affectability and decline the rate of type 2 diabetes^[77].

ACEIs or ARBs are renoprotective and slight strengthen of endless nephropathies in animals and sufferers. However, they have little impact on basal glucose and insulin stages, in animals^[78].

Renin Inhibitors

Renin is the foremost catalyst in RAAS, it assumes an element in BP manipulation. It converts Ang to Ang I, that's thusly modified over by ACE to AngII. AngII has both immediate and backhanded impacts on BP. It straightforwardly makes blood vessel smooth muscle contract, prompting vasoconstriction and expanded BP. Ang II moreover invigorates the creation of aldosterone from the adrenal cortex, which causes the tubules of the kidneys to build reabsorption of sodium, with water following, in the end increasing plasma extent, and consequently BP. Renin inhibitor ties to renin; this coupling keeps the transformation of Ang to Ang I. Aliskiren is the first approved drug in the renin inhibitor class^[79] (Figures 2, 3).

- Superiority of renin inhibitors over ACEIs

ACEIs resulted in a decrease in AngII level; however they have a limited efficacy due to incomplete block of

ACE and generation of AngII by other pathways. ACEI also interfere with bradykinin breakdown. Chronic ACEI use resulted in a return increase in circulating AgII due to interruption of AgII feedback on renin secretion^[80]. However, renin inhibitors do not affect bradykinin release and block the action of AgII chronically at the AT-1 receptor^[80].

Previous clinical study reported that renin inhibition by aliskiren resulted in the same effectiveness as ACEI with fewer side effects. ACEI produce severe cough due to kinins increase. Aliskiren considered a good substitute in this condition^[81]. Added advantage for aliskiren is its extended t1/2 with ideal pharmacokinetic properties^[82].

- Renoprotective impacts of renin inhibitors

One month treatment with aliskiren (the principal drug in renin inhibitor class) after DN induction by STZ lead to euglycemia and normalized serum insulin when compared with diabetic group^[48, 83]. This impact was emphasized by the in-vitro experiment where aliskiren leads to dose-dependent stimulation of insulin secretion from isolated pancreatic islets of normal rats. Likewise, aliskiren synergized gliclazide-initiated insulin secretion in this in-vitro experiment. Furthermore, standardization of adiponectin when compared with the diabetic group was reported; this is related with bringing down insulin resistance. The stated antidiabetic impact of aliskiren on this investigation might be because of its capacity to empower insulin secretion or faded insulin resistance with the aid of normalizing serum adiponectin degree and antioxidant effects^[48].

Habibi *et al.* (2008)^[84] exhibited that renin restraint by using aliskiren weakened insulin resistance in transgenic Ren2 rats that overexpress renin. on this manner, an achievable connection among coordinate renin hindrance by using aliskiren and insulin changed into proposed.

Renoprotective impacts of aliskiren were showed by its capacity to standardize BUN and serum creatinine^[48]. Stanton 2003 and Scherthamer 2008^[85, 86] introduced that restraint of the main step in the RAAS (transformation of Ang to Ang I by using renin) by using aliskiren prompting extreme renoprotective effect isn't always just by way of blocking angiotensin II (Ang II) completely, but in addition via hindering the effects created thru activation of (pro) renin Receptor (P) RR. Likewise, aliskiren can diminish the gene expression of (P) RR and may change the three-dimensional setup of renin^[87]. Also aliskiren was accounted for to enhance glomerular filtration in past examinations^[88, 89]. Aliskiren additionally increased serum adiponectin to ordinary level when compared with the diabetic group^[48].

Prior investigations detailed that treatment with aliskiren brought about oxidation prevention impacts which were showed by critical reduction in renal MDA, serum NO and increment GSH, SOD. This proposes its

renoprotective impact could be by means of combating oxidative stress created by STZ^[48, 90 - 92].

Aliskiren decreased some antiinflammatory biomarkers, this showing its anti-inflammatory activity and this is steady with its renoprotecting activity. This was seen by critical decline in kidney TNF- α and TGF- β when compared with diabetic group^[48, 83]. Aliskiren; as a renin inhibitor represses the binding of renin to renin receptors, which empowers the generation of inflammatory mediators as TGF- β , TNF- α , PAI-1, fibronectin, and collagen through the Ang-independent extracellular signal-regulated kinase 1 and 2 pathways^[93-95].

Aliskiren also prevents TNF- α dependent apoptotic pathway by decreasing elevated TNF- α and decreasing caspase 3^[48, 96]. Inhibition of TGF- β 1 by aliskiren suggests that aliskiren prevents apoptosis and tubular atrophy through the inhibition of TGF- β 1 and TNF- α expression^[48].

Streptozotocin-induced histopathological and ultrastructural changes were markedly improved by treatment with aliskiren. There was restoration of epithelial integrity. Attenuation of glumerosclerosis and tubulointerstitial fibrosis are considered as other important predictors of renal dysfunction^[97]. This finding confirms the previous studies suggesting that RAAS blockade leads to preservation of podocyte architecture, mitochondrial function and epithelial integrity^[90, 98, 99].

CONCLUSIONS

All the mentioned research papers above, reporting the novel mechanisms and benefits of targeting the RAAS by the use of ACEIs; ARB or direct renin inhibitors in glucose lowering, antioxidant, anti-inflammatory and renoprotection. They can be used as monotherapy or combination therapy in hypertensive-diabetic patients to protect them from DN. Also, new preclinical and clinical examinations are important to explore the safety of these agents and the effect of blend treatment.

COMPLIANCE WITH ETHICAL STANDARDS

- No Funding sources available
- Authors declare that they have no conflict of interest.
- This article does not contain any studies with human participants or animals performed by the author.

LIST OF ABBREVIATIONS

AT2R	Angiotensin type 2 receptor.
ARBs	Angiotensin receptor blockers.
DN	Diabetic nephropathy.
ET 1	Endothelin 1.
eNOS	Endothelial nitric oxide synthase.
ESRD	End stage renal disease.
GLUT2	Glucose transporter 2.

GSH	Reduced glutathione.
H	Hour
IL6	Interleukin 6.
IgA	Immunoglobulin A.
IgG	Immunoglobulin G.
MDA	Malondialdehyde.
RAAS	Renin angiotensin aldosterone system.
ROS	Reactive Oxygen Species.
RR	Renin receptor.
SOD	Superoxide dismutase activity.
STZ	Streptozotocin.
TBARS	Thiobarbituric acid reactive substance.
TNF- α	Tumor necrosis factor - α .

CONFLICT OF INTEREST

There are no conflicts of interest.

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