Lessons from the Spontaneous Mouse Models
for Treatment of Type 2 Diabetic Nephropathy and IgA Nephropathy

YASUHIKO TOMINO*

Abstract

This review introduces a new strategy for treatment of type 2 diabetic nephropathy and IgA nephropathy based on the findings in animal models, i.e. KK-A'/Ta mouse and ddY mouse. Diabetic nephropathy is a major cause of end-stage kidney disease (ESKD) in patients with diabetes. In human glomeruli, expansion of diffuse mesangial matrices, exudative lesions and/or segmental nodular sclerosis are pathological features of diabetic nephropathy.

There have been many reports using various models of type 2 diabetes. Ito et al., my colleagues, reported that the pathological changes of glomeruli in KK-A'/Ta mice were consistent with those in the early stage of human diabetic nephropathy. Advanced glycation end products (AGEs) and transforming growth factor-beta (TGF-β) protein appeared to be localized in the glomerular mesangial matrices. It appears that KK-A'/Ta mice, especially in terms of histopathological findings, are a suitable animal model for type 2 diabetic nephropathy.

For therapeutic interventions to reduce AGEs, many compounds have been reported to be AGE inhibitors, such as aminoguanidine, phenacyl thiazolium bromide, 2-isopropylidenedihydrazone-4-oxothiazolidine-5-yl-acetanilide (OPB-9195), 2, 3-diaminophenazine, vitamin C, vitamin E, angiotensin II receptor inhibitor and pyridoxamine. It is indicated that pyridoxamine ameliorated lipid peroxidation and insulin resistance in KK-A'/Ta mice. Eicosapentaenoic acid (EPA) showed multiple effects such as anti-thrombotic, hypolipidemic, anti-atherogenic, anti-inflammatory and anti-mitogenic actions. EPA improved type 2 diabetic nephropathy in such mice.

IgA nephropathy is the most common primary chronic glomerulonephritis, which was described by J. Berger. Histopathologically, IgA nephropathy is characterized by expansion of glomerular mesangial matrix with mesangial cell proliferation. Glomeruli typically contain generalized-diffuse granular mesangial deposits of IgA (IgA1), IgG and C3. In 1985, Imai et al. first reported that the ddY strain of mouse can serve as a spontaneous animal model for IgA nephropathy. These mice show mild proteinuria without hematuria, and mesangioproliferative glomerulonephritis with severe glomerular IgA deposits in association with an increase of serum IgA level. Electron dense deposits are observed in the glomerular mesangial areas by electron microscopy. These immunohistopathological findings in ddY mice resemble those in IgA nephropathy patients. These findings from the ddY mouse appear to be useful in determining the pathogenesis and treatment of patients with IgA nephropathy. Although glucocorticoids and immunosuppressants are effective for IgA nephropathy patients demonstrating minor to moderate glomerular injuries, it is necessary to use large doses of these drugs for prolonged periods, which causes severe adverse effects. It appears that PSL-liposome-treated ddY mice showed a marked decrease. Treatments with mizoribine (an immunosuppressant), a monoclonal antibody to murine CD4 molecules and bone marrow transplantation (BMT) also improved glomerular injury in IgA nephropathy in ddY mice. BMT from quiescent ddY mice resulted in the reduction of not only glomerular injury but also mesangial IgA and IgG depositions in recipient-quiescent ddY mice. It appears that bone marrow cells assumed to be IgA-producing cells, may initiate IgA nephropathy.

Key words : KK-A'/Ta mouse, ddY mouse, diabetic nephropathy, IgA nephropathy, treatment

*Division of Nephrology, Department of Internal Medicine, Juntendo University School of Medicine, Tokyo, Japan

[Received June 23, 2009] [Accepted June 29, 2009]
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, expansion of diffuse mesangial matrices, exudative lesions and/or segmental nodular sclerosis are pathological features of diabetic nephropathy. There have been many reports using various models of type 2 diabetes (Table-1).

The KK-/Ta mouse, one of the type 2 diabetic nephropathy mouse models, was established from a Japanese native mouse as an inbred mouse by Kondo et al. in 1957). Since the phenotypic characteristics of KK-/Ta mice are not especially marked, the KK-A'/Ta mouse was established by Nishimura et al. in 1969). This mouse was produced by transfer of the yellow obese gene (A' allele) into the KK-/Ta mouse. The diabetic phenotype in the KK-A'/Ta mouse is more severe than that in KK-/Ta mouse. In 2006, Ito et al., my colleagues, reported that the pathological changes in glomeruli of KK-A'/Ta mice were consistent with those in the early stage of human diabetic nephropathy) (Table-2). Advanced glycation end products (AGEs) and transforming growth factor-β (TGF-β) protein appeared to be localized in the glomerular mesangial matrices). The KK-A'/Ta mouse, especially in terms of histopathological findings, is considered to be a suitable animal model for type 2 diabetic nephropathy.

On the other hand, IgA nephropathy is the most common primary chronic glomerulonephritis, which was described by J. Berger). Histopathologically, IgA nephropathy is characterized by expansion of the glomerular mesangial matrix with mesangial cell proliferation. Glomeruli typically contain generalized-diffuse granular mesangial deposits of IgA (IgA1), IgG and C3. Since pathogenesis of IgA nephropathy is still obscure, it is important to determine the initiation and progression of this disease using a suitable animal model. Several investigators, including Rifai’ s group (Providence, RI, USA) and Emancipator’ s group (Cleveland, OH, USA) reported various experimental animal models for this disease. In 1985, Imai et al.) first reported that the ddY strain of mouse can serve as a spontaneous animal model for IgA nephropathy. These mice show mild proteinuria without hematuria, and mesangio-roliferative glomerulonephritis with severe glomerular IgA deposits in association with an increase of serum IgA level) (Figure-1). Electron dense deposits are observed in the glomerular mesangial areas by electron microscopy. Furthermore, Muso’ s group succeeded in generating a mouse model of IgA nephropathy with a high incidence and early onset of glomerular IgA deposition). These immunohisto-pathological findings in ddY mice resemble those in

![Figure-1 ddY mouse and IgA staining in a glomerulus by immunofluorescence](image-url)
IgA nephropathy patients. Such findings from the ddY mouse appear to be useful in determining the pathogenesis and treatment of patients with IgA nephropathy.

The objectives of this review are to introduce new strategies for treatment of type 2 diabetic nephropathy and IgA nephropathy using animal models, i.e. the KK-A'/Ta mouse and ddY mouse. It is necessary to confirm whether the treatments studied in the KK-A'/Ta mouse and ddY mouse are effective in patients with type 2 diabetic nephropathy and IgA nephropathy.

I. Treatment for Diabetic nephropathy

a. Pyridoxamine (K-164)

Non-enzymatic glycation has been implicated in the pathogenesis of diabetic nephropathy. There are multiple pathways for the formation of AGEs, including N'- (carboxymethyl) lysine (CML), N'- (carboxyethyl) lysine (CEL) and pentosidine, from glucose, products of antioxidation of glucose, Schiff bases and Amadori products. The presence of AGEs is closely related to hyperglycemia and their pathobiochemistry could explain diabetic nephropathy. Specific AGEs, such as CML, are major products of glycoxidation reactions. In therapeutic interventions for reducing AGEs, many compounds have been reported as AGE 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 inhibitors and pyridoxamine. Pyridoxamine was introduced by Khalifah et al. as an inhibitor of AGE formation from Amadori products. Degenhardt et al. 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. reported prevention of the development of type 2 diabetic nephropathy in KK-A'/Ta mice by pyridoxamine (K-163), an AGE inhibitor. AGEs have been associated with increased oxidative and nitrosative stresses in both in vitro and in vivo studies. Pyridoxamine, especially at 400mg/kg body weight per day, improved levels of the urinary albumin/creatinine ratio (ACR), fasting serum triglyceride (TG) and 3-deoxyglucosone (3 DG) in KK-A'/Ta mice. CML and nitrotyrosine accumulation in glomeruli were decreased. TGF-beta 1 and laminin-beta 1 messenger RNA expressions in the kidneys were significantly lower than those in the controls. This effect of pyridoxamine was related to improvement of CML and nitrotyrosine accumulation in the kidneys by anti-AGE and/or antioxidant effects.

Furthermore, Murakoshi et al., my colleagues, reported the pleiotropic effect of pyridoxamine on diabetic complications via CD36 expression in KK-A'/Ta 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 TG, especially VLDL, and fasting serum insulin. Accumulation of malondialdehyde (MDA), an advanced lipoxidation end product, in the pyridoxamine treated group was significantly lower than that in the non-treatment group. CD36 accumulation and mRNA expression in kidneys and adipose tissues in the treatment group were significantly higher than those in the non-treatment group. It is indicated that pyridoxamine ameliorated lipid peroxidation and insulin resistance in KK-A'/Ta mice. This pleiotropic effect of pyridoxamine was related to CD36 expression in the kidneys and adipose tissues.

b. Eicosapentaenoic acid (EPA)

Previous studies reported that eicosapentaenoic acid (EPA) was effective against all renal diseases such diabetic nephropathy. EPA is one of the n-3 polyunsaturated fatty acids (PUFA) which is present in fish oil. It was shown that EPA has many effects such as anti-thrombotic, hypolipidemic, anti-atherogenic, anti-inflammatory and anti-mitogenic actions. Monocyte chemoattractant protein-1 (MCP-1) is a regulating macrophage recruitment protein, which is up-regulated in patients with diabetic nephropathy. In KK-A'/Ta mice injected with EPA ethyl ester (1g/kg/day), Zhang et al. and Hagiwara et al. reported that EPA improved type 2 diabetic nephropathy in the KK-A'/Ta mice by decreasing hypertriglyceridemia, glucose tolerance and albuminuria. Glomerular mesangial matrix expansion and segmental sclerosis, and interstitial fibrosis were markedly decreased by
EPA treatment. Diabetes-induced up-regulation of MCP-1 and TGF-beta expressions was inhibited by EPA, together with the reduction of glomerular macrophage infiltration and oxidative stress. It appears that EPA might be a therapeutic agent for diabetic nephropathy patients.

II. Treatment for IgA nephropathy

a. Steroid-liposome

Although glucocorticoids are effective in IgA nephropathy patients associated with minor to moderate glomerular injuries, it is necessary to use large doses of the drug for long periods. There are severe adverse effects such as diabetes, peptic ulcer and aseptic necrosis of the bones. Drug delivery systems (DDS) that can target drugs to specific body sites have long been sought, because they have potential advantages for improved drug delivery such as reduction of harmful adverse reactions and potentially decreased drug doses required for targeted delivery to a particular cell type.

The purpose of this review is to compare the improvement of immunopathologic findings between PSL-liposome and ordinary PSL treatment of IgA nephropathy in ddY mice. Immunopathological studies were performed to determine whether glomerular injuries in ddY mice are influenced by treatment with a newly developed liposome loaded with prednisolone phosphate (PSL-liposome) \(^\text{10}\). The synthesized novel cationic lipid 3, 6-dipentadecyloxy-1-amizino-benzene (TRX-20) was employed to obtain selective affinity to the anionic cell surface and ECM in glomerular mesangial lesions. ddY mice were treated intravenously with 1.0 \(\text{mg/kg}\) of PSL-liposome once a week from 45 weeks to 61 weeks of age. ddY mice were also intravenously treated with 1.0 \(\text{mg/kg}\) of ordinary PSL once a week. In immunofluorescence, mean intensity of IgA and C3 depositions in glomeruli of PSL-liposome-treated ddY mice were markedly decreased when compared with those of ordinary PSL-treated and untreated control ddY mice. Glomerular mesangial expansion in PSL-liposome-treated ddY mice was milder than that in ordinary PSL-treated ddY mice or untreated control ddY mice. It appears that treatment with PSL-liposome is effective in improving glomerular IgA and C3 depositions and glomerular expansion in IgA nephropathy of ddY mice.

b. Immunosuppressants

Mizoribine, an immunosuppressant, was developed in Japan and shown to prevent the proliferation of lymphocytes \textit{in vitro} and to possess immunosuppressive action \textit{in vivo}. Since mizoribine also has a suppressive effect on antibody formation via direct inhibition of B-cell function, it has beneficial effects in patients with chronic glomerulonephritides, lupus nephritis, nephrotic syndrome and renal transplantation. Shimizu \textit{et al.} \(^\text{15}\), my colleagues, determined the clinical and immunopathological effects of mizoribine in ddY mice. The ddY mice were treated with 0.05 \(\text{mg/ml}\) (low dose) or 0.1 \(\text{mg/dl}\) (high dose) of mizoribine for 35 weeks. Numbers of total T cells (CD3+ T cells), CD4+ T cells, CD8+ T cells and CD11b+ cells among the spleen cells were measured by flow cytometry. Numbers of IgA-, IgG- or IgM-bearing B cells among the spleen cells were also counted. Immunohistopathological changes were examined by light microscopy and immunofluorescence. After 20 weeks of treatment, levels of urinary protein excretion in the ddY mice treated with the high dose of mizoribine were lower than those treated with the low dose. Expansion of glomerular mesangial areas in ddY mice treated with the high dose of this drug was significantly decreased compared with the low dose or with the drinking water control. Numbers of B cells and IgA-bearing B cells among the spleen cells of ddY mice treated with the low or high dose of mizoribine were lower than in those given only drinking water. It appears that treatment with mizoribine might affect B cells, especially IgA-bearing B cells, and improve glomerular injury in IgA nephropathy of ddY mice.

c. Treatment with a monoclonal antibody to murine CD4 molecules

Immunopathological studies were performed to determine whether glomerular injuries in ddY mice are influenced by treatment with a monoclonal antibody (mAb) to murine CD4 molecules \(^\text{16}\). The ddY mice were initially treated with intravenous injections, followed by weekly intraperitoneal injections of mAb CD4. Flow cytometry showed that there was a marked decrease in the number of CD4+ T cells. In immunofluorescence, the mean intensity of IgA
deposits in the glomerular mesangial areas and capillary walls of treated ddY mice was significantly lower than that in saline-treated control ddY mice of comparable age. Glomerular mesangial expansion in the treated ddY mice was milder than that in the same control ddY mice. However, no significant differences in the levels of serum IgA, urinary protein excretion, and average number of intraglomerular cells were observed between the treated and control ddY mice. It appears that although CD4+ T cells control the amount of IgA deposits in glomeruli, other factors may be involved in the evolution of IgA nephropathy in ddY mice. It is not known whether the increase observed in the number of intraglomerular cells in both treated and control ddY mice is due to resident glomerular cells or mononuclear cells infiltrating the glomeruli. Our studies showed that increased glomerular cells in ddY mice are positive for markers of Thy-1, 2 (total T cells), CD8 (killer/suppressor T-cells) and CD11 (mac-1, macrophages/monocytes), suggesting that the majority of these cells are likely to be infiltrating cells and glomerular mesangial cells. Since several cytokines or growth factors such as IL-1, IL-6, TNF α and platelet-derived growth factor (PDGF) have been shown to be involved in mesangial cell proliferation, it was suggested that, in addition to the effect of CD4+ T cells that may modulate the amount of glomerular IgA deposits, these factors may be involved in the progressive mechanism of IgA nephropathy.

d. Bone marrow transplantation (BMT)

A previous report demonstrated that in a patient with IgA nephropathy and chronic myeloblastic leukemia, bone marrow transplantation (BMT) resulted not only in remission of leukemia but also in remission of IgA nephropathy17). Imasawa et al. also reported that BMT from normal mice attenuated glomerular lesions in a murine model of IgA nephropathy, HIGA (high serum IgA ddY) mice, while the glomerular lesion associated with IgA deposition was reconstituted in normal recipient mice after BMT from HIGA mice. These findings indicated that IgA nephropathy may involve disorders of stem cells. The ddY mouse is known as a spontaneous murine IgA nephropathy model, but the incidence of IgA nephropathy is highly variable. Suzuki et al., my colleagues, recently observed that ddY mice could be classified into three groups, the early onset (20 weeks), late onset (40 weeks), and quiescent groups by serial renal biopsies that confirm glomerular lesions and IgA deposition19). A genome-wide association study of the early onset and the quiescent mice revealed that the susceptibility to murine IgA nephropathy is partly regulated by specific loci syntenic to the IgAN1 gene known as a candidate gene of human familial IgA nephropathy19) 20). These results indicated the suitability of the grouped ddY mouse model for examining the pathogenesis of IgA nephropathy. Although the potential of bone marrow derived cells (BMC) to differentiate to glomerular cells has been discussed, the role of BMC in the kidney is still obscure. The mechanism of glomerular immune-complex deposition and the role of BMC in the kidneys were examined using ddY mice. In 2007, Suzuki et al. also reported that BMC are responsible for the induction of IgA nephropathy21). BMT from the early onset ddY mice resulted in mesangio-proliferative glomerular injury with mesangial IgA and IgG depositions in the recipient-quiescent ddY mice. In contrast, BMT from quiescent ddY mice resulted in reduction of not only glomerular injury but also mesangial IgA and IgG depositions in the recipient-early onset ddY mice (Figures-2 and 3). BMT from the early onset ddY mice caused progression of urinary albumin levels in the recipient-quiescent ddY mice, and also caused a marked increase of urinary albumin levels in the recipient-early onset ddY mice. It appears that BMC, presumed to be IgA producing cells, may initiate IgA nephropathy.

Acknowledgments : I sincerely thank my col-
leagues in the Division of Nephrology, Department of Internal Medicine at Juntendo University School of Medicine, Tokyo, Japan.

References

 Lessons from the spontaneous mouse models for treatment of type 2 diabetic nephropathy and IgA nephropathy

2型糖尿病腎症・IgA腎症治療について
自然発症マウスモデルから学ぶこと

富 野 康巳*  
YASUHIKO TOMINO

抄録

本論文では、2型糖尿病腎症とIgA腎症に対する新しい治療法について自然発症動物モデルであるKK-A'／TaマウスとddYマウスを用いて検討したものである。糖尿病腎症は、末期腎不全の主要な原因疾病で、ヒト腎症の糸球体では、メサンギウム基質の拡大、滲出性病変、分節性硬化が特徴である。当教室では、KK-A'／Taマウスの腎病変は、ヒトの糖尿病腎症の初期病変に酷似し、糸球体メサンギウム領域には、終末糖化産物（AGEs）やTGF-βの蓄積が認められたことを明らかにした。このことから、KK-A'／Taマウスは、2型糖尿病腎症の自然発症モデルとして、使用可能であると考えられる。現在、AGEs産生抑制効果のある薬物として、aminoguanidine, phenacyl thiazoilum bromide, OPB-9195, 2, 3-diaminophenazine, vitamin C・E, ARBやpyridoxamineが知られている。また、EPA製剤には、抗血栓作用、脂質低下作用、抗動脈硬化・炎症・分縦作用があるといわれている。私達は、pyridoxamineとEPAの投与は、KK-A'／Taマウスの糖尿病腎症を改善させることを報告した。IgA腎症は、最も頻度の高い慢性糸球体腎炎であり、糸球体メサンギウム領域にIgA（IgA1）、IgG, C3の顆粒状沈着とメサンギウム基質の拡大、メサンギウム細胞の増殖が特徴である。1985年Imaiらが、ddYマウスがIgA腎症に非常に類似した病変を呈することを報告して以来、実験に広く用いられている。私達は、ddYマウスにPSL封入リポソーム（PSL-liposome）,免疫抑制薬であるmizoribine, モノクローナルマウスCD4抗体のそれぞれを投与し、腎病変を改善させることを報告した。また、早期発症ddYマウスに沈着するIgAは、末発症ddYマウスの骨髄を移植することで減少した。このことから、本症の発症に骨髄細胞の関与が示唆される。

キーワード：KK-A'/Taマウス, ddYマウス, 糖尿病腎症, IgA腎症, 治療