PATHOGENESIS OF DIABETIC NEPHROPATHY

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ABSTRACT

Diabetic nephropathy is associated with increased mortality in diabetic patients and is a major cause of end stage renal disease in most countries. Diabetic nephropathy seems to occur as a result of an interaction between metabolic and hemodynamic factors. Hemodynamic factors that contribute to the development of diabetic nephropathy include increased systemic and intraglomerular pressure, as well as activation of vasoactive hormone pathways including the renin-angiotensin system and endothelin. These hemodynamic pathways activate intracellular second messengers such as protein kinase C (PKC), Mitogen-activated protein (MAP) kinase, nuclear transcription factors such as NF-kB and various growth factors such as the proinflammatory cytokines, TGF-β and the permeability enhancing growth factor, vascular endothelial growth factor, VEGF. Glucose dependent pathways are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation and the accumulation of advanced glycation end products (AGEs). In combination, these pathways ultimately lead to increased renal albumin permeability and extracellular matrix accumulation, resulting in increasing proteinuria, glomerulosclerosis and ultimately tubulointerstitial fibrosis.

INTRODUCTION

Diabetic nephropathy is also known as Kimmelstiel Wilson syndrome and it was discovered in 1936 by Clifford Wilson and Paul Kimmelstiel. Diabetic nephropathy occurs as a result of an interaction between hemodynamic and metabolic factors. Hemodynamic factors that contribute to the development of diabetic nephropathy include increased systemic and intraglomerular pressure, as well as activation of vasoactive hormone pathways including the renin angiotensin system and endothelin. These hemodynamic pathways activate intracellular second messengers such as protein kinase C (PKC), Mitogen-activated protein (MAP) kinase, nuclear transcription factors such as NF-kB and various growth factors such as the proinflammatory cytokines, TGF-β and the permeability enhancing growth factor, vascular endothelial growth factor, VEGF. Glucose dependent pathways are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation and the accumulation of advanced glycation end products (AGEs). In combination, these pathways ultimately lead to increased renal albumin permeability and extracellular matrix accumulation, resulting in increasing proteinuria, glomerulosclerosis and ultimately tubulointerstitial fibrosis.

HEMODYNAMIC PATHWAYS

Glomerular hyperfiltration and hyperfiltration are the early signs resulting from decreased resistance in both the afferent and efferent arterioles of the glomerulus. Afferent arteriole seems to have a greater decrease in resistance than the efferent. Many factors have been reported to be involved in this faulti autoregulation, including nitric oxide, prostaglandins, vascular endothelial growth factor (VEGF), TGF-β1, and the rennin angiotensin system, specifically angiotensin II. These early hemodynamic changes alleviate albumin leakage from the glomerular capillaries and overproduction of mesangial cell matrix, as well as thickening of the glomerular basement membrane and injury to podocytes. In addition, increased mechanical strain from these hemodynamic changes can induce localized release of certain cytokines and growth factors. The action of vasoactive hormone such as angiotensin II and endothelin are mediator of renal hemodynamic changes. Glomerular hypertension and hyperfiltration contribute to the development of diabetic nephropathy because use of renin-angiotensin blockers preserves kidney function and morphology. Blockade of the renin-angiotensin–aldosterone system antagonizes the profibrotic effects of angiotensin II by reducing its stimulation of TGF-β1. Support that such profibrotic effects underlie diabetic nephropathy has also been provided by study of an animal model of diabetic nephropathy. Transient blockade of the renin-angiotensin system for 7 weeks in prediabetic rats reduced proteinuria and improved glomerular structure. Additionally, the administration of an angiotensin-converting-enzyme inhibitor to patients with type-1 diabetes and nephropathy appears to reduced serum concentrations of TGF-β. A correlation exists between decreased levels of TGF-β1 in serum and urine and renoprotection, as determined by changes in the glomerular filtration rate.

Fig. 1: Interaction of hemodynamic and metabolic pathway, cytokines and intracellular signalling molecules mediating diabetic nephropathy

Renin-angiotensin system in nephropathy

The renin–angiotensin system (RAS) has been extensively studied in diabetes. Earlier studies centered on the systemic RAS, and the data obtained have been conflicting, with stimulation, suppression, and no change in the system being reported. The factors that influence the systemic RAS in addition to the different stages of disease and species studied may explain many of these divergent findings. However in various diabetic models, increased renal renin content relative to plasma renin levels has generally been found, thus suggesting impaired renal renin release into the circulation. In clinical diabetic nephropathy there is decreased plasma renin activity which may be due to nonenzymatic glycation of prorenin with decreased conversion to active renin. Thus, diabetic nephropathy has traditionally been considered a "low renin" state. However, plasma renin activity may not accurately reflect activity of the RAS in the kidney.

Another problem has been the difficulty of accurate measurement of plasma angiotensin II (Ang II), which is an important issue because discordance can exist between plasma renin and Ang II levels. More recently, the intrarenal RAS has been the focus of extensive study. Abundant evidence indicates the existence of local tissue RASs that
are regulated is independent of plasma RAS. It was reported that decrease in glomerular Ang II receptors in the diabetic rat 3 to 4 weeks after induction of the disease. Downregulation of glomerular Ang II receptors implies that intra-renal Ang II generation may be increased. The density of Ang II receptors in the proximal tubules was reported to be reduced in diabetic rats and was accompanied by decreased mRNA expression for the AT1 receptor. Recently, AT1 receptor density has also been shown to be decreased in mesangial cells when incubated in high-glucose media. ACE activity in whole kidney is low in diabetes.

However, this is probably due primarily to mesangial RAS. Ang II is produced by primary cultures proximal tubule suppression because staining for ACE has been found to be enhanced in glomeruli and vasculature of diabetic rats and in patients with diabetic nephropathy. These data suggest that the term "intra renal" RAS is an oversimplism, in as much as the vascular RAS (vessels and glomeruli) appears to be regulated differently from the tubulointerstitial RAS. Angiotensin receptor blockers (ARBs) enhance the renal vasoconstriction in patients with diabetes (despite the presence of low plasma rennin activity), again supporting the concept that the renal vascular RAS is activated despite suppression of the circulating RAS. In several intrarenal compartments including the glomeruli have been found to be several orders of magnitude higher than those found systemically. This shows the existence of both local RAS acting independently of the systemic RAS and also is consistent with the finding that in most renal cell culture studies, effects of Ang II are observed at sub-stagnantly higher concentrations (about 0.01–1.0 mmol/L) than those found in the systemic circulation.

**Vaso-active hormones**

Endothelium is an interior covering of blood vessels. There are various biological functions of endothelium and it regulates vascular tone and maintain free flow of blood in vessels. The luminal surface of every blood vessel, forming a physical and metabolic barrier to circulating elements. The endothelium is an important endocrine organ and release a number of vasoactive hormones, including endothelin (ET-1) and endothelin-deriv hyperpolarizing factor (EDHF: nitric oxide and prostacyclin). Endothelin-1 is a potent vasoconstrictor. Endothelium-derived hyperpolarizing factor is still in controversial subject of vascular biology. Endothelial cells of every blood vessel release Nitric oxide and prostacyclin they form a particular partnership in the regulation of vascular and platelet function.

**Nitric Oxide**

NO, originally identified as "endothelial derived relaxing factor," is a ubiquitously utilized signaling molecule that regulates a wide variety of organ and cellular functions, including renal hemodynamics and salt and water regulation. NO is generated enzymatically from the amino acid L-arginine by one of three specific nitric oxide synthases: "neuronal" (NOS1 or nNOS), "endothelial" (NOS3 or eNOS), or "inducible" (NOS2 or iNOS). Many, but not all, of the intracellular signaling pathways activated by NO are mediated by activation of guanylate cyclase, which increases intracellular levels of cyclic guanosine monophosphate.

All three NOS isoforms are present in the mammalian kidney, with both distinct and overlapping patterns of distribution. In normal kidney, NOS1 is highly expressed in the macula densa and glomerular parietal epithelium, as well as in the medulla in the collecting ducts and thin ascending limb. NOS2 is expressed in the endothelium of glomerular capillaries and afferent and efferent arterioles, renal arteries, and descending vasa recta, as well as in proximal tubule and medullary thick ascending limb. NOS3 is also expressed in tubules, including S3 segments of the proximal tubule, medullary thick ascending limb, and collecting duct, in addition to arcuate arteries and vasa recta bundles. Both in vivo and in vitro studies have provided conflicting results regarding NOS expression and NO production in diabetes. Most but not all studies in cultured renal cells have determined decreased NO production in response to hyperglycemia.

**Prostacyclin**

The first step in prostacyclin synthesis is the liberation of arachidonic acid from membrane-bound lipids via the enzymatic actions of phospholipase A2 (PLA2). In endothelial cells, phospholipase A2 activation is a calcium-dependent step. Once liberated, arachidonic acid is available for metabolism by cyclooxygenase (COX). Cyclo-oxygenase is present in two isoforms: COX-1 and COX-2. Cyclo-oxygenase-1, like NOSI or NOSII, is constitutively expressed, while COX-2, like NOSII, is induced at sites of inflammation and/or by PAMPs. In healthy endothelial cells, COX-1 is the predominate isoform. Cyclo-oxygenase has two enzymatic activities: firstly, an oxygenase step forms prostaglandin (PG) G2; and secondly, a peroxidase step, which forms PGH2 from PGG2. Prostaglandin H2 is the substrate for a range of downstream prostaglandin synthase enzymes, including prostacyclin synthetase (PGIS), the actions of which result in the formation of prostacyclin. Endothelial cells are enriched in cyclooxygenase-1 (COX-1) and PGIS, which is why, when phospholipase A2 is activated, prostacyclin is the predominant metabolite made. It is important to note that in platelets, which also express predominantly COX-1, thromboxane is the principal product made. This is because platelets express mainly thromboxane synthase with negligible levels of PGIS.

**Endothelin-1**

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by vascular endothelium from big ET-1 via specific cleavage by endothelin converting enzyme (ECE). ET-1 produces its actions in the course of the disease decreased production may predominate. However, not all studies have shown this pattern, and the reader is referred to an extensive recent review of the subject. In some studies, administration of nonspecific NOS inhibitors induced more severe renal vasoconstriction and decreases in GFR in early diabetic rats than control rats suggesting that NO may be a mediator of the hyperfiltration seen at this point in the disease; however, other studies have failed to detect such differences. Specific inhibitors of NOSI normalized GFR in hyperfiltering diabetic rats.

![Fig. 2: Schematic representation of NO changes in diabetic nephropathy and various pathophysiological triggers for such changes.](image-url)
The activation of ETA receptor induced the renal TGF-β production and inflammation in diabetic rats. Treatment with CP0213, a dual ETA/ETB receptor antagonist has been found to improve the renal function in rats with diabetic nephropathy by suppressing Nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase, suggesting that ETA contributes to the pathogenesis of diabetic nephropathy via upregulation of NAPDH oxidase mediated ROS production in renal cells. Thus under pathological conditions, prevention of endothelin-mediated various signalling pathways may provide an alternative approach to treat diabetic nephropathy.

**Urotensin**

Urotensin II (UII) is an 11-amino acid vasoactive peptide, recently identified as the ligand for a novel G protein-coupled receptor, GPR-14 (renamed urotensin receptor [UT]). In addition to its potent vasoconstrictive actions, UII also has trophic and profibrotic effects leading to its implication in the pathogenesis of heart failure. However, it has been noted that elevated plasma levels of UII in association with renal impairment and diabetes and diabetic nephropathy (urotensin-II, an endogenous vasconstrictor, has been suggested to be involved in the pathogenesis of vascular endothelial dysfunction [VED]. UII increases the activity of NADPH oxidase and plasminogen activator inhibitor-1 (PAI-1) and cause decrease in endothelium dependent relaxation. The overexpression of urotensin-II in endothelial cells cause VED by increasing the expression of type I collagen and formation of ROS.

**METABOLIC PATHWAY**

**Advanced glycosylation end products**

AGEs are a chemically heterogeneous group of compounds formed as a result of the "Maillard reaction" when reducing sugars react non-enzymatically with amine residues, predominantly lysine and arginine, on proteins, lipids and nucleic acids. While the initial stage of the reaction leading to the formation of reversible glycosylation proteins termed Schiff bases is rapid and glucose dependent, a much slower reaction over a period of days results in the formation of the more stable Amadori product. These early glycosylation products accumulate predominantly on long lived proteins such as vesicular collagen and crystallines, undergoing a series of in vivo rearrangements to form irreversible, complex compounds and cross-links, termed AGEs. Once AGE related cross-links form on proteins, they become resistant to proteolytic degradation. As well as their non receptor mediated effects, AGEs can exert their biological effects through receptor mediated mechanisms, the most important of which is the receptor for advanced glycation end products (RAGE). RAGE is a signal transduction receptor which belongs to the immunoglobulin superfamily and is expressed on a number of cell types including monocytes/macrophages, endothelial cells, renal mesangial cells and podocytes. Binding of AGES to the RAGE receptor activates a number of pathways implicated in the development of diabetic complications, specifically diabetic renal disease. These include enhanced cytosolic reactive species formation, stimulation of intracellular molecules such as PKC and NF-kB and the activation and expression of a number of growth factors and cytokines such as TGF-β and VEGF. Indeed, strategies to inhibit the formation of AGEs have been shown to ameliorate diabetic nephropathy.

In the initial study by Soulis-Liparota et al. aminoguanidine, an inhibitor of AGE formation, which acts by scavenging intermediates in the advanced glycation pathway attenuated the rise in albuminuria observed in diabetic rodents, while preventing increases in collagen related fluorescence in isolated glomeruli and renal tubules. Similar results have been obtained with alagebrium, a putative AGE cross-link breaker. In an experimental study performed by our group, both aminoguanidine and alagebrium attenuated the albuminuria observed in diabetic rodents. Furthermore, alagebrium was shown to reduce albuminuria in a type 1 diabetic rodent model. Indeed, group treated with alagebrium demonstrated increases in collagen and other extracellular matrix components in experimental diabetic nephropathy, this study also sought to determine the mechanisms surrounding the improvements in microalbuminuria in diabetic rodent kidneys. The compound was shown to reduce diabetes induced increases in the gene expression of TGF-β1, connective tissue growth factor (CTGF) and collagen IV. Early treatment with alagebrium was also associated with significant structural improvement in the kidney including a reduction in the glomerulosclerotic index and tubulointerstitial area, in conjunction with a reduction in AGE peptide fluorescence in serum and tissue. Furthermore, a reduction in renal accumulation of the specific AGE, carboxymethyllysine (CML) and decreased RAGE immunostaining was also seen, providing further evidence that accumulation of AGEs is implicated in renal extracellular matrix accumulation in disease. In the setting of diabetes mellitus and long-term hyperglycemia, nonenzymatic modifications of proteins (or lipids) by glucose or its metabolic products, results in their stable modification and altered function. This process is thought to underlie a major pathogenic pathway leading to tissue injury in diabetes. A major pathway for AGE formation involves triose phosphate intermediaries derived from metabolism of glucose. Triose phosphates build up as intracellular glucose increases and can nonenzymatically form the early glycosylation product methyglyoxal by spontaneous decomposition. Amine-catalyzed sugar fragmentation reactions then modify protein lysine residues directly, forming N- (epsilon) - (carboxymethyl)lysine (CML), a major product of oxidative modification of glycoproteins. Alternatively, reaction of terminal amino groups (e.g., on lysine) with glucose itself may form from early glycation products (i.e., Amadori products) which rearrange to produce stable moieties that possess distinctive chemical cross-linking and biologic properties, designated AGEs. Other glucose-derived Amadori products and fructose are thought to be potential precursors of 3-deoxyglucosone (3-DG) in vivo. Fructose generated by the aldose reductase pathway is converted into fructose-3-phosphate by the action of 3-phosphokinase (3- PK). This leads to the generation of 3-deoxyglucosone, a central precursor in the generation of an array of AGEs, in particular, CML-adducts and others (3-Deoxyglucosone, 1999) 3-DG can further react with proteins to form pyrrolines or pentosidine. AGEs have been suggested to represent a general marker of oxidative stress and long-term damage to proteins in aging, atherosclerosis, and diabetes. Renal CML-AGE is increased in diabetes. Immunolocalization of CML in skin, lung, heart, kidney, intestine, intervertebral discs, and particularly in arteries provide evidence for age-dependent increases in CML accumulation in distinct locations, and acceleration of this process in diabetes. Immunostaining and immunoblots of diabetic human kidneys show increased CML in diabetic glomeruli, especially in the mesangial matrix and capillary walls.

**Oxidative stress**

Generally, large amount of reactive oxygen species are generated with in nephron by metabolic activity which is counter balanced by a large number of antioxidant enzymes and free radical scavenging systems. Peroxidation of cell membrane lipids, oxidation of proteins, renal vasoconstriction and damage to DNA are the negative biological effects of reactive oxygen species. Unfortunately, hyperglycemia tips the balance towards production of reactive oxygen species, most of which seem to be generated in the mitochondria. The metabolism of glucose through destructive alternate pathways, such as via PKC activation and advanced...
glycation end-product formation, in the setting of hyperglycemia also seems partly dependent on reactive oxygen species. Oxidative stress specifically induced by hyperglycemia even before diabetes becomes clinically apparent. DNA damage marker induced by reactive oxygen species are higher in patients with more-severe nephropathy (ie, proteinuria versus microalbuminuria). Diabetic nephropathy is linked with severe oxidative stress. This pathway may be responsible for the decreased bioavailability of nitric oxide in the kidney.

**Reactive oxygen species**

Diabetic nephropathy is characterized by excessive deposition of extracellular matrix (ECM) in the kidney, leading to glomerular mesangial expansion and tubulointerstitial fibrosis. Clinical studies have demonstrated that high blood glucose is the main cause of initiation and progression of diabetic vascular complications including nephropathy. High reactive oxygen species (ROS) induced by glucose and upregulates TGF-β1 and extra cellular matrix protein (ECM) expression in glomerular mesangial cells. Hyperglycemia induced ROS generation and ROS-induced activation of signal transduction cascade and transcription factors and overexpression of genes and proteins in glomerular mesangial and tubular epithelial cells leading to ECM accumulation in diabetic kidney.

**Nephrin**

Podocytes (specialized visceral epithelial cells) are important for the maintenance of the dynamic functional barrier. Nephrin, a protein found in these cells, is crucial for maintaining the integrity of the intact filtration barrier. The renal expression of nephrin might be impaired in diabetic nephropathy. Patients with diabetic nephropathy have markedly reduced renal nephrin expression and fewer electron-dense slit diaphragms compared with patients without diabetes and minimal nephropathic changes or controls. Furthermore, nephrin excretion is raised 17–30% in patients with diabetes (with and without albuminuria) compared with that in individuals without diabetes.

Thus, nephrin excretion could be an early finding of podocyte injury, even before the onset of albuminuria. Treatment with blockers of the renin-angiotensin-aldosterone system might help protect nephrin expression. In a study of patients with type 2 diabetes, treatment with an angiotensin-converting enzyme inhibitor for 2 years maintained nephrin expression at control levels compared with that in untreated patients with diabetes. By contrast, the expression of two other important podocyte and slit diaphragm proteins, podocin and CD2AP, was similar in the three groups. Comparable decreases in renal nephrin expression were reported in other studies of diabetic nephropathy.

**Renal syndromes of nephropathy**

Tubulopathies
Renal Tubular Acidosis/Fanconi Syndrome
Sodium Wasting
Potassium Wasting
Nephrogenic Diabetes Insipidus
Nephrotic Syndrome/Proteinuria
Glomerular Disease
Minimal Change Glomerulonephritis
Focal Segmental Glomerulosclerosis
Membranous Glomerulonephritis
Other
Thrombotic Microangiopathy
Hemolytic uremic syndrome/Thrombotic thrombocytopenic purpura
Acute Kidney Injury
Hemodynamic Disturbances
Parenchymal Kidney Disease
Collecting System Disease
Chronic Kidney Disease
Analgescic Nephropathy
Chronic Tubulointerstitial Nephritis
Secondary Progression of Toxin-Induced Kidney Disease

**Treatment for diabetic nephropathy**

Captopril, lisinopril, quinapril and fosinopril are currently used ACE inhibitors to treat diabetic nephropathy. ACE inhibitors increase the bioavailability of NO and activate eNOS by and inhibit synthesis of ang-II. Lisinopril inhibits the formation of TGF-β and tubulointerstitial fibrosis in diabetic nephropathy patient. Proteinuria, glomerular hypertrophy and tubulointerstitial fibrosis reduced by Fosinopril in experimental diabetic nephropathy. Quinapril reduces proteinuria, cholesterol levels, glomerular lesions and podocyte damage in diabetic nephropathy. Angiotensin-II AT1 receptor blockers like candesartan and telmisartan have been noted to attenuate diabetic nephropathy by reducing proteinuria. ABT-62 an endothelin (ETA) receptor antagonists have been noted to reduce proteinuria in experimental diabetic nephropathy. Palosuran, a novel and selective uricosuric-II receptor blocker was noted to reduce albuminuria in renal disease. Fenofibrate Activate the of peroxisome proliferator activated receptor-α (PPAR-α) and produces renoprotective effect by suppressing renal PAI-1 in experimental diabetic nephropathy. PPAR-γ agonists such as pioglitazone and rosiglitazone significantly reduced glomerulosclerosis and tubulointerstitial fibrosis in patients with diabetic nephropathy. In addition, pioglitazone markedly reduced glomerular hypertrophy, mesangial expansion and urinary albumin excretion in patients with diabetic nephropathy. Recently, it has been suggested that suppression of the Rho-kinase pathway by fasudil, a selective rho kinase inhibitor could be a novel strategy to treat diabetic nephropathy by downregulating TGF-β and reducing ROS formation. Inhibition of HMG-CoA-Reducase by statins like atorvastatin, pravastatin and cerivastatin was noted to activate eNOS, maintain GFR and renal cortical blood flow and consequently reduce glomerular lesion. Resveratrol, a polyphenolic phytoalexin a potent antioxidant present in red wine, attenuated renal dysfunction by reducing proteinuria and ROS formation in rats with diabetic nephropathy. On the basis of this discussion, it may be suggested that the above-mentioned drugs may have improved renal function in nephropathy due to their properties of protecting the function of vascular endothelium.

**Conclusion**

Appearance of complex interaction between hemodynamic and metabolic pathways resulting in the development of diabetic nephropathy. While established therapy used in the development of the disease include strict hyperglycemia control and the use of the renin angiotensin system inhibitors, various metabolic pathways such as advanced glycation and specific protein kinase C isoforms provide important promise for the future. It is anticipated that a combination of therapies that directly affect different steps in the pathophysiology of diabetic nephropathy will be required to further reduce the progression and prevent the development of diabetic nephropathy in the future.

**REFERENCES**


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