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ORIGINAL ARTICLE

Lipoprotein glomerulopathy induced by ApoE-Sendai is different from glomerular lesions in aged apoE-deficient mice

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Abstract
Objective A mutant of apolipoproteinE (apoE), ApoE-Sendai (Arg145Pro), is one of the major causative factors of human lipoprotein glomerulopathy (LPG). An apoE-deficient mouse with introduced ApoE-Sendai gene (ApoE-Sendai mouse) developed a murine counterpart of LPG, whereas it was also reported that apoE-deficient mouse (apoE KO mouse) spontaneously developed LPG-like lesion regardless of introduction of ApoE-Sendai gene. In the present study, we differentiated renal lesions between these two models by detailed analyses of histology and lipoprotein profile, and clarified the role of apoE variants.
Method ApoE-Sendai mice were induced by injection of adenovirus vectors. The kidneys showing LPG-like lesions in apoE-Sendai and apoE KO mice were histopathologically evaluated. Plasma lipids and lipoproteins of both mice were also examined.
Results Histological alteration of the kidney in ApoE-Sendai mice was observed with light microscopy (in 40 out of 50 mice; mild 24, moderate 13, severe 3). Characteristic lesions were dilated vascular lumens mimicking lipoprotein thrombi in human LPG. Similar changes were found in hematoxylin–eosin stained sections of aged apoE KO mice. Meanwhile, periodic acid–Schiff, Azan Mallory, and Oil red O/Sudan III stained sections revealed that the dilated lumens of ApoE-Sendai mice mainly contained lipids and lipoproteins but those of aged apoE KO mice contained much other materials, e.g., proteins and fibrils. These findings were supported by electron micrographs, in which round-shaped droplets indicating lipoproteins were observed in ApoE-Sendai mice but not in aged apoE KO mice. In the kidney of apoE KO mice many anti-mouse CD68 Ab positive cells were detected. This contrasts with the result seen in ApoE-Sendai mice. The plasma lipoprotein compositions of the two types of mice were totally different.
Conclusion It was certain that the kidneys of aged apoE KO mice showed morphological alteration, but the histological findings of glomerular lesions were different from those seen in the kidneys of ApoE-Sendai mice. According to the histological findings and plasma lipoprotein profile, ApoE-Sendai mice, not apoE KO mice, is a murine model for human LPG. This means that apoE variants are essential to LPG.

Keywords Lipoprotein glomerulopathy · ApoE-Sendai · ApoE KO mice

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Introduction

Lipoprotein glomerulopathy (LPG) is a disease characterized by deposition of thrombus-like lipoprotein in glomerular capillaries, and showing type III hyperlipoproteinemia with high concentration of plasma apoE [1–3]. An apoE variant, Apo E2(Arg145Pro) Sendai, was initially identified [4], and subsequently several other variants were also discovered in LPG patients [5–9]. These evidences have

indicated that abnormal apoE is responsible for LPG [2, 10], and that the structural changes of the binding site with low-density lipoprotein (LDL) receptor, 140–150 residues of apoE, result in type III hyperlipoproteinemia [5, 11–13].

However, in contrast to patients with familial type III hyperlipoproteinemia, carriers of ApoE-Sendai did not show any characteristic symptoms of systemic lipid abnormalities, such as corneal arcus, xanthoma, and Achilles tendon thickness, and the degree of hyperlipidemia was not severe [2, 14, 15]. Some cases with specific renal lesions for LPG showed normal lipid profile [2, 16]. Therefore, LPG is considered not to be caused by hyperlipoproteinemia due to reduced LDL receptor binding capacity by apoE variants, but to be caused by specific deposition of abnormal lipoproteins including apoE variants in the kidneys [10, 17]. From this point of view, renal histology, especially electron microscopy [18] and lipid staining [19], is the most reliable diagnostic tool for LPG. This fact is authorized by many standard textbooks [20–23].

ApoE-deficient mice (apoE KO mice) received virus-mediated transduction of ApoE Sendai (ApoE-Sendai mice) developed the murine counterpart of LPG, but not by induction of any apoE isoforms [24], and that study suggested that ApoE-Sendai was etiologic cause of LPG. On the other hand, it was reported that aged apoE KO mice (apoE KO mice) spontaneously develop LPG and that abnormal ApoE-Sendai is not necessarily required for onset of the disease [25].

In the present study, we differentiated renal lesions between these two models by detailed analyses of histology and lipoprotein profile, and clarified the role of apoE variants in LPG.

Materials and methods

Animals

ApoE KO [26] and C57BL/6 mice (wild type) were obtained from Jackson Laboratory (Bar Harbor, Maine, USA), and the generation of apoE KO mice in the C57BL/6 background was performed by Dr. Oikawa, Nippon Medical School. Mice were bred and maintained in specific pathogen free (SPF) facility of Fukuoka University under permission of Dr. Oikawa. Mice were maintained in a temperature-controlled room (22°C) with a 12-h light/dark cycle. Mice were allowed free access to standard food (CRF-1 food, containing normal 5.7% fat and 0% cholesterol, Japan Chares River Ltd. Yokohama, Japan) and water.

Experimental protocol

The recombinant adenoviruses designated ApoE-Sendai were processed according to the method reported by Ishigaki et al. [18]. Fifty 20-week-old mice were injected with adenoviruses via tail vein. At day 30 after injection, blood samples were collected for lipoprotein analyses and the mice were sacrificed. Kidneys were removed for histopathological observations. Food was removed from the mice 4 h before collection of blood from the retro-orbital plexus. Plasma was stored at −80°C until analysis. Twenty 24-week-old (24-wo) and three 86-week-old (86-wo) apoE KO mice were subjected to comparison of lipoprotein profiles and histopathology with ApoE-Sendai-injected mice. The experimental protocol was approved by the Institutional Review Board of Fukuoka University.

Plasma cholesterol, triglyceride, and lipoprotein analyses

Plasma total cholesterol (TC) and triglyceride (TG) were assessed. Plasma lipoproteins were analyzed by online dual enzymatic method for simultaneous quantification of cholesterol and triglycerides by high-performance liquid chromatography (HPLC) as previously described [27, 28].

Histopathological observations of kidneys

Mice were killed on day 30 after infection of the viral vector. The kidney was removed and fixed in 10% neutral buffered formalin, methyl Carnoy's solution, and Karnovsky's solution. The fixed kidneys were embedded in paraffin following routine methods. Paraffin-embedded samples were sliced at 2–3 μm and stained with hematoxylin and eosin (HE), periodic acid–Schiff (PAS), and Azan–Mallory (AM). In frozen sections, lipids were studied with Oil red O and Sudan III stain. For electron microscopy (EM), small blocks of tissues were fixed with 2.5% glutaldehyde and 2% paraformaldehyde followed by postfixation in 1% osmium tetroxide, and embedded in Epon812 by our standard procedure. Epon-embedded blocks were cut at 80 nm with a diamond knife. Ultrathin sections were double-stained with uranyl acetate and lead citrate for electron microscopy. The same faces of blocks were cut at 1 μm with a sapphire knife replacing a diamond knife. These semithin sections were fixed onto lysine-coated slide glasses laying on hot plate at 60°C to 70°C. We also examined lipids using Sudan III stain in these sections [19]. Histopathological diagnosis was done by third-party experts familiar with pathology of LPG without knowledge regarding the sources of tissue. AM stain was used for evaluation of LPG. The tissue specimens were classified into no change, slight, mild, and severe as follows: no change, all glomeruli do not have lipoprotein thrombi and foam cells; mild, mesangium areas show hyperplasia by vacuolar degeneration, but does not show so-called lipoprotein thrombi; moderate, as for mild, but some glomeruli have lipoprotein thrombi and foam cells; severe,

many glomeruli clearly have lipoprotein thrombi and foam cells. Lipoprotein thrombi were confirmed by lipid stain technique and EM using frozen sections and Epon-embedded blocks.

Immunopathological studies of the kidneys

After deparaffinization of formalin-fixed sections in xylene and ethanol, and washing in phosphate-buffered saline (PBS), the sections were incubated with anti-mouse CD68 antibody (Ab) (Hycult biotechnology, Uden, The Netherlands) at concentration of 1 μg IgG/ml PBS including 1% bovine serum albumin (BSA-PBS). Staining was performed using anti-mouse IgG-HRP labeled polymer (DakoCytomation Inc., Carpinteria, USA).

Statistical analysis

Quantitative data are given as mean ± standard deviation (SD). Analysis of variance (ANOVA) followed by Fisher's method (StatView version 5.0) was performed to analyze the differences between groups. P value of less than 0.05 was considered statistically significant.

Results

Histopathological analyses

Histological alterations of the kidney in 24-wo ApoE-Sendai and 86-wo apoE KO mice were observed with light microscopy. Histopathological findings of LPG in ApoE-Sendai and 86-wo apoE KO mice were varied, and were assigned to four categories (Fig. 1). Characteristic lesions were dilated vascular lumens mimicking lipoprotein thrombi in human LPG (Fig. 2a–c). Similar changes were found in HE stained sections of three of eight 86-wo apoE KO mice (Fig. 2d). However, such changes were hardly found in 24-wo apoE KO mice. Meanwhile, PAS positive (Fig. 2e arrow) and blue-stained (Fig. 2f arrow) substances

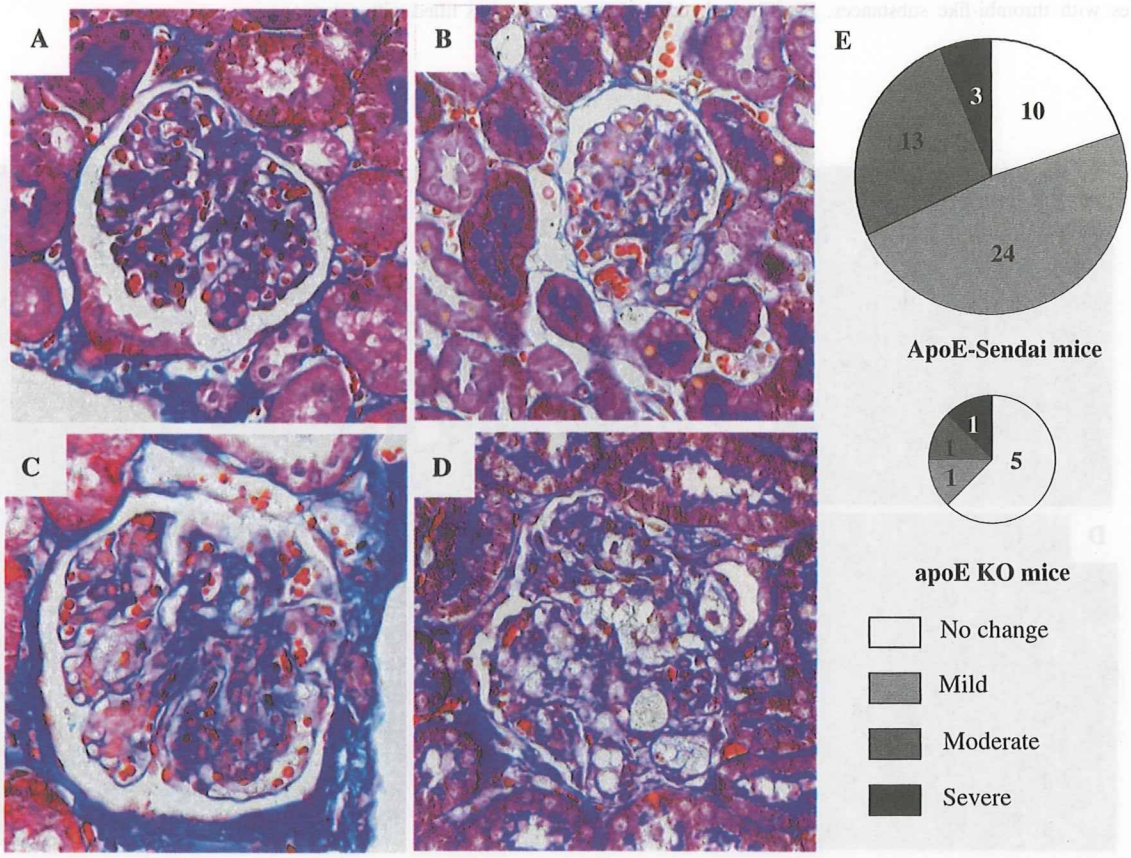

Fig. 1 Pathohistological alterations in kidneys of ApoE-Sendai and apoE KO mice were evaluated and assigned to one of four categories; representative AM stained glomerulus from each category are shown. **a** No change: all glomeruli showed neither lipoprotein thrombi nor foam cells. **b** Mild: a part of mesangium area showed hyperplasia and stained pale with aniline blue, but lipoprotein thrombi were not seen. **c** Moderate: most of mesangium area showed hyperplasia and vacuolized, and some glomeruli have lipoprotein thrombi and foam cells. **d** Severe: many glomeruli clearly have lipoprotein thrombi and foam cells. **e** Distributions of 50 ApoE-Sendai and 8 apoE KO mice in the four categories (number of mice in each category is indicated)

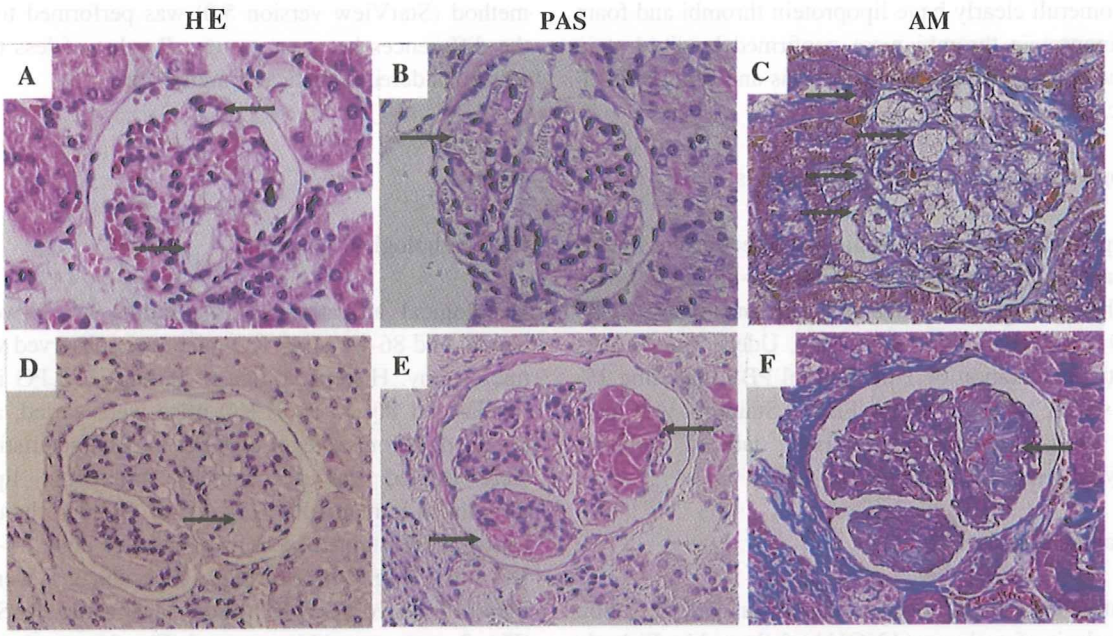

Fig. 2 Histological findings in the kidney of ApoE-Sendai and apoE KO mice showed characteristic marked dilatation of glomerular capillaries with thrombi-like substances. Paraffin-embedded kidney sections were stained with HE, PAS, and AM (ApoE-Sendai mice: **a–c**, apoE KO mice: **d–f**. *Arrows* indicate findings of dilated vascular lumens filled with substances

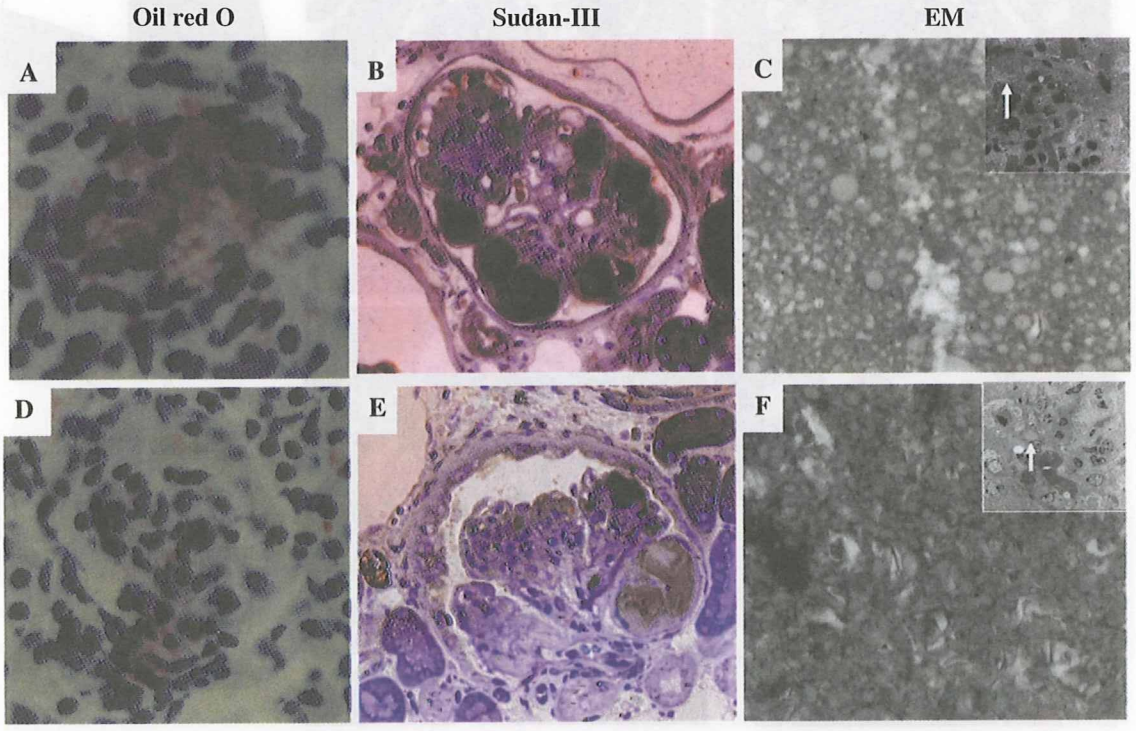

Fig. 3 The thrombi-like substances located in the lumen in the kidney of apoE KO mice were different from those of ApoE-Sendai mice. In frozen sections, lipids were stained with Oil red O and Sudan III (ApoE-Sendai mice: **a, b**, apoE KO mice: **d, e**, respectively). Electron-microscopic findings of thrombi-like substances in ApoE-Sendai mice **c** and apoE KO mice **f**. *White arrows* in the low-magnification images indicate the areas examined at high magnification. Magnifying powers were ×2,000 and ×40,000, respectively

were located in the vascular lumens of PAS and AM stained sections of 86-wo apoE KO mice, respectively. These findings were negative for ApoE-Sendai mice (Fig. 2b, c).

On the other hand, the substances in dilated lumens of ApoE-Sendai mice were demonstrated by Oil red O stain in frozen sections (Fig. 3a) and Sudan III stain in Epon-embedded semithin sections (Fig. 3b). The substance in 86-wo apoE KO mice were palely stained by Oil red O and Sudan III, respectively (Fig. 3d, e).

EM showed that the substances in ApoE-Sendai mice were mainly composed of numerous and various sized round-shaped droplets, which were similar to these in human LPG (Fig. 3c), while those in apoE KO mice showed chaotic small fibrous aggregated feature (Fig. 3f).

To summarize the histopathology results, the substances in the dilated lumens of ApoE-Sendai mice mainly contained lipids and lipoproteins, whereas those of aged apoE KO mice had much other materials, e.g., proteins and fibrils.

Immunopathological studies of the kidneys

In addition to the histological finding of dilated vascular lumens filled with the substance, the presence of glomerular foam cells in mesangial areas was conspicuous in the kidney of 86-wo apoE KO mice, as documented by immunohistological examination with anti-mouse CD68 Ab (Fig. 4a). However, in the kidney of ApoE-Sendai mice, foam cells positive for CD68 identifying macrophage were rarely confirmed within glomerulus (Fig. 4b). In fact there was a significant differences in the average numbers of anti-CD68 Ab positive cells per glomerulus between the two mice (Fig. 4c).

Plasma cholesterol, triglyceride, and lipoprotein analyses

The values of TC of 24-wo apoE KO and ApoE-Sendai mice were 713.5 ± 223.7 mg/dl and 308 ± 158 mg/dl, respectively. That of 86-wo apoE KO mice was

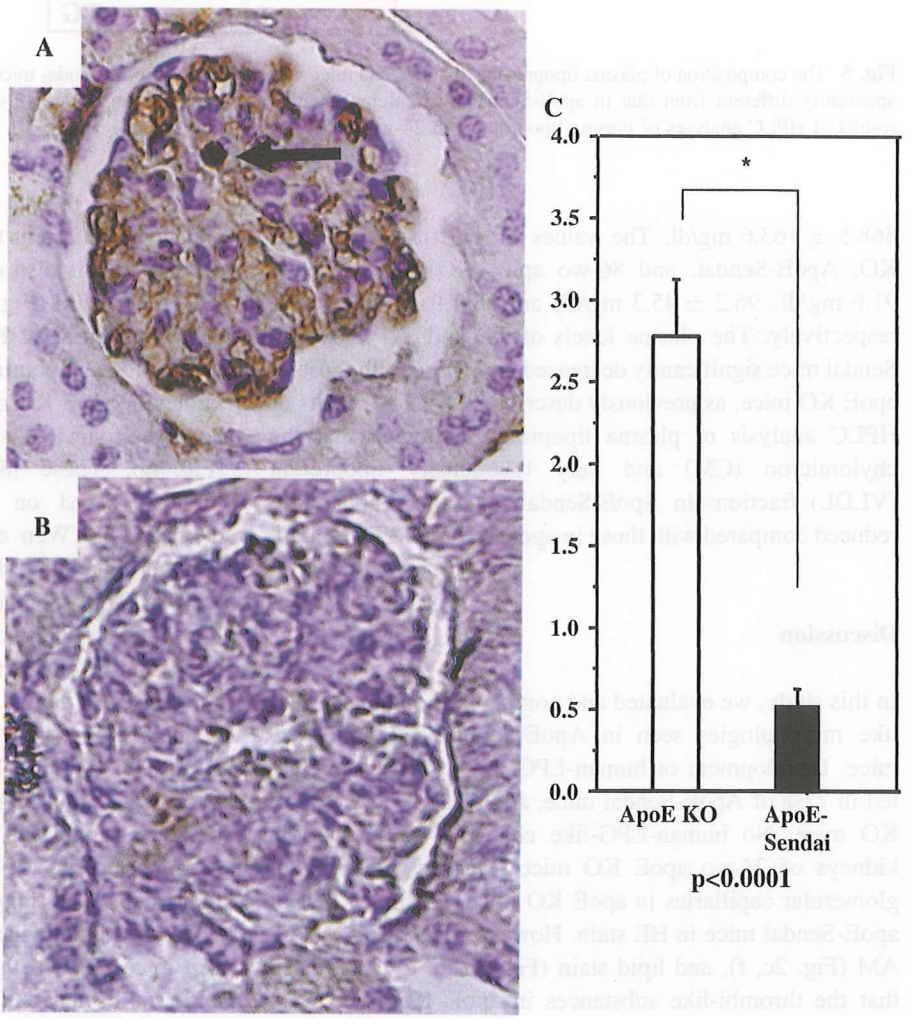

Fig. 4 The presence of glomerular foam cells in mesangial areas was conspicuous in the kidney of apoE KO mice. Representative findings of immunohistologically examined glomeruli with anti-mouse CD68 Ab. The foam cells (*arrow*) were detected in apoE KO mice (**a**), but hardly seen in apoE-Sendai mice (**b**). **c** The average numbers of anti-CD68 Ab positive cells per glomerulus between both mice

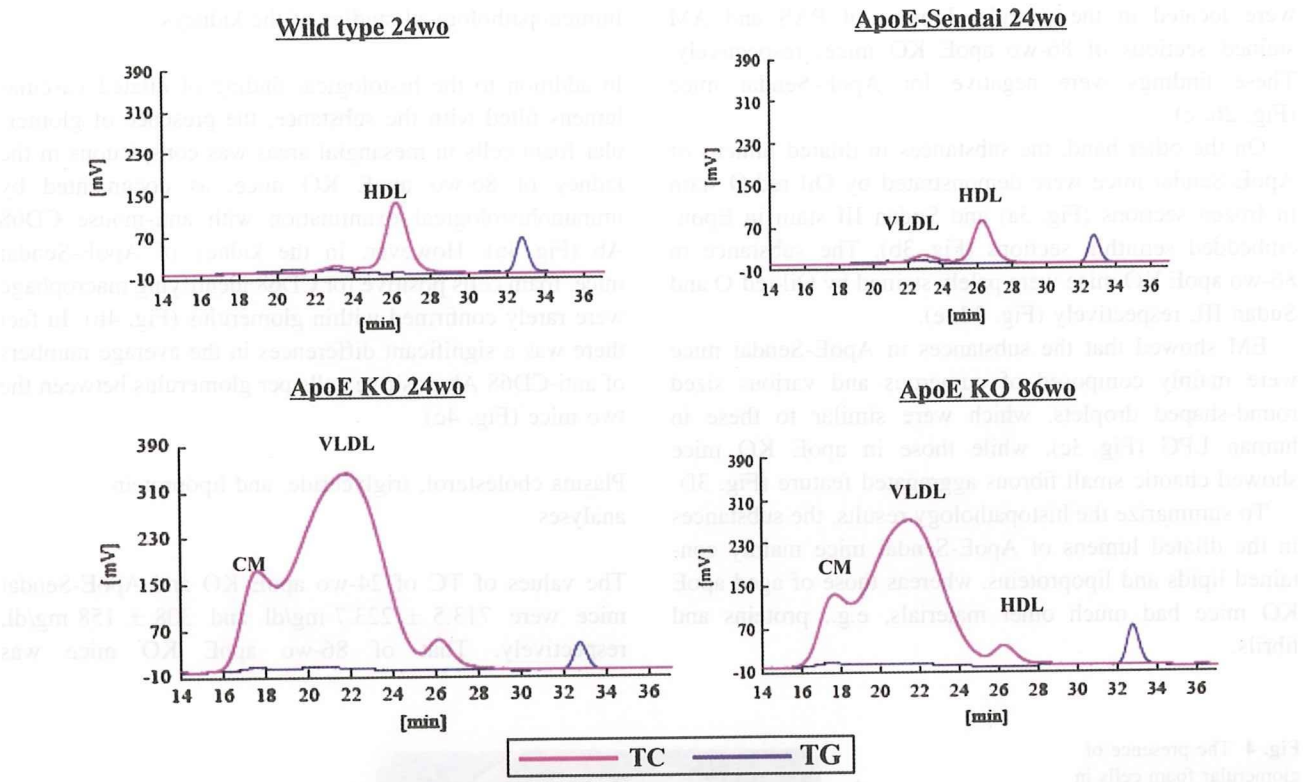

Fig. 5 The composition of plasma lipoproteins in apoE KO mice was apparently different from that in apoE-Sendai mice. Representative results of HPLC analyses of plasma lipoproteins in 24-wo wild-type, 24-wo ApoE-Sendai mice, and 24-wo and 86-wo apoE KO mice. *CM* chylomicron, *VLDL* very low-density lipoprotein, *HDL* high-density lipoprotein

868.5 ± 163.6 mg/dl. The values of TG of 24-wo apoE KO, ApoE-Sendai, and 86-wo apoE KO were 159.1 ± 91.6 mg/dl, 96.2 ± 45.3 mg/dl, and 134.9 ± 47.8 mg/dl, respectively. The plasma levels of TC and TG in ApoE-Sendai mice significantly decreased compared with those of apoE KO mice, as previously described [24]. The results of HPLC analysis of plasma lipoproteins showed that the chylomicron (CM) and very low-density lipoprotein (VLDL) fractions in ApoE-Sendai mice were apparently reduced compared with those in apoE KO mice (Fig. 5a–c).

Discussion

In this study, we evaluated and compared the human-LPG-like morphologies seen in ApoE-Sendai and apoE KO mice. Development of human-LPG-like lesion was detected in 77% of ApoE-Sendai mice, and 38% of 86-wo apoE KO mice. No human-LPG-like change was detected in kidneys of 24-wo apoE KO mice. Marked dilatation of glomerular capillaries in apoE KO mice resembled that of apoE-Sendai mice in HE stain. However, PAS (Fig. 2b, e), AM (Fig. 2c, f), and lipid stain (Fig. 3a, b, d, e) revealed that the thrombi-like substances in apoE KO mice were apparently different from those in apoE-Sendai mice. The difference was also observed in the feature of substances detected by EM (Fig. 3c, f). These findings suggested that substances in apoE KO mice were not mainly constructed with lipids or lipoproteins. In immunohistological study of kidney of apoE KO mice with anti-mouse CD68 Ab, many positive foam cells were detected in mesangial area (Fig. 4a). These histological and immunopathological findings based on aging were consistent with those described by Wen et al. [19], although their mice were younger than ours. Moreover, this contrasts with the results seen in ApoE-Sendai mice. The compositions of plasma lipoprotein of 86-wo apoE KO and 24-wo ApoE-Sendai were totally different (Fig. 5). Based on the results, the substances in dilated capillaries in ApoE Sendai mice may be induced by in situ lipid abnormality but those in old apoE KO mice may be related to various factors generated by aging. Aging might generally cause various nephropathies, in support of the conventional mechanism of glomerulosclerosis [29–32]. Infiltration of CD68 positive foam cells also suggests the development of glomerulosclerosis as analogue to atherosclerosis [33]. In fact apoE KO mice used in the present study show hyperlipidemia and arteriosclerosis [34].

Human LPG showed distinct peculiar histology and abnormal lipoprotein profiles mimicking type III hyperlipoproteinemia. Histological findings are the most important evidence for making a diagnosis of LPG. On light microscopy, capillary lumina in the glomerulus are markedly dilated by pale-stained substances. Foam cells characterizing lipidosis are rarely seen in either the glomeruli or the interstinum. On electron microscopy, thrombus-like substances in the glomerular capillaries are composed of granules and vacuoles of various sizes. Immunohistochemical study defines the deposition of apoB and apoE, and Sudan or Oil red O staining reveals lipid droplets in the thrombus-like substances [1]. Actually, apoE is an important apolipoprotein forming VLDL and LDL and has a pivotal role in the development of lipoprotein thrombi in human LPG [28, 29]. Therefore, the findings of the kidney of 86-wo apoE KO mice obtained in this study were different from those in ApoE-Sendai mice and were not appropriate for murine model of human LPG [35, 36]. Although it has not been clarified how apoE mutations cause LPG, this study indicates that mutation of ApoE-Sendai plays a crucial role in the pathogenesis of LPG in animal as well as in human.

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ORIGINAL ARTICLE

Anemia and hypertension are risk factors for both renal prognosis and survival in patients with diabetes mellitus

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Abstract
Background Diabetic nephrosclerosis is the most common cause of renal failure in the industrialized countries. At the same time, the mortality rate of patients with diabetes mellitus is high.
Methods To clarify the factors influencing the prognosis and survival of patients with diabetic nephrosclerosis, we carried out a retrospective follow-up study of 166 cases (age, 55.6 ± 1.0 years; male/female, 110/56) by simple and multifactorial analyses of clinical data recorded at time of renal biopsy, including survival after diagnosis of diabetic mellitus (months), body mass index (BMI) (kg/m^2) [body weight/(body height)^2], age (years), mean blood pressure (mBP) (mmHg) [diastolic BP + (systolic BP − diastolic BP)/3], serum levels of albumin (mg/dl), urea nitrogen (BUN) (mg/dl), serum creatinine (s-Cr) (mg/dl), total cholesterol (mg/dl), triglyceride (mg/dl), and fasting blood sugar (FBS) (mg/dl), hematocrit (%), HbA1c (%), urinary protein secretion (g/day), insulin resistance, BP control (good, <140/90 mmHg or poor, ≥140/90 mmHg) after biopsies, and pathomorphological parameters at the biopsy.
Results We found a significant association between renal prognosis and several factors, e.g., hypoalbuminemia, anemia, high levels of BUN and s-Cr, hypercholesteremia, hypertriglyceridemia at biopsy, poor control of BP after biopsies, Kimmelstiel–Wilson nodule, and severe glomerular and tubulointerstitial damages at the biopsy. In addition, associations between survival and factors such as low value of BMI, elderly age at the biopsy, and poor control of BP after biopsies were significant. By multivariate analysis we also found a significant association of renal prognosis with anemia, BUN, severe glomerular damage at the biopsy, and poor control of BP after biopsies. At the same time, poor control of BP after biopsies had a significant association with survival. On Kaplan–Meier analysis, anemia at biopsy and hypertension after biopsies are risk factors for both renal prognosis and survival in diabetes mellitus patients.
Conclusions Our data strongly suggest that good control of BP after biopsies and anemia at the biopsy play pivotal roles in the prognosis and survival of patients with diabetic glomerulosclerosis.

Keywords Diabetic nephrosclerosis · Renal prognosis and survival · Anemia · Hypertension

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Introduction

Diabetic nephrosclerosis is the most common cause of renal failure in industrialized countries [1]. At the same time, the survival of patients with diabetes mellitus is poor [2–4]. These are issues not only of medicine but also of finance [5, 6]. Hyperglycemia [7, 8], hypertension [9, 10], anemia [11, 12], protein-rich diet [13–15], and severe proteinuria [16] have been reported as the prognostic factors in patients with diabetic nephrosclerosis. Also,

cardiovascular diseases have been reported as contributing to the survival factors for patients with this disease. In this study, we investigated whether hyperglycemia, hypertension, anemia, and severe proteinuria could be prognostic factors for the course and outcome of diabetic nephrosclerosis and survival in patients with the disease [2–4].

Subjects and methods

Subjects

Of some 12,000 serial renal biopsy cases in the Department of Pathology, Fukuoka University School of Medicine, in 25 years, 276 patients (male/female: 177/99; mean age at renal biopsy: 55.9 ± 0.8 years; range: 16–87 years) were diagnosed with diabetic nephrosclerosis. Prognostic outcomes were available for 166 of these patients (60.1%), of whom 110 were males and 56 were females, with patient age ranging from 18 to 78 years (mean ± SE: 55.6 ± 1.0 years). Of 166 cases, the survival prognosis of only one patient was not known. Mean duration from biopsy to end of follow-up was 7.9 ± 4.5 years in all patients. The diagnosis of diabetic nephrosclerosis in the renal biopsy cases was made as follows: after samples were embedded in paraffin, hematoxylin–eosin, periodic acid Schiff, and periodic acid methenamine silver stainings were performed. Kimmelstiel–Wilson nodules (KW nodules) or diffuse mesangial proliferation, both recognized as hallmarks of diabetic nephrosclerosis under polarized light microscopy, and glomerular basement membrane (GBM) thickening (≥400 nM) under electron microscope (EM) were seen in all 166 cases. However, no immunoglobulin deposits were seen by the immunofluorescence method in any of the 166 cases.

Methods

The following parameters were reviewed as candidate prognostic factors: survival after the diagnosis of diabetes mellitus (months), body mass index (BMI) (kg/m^2) [body weight/(body height)^2], age (years), mean blood pressure (mBP) (mmHg) [diastolic BP + (systolic BP − diastolic BP)/3], serum levels of albumin (mg/dl), urea nitrogen (BUN) (mg/dl), creatinine (s-Cr) (mg/dl), total cholesterol (mg/dl), triglyceride (mg/dl), and fasting blood sugar (FBS) (mg/dl), hematocrit (%) (anemia was defined as ≤40%), HbA1c (%), urinary protein secretion (g/day), insulin resistance [homeostasis model assessment insulin resistance index (HOMA-R); fasting blood sugar (mg/dl)/18 × fasting serum insulin (μU/ml)/22.5] at the biopsy, and BP control after biopsies, KW nodules, index of diabetic glomerulosclerosis (DGS index: [0 × Number(N)0 + 1 × N1 + 2 × N2 + 3 × N3 + 4 × N4]/(N0 + N1 + N2 + N3 + N4); mild: DGS index ≤ 2.0; moderate: 2.0 < DGS index ≤ 3.0; advanced: 3.0 < DGS index ≤ 4.0) [17] using the Gellman classification (grade 0–4) [18], and grade of tubulointerstitial damage (TI damage, 0 to +3) at the biopsy [19]. We used the point-counting method to evaluate damage in the tubular basement membrane and/or interstitium. Under high magnification (×400) using an 81-point (100-square) eyepiece micrometer, we analyzed at least ten consecutive nonoverlapping cortical fields from each biopsy section. The numbers of points overlying damages in the tubular basement membranes and interstitial space were counted. The result was expressed according to the following formula: (number of grid intersections in areas of damage in the cortical interstitium/total number of grid intersections) × 100. We divided the cases semiquantitatively into four grades of TI damage: 0, no TI damage; 1+, <25% TI damage; 2+, ≥25 to <50% TI damage; 3+, ≥50% TI damage. The follow-up data (BP control situation and prognosis after biopsies) were

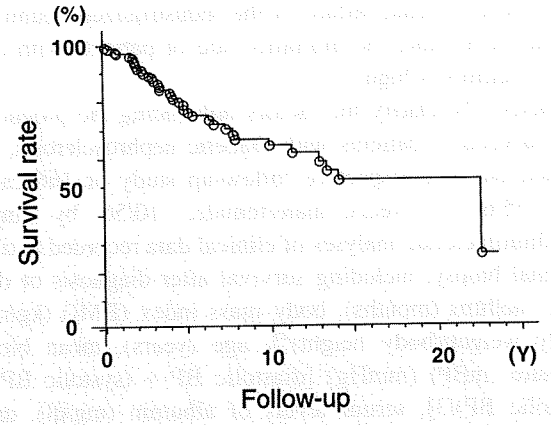

Fig. 1 Survival curve of the patients with diabetes mellitus was poor: 64.7% of diabetic nephrosclerotic patients survived at least 10 years and 52.4% survived at least 20 years after diagnosis

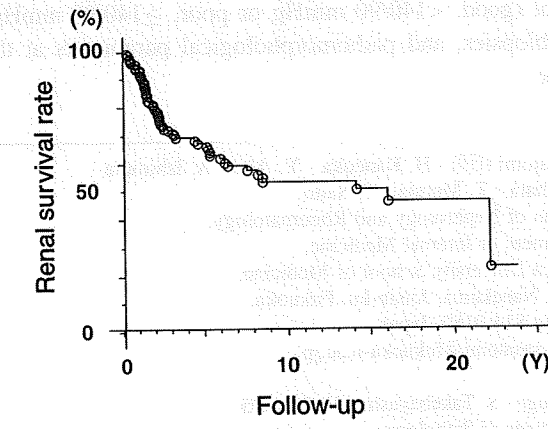

Fig. 2 Renal survival curve of the patients with diabetic nephrosclerosis was poor: 53.8% of the patients with 10-year survival and 46.7% if the patients with 20-year survival were free from hemodialysis

collected by reviewing medical charts as well as administering questionnaires via phone calls or letters. Good BP control means <140/90 mmHg; poor BP control means ≥140/90 mmHg. The clinical data regarding the BP control situation were collected for 129 patients (77.7%). At the same time, 22 sets of BP and anemia data including medications were collected in the present, that is, at the end of the follow-up period (13.3%).

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD). Comparison of continuous variables between groups were performed using nonpaired Student's t test and generalized Wilcoxon test. Categorical and nominal data were compared using the χ^2 test. The multivariate association of presumed risk factors with death and renal death was performed by a multiple logistic model. Survival and kidney survival curves were calculated by Kaplan–Meier method, comparing groups using the log-rank test. All analysis were performed using the StatView software program 5.0. $P \leq 0.05$ was considered significant.

Results

In our population, 64.7% of diabetic nephrosclerotic patients survived at least 10 years, and 52.4% survived at least 20 years after diagnosis (Fig. 1). In addition, 53.8% of the patients with 10-year survival and 46.7% of the patients with 20-year survival were free from hemodialysis (Fig. 2). We evaluated factors using the χ^2 test or Student's t test and found that the significant survival clinical factors were low BMI value, elderly status, and poor BP control after renal biopsy (Table 1). Among these factors, poor BP control after renal biopsy was the risk factor that was determined by multivariate analysis, including a multiple logistic model for survival (Table 2). Survival of patients with both anemia at the biopsy and poor BP control after biopsies was significantly shorter than that of patients without them in analysis using the Kaplan–Meier method (Fig. 3). We found that the significant renal prognostic clinical factors were hypoalbuminemia, anemia, high levels of BUN and s-Cr, hypercholesteremia and hypertriglyceridemia at the biopsy, and poor BP control after renal biopsy (Table 3). KW nodules, severe glomerular damage, and TI damage at the biopsy were indicated as pathological parameters (Table 4). Among these factors, anemia, BUN at the biopsy, poor BP control after renal biopsy, and severe glomerular damage at the biopsy were renal risk factors determined by the multivariate analysis including a multiple logistic model (Table 5). Renal survival of patients with both anemia at the biopsy and poor BP control after biopsies was significantly shorter than that in patients without them (Fig. 4). In the present data collected at the end of the follow-up period, there were tendencies toward anemia in the group of patients that had died, but because the number of patients in this group was small, the association between BP, anemia, and survival was not seen in detail. The drugs taken for disease treatment did not show any significant differences in outcome (Table 6). Both of the patients that died had been taking angiotensin converting enzyme inhibitor (ACE-I) and Ca antagonist. Of 20 patients in the group of patients that survived, 8 patients had been taking Ca antagonist, 3 patients had been taking angiotensin II receptor blocker (ARB), and Ca antagonist, 1 patient

Table 1 Simple analysis (clinical parameters)

	Death ($N = 41$)	Survival ($N = 124$)	P
Male/female	32/9	78/46	0.0674
BMI (BW/BH^2)	21.5 ± 0.6	23.1 ± 0.4	0.0301
Age (years)	60.1 ± 1.8	54.1 ± 1.2	0.0089
Mean BP (mmHg)	105.7 ± 2.6	107.5 ± 1.4	0.5341
History of disease (months)	156.4 ± 22.8	125.1 ± 8.6	0.1287
Albumin (g/dl)	3.2 ± 0.2	3.5 ± 0.1	0.0521
Hematocrit (%)	33.8 ± 1.1	34.8 ± 0.6	0.4347
BUN (mg/dl)	27.7 ± 2.7	25.4 ± 1.6	0.4888
s-Creatinine (mg/dl)	1.6 ± 0.2	1.7 ± 0.1	0.9350
T.cholesterol (mg/dl)	221.9 ± 11.1	227.1 ± 8.2	0.7386
Triglyceride (mg/dl)	152.0 ± 14.8	166.0 ± 12.1	0.5308
FBS (mg/dl)	147.2 ± 10.0	164.4 ± 10.0	0.3215
HbA1c (%)	7.4 ± 0.6	7.6 ± 0.4	0.7776
U-protein (g/day)	3.7 ± 0.5	5.1 ± 0.8	0.3248
BP control (good/poor) (%)	15.2/84.8 (31)[a]	46.0/54.0 (98)[a]	0.0009
HOMA-R	3.7 ± 1.9 (3)[a]	1.3 ± 0.3 (7)[a]	0.0882

[a] Observative number in BP control and HOMA-R

Table 2 Survival factors (multivariant analysis)

	P value	Exp.
BMI	0.1474	0.921
Age	0.3397	1.017
Poor BP control	0.0035	6.075

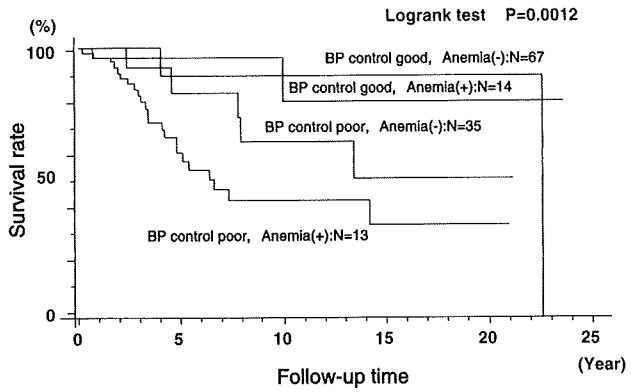

Fig. 3 Survival rate of patients with poor control of BP after biopsies and anemia at the biopsy was significantly lower than that of patients in the other groups: 43.7% of these patients survived at least 10 years and 35.0% survived at least 20 years after diagnosis

had been taking ARB, ACE-I, and Ca antagonist, 2 patients had been taking ACE-I, 1 patient had been taking ARB, and 5 patients did not know what medications they were receiving.

Discussion

Low BMI, elderly status at the biopsy, and poor control of blood pressure after renal biopsy were demonstrated to be significant factors for poor survival prognosis. The control of blood pressure decreases in correlation with the presence of cardiovascular and cerebral diseases. As a result, survival rate is prolonged [20–22]. At the same time, hypoalbuminemia, anemia, high levels of BUN and s-Cr, hypercholesteremia, hypertriglyceridemia at the biopsy, poor control of blood pressure after renal biopsy, KW nodules, and severe glomerular and TI damages at the biopsy were demonstrated to be significant factors for poor renal prognosis. These renal prognostic factors have been frequently reported as prognostic. Some researchers also pointed out that hyperlipidemia, especially hypertriglyceridemia, was associated with progression of renal damage [23–25]. It has been suggested that hypercholesteremia [16, 26], probably due to hyperglycemia [27], is likely associated with the progression of diabetic nephrosclerosis. In this study, our results indicated that hypercholesteremia and hypertriglyceridemia at the biopsy were prognostic factors in the development of diabetic nephrosclerosis. Insulin resistance plays a pivotal role in hyperglycemia and hyperlipidemia. The insulin resistance introduces reduced activity of lipoprotein lipases, reduced resolution of very low-density lipoprotein (VLDL) into body fluid, and high concentration of VLDL in serum, resulting in hypertriglyceridemia with hypo high-density lipoprotein (HDL)-cholesterolemia [28, 29]. Therefore, there is a need for the control or correction of insulin resistance [30, 31]. We failed to demonstrate insulin resistance as a prognostic factor mainly because of the small number tested, but it was interesting that insulin resistance had a tendency to become a survival factor. Suzuki et al. [32] reported that diabetic nephrosclerosis with nodular lesions was a marker of poor prognosis. With multivariate analysis we found that anemia, BUN at the

Table 3 Simple analysis (clinical parameters)

	Renal death ($N = 61$)	Survival ($N = 105$)	*P*
Male/female	42/19	68/37	0.591
BMI (BW/BH^2)	23.1 ± 0.5	22.6 ± 0.4	0.4524
Age (years)	56.9 ± 1.4	54.8 ± 1.3	0.3038
Mean BP (mmHg)	108.7 ± 1.9	106.0 ± 1.5	0.2756
History of disease (months)	153.0 ± 16.1	119.5 ± 9.1	0.0532
Albumin (g/dl)	3.1 ± 0.1	3.7 ± 0.1	0.0001
Hematocrit (%)	31.4 ± 0.8	36.4 ± 0.6	<0.0001
BUN (mg/dl)	33.2 ± 2.8	21.5 ± 1.3	<0.0001
s-Creatinine (mg/dl)	2.1 ± 0.2	1.4 ± 0.1	<0.0001
T.cholesterol (mg/dl)	244.5 ± 13.9	216.5 ± 6.8	0.0453
Triglyceride (mg/dl)	188.6 ± 21.7	148.3 ± 8.0	0.0435
FBS (mg/dl)	155.6 ± 10.5	162.5 ± 11.2	0.6573
HbA1c (%)	8.0 ± 0.6	7.2 ± 0.4	0.2353
U-protein (g/day)	4.9 ± 0.6	4.6 ± 0.9	0.7963
BP control (good/poor) (%)	21.4/78.6 (54)[a]	51.3/48.7 (75)[a]	0.0032
HOMA-R	2.5 ± 1.0 (6)[a]	1.3 ± 0.5 (4)[a]	0.3861

[a] Observative number in BP control and HOMA-R

Table 4 Pathologic prognostic factors (simple analysis)

	Renal death ($N = 61$)	Survival ($N = 105$)	P
KW nodule (+)/(−) (%)	64.5/35.5	39.0/61.0	0.0016
Index of DGS	2.7 ± 0.1	2.0 ± 0.1	<0.0001
Mild (%)	14.5	48.3	<0.0001
Moderate (%)	46.8	34.8	
Advanced (%)	38.7	16.9	
TI damage (0/1+/2+/3+) (%)	1.7/15.5/43.1/39.7	9.3/36.0/40.0/14.7	0.0026

K–W Kimmelstiel–Wilson, *TI* tubulointerstitial

Table 5 Renal prognostic factors (multivariant analysis)

	P value	Exp.
Albumin	0.6400	1.216
Total cholesterol	0.7264	1.001
Triglyceride	0.9538	1.000
Hematocrit	0.0113	0.886
BUN	0.0326	0.950
Serum creatinine	0.4498	1.169
Poor BP control	0.0044	4.608
KW nodule (+)	0.9392	0.966
Index of DGS	0.0149	3.273
TI damage	0.9598	1.021

K–W Kimmelstiel–Wilson, *TI* tubulointerstitial

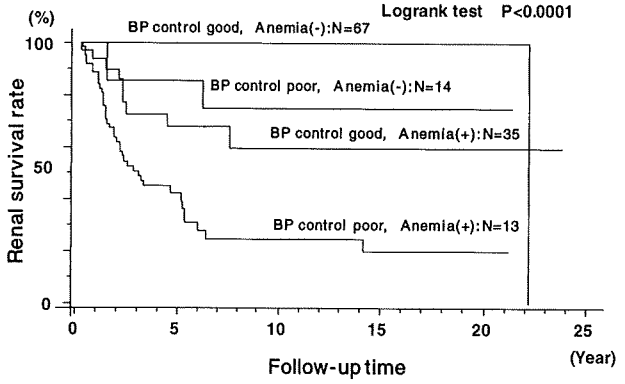

Fig. 4 Renal survival rate of patients with poor control of BP after biopsies and anemia at the biopsy was significantly lower than that of patients in the other groups: 24.7% of these patients with 10-year survival and 19.7% of the patients with 20-year survival were free from hemodialysis

biopsy, hypertension after renal biopsy, and severe glomerular damage at the biopsy were more important renal prognostic factors than the other factors were. Anemia has been reported as a prognostic factor in diabetic nephrosclerosis [11, 12]. The mechanisms of anemia were reported to be erythropoietin (epo) secretory cell reduction by tubulointerstitial damage [11, 33], low epo secretion by diabetic autonomic nervous system damage [34–36], and epo loss into the urine by nephrotic syndrome [34, 36]. However, the mechanisms of anemia are still unclear. Also, there are many

Table 6 Simple analysis (clinical parameters in the present data collected at the end of the follow-up period)

	Death ($N = 2$)	Survival ($N = 20$)	P
Male/female	1/1	12/8	0.7855
Age (years)	69.5 ± 3.5	62.4 ± 2.5	0.4006
Mean BP (mmHg)	89.7 ± 3.7	104.9 ± 3.6	0.2116
RBC ($\times 10^4/\mu l$)	289.0 ± 32.0	342.7 ± 14.8	0.2714
Hb (mg/dl)	8.7 ± 0.2	10.6 ± 0.5	0.2184
Hematocrit (%)	26.3 ± 1.2	31.3 ± 1.4	0.2800
Epo (+/−) (%)	33.3/66.7	42.9/57.1	0.7518
Antihypertensives (+/−) (%)	100.0/0.0	75.0/25.0	0.4212

pieces of evidence that show blood pressure control by ACE-I [37–39] or ARB [40–42] introduces good prognosis in diabetic nephrosclerosis [9, 10]. As we found in our analysis using the Kaplan–Meier method, anemia at the biopsy is a survival factor along with hypertension after biopsies. Lately, it has been reported that anemia was associated with higher mortality and hospitalization in hemodialysis patients [43, 44].

In conclusion, researchers have demonstrated that anemia at the biopsy and hypertension after renal biopsy are the most important renal prognostic and survival factors in patients with diabetes mellitus. Only control of blood sugar could not be reduced in correlation with the presence of macrovascular diseases, including myocardial infarction and cerebral infarction [45]. Therefore, the control of hypertension and anemia are important for the prevention of loss of renal function and shortened survival in diabetic patients. At the same time, researchers are expecting to reduce introduction to hemodialysis and improve the quality of life of diabetic patients by correction of these risk factors.

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BRIEF REPORT

Efficacy and safety of lisinopril for mild childhood IgA nephropathy: a pilot study

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Abstract Even in children with mild immunoglobulin (Ig) A nephropathy (IgA-N) showing minimal/focal mesangial proliferation, persistent proteinuria seems to be a risk factor for progression of the disease, indicating the need for an effective and safe treatment even in such cases. Studies carried out to date have indicated that angiotensin-converting enzyme inhibitors (ACEIs) reduce urinary protein excretion and preserve renal function in adult IgA-N. However, no prospective study of ACEI only for childhood IgA-N has yet been carried out. In this prospective single-arm pilot trial, we administered lisinopril (0.4 mg/kg per day) as therapeutic treatment to 40 children with mild IgA-N with proteinuria [morning urinary protein/creatinine ratio (uP/Cr) $\geq$ 0.2 g/g]. Thirty-three patients reached the primary endpoint (uP/Cr < 0.2) during the 2-year treatment period. The cumulative disappearance rate of proteinuria determined by the Kaplan–Meier method was 80.9%. Mean uP excretion was reduced from 0.40 to 0.18 g/m^2/day ($p<0.0001$). Of the 40 patients treated, five (12.5%) showed dizziness, and four of these five needed the lisinopril dose reduced. However, lisinopril therapy was continued in all patients during the 2-year treatment period. No other side effect, such as cough, was observed. We conclude that the efficacy and safety of lisinopril is seemingly acceptable for the treatment of children with mild IgA-N.

Keywords Angiotensin-converting enzyme inhibitor · Focal mesangial proliferation · Minimal change · Proteinuria · Renin-angiotensin system · Urinary protein to creatinine ratio · Urinary protein excretion

The participants in the Japanese Pediatric IgA Nephropathy Treatment Study Group are listed in the Appendix.

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Introduction

Even in children with mild IgA nephropathy (IgA-N) showing minimal or focal mesangial proliferation, persistent proteinuria seems to be a risk factor for progression of the disease [1], indicating the need for an effective and safe treatment even in such cases. Studies carried out to date have indicated that angiotensin-converting enzyme inhibitors (ACEIs) reduce urinary protein excretion and preserve renal function in adult patients with IgA-N [2–6]. A randomized controlled trial (RCT) has recently proved that ACEI treatment can have a significant effect on the progression of IgA-N in children and young people [7]. However, no prospective study of ACEI only for childhood IgA-N has yet been carried out. In the pilot study reported here, we administered lisinopril as therapy for mild childhood IgA-N and evaluated the efficacy and safety of lisinopril.

Methods

The study was a prospective single-arm open labeled pilot trial involving the ten Japanese pediatric renal centers comprising The Japanese Pediatric IgA Nephropathy Treatment Study Group.

Patients

Patients were assessed for eligibility for the study if they were newly diagnosed as having IgA-N with minimal or focal mesangial proliferation by renal biopsy. Inclusion criteria were: (1) age≤18 years at study entry; (2) no previous treatment; (3) sufficient renal biopsy tissue available for histological evaluation (minimum of ten glomeruli); (4) early morning urinary protein to creatinine ratio (uP/Cr) of ≥ 0.2 g/g continuously during the period between renal biopsy and study entry. The upper limit of uP/Cr was not prescribed.

The diagnosis of IgA-N was based on the presence of IgA as the sole or predominant immunoglobulin in the glomerular mesangium without systemic disease [8]. Minimal or focal mesangial proliferation was defined on the basis of the World Health Organization criteria [9].

Study design

After determining study eligibility and obtaining informed consent, we administered the patients lisinopril for 24 months. Lisinopril was started orally at a single daily dose of 0.2 mg/kg body weight (maximum 10 mg/day) given every day and, after confirmation of reliance for 1 week, it was increased to a single daily dose of 0.4 mg/kg (maximum 20 mg/day).

Study endpoints

The primary endpoint was the disappearance of proteinuria, as defined by an early morning uP/Cr of < 0.2 g/g [10], and the secondary endpoints were urinary protein excretion per day at the end of treatment and side effects.

Table 1 Baseline characteristics

Baseline characteristics of children with mild IgA nephropathy (n=40)	
Demographic	
Age, years, mean (range)	11.4 (4.4–15.4)
Sex (M/F)	17/23
Months of disease, mean (range)	19.3 (3.4–108.3)
Months from biopsy, mean (range)	1.2 (0.4–4.2)
Initial presentation	
School screening	32 (80.0%)
Chance hematuria	5 (12.5%)
Macroscopic hematuria	3 (7.5%)
Blood pressure	
Systolic mmHg, mean (SD)	107 (13)
Diastolic mmHg, mean (SD)	57 (9)
Renal function	
Urinary protein excretion, g/m^2/day, mean (range)	0.39 (0.08–1.35)
Morning urinary protein/creatinine ratio, g/g, mean (range)	0.58 (0.21–2.36)
Hematuria in morning urine[a], mean (SD)	2.3 (1.0)
Blood urea nitrogen, mmol/L, mean (SD)	4.8 (1.0)
Serum creatinine, μmol/L, mean (SD)	49 (14)
Estimated creatinine clearance, mL/min per 1.73 m^2, mean (SD)	120 (16)
Serum IgA, mg/dL, mean (SD)	260 (113)
Renal biopsy	
Number of glomeruli, mean (range)	26.3 (10–76)
Glomeruli showing sclerosis, %, mean (range)	1.5 (0.0–16.7)
Glomeruli showing crescents, %, mean (range)	8.3 (0.0–33.3)
Glomeruli showing capsular adhesions, %, mean (range)	2.9 (0.0–20.0)
Intensity of mesangial IgA deposits[b], mean (SD)	2.1 (0.6)

IgA Immunoglobulin A; *SD* standard deviation

[a] Hematuria was quantified using dipsticks and macroscopic hematuria was quantified as 4

[b] The intensity of deposits on immunofluorescence microscopy was graded semiquantitatively on a scale from 0 to 3+: no, 0; slight, 1+; moderate, 2+; and intense, 3+

Table 2 Effect of the 2-year treatment with lisinopril

Clinical parameters	Treatment with lisinopril (n=38)		
	Start	Endpoint	p
Urinary protein excretion, g/m^2 per day, mean (range)	0.40 (0.08–1.35)	0.18 (0.00– 0.89)	< 0.0001
Urinary protein to creatinine < 0.2 (g/g)	0	33 (80.9%[a])	
Hematuria in morning urine[b] (mean [SD])	2.3 (1.0)	1.0 (1.3)	0.0012
Estimated creatinine clearance, mL/min per 1.73 m^2, mean (SD)	119 (16)	121 (16)	0.0537
Estimated creatinine clearance < 60 mL/min per 1.73 m^2	0	0	
Serum IgA, mg/dl, mean (SD)	256 (105)	255 (104)	0.4813
Blood pressure			
Systolic mmHg, mean (SD)	106 (12)	106 (10)	0.4768
Diastolic mmHg, mean (SD)	56 (9)	57 (8)	0.8645

[a] The cumulative disappearance rate of proteinuria determined by the Kaplan-Meier method
[b] Hematuria was quantified using dipsticks, and macroscopic hematuria was quantified as 4

Sample size

An acceptable baseline cumulative proteinuria disappearance rate of 50% at the end of the 2-year treatment period with lisinopril was estimated from the data of our previous RCT for children with mild IgA-N [11]. The calculated sample size of 40 patients was based on a one-sided significance level of 0.05, a statistical power of 80%, an expected cumulative proteinuria disappearance rate of 70% (+ 20% of a baseline) at the end of the 2-year treatment period with lisinopril, and an attrition rate of 10%.

Data analysis and statistics

Differences between study entry and study end were tested by the Wilcoxon signed rank test. The disappearance rate of proteinuria was analyzed by the Kaplan–Meier method.

Results

Between August 1998 and July 2001, 54 children were newly diagnosed as having IgA-N showing minimal or focal mesangial proliferation. Of these 54 pediatric patients, 52 were willing to enter the study. Of these 52 children, 40 met the criteria for inclusion in the trial. The clinical and laboratory characteristics of the patients are shown in Table 1.

Thirty-eight patients were followed for the 2-year treatment period. Two patients were lost to follow-up in the 14th and 17th month, respectively. In one of these patients (lost in the 17th), proteinuria disappeared during the first month of treatment; the other showed non-compliance of lisinopril and demonstrated no disappearance of proteinuria during the follow-up period. A third patient showed non-compliance of lisinopril, and there was no disappearance of proteinuria during the 2-year treatment period. Data from all 40 patients were included for the analysis of the disappearance rate of proteinuria managed in an intention-to-treat manner.

Changes in proteinuria, hematuria, renal function, and serum IgA concentrations

At the end of the 2-year treatment period, 33 patients reached the primary endpoint (uP/Cr < 0.2) (Table 2). The cumulative disappearance rate of proteinuria determined by the Kaplan–Meier method was 80.9%. Figure 1 shows the Kaplan–Meier analysis of the disappearance rate of proteinuria.

Side effects

Five of the 40 patients (12.5%) showed dizziness and four of the five patients needed to have the dose of lisinopril reduced (to between 1/2 and 3/4 of maximum dose). However, lisinopril was continued in all patients during the 2-year treatment period. No other side effect, such as cough, was seen.

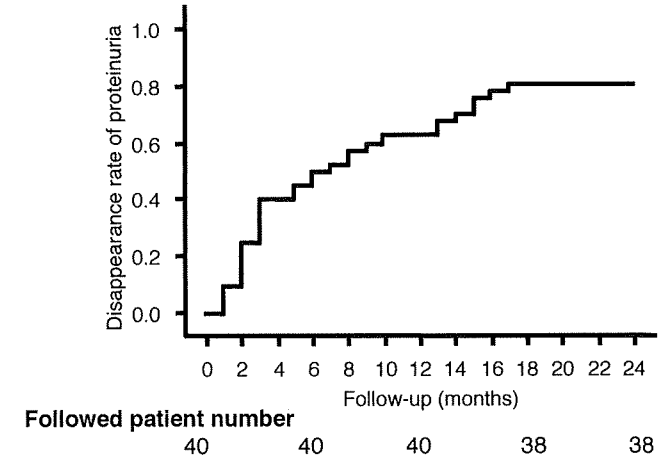

Fig. 1 Disappearance of proteinuria

Discussion

This study was planned as a prospective single-arm pilot trial of lisinopril, and its aim was to evaluate the efficacy and safety of the treatment regimen. Another significant aim of this study was to establish a standard regimen for future RCTs in children with mild IgA-N. Based on the data obtained in this study, the use of lisinopril for 2 years seems to be an acceptable treatment for children with mild IgA-N; consequently, a new RCT for mild IgA-N in children comparing lisinopril and lisinopril plus losartan potassium is currently being conducted by the Japanese Study Group of Kidney Disease in Children (JSKDC; UMIN ID C-6, http://www.umin.ac.jp/).

Although this study was successful in testing the safety of lisinopril in children with mild IgA-N, it is still a preliminary uncontrolled study and, as such, it provides limited information on efficacy. It is important to note that there is a possibility of spontaneous remission of proteinuria in IgA-N, particularly in mild cases. Therefore, the decrease of proteinuria observed in this study may not be due only to the effect of lisinopril. In Japan, a variety of factors constrain us from including a no treatment group in studies of childhood IgA-N, even in mild cases. Therefore, we cannot conduct a RCT with a no treatment control group. However, we can use data from our previous RCT in which there was a control (no drug) group as a historical control [11]. Based on data from the previous RCT and following a matching of entry criteria, we determined that only five of 27 patients reached the primary endpoint of the study reported here (uP/Cr < 0.2) at the end of the 2-year follow-up period without treatment. The cumulative disappearance rate of proteinuria determined by the Kaplan–Meier method was only 20.5%. The ratio is significantly low compared to that of the current study (vs. lisinopril, log-rank $p<0.0001$). This fact supports the efficacy of lisinopril.

The importance of changes in proteinuria has recently been emphasized in IgA-N. A RCT of adult patients with IgA-N confirmed an independent renal protective effect of ACEI (enalapril) and, by multivariate analysis, confirmed the independent value of proteinuria reduction over the course of the trial—but not the presenting proteinuria [6]. A predictive algorithm in adult patients with IgA-N found that only lower blood pressure and lower levels of proteinuria measured over time—and not the values at presentation—predicted outcome [12]. It has also been shown recently that even partial remission of proteinuria is associated with better renal outcome in patients with IgA-N [13]. The importance of the renin–angiotensin–aldosterone system blockade on proteinuria is also supported by the results of a recent RCT in Asian patients with IgA-N using ARB valsartan [14]. These studies support the importance of the study reported here.

The only side effects in our study was dizziness (5/40, 12.5%), which was dose-dependent. Lisinopril was continued in all patients during the 2-year treatment period. To reduce the frequency and severity of the dizziness, we may be able to modify the regimen of lisinopril. There is a possibility that the same result could be obtained with a lower dose or that at least the remission could be maintained for long-term treatment with a lower dose. In a multicenter study of the pharmacokinetics of lisinopril in 52 pediatric patients with hypertension, no serious adverse events related to lisinopril were reported [15]. These findings support the safety of lisinopril in children.

Taken together, the available evidence indicates that the use of lisinopril in children with mild IgA-N for 2 years early in the course of disease may reduce proteinuria. The efficacy and safety of lisinopril seems to be acceptable for treatment of children with mild IgA-N. In conclusion, these findings suggest that lisinopril may be a reliable option as a treatment of children with mild IgA-N.

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BRIEF REPORT

Membranous nephropathy associated with thyroid-peroxidase antigen

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Abstract A 6-year-old previously healthy Japanese girl was found to have dipstick 2+ proteinuria and a goiter based on the results of a routine school medical examination. Her serum free-thyroxine level was 4.98 ng/dL (normal range 0.95–1.74 ng/dL), thyroid-stimulating hormone (TSH) was less than 0.003 μU/mL (0.34–3.88 μU/mL), anti-microsomal (anti-thyroid-peroxidase) antibody was 1600 T (up to 100), anti-thyroglobulin antibody was 400 T (up to 100), and TSH-receptor antibody was 84% (up to ±10%). These results are consistent with a diagnosis of Graves' disease. Electron microscopy examination of a renal biopsy specimen revealed electron-dense deposits located in the subepithelial spaces, and immunofluorescence microscopy examination demonstrated bright granular stainings of immunoglobulin G along the glomerular capillary walls. These findings are characteristic of membranous nephropathy. To investigate the relationship between the membranous nephropathy and Graves' disease, we carried out a second immunofluorescence study, which revealed that the immunoglobulin G granular deposits corresponded to glomerular granular staining of thyroid-peroxidase, whereas staining for thyroglobulin was absent. It was therefore assumed that the deposition of immune complexes mediated by thyroid-peroxidase had caused the membranous nephropathy in this patient. This is the first report of membranous nephropathy associated with Graves' disease in which deposits of thyroid-peroxidase, rather than thyroglobulin, have been confirmed in the kidney.

Keywords Goiter · Immune complex · Lisinopril · Proteinuria · Thiamazole · Thyroglobulin

Introduction

Membranous nephropathy (MN) is a chronic glomerular disease characterized by nephrotic or non-nephrotic proteinuria [1, 2]. The characteristic histopathological features of MN are diffusely thickened glomerular capillary walls exhibiting projections or spikes under light microscopy, fine granular staining for immunoglobulin G (IgG) and complement along the glomerular capillary walls on immunofluorescence microscopy, and electron-dense deposits located in the subepithelial area as seen on electron microscopy [1]. The presumed cause of MN is deposition of immune complexes. Compared with adults, a greater percentage of cases of MN in children are secondary [2]. Previously in Japan, hepatitis B was the most prevalent cause [3].

Membranous nephropathy has rarely been reported in association with Graves' disease. We describe here a girl with MN associated with Graves' disease, which was probably caused by thyroid-peroxidase (TPO) antigen-antibody immune complexes.

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