Review Article

Drug Therapy Targets for Diabetic Nephropathy: An Overview

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ABSTRACT

Diabetic nephropathy is a leading cause of chronic kidney disease and end stage renal disease and accounts for significant morbidity and mortality in diabetic patients. Hyperglycemia may lead to end stage renal damage through both metabolic and non metabolic pathways. The non-enzymatic glycation of proteins with irreversible formation and deposition of reactive advanced glycation end products (AGE) have been noted to play a major role in the pathogenesis of diabetic nephropathy. Further, diabetic nephropathy is associated with hyperactivity of sorbitol aldose reductase pathway, hyperactivity of hexosamine biosynthetic pathway, activation of protein kinase C and MAPK and overexpression of growth factors and cytokines i.e. transforming growth factor-β, vascular endothelial growth factor, platelet-derived growth factor and insulin-like growth factor. Moreover, high glucose concentration in diabetes has been noted to induce oxidative and nitrosative stress, activate intracellular RAAS and release endothelin-1 and prostaglandins to deteriorate the function of kidney. In addition, up-regulation of transforming growth factor-β (TGF-β) and consequent overproduction of extracellular matrix molecules have been implicated in the progression of diabetic nephropathy. The present review study the various drug targets and drug therapy in diabetic nephropathy.

Keywords: Diabetic nephropathy, drug therapy, glycemic control, renal diseases.

INTRODUCTION

Diabetic nephropathy is characterized by thickening of basement membranes and mesangial expansion with progression into glomerulosclerosis, tubular necrosis and interstitial fibrosis, which ultimately result in renal failure[1]. Hyperglycemia may lead to end stage renal damage through both metabolic and non metabolic pathways. The non-enzymatic glycation of proteins with irreversible formation and deposition of reactive advanced glycation end products (AGE) have been noted to play a major role in the pathogenesis of diabetic nephropathy[2]. Further, diabetic nephropathy is associated with hyperactivity of sorbitol-aldose reductase pathway[3] (Cooper *et al.*, 1998), hyperactivity of hexosamine biosynthetic pathway, activation of protein kinase C[4] and MAPK[5] and overexpression of growth factors and cytokines i.e. transforming growth factor-β, vascular endothelial growth factor, platelet-derived growth factor and insulin-like growth factor[6]. Moreover, high glucose concentration in diabetes has been noted to induce oxidative and nitrosative stress[7] activate intracellular RAAS and release endothelin-1 and prostaglandins[8] to deteriorate the function of kidney. In addition, up-regulation of transforming growth factor-β (TGF-β) and consequent overproduction of extracellular matrix molecules have been implicated in the progression of diabetic nephropathy.

GLYCEMIC CONTROL

Improved glycemic control has been shown to prevent the development of microalbuminuria in both type 1 and type 2 diabetes[9] and it can also stabilise or normalise established microalbuminuria. Glycemic level is also important for the rate of progression of overt diabetic nephropathy. Some previous studies have suggested a glycemic threshold for the development of diabetic nephropathy. However, no evidence for such a threshold was found in the Diabetes Control and Complications Trial (DCCT), although the magnitude of the absolute risk reduction is greatest with reductions in HbA1c from a high level. Thus, there is still a clinically relevant risk reduction when HbA1c is reduced further towards the normal range. On the other hand, an intensified glycemic control also increases the risk of hypoglycaemia, particularly in type 1 diabetes. A clinical observation in patients with diabetic nephropathy is that glycemic control tends to deteriorate during the progression of nephropathy. Some studies have suggested that rapid acting insulin analogues may be beneficial in patients with overt nephropathy[10] and in patients on haemodialysis treatment[11].

TREATMENT OF HYPERTENSION AND MICROALBUMINURIA

Early in the 1980's the progression of diabetic nephropathy was shown to be delayed with long-term treatment with blood pressure lowering agents[12]. A close correlation between arterial blood pressure and the rate of decline in GFR has also been shown in overt diabetic nephropathy. Statistical analyses did not reveal a lowest threshold for the adverse effect of high systemic blood pressure on the fall in GFR[13]. A meta-analysis of trials with ACE-I in diabetic nephropathy showed that these agents lead to a reduction in the risk of progression from microalbuminuria to overt nephropathy and they seem to be superior, in terms of renal protection, to other anti-

hypertensive agents. Treatment with ACE inhibitor also seemed to have long-term effects with preservation of diabetic nephropathy function, i.e. GFR, over at least eight years[14]. Early intervention with ACE-I and blood pressure reduction already in normotensive patients with microalbuminuria has been shown to be beneficial both in type 1 and type 2 diabetes[15]. Several clinical trials using angiotensin receptor blockers (ARB) in patients with diabetes have shown similar renoprotective effects as ACE-I, both in type 1 and in particular, in type 2 diabetes[16]. The use of ACE inhibitor and ARB can lead to renal protection in diabetic nephropathy but still nephropathy may continue to progress but at a slower rate. A more effective blockade of the effects of AT II by reducing both synthesis and its binding to the AT-II type 1 receptor, i.e. a combination therapy with ACE-I and ARB has shown an additive effect on blood pressure and markers of renal function such as albuminuria[17]. Clinical trials have also indicated that ACE-I and ARB may be more effective than traditional antihypertensive treatment in reducing the progression towards ESRD[18]. It is nevertheless clear that treatment of hypertension is an important task in preventing and postponing the development of diabetic nephropathy. In the UKPDS trial more than 2/3 of the patients with type 2 diabetes and hypertension needed at least two antihypertensive agents to achieve a mean blood pressure of 144/82. A combination of blood pressure-lowering agents will thus be required in most hypertensive patients with type 2 diabetes. In the Swedish national guidelines for diabetes care, ACE-I has been recommended as a first choice in patients with type 1 diabetes and signs of incipient or overt diabetic nephropathy. A blood pressure target ≤130/80, or perhaps even lower, is recommended in these patients[19].

The rate of synthesis for AT II seems to play an important role in initiation and progression of diabetic nephropathy by affecting hemodynamic and non-hemodynamic mechanisms. In the future perhaps determination of I/D polymorphisms of the ACE gene or other candidate gene polymorphisms within the RAS may improve the evaluation of the individual risk profile and help us to individualise antihypertensive treatment.

AGE Inhibitors

Aminoguanidine the first targeted AGE product therapy, it is a hydrazine derivative that prevents AGE product formation by binding reactive carbonyl intermediates. Aminoguanidine affects nephropathy and retinopathy in 690 type 1 diabetics evaluated in a randomized double blinded, placebo controlled trials. Progression of glomerular filtration rate declined, proteinuria, and retinopathy was significantly improved. Although three patients given high dose aminoguanidine developed glomerulonephritis. A similar follow up study was halted due to safety concern and an apparent lack of efficacy[20]. A newer agent alagebrium chloride (ALT 711) cleaves AGE product and protein cross links. Thereby facilitating AGEP clearance two clinical trials with mixed diabetic populations with atherosclerosis and heart failure reveals that ALT 711 can improve vascular and left ventricular compliance with an adverse effect similar to placebo[21]. In animal studies, ALT 711 was beneficial in treating diabetic renal complications.

AGE inhibitors such as aminoguanidine[22], OPB-9195[23] and ALT-946[24] and a RAGE-specific neutralizing antibody[25] attenuated the development of diabetic nephropathy.

Aldose Reductase Inhibitors

Aldose reductase inhibitor (ARI) an ideal target for reducing the deleterious effects associated with polyol pathway activation. However clinical trials with ARIs have shown lack of efficacy or adverse effects. In 1980s sorbinil became the first ARI to undergo clinical trials after promising preclinical results. Results from several studies on neuropathy were mixed, but the majority suggested a lack of significant effects[26-27]. Sorbinil evaluated for treating retinopathy and nephropathy in early 1990s but again showed a lack of efficacy. Tolrestat or lidorestat were halted due to toxicities before their efficacy could be definitely evaluated. Ponalrestat and zopolrestat were ineffective despite of having more favourable side effects profiles[28] suggested the utility of epalrestat in ameliorating autonomic neuropathies related to cardiac fumction and gastric and esophageal motility[29-31]. Epalrestat effects on nephropathy were evaluated via a placebo-controlled, randomized trial over 5 years. Thirty five type 2 diabetics with baseline microalbuminuria were studied, and microalbuminuria was found to be unchanged in the treatment group, but significantly increased in the placebo controlled arm suggesting a benefit in treating nephropathy[32].

Lipid Lowering Treatment

Hypercholesterolemia plays an important role in aggravating renal damage in experimental diabetes in animals and this can be prevented by lipid-lowering therapies[33]. An independent association between hypercholesterolemia and rapid loss of renal function in patients with type 1 diabetes and diabetic nephropathy has been demonstrated[34]. Similar results are seen in patients with type 2 diabetes[35]. Intervention with the cholesterol-lowering agents, statins, over a long period in type 2 diabetes slowed the decline in GFR and reduced albumin excretion[36-37]. There are few trials in type 1 diabetes but in one trial lipid-lowering treatment tended to have a beneficial effect on microalbuminuria[38].

Erythropoietin (EPO) Treatment

Anemia due to a relative erythropoietin deficiency may occur early in some patients with diabetic nephropathy. The mechanisms for this have not been elucidated but an association with autonomic neuropathy[39] or early renal interstitial damage has been suggested. Anemia in chronic renal failure is normally not observed until GFR drops to 20-40 ml/min. Many of these patients are also treated with ACE inhibitors, which may cause a small decrease in serum erythropoietin level as a side-effect[40].

Early EPO substitution is probably beneficial as anemia may contribute to insulin resistance and left ventricular hypertrophy[41], both of which are strong risk factors for cardiovascular disease, and maybe also to the progression of renal disease[42].

Vitamins

No alterations in vitamin D metabolism have been shown in patients with diabetes and normal renal function[43] even though an early loss of bone density has been observed in type 1 diabetes[44]. A more pronounced bone loss is seen in uremic patients with diabetes com-pared to non-diabetic patients[45]. In chronic renal failure an early elevation of PTH, partly due to hyperphosphatemia and hypocalcemia is seen[46]. The activation of 1-α hydroxylation of 25(OH) D-vitamin is reduced in renal failure, and hence, early substitution with active D-vitamin and dietary phosphate restriction in early chronic renal failure may be recommended, particularly in patients with diabetes. Vitamin E has been suggested to prevent microvascular complications patients with type 1 diabetes[47]. On the other hand, no beneficial effect of E-vitamin supplementation on cardiovascular events could be confirmed in a large clinical trial, the HOPE study[48]. Elevated levels of homocysteine are observed in more than 90% of patients with ESRD[49], but they do not seem to be altered by diabetes[50] or isolated microalbuminuria[51]. High levels of homocystein have been linked to atherosclerosis, but not to the rate of progression of renal impairment[52]. It is speculated that a lowering of the homocystein levels, using a folic acid and B-vitamin regimen in renal disease, could reduce the excess incidence of cardiovascular disease and this is explored in ongoing trials.

Growth Factors and Cytokines Modulator

Mesangial cell proliferation inhibitors like LTP4 antagonist, PDGF inhibitors MMP inhibitors, CDK inhibitor etc. are the potent targets, used in the treatment of diabetic nephropathy[53]. Various growth factors like HGF and BMP-7 has been reported to have protective effect in the progression of nephropathy where as other factors like TGF, PDGF, EGF and VEGF inhibitors shown to have protective effect in the prevention of diabetic nephropathy[54].

mTOR Pathway Inhibitor

mTOR has been shown to be a central regulator of cell growth and is regulated by a large number of signals including nutrients such as glucose and amino acids and growth factors such as insulin and IGF-1. mTOR activation stimulates protein synthesis and cellular hypertrophy in various types of cells and organs. mTOR activation plays an important role in insulin resistance in insulin target tissues such as fat, muscle and liver[55]. Moreover, renal cortical homogenates from diabetic db/db mice showed decrease in eEF2 phosphorylation and increment in eEF2 kinase phosphorylation probably due to mTOR activation[56]. p70S6-kinase, a downstream of mTOR, was highly activated in mesangial cells in diabetic obese db/db mice. Furthermore, systemic administration of rapamycin, a specific and potent inhibitor of mTOR markedly ameliorated pathological changes and renal dysfunctions, where mTOR plays a pivotal role in the development of ESRD[57]. Rapamycin is an FDA approved drug and has been used clinically to inhibit both host rejection following organ transplantation, such as kidney and islets and the re-stenosis of coronary arteries post angioplasty[58]. Recent studies also found that rapamycin has potent growth inhibitory activity against the development of various types of tumors and has potential as an anti-cancer treatment. Rapamycin specifically inhibits the mammalian target of rapamycin (mTOR), a protein kinase[59].

Vascular Endothelial Growth Factor (VEGF) Inhibitors

VEGF also has a role in the pathogenesis of diabetic nephropathy. Increased production of VEGF from podocytes causes glomerular hypertrophy and is associated with proteinuria[60]. SU5416 is a selective small-molecule inhibitor that blocks all VEGF receptors at the level of the tyrosine kinase[61]. Inhibition of VEGF by SU5416 ameliorated albuminuria in an experimental model of diabetic nephropathy[62].

TGF-β Inhibitors

Administration of anti- TGF-β 2 IgG4, a neutralising antibody suppresses renal fibrosis and reduce albuminuria by maintaining the procollagen-I C-propeptide expression at normal level in diabetic rats with nephropathy[63]. Further, administration of murine (1D11), an anti- TGF-β antibody in combination with lisinopril have renoprotective effect by reducing proteinuria in diabetic rats with nephropathy[64]. Moreover, administration of circular antisense TGF-β oligodeoxynucleotides (ODNs) have renoprotective potential by inhibiting the overexpression of TGF-β in the kidney of diabetic rats[65].

PKC Inhibitors

PKC isoforms, PKC-α and PKC-β appear to be the most relevant and most likely targets of new therapeutic agents for diabetic nephropathy. Ruboxistaurin (RBX), a highly selective PKC-β inhibitor, shows most promising effects in the prevention of diabetic vascular complications[66] and specifically inhibits the activation of the PKC-βI isoform without affecting PKC-α isoform activation and prevents the increased mRNA expression of TGF-β1 and extracellular matrix components, such as fibronectin and alpha1 (IV) collagen in the glomeruli[67]. RBX also improves the glomerular filtration and albumin excretion rates[68]. RBX can abrogate the glucose-induced oxidative stress in the glomeruli of streptozotocin-induced diabetic rats through a decreased activation of critical NADPH subunits[69]. It can also normalize eNOS activity in glomerular endothelial cells that is decreased in response to glucose induced PKC activation[70]. eNOS plays an important role in renal protection by maintaining normal glomerular function through the inhibition of

thrombosis, leukocyte adhesion/activation, apoptosis and oxidative stress.

Aldosterone Antagonists

Aldosterone accelerates renal damage by inducing the production of growth factors and ROS and interfering in the process of extra cellular matrix degradation[71]. Treatment with spironolactone, an aldosterone receptor antagonist, downregulates renal expression of matrix regulating genes such as TGF-β, matrix metalloproteinase, VEGF and insulin growth factor (IGF) and thus decreases albuminuria and mitigates glomerulosclerosis in experimental DN and possess renoprotective effect by reducing the oxidative stress and attenuating the overexpression of monocyte chemoattractant protein-1 (MCP-1) in patients with DN[72] (Takebayashi *et al.*, 2006). In a recent study, eplerenone, an aldosterone receptor antagonist has been noted to improve GFR and inhibit glomerulosclerosis in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Moreover, the combined treatment of eplerenone with enalapril markedly decreased the renal expression of TGF-β, type IV collagen and PAI-1 in OLETF rats[73].

Glycosaminoglycans

Glycosaminoglycans are important components of plasma membranes and are believed to be important determinants of glomerular basement membrane permeability. It is assumed that critical loss of glycosaminoglycans has a significant role in the pathophysiologic process of albuminuria and proteinuria[74]. Sulodexide (80% low-molecular-weight heparin, 20% dermatan sulfate) is an oral formulation of the natural polysaccharide glycosaminoglycan. The nephroprotective effects of sulodexide have not been fully explained yet. It is believed that this drug preserves the ionic charge of the glomerular barrier and decreases proliferation and fibrosis in the diabetic kidney[75].

Endothelin Receptor Antagonists

Endothelins comprise a 3-member family of 21–amino acid peptides and regulate water and sodium excretion and acid-base balance and maintain normal renal cell proliferation and tonic vasoconstriction in the kidney[76]. However, an increase in endothelin production causes kidney damage[77]. Diabetic nephropathy is associated with enhanced renal synthesis of endothelins. Avosentan (SPP301) is a new orally available endothelin 1 antagonist.

Calcium Channel Blockers

Nondihydropyridine CCBs have been shown to be renoprotective by decreased urinary albumin excretion (UAE) and improved glomerular barrier size-selectivity in patients with type 2 diabetes mellitus and overt nephropathy[78]. Nondihydropyridine CCBs combined with an ACE inhibitor may produce greater reductions in UAE than either agent alone. Diltiazem and lisinopril significantly reduced urinary protein excretion compared with baseline ($P < 0.05$). Urinary protein excretion was not statistically significant between the agents during either period. The results of this study suggest that diltiazem may be an appropriate alternative agent for patients who cannot tolerate an ACE inhibitor due to adverse effects. The use of lisinopril and diltiazem over a loop diuretic plus beta-adrenergic antagonist for attenuating the progression of diabetic renal disease was also supported[79]. In comparison of nondihydropyridine CCB, diltiazem, with a dihydropyridine CCB, nifedipine, to determine the effect on proteinuria and glomerular membrane permeability, diltiazem reduced proteinuria and improved glomerular permselectivity, thus slowing nephropathy progression. The nondihydropyridine CCBs have been found to be as effective as ACE inhibitors in clinical trials and should be considered an alternative if there is a contraindication to the use of an ACE inhibitor or ARB or if adequate blood pressure control is not obtained with ACE inhibitor or ARB monotherapy[80].

PPAR Ligands in the Management of Diabetic Nephropathy

Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors of nuclear hormone receptor superfamily, which comprises of three members such as PPAR α, PPAR γ and PPAR β/δ[81]. PPAR ligands are promising agents to prevent the progression of diabetic nephropathy. Activation of PPARα induces gene expressions that promote lipid metabolism[82]. PPARα plays an important role in the oxidation of fatty acids. Fibrates class of interventions such as bezafibrate, fenofibrate and gemfibrozil are well-known hypolipidemic agents and they are ligands of PPARα[83]. Reducing the circulating lipids in diabetic patients may provide a new therapeutic option in managing diabetic nephropathy. Bezafibrate provide renoprotection by reducing albuminuria and circulating lipid levels in diabetic patients with nephropathy[84]. Treatment with fenofibrate affords renoprotection by reducing the occurrence of albuminuria and glomerular lesions in experimental diabetic mice[85]. Further, fenofibrate provide renoprotection by decreasing renal COX-2 expression and reducing nitrosative stress in the kidney of diabetic rats with early stages of nephropathy. The administration of gemfibrozil reduce albuminuria, glomerulosclerosis and tubulointerstitial fibrosis in diabetic mice with nephropathy[86]. Activation of PPARγ regulates gene expressions that promote insulin sensitization and glucose metabolism. Thiazolidinedione class of interventions such as troglitazone, rosiglitazone, ciglitazone and pioglitazone are well-known PPARγ agonists employed as insulin sensitizing antidiabetic agents. Chronic diabetes mellitus reduces the mRNA levels of PPARγ in the glomeruli[87]. Therapeutic potential of PPARγ agonists in preventing the development of diabetic nephropathy. Troglitazone have renoprotective effect by reducing urinary albumin/creatinine ratio and downregulating angiotensin-II-induced expression of plasminogen activator inhibitor-1 (PAI-1) in mesangial cells of diabetic rats with nephropathy[88] and also

decrease TGF-β mediated upregulation of type-1 collagen in mouse mesangial cells. Treatment with rosiglitazone possess renoprotective effect as it reduces albuminuria and prevents renal endothelial dysfunction in diabetic patients with nephropathy[89] and markedly reduced high glucose mediated over expression of intracellular adhesion molecule-1 (ICAM-1) and β-integrin in glomerular mesangial cells of rats. Treatment with pioglitazone markedly reduced the occurrence of albuminuria and prevented the development of glomerulosclerosis and glomerular hypertrophy by suppressing the expression of TGF-β, VEGF, PAI-1, type-IV collagen and ICAM-1 in the kidney of diabetic rats with nephropathy.

Herbal Drugs

Sun ginseng at dose of 50 or 100 mg/kg/day for 15 day attenuated water intake and urine excretion induced by diabetes. In addition, the diabetic rats given *Sun ginseng* at a dose of 100 mg/kg body weight showed significant decreases in serum glucose, serum glycosylated protein and urinary protein levels, suggesting that *Sun ginseng* improves the abnormal conditions that lead to oxidative stress. Furthermore, *Sun ginseng* significantly reduced AGE product formation and thiobarbituric acid-reactive substance levels elevated in the kidneys of diabetic rats[73]**.** Green tea is a useful agent to protect against protein oxidation and glycation associated diseases[90]. It also indicated as beneficial agents to manage the development of diabetic nephropathy induced by subtotal nephrectomy plus streptozotocin injection[91]. Corni Fructus (*Cornus officinalis*), had an effect on STZ-induced diabetic rats as compared aminoguanidine Treatment with Corni Fructus for 10 day suppressed hyperglycemia, proteinuria, renal AGE formation, and related protein expressions, *i.e.*, receptor for AGEs, nuclear factor-kB, TGF-β and *N*e-(carboxymethyl) lysine, in the same way as with aminoguanidine[92]**.** *E. jambolana* preparations employed by practitioner of natural health for treatment of diabetes and related complications, antioxidant, anti-inflammatory, and antifertility agents. *Eugenia jambolana* plant serves varies purposes in diabetic patients such as lowering blood glucose level, delaying diabetic complications such as neuropathy and cataract[93]**.** Moreover 1.5% *C. aromatica*-containing diet for 1 wk before and 8 wk after administration of streptozotocin, improves all the events induced by streptozotocin except for hyperglycemia decreased markedly. Thus, *C. aromatica* may have therapeutic potential for the prevention of hyperglycemia-associated diabetic complications[94].

CONCLUSION

Nephropathy involves complex multifactorial signaling mechanism in pathological condition which make it a significant cause of morbidity and mortality in diabetes. Optimal glucose and blood pressure control is the primary goal to delay diabetic nephropathy progressions. But, these therapeutic interventions are insufficient to control the symptoms of diabetic nephropathy. Further studies are warranted to explore the effect of various pharmacological interventions modulate these target site involved in progression of diabetic nephropathy.

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