Pathogenesis and Treatment of Diabetic Nephropathy : Lessons from Animal Models for the Pathogenesis and Treatment of Human Type 2 Diabetic Nephropathy

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Diabetic nephropathy is a major cause of end-stage kidney disease (ESKD) in patients with both type 1 and type 2 diabetes. Almost 30% of type 1 or 2 diabetic patients develop diabetic nephropathy despite strict blood glucose and/or blood pressure control. In human glomeruli, the expansion of diffuse mesangial matrices, exudative lesions and/or segmental nodular sclerosis are pathological characteristics of diabetic nephropathy. The spontaneous mouse model, i.e. the KK-A^y mouse, was produced through the transfer of the yellow obese gene (A^y allele) into the KK mouse. The diabetic phenotype in this mouse is more severe than that in KK mouse. In 2006, Ito *et al.* reported that the pathological changes in the glomeruli of KK-A^y mice were consistent with those in the early stage of human diabetic nephropathy¹⁾. In electron microscopy, a diffuse thickening of the glomerular basement membrane (GBM) was observed in this model mouse. Advanced glycation end products (AGEs) and the transforming growth factor- β (TGF- β) protein appeared to be localized in the glomerular mesangial matrices. The KK-A^y mouse, especially in terms of histopathological findings, is considered to be a suitable animal model for type 2 diabetic nephropathy²⁾.

The objective of this review is to introduce a new strategy for the treatment of type 2 diabetic nephropathy using the KK-A^y mouse.

Keywords : treatment, animal model, KK-A^y mouse, type 2 diabetic nephropathy

Treatment for Diabetic Nephropathy

In the 1970s, it was generally thought that only hyperglycemia might induce diabetic nephropathy. Recently, it appears that the various factors, such as genes, advanced glycation end products (AGEs), reactive oxygen species (ROS), systemic and/or glomerular hypertension, dyslipidemia, inflammation, protein kinase C (PKC) and/or nitrogen oxide (NO) activity, and a high protein diet, might be interrelated to the initiation and progression of diabetic nephropathy. Many compounds have been reported to be AGEs inhibitors, such as aminoguanidine, phenacyl thiazolium bromide, 2-isopropylidenehydrazono-4-oxo-thiazolidine-5-yl-acetanilide (OPB-9195), 2,3-diaminophenazine, vitamin C, vitamin E, angiotensin II receptor blocker (ARB) and pyridoxamine (K-164).

In human diabetic nephropathy, recommended treatment regimes include the strict control of blood glucose (HbA1c of less than 6.9%, NGSP value), strict blood pressure control (less than 130/80 mmHg), the restriction of dietary salt and

protein, adequate exercise, and non-smoking. Recently, it has been observed that the dipeptidyl peptidase-4 (DPP-4) inhibitor may play an important role in increasing active glucagon-like peptide-1 (GLP-1) in diabetic patients. GLP-1 may stimulate insulin secretion from islet β -cells and suppress glucagon secretion from islet α -cells. Thus, the DPP-4 inhibitor is also effective for blood glucose control in patients with diabetic nephropathy. It is necessary to evaluate the efficacy of new anti-diabetic drugs using spontaneous animal models.

There have been many reports on the pathogenesis and treatment of type 2 diabetic nephropathy using various animal models. It appears that KK-A^y mice are a useful spontaneous animal model for the evaluation of the pathogenesis and treatment of patients with type 2 diabetic nephropathy^{1) 2)}. The objective of this review is to introduce a new strategy for the treatment of type 2 diabetic nephropathy using KK-A^y mice.

1. Anti-hypertensive and anti-ROS effects of ARB

The activation of ROS and oxidative stress may

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[Received July 12, 2013]

induce kidney injury, such as glomerulosclerosis and tubulointerstitial fibrosis, in patients with diabetic nephropathy. It is generally considered that angiotensin (ANG) II may stimulate ROS, contribute to the appearance of proteinuria, and induce renal fibrosis via several signaling pathways. Heavy proteinuria is an independent risk factor for the progression of renal diseases. Angiotensin converting enzyme inhibitors (ACEIs) or ARB decreased the levels of urinary protein excretion. In 2004, Fan *et al.*³⁾ reported that candesartan, one type of ARB, reduced an accumulation of AGEs and reduced urinary albumin excretion by downregulating NADPH oxidase p47phox and iNOS expression and by attenuating RAGE (receptor for AGEs) expression in KK mouse with diabetic nephropathy. Moon *et al.*⁴⁾ also indicated that angiotensin (ANG) - (1-7) may attenuate ANG-stimulated NAD(P)H dependent ROS-mediated injury in type 2 diabetic nephropathy using KK-A^y mice.

2. The anti-glycation and anti-ROS effects of pyridoxamine (K-164)

Persistent hyperglycemia and hyperproduction of AGEs may induce glomerulosclerosis in patients with type 2 diabetic nephropathy. Non-enzymatic glycation has been implicated in the pathogenesis of diabetic nephropathy. There are multiple pathways for the formation of AGEs, including N ϵ -(carboxymethyl) lysine (CML), N ϵ -(carboxyethyl) lysine (CEL) and pentosidine, from glucose, products of autooxidation of glucose, Schiff bases, and Amadori products. The presence of AGEs is closely related to hyperglycemia in diabetic nephropathy. Specific AGEs, such as CML, are major products of glycoxidation reactions. Immunohistologically, CME was observed in the glomerular mesangial areas, capillary walls and Bowman's capsules from patients with type 2 diabetic nephropathy (Figure-1).

Many compounds have been reported as AGE inhibitors, such as aminoguanidine, phenacyl thiazo-

Figure-1 Histopathology of human type 2 diabetic nephropathy

lium bromide, 2-isopropylidenehydrazono-4-oxo-thiazolidine-5-yl-acetanilide (OPB-9195), 2,3-diaminophenazine, vitamin C, vitamin E, angiotensin II receptor inhibitors, and pyridoxamine²⁾. Pyridoxamine was introduced by Khalifah *et al.*⁵⁾ as an inhibitor of AGE formation from Amadori products. Degenhardt *et al.*⁶⁾ also reported that pyridoxamine inhibited AGE formation and retarded the development of diabetic nephropathy in streptozotocin-treated rats, an animal model for type 1 diabetes mellitus. In 2007, Tanimoto *et al.*⁷⁾ reported the prevention of the development of type 2 diabetic nephropathy in KK-A^y mice by pyridoxamine (K-163). AGEs have been associated with increased oxidative and nitrosative stresses in both *in vitro* and *in vivo* studies. CML and nitrotyrosine accumulation in glomeruli were decreased by pyridoxamine treatment. TGF- β and laminin- β 1 messenger RNA expressions in the kidneys were significantly lower than those in the controls. This effect of pyridoxamine was related to the improvement of CML and nitrotyrosine accumulation in the kidneys by anti-AGE and/or antioxidant effects¹⁰⁾.

Furthermore, Murakoshi *et al.*⁸⁾ reported on the pleiotropic effect of pyridoxamine on diabetic complications via CD36 expression in KK-A^y mice. CD36 is an 88-kDa membrane glycoprotein belonging to the class B scavenger receptor family, which possess one long extracellular loop between the two transmembrane domains. Pyridoxamine decreased levels of serum triglyceride (TG), especially VLDL, and fasting serum insulin. The accumulation of malondialdehyde (MDA), an advanced lipoxidation end product, in the pyridoxamine treated group was significantly lower than that in the non-treatment control group. CD36 accumulation and mRNA expression in the kidneys and adipose tissues of the treatment group were significantly higher than those in the control group. It appears that pyridoxamine ameliorated lipid peroxidation and insulin resistance in KK-A^y mice. This pleiotropic effect of pyridoxamine was related to CD36 expression in the kidneys and adipose tissues.

3. The anti-microinflammatory effect of eicosapentaenoic acid (EPA)

Previous studies reported that eicosapentaenoic acid (EPA) was effective against all renal diseases, such as diabetic nephropathy and IgA nephropathy.

EPA is one of the n-3 polyunsaturated fatty acids (PUFA) that are present in fish oil. EPA has many effects, such as anti-thrombotic, anti-dyslipidemic, anti-atherogenic, anti-inflammatory, and anti-mitogenic effects. Monocyte chemoattractant protein-1 (MCP-1) is a regulating macrophage recruitment protein, which is upregulated in patients with type 2 diabetic nephropathy. In KK-A^y mice injected with EPA ethyl ester, Zhang *et al.*⁹⁾ and Hagiwara *et al.*¹⁰⁾ reported that EPA improved type 2 diabetic nephropathy in the KK-A^y mice by decreasing hypertriglyceridemia, glucose tolerance and albuminuria. Glomerular mesangial matrix expansion and segmental sclerosis, and interstitial fibrosis were markedly decreased in the EPA treatment group. The diabetes-induced upregulation of MCP-1 and TGF- β expressions were inhibited by EPA, together with the reduction of glomerular macrophage infiltration and oxidative stress in such mice. It appears that EPA might be a therapeutic agent for diabetic nephropathy patients.

Conclusions

It is shown that KK-A^y mice are a useful spontaneous animal model for the evaluation of treatments in type 2 diabetic nephropathy patients. It has been confirmed that the treatments examined in this model mouse are effective for such patients in clinics (Figure-2). A part of this review was presented to the students of the Juntendo University Graduate School on May 17, 2013.

Acknowledgments : I sincerely thank my colleagues in the Division of Nephrology, Department of Internal Medicine at the Juntendo University Faculty of Medicine, Tokyo, Japan.

Financial Disclosures : None declared.

Funding/Support : None declared.

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Figure-2 New insights in management of type 2 diabetic nephropathy

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