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Effect of pyridoxamine (K-163), an inhibitor of advanced glycation end products, on type 2 diabetic nephropathy in KK-A^y/Ta mice

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Abstract

Advanced glycation end products (AGEs) from the Maillard reaction contribute to the pathogenesis of diabetes-associated complications such as diabetic nephropathy. In therapeutic interventions for reducing AGEs, many compounds have been reported as AGE inhibitors. The objective of the present study was to examine the effect of pyridoxamine (K-163), an AGE inhibitor, in type 2 diabetic KK-A^y/Ta mice. KK-A^y/Ta mice were given pyridoxamine (200 or 400 mg/kg per day) starting at 8 weeks of age for 12 weeks. They were divided into 3 groups as follows: pyridoxamine 200 mg/kg per day treatment group (n = 10), pyridoxamine 400 mg/kg per day treatment group (n = 10), and a tap water group as the control group (n = 20). The urinary albumin/creatinine ratio (ACR), body weight (BW), levels of fasting and casual blood glucose, blood glycated hemoglobin (HbA_{1c}), fasting serum insulin, triglyceride (TG), total cholesterol (T-Cho), and 3-deoxyglucosone (3DG), and systemic blood pressure were measured as biochemical parameters. N^{ε}-(Carboxymethyl)lysine (CML) and nitrotyrosine accumulations in glomeruli were evaluated by immunohistochemical analyses. Transforming growth factor β1 (TGF-β1) and laminin-β1 messenger RNA expressions in the kidneys were evaluated by real-time polymerase chain reaction. Pyridoxamine, especially at 400 mg/kg per day, improved the levels of urinary ACR, fasting serum TG, and 3DG. CML and nitrotyrosine accumulations in glomeruli were decreased. Furthermore, large doses of pyridoxamine prevented not only urinary ACR but also increases of BW, casual blood glucose, and HbA_{1c}. TGF-β1 and laminin-β1 messenger RNA expressions in kidneys were significantly lower than those in the controls. There were no significant changes in the levels of fasting blood glucose, serum T-Cho, and systemic blood pressure among all groups. It appears that pyridoxamine improved urinary ACR by its anti-AGE and anti-oxidant effects in the kidneys of KK-A^y/Ta mice.

1. Introduction

Nonenzymatic glycation has been implicated in the pathogenesis of diabetic nephropathy [1]. The Maillard reaction between sugar and protein proceeds through a labile Schiff base, which isomerizes to a ketoamine adduct, the Amadori product, including glycated hemoglobin (HbA_{1c}) and 3-deoxyglucosone (3DG). Oxidative decomposition and further reaction of the Amadori products produce advanced glycation end products (AGEs), including N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl)lysine (CEL) and pentosidine [2,3]. There are multiple pathways to the formation of AGEs from glucose, products of autoxidation of glucose, Schiff bases, and Amadori products. Specific AGEs, such as CML, are major products of glycoxidation reactions. They may also be formed from multiple carbohydrate precursors, including glucose, fructose, and metabolic intermediates. Through its effects on protein structure, function, and turnover, the accumulation of CML in tissue proteins is thought to contribute to the development of diabetic nephropathy [2]. The presence of AGEs is closely related to hyperglycemia and their pathobiochemistry could explain diabetic nephropathy. Histopathologic studies have shown AGE accumulation in a variety of tissue types including renal cortex, glomerular mesangium, and basement membrane [4].

In diabetic nephropathy, glomerular hypertrophy is associated with an overexpression of transforming growth

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factor β1 (TGF-β1), laminin-β1, type IV collagen, and platelet-derived growth factor [5-7]. Oxidative and nitrosative stresses are also recognized as key factors in the development of diabetic nephropathy [8]. AGEs have been associated with increased oxidative and nitrosative stresses in both in vitro and in vivo studies [9,10]. Nagai et al [11] reported that peroxynitrite can induce CML formation through oxidative cleavage of the Amadori products and through generation of reactive α-oxoaldehydes from glucose.

In therapeutic interventions for reducing AGEs, many compounds have been reported as AGE inhibitors, such as aminoguanidine, phenacyl thiazolium bromide, 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-yl-acetanilide (OPB-9195), 2,3-diaminophenazine, vitamin C, vitamin E, angiotensin II receptor inhibitor, and pyridoxamine [12-15]. Pyridoxamine was introduced by Khalifah et al [16,17] as an inhibitor of AGE formation from Amadori products. Degenhardt et al [18] reported that pyridoxamine inhibited AGE formation and retarded the development of diabetic nephropathy in streptozotocin-treated rats, the animal model for type 1 diabetes mellitus. However, the effects of this agent have not been fully described in type 2 diabetic nephropathy.

The KK-A^y/Ta mouse was established by Nishimura [19] in 1969. This mouse was produced by the transfer of the yellow obese gene (A^y allele) into the KK/Ta mouse [19]. Because the diabetic feature in the KK-A^y/Ta mouse is more severe than that in the KK/Ta mouse, this mouse is widely used as an experimental model for type 2 diabetes mellitus [20]. The KK-A^y/Ta mouse spontaneously exhibits type 2 diabetes mellitus associated with hyperglycemia, glucose intolerance, hyperinsulinemia, obesity, and microalbuminuria. Renal lesions in the KK-A^y/Ta mouse closely resemble those in human diabetic nephropathy. Glomeruli of the KK-A^y/Ta mouse show diffuse-type hyperplasia of mesangial areas with mesangial cell proliferation, segmental sclerosis, overexpression of TGF-β1, and AGE accumulation at 20 weeks of age [15,21]. Therefore, this mouse is considered as a suitable model for the study of type 2 diabetic nephropathy in humans.

In the present study, we investigated the prevention of the development of type 2 diabetic nephropathy in KK-A^y/Ta mice by pyridoxamine (K-163), an AGE inhibitor.

2. Research design and methods

2.1. Animals

Male diabetic KK-A^y/Ta Jcl mice (6 weeks old) were purchased from CLEA Japan (Tokyo, Japan). The mice were individually housed in plastic cages with free access to food (rodent pellet diet NMF, 1456 kJ/100 g, containing 5.5% crude fat) and water throughout the experimental periods. All mice were maintained in the same room under conventional conditions with a regular 12-hour light/dark cycle and temperature controlled at 24°C ± 1°C.

2.2. Reagents and treatments

Pyridoxamine dihydrochloride (K-163; 4-aminomethyl-3-hydroxy-2-methyl-5-oxymethylpyridine dihydrochloride) was kindly provided by Kowa (Tokyo, Japan). Because gavage can increase the blood glucose levels and mortality, hyperglycemic KK-A^y/Ta mice were given pyridoxamine (1 g/L [200 mg/kg per day] or 2 g/L [400 mg/kg per day]) in the drinking water starting at 8 weeks of age for 12 weeks. They were divided into 3 groups as follows: pyridoxamine 200 mg/kg per day treatment group (n = 10), pyridoxamine 400 mg/kg per day treatment group (n = 10), and tap water group as a control group (n = 20).

2.3. Biochemical characterization

The albumin/creatinine ratio (ACR), body weight (BW), and levels of fasting and casual blood glucose and HbA_{1c} were measured at 8, 12, 16, and 20 weeks of age. Systemic blood pressure was measured at 12, 16, and 20 weeks of age. Levels of fasting serum insulin, triglyceride (TG), total cholesterol (T-Cho), and 3DG were measured at 20 weeks of age.

Urinary samples were collected for 24 hours by using a mouse metabolic cage (CLEA Japan). Urinary albumin and creatinine samples were measured by immunoassay (DCA 2000 system, Bayer Diagnostics, Elkhart, IN) [20,21]. Glucose levels in blood obtained from the retro-orbital sinus were measured by using Glucocard (Kyoto Daiichi Kagaku, Kyoto, Japan). HbA_{1c} was measured by immunoassay (DCA 2000 system, Bayer Diagnostics) [20,21]. Fasting serum insulin was measured with a Morinaga mouse insulin enzyme-linked immunosorbent assay kit (Seikagaku, Tokyo, Japan). Serum TG and T-Cho were measured by an autoanalyzer (Fuji Dry-Chem 5500, FUJIFILM, Tokyo, Japan). Serum 3DG was measured by high-performance liquid chromatography (Fushimi Pharmaceutical, Kagawa, Japan [22]). Blood pressure was measured at 11:00 AM by a noninvasive tail cuff and pulse transducer system (Softron BP-98A, Tokyo, Japan) after the mice were externally prewarmed at 38°C for 10 minutes. At least 3 to 6 recordings were taken for each measurement. Standard deviations of less than 5.0 were defined as levels of blood pressure as previously described [23].

2.4. Immunohistochemical staining for CML and nitrotyrosine

The mice were sacrificed at 20 weeks of age. The kidneys were retrogradely perfused by saline via the abdominal aorta for 5 minutes at a pressure of about 150 mm Hg without prior flushing of the vasculature. Immunohistochemical analysis for CML in renal tissue sections was performed by using the ABC kit (Vector Laboratories, Burlingame, CA). Cryostat kidney sections (3 μm) were air-dried for 10 minutes and then fixed in cold acetone for 10 minutes. To reduce the background, nonspecific binding was blocked by incubating with 10% normal goat serum in blocking solution

(phosphate-buffered saline [pH 7.2] containing 2% bovine serum albumin, 2% fetal calf serum, and 0.2% fish gelatin) for 30 minutes. Nonspecific staining was blocked by incubation for 15 minutes with avidin and then biotin by using the avidin-biotin blocking kit (Vector Laboratories). Endogenous peroxidase activity was inhibited by incubation for 10 minutes with methanol containing 0.3% hydrogen peroxide. The sections were then incubated with the primary antibody (Ab) diluted 1:100 in blocking solution at 4°C overnight. The primary Ab used was horseradish peroxidase–conjugated anti-CML Ab (Trans Genic, Kumamoto, Japan). The sections were then incubated with a tyramide signal amplification (TSA) Biotin System (PerkinElmer, Boston, MA) for 10 minutes. Biotinylated horseradish peroxidase was applied for 30 minutes. Peroxidase activity was developed in 3,3-diaminobenzidine. Finally, Mayer's hematoxylin was added as a counterstain. Immunohistochemical staining for nitrotyrosine was performed as described previously by using an analogous protocol [15]. The primary Ab was polyclonal rabbit antinitrotyrosine Ab (Upstate Biotechnologies, Lake Placid, NY). The secondary Ab was antirabbit Envision + polymer reagents (DAKO, Carpinteria, CA). Intensities of 3,3-diaminobenzidine in at least 10 glomeruli from each mouse were quantitated by using a KS-400 version 4.0 image analysis system (Carl Zeiss Vision, Munich, Germany). These examinations were performed by 2 investigators without knowledge of the origin of the slides; mean values were calculated [15,21].

2.5. Quantitative messenger RNA analyses of TGF-β1 and laminin-β1 by real-time polymerase chain reaction

Kidneys were dissected and snap-frozen in liquid nitrogen for total RNA extraction. RNA was extracted with Trizol (Total RNA Isolation Reagent, Life Technologies, Rockville,

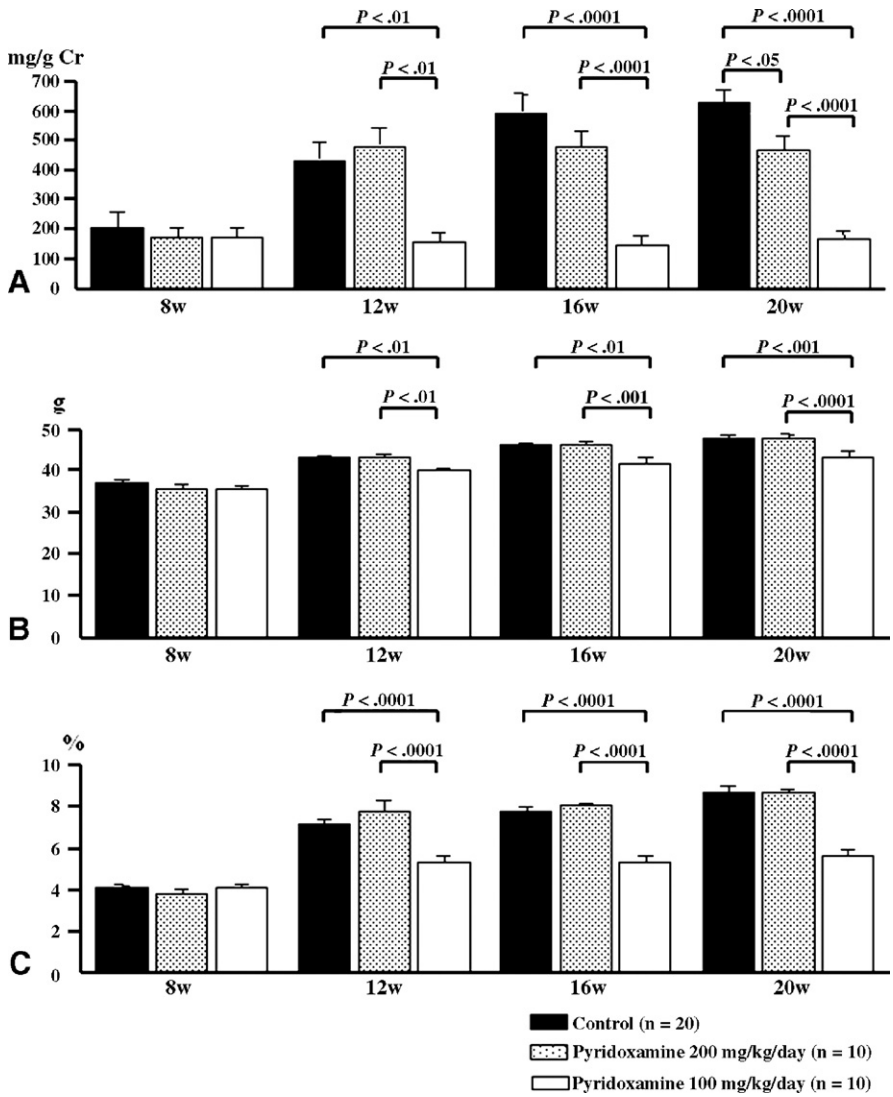

Fig. 1. Biochemical characterizations at each age (8, 12, 16, and 20 weeks of age) in KK-A^y/Ta mice treated with pyridoxamine. A, Mean level of urinary ACR. B, BW. C, Mean level of HbA_{1c}. Data are expressed as means ± SE.

Table 1
Biochemical parameters in KK-A^y/Ta mice treated with pyridoxamine

	Age (wk)	Control (n = 20)	200 mg/kg per day (n = 10)	400 mg/kg per day (n = 10)
Urinary albumin (mg/L)	8	64.7 ± 11.6	68.2 ± 10.2	60.9 ± 8.5
	12	116.2 ± 6.4	84.4 ± 7.9*	55.2 ± 6.8*
	16	113.3 ± 4.3	86.1 ± 5.4*	58.4 ± 6.7*
	20	108.2 ± 4.8	94.4 ± 3.6	67.3 ± 4.5*
Urinary creatinine (mg/dL)	8	33.2 ± 2.6	30.6 ± 2.2	46.7 ± 3.7
	12	27.9 ± 2.6	19.3 ± 1.8	37.9 ± 4.2
	16	19.3 ± 2.0	19.7 ± 2.0	56.0 ± 7.8*
	20	17.2 ± 0.8	21.9 ± 2.0	63.3 ± 8.5*
Fasting blood glucose (mg/dL)	8	112.7 ± 4.8	113.8 ± 4.2	113.2 ± 3.5
	12	95.6 ± 8.7	92.6 ± 3.6	98.2 ± 5.5
	16	93.8 ± 1.9	93.0 ± 2.7	94.2 ± 2.1
	20	94.6 ± 7.5	114.5 ± 6.9	100 ± 3.8
Casual blood glucose (mg/dL)	8	258.2 ± 22.0	269.5 ± 23.6	265.4 ± 25.1
	12	465.2 ± 32.9	422.4 ± 31.9	227.2 ± 24.9*
	16	506.0 ± 14.9	502.8 ± 30.4	275.8 ± 32.9*
	20	539.3 ± 35.2	521.8 ± 20.3	216.8 ± 25.8*
Heart rate (beats/min)	12	615.3 ± 22.3	606.8 ± 6.9	599.1 ± 28.1
	16	611.9 ± 25.9	603.6 ± 6.9	587.6 ± 20.3
	20	630.9 ± 17.3	619.2 ± 6.9	601.4 ± 19.5
Systolic blood pressure (mm Hg)	12	116.7 ± 2.9	114.0 ± 3.5	115.8 ± 3.4
	16	115.8 ± 4.2	114.5 ± 2.9	114.9 ± 3.2
	20	113.5 ± 2.7	113.1 ± 1.7	114.1 ± 2.0
Diastolic blood pressure (mm Hg)	12	76.5 ± 2.3	76.3 ± 2.4	75.1 ± 2.3
	16	74.6 ± 3.2	73.6 ± 3.1	74.5 ± 3.2
	20	73.2 ± 3.4	72.6 ± 2.7	74.5 ± 2.3
Mean blood pressure (mm Hg)	12	87.5 ± 2.7	88.8 ± 2.6	88.5 ± 2.3
	16	89.0 ± 3.2	87.2 ± 2.8	88.0 ± 2.9
	20	85.5 ± 2.8	85.3 ± 2.1	87.6 ± 1.9

Data are expressed as means ± SE.
* $P < .001$ vs control.

MD). Complementary DNA was synthesized by using random hexamers (Quantum RNA kit, Ambion, Austin, TX) and Superscript II RNaseH reverse transcriptase (Life Technologies, Rockville, MD). Primers and fluorogenic probes of TGF-β1 and laminin-β1 were obtained from a commercial base (TaqMan Gene Expression Assays, Applied Biosystems, Foster City, CA). The assay IDs of TGF-β1 and laminin-β1 were Mm00441724m1 (GenBank no. NM_011577) and Mm00801853m1 (GenBank no. NM_008482), respectively. The complementary DNA obtained was further amplified by a real-time polymerase chain reaction (PCR) system (ABI Prism 7500 Real Time PCR System, Perkin-Elmer, Foster City, CA). Initial template concentration was derived from the cycle number at which the fluorescent signal crosses a threshold in the exponential phase of the PCR reaction. Relative gene expression was determined based on the threshold cycles (Ct values). The PCR parameters were 95°C for 10 minutes, followed by 50 cycles at 95°C for 15 seconds and 60°C for 60 seconds.

2.6. Statistical analysis

All data were presented as mean ± SE. Comparisons between 2 parameters were analyzed by Student unpaired *t* test. Comparisons among 3 or more parameters were analyzed by 1-way analysis of variance. *P* values of less than .05 were defined as statistically significant.

3. Results

3.1. Biochemical characterization

The mean level of ACR at 12, 16, and 20 weeks of age in both the 200 and 400 mg/kg per day treatment groups were significantly lower than those in the control group ($P < .05$) (Fig. 1A; Table 1). The mean levels of BW, casual blood glucose, and HbA_{1c} in the 400-mg/kg per day treatment group were significantly lower than those in the controls ($P < .01$). However, there were no statistically significant changes in the levels of BW, casual blood glucose, and HbA_{1c} between the 200 mg/kg per day treatment and control groups (Fig. 1B and C; Table 1). The fasting serum TG levels at 20 weeks of age in the 400-mg/kg per day treatment group were significantly lower than those in the control group ($P < .005$). However, there were no statistically significant changes in the levels of fasting serum TG between the 200 mg/kg per day treatment and control groups (Fig. 2A). There were no significant changes in the levels of fasting T-Cho among all groups (Fig. 2B). The fasting serum insulin levels at 20 weeks of age in the

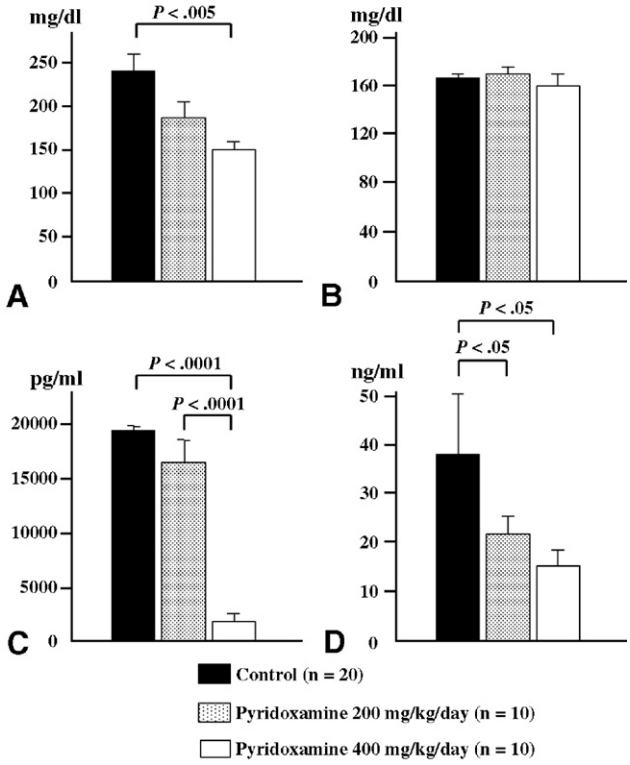

Fig. 2. Mean levels of fasting serum insulin and serum 3DG at 20 weeks of age in KK-A^y/Ta mice treated with pyridoxamine. A, Fasting serum TG levels. B, Fasting serum T-Cho levels. C, Fasting serum insulin levels. D, Serum 3DG levels. Data are expressed as means ± SE.

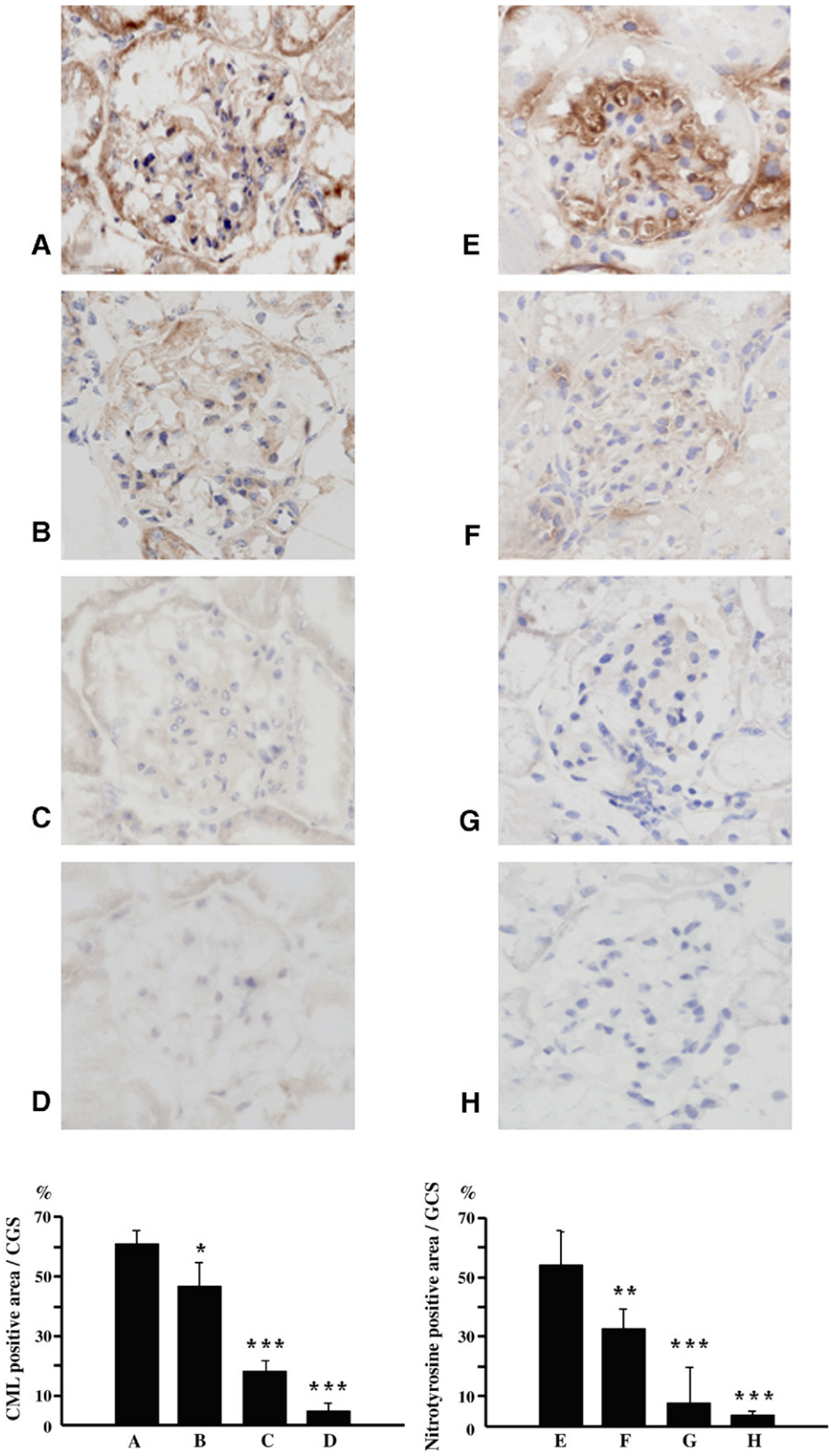

Fig. 3. Immunohistochemical staining for CML and nitrotyrosine at 20 weeks of age in renal tissue sections treated with pyridoxamine. A-D, CML. E-H, nitrotyrosine. A and E, Control group; B and F, 200 mg/kg per day treatment group; C and G, 400 mg/kg per day treatment group; D and H, negative control (control group without the primary Ab). Original magnification ×400. Data are expressed as means ± SE. *$P < .05$ vs control; **$P < .001$ vs control; ***$P < .0001$ vs control. GCS indicates glomerular cross sections.

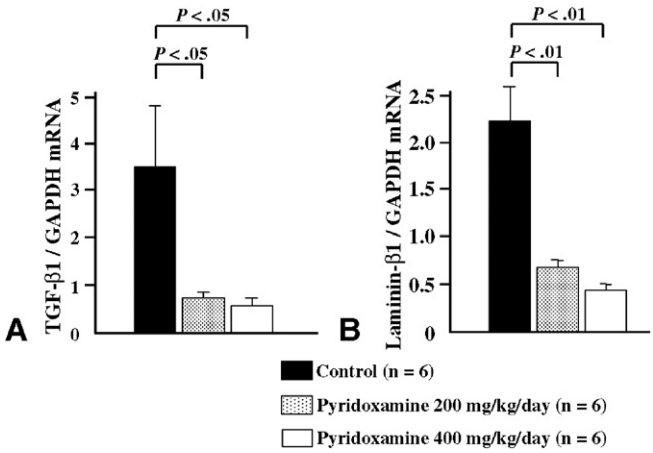

Fig. 4. Mean expressions of mRNA for TGF-β1 and laminin-β1 in renal tissue by real-time PCR at 20 weeks of age. A, Expression of TGF-β1 mRNA. B, Expression of laminin-β1 mRNA. Data are expressed as means ± SE.

400-mg/kg per day treatment group were significantly lower than those in the control group ($P < .0001$). However, there were no statistically significant changes in the levels of fasting serum insulin between the 200 mg/kg per day treatment and control groups (Fig. 2C). The mean level of serum 3DG in both 200 and 400 mg/kg per day treatment groups were significantly lower than those in the control group ($P < .05$) (Fig. 2D).

There were no significant changes in the levels of fasting blood glucose and systemic blood pressure among all groups (Table 1).

3.2. Immunohistochemical staining for CML and nitrotyrosine

In immunohistochemical staining, CML and nitrotyrosine were localized in the glomerular mesangial areas. In the pyridoxamine treatment groups, especially in the 400-mg/kg per day treatment group, accumulations of CML and nitrotyrosine at 20 weeks of age were decreased compared with those in the control groups ($P < .05$) (Fig. 3).

3.3. Quantitative messenger RNA analyses of TGF-β1 and laminin-β1 by real-time PCR

The messenger RNA (mRNA) levels of TGF-β1 and laminin-β1 in both the 200 and 400 mg/kg per day treatment groups were significantly lower than those in the control group ($P < .05$) (Fig. 4A and B).

4. Discussion

In the present study, we demonstrated that pyridoxamine (K-163), one of the AGE inhibitors, ameliorates the levels of urinary ACR and serum 3DG in KK-A^y/Ta mice without changing systemic blood pressure. Furthermore, pyridoxamine prevented accumulations of CML, nitrotyrosine, TGF-β1, and laminin-β1 in the kidney tissues.

Pyridoxamine was first described as a post-Amadori inhibitor of AGE formation [16,17]. It also has a potent nucleophile and/or trap dicarbonyl intermediates during AGE formation [24,25]. However, the detailed inhibitory mechanism on the progression of diabetic nephropathy by pyridoxamine remains elusive. Previous reports have shown the possible mechanisms of pyridoxamine in vitro. Voziyan et al [26] reported that pyridoxamine reacts directly with Maillard reaction intermediates, such as glyoxal and glycolaldehyde, and inhibits formation of CML. Onorato et al [27] demonstrated that pyridoxamine inhibits chemical modification of proteins during metal-catalyzed oxidation reactions, the formation of AGEs (CML and CEL) and advanced lipoxidation end products (ALEs). In the 200 mg/kg per day treatment group, we observed that pyridoxamine improved the levels of urinary ACR without changing BW, casual blood glucose, and HbA_{1c} compared with those in the control group. These findings in the 200 mg/kg per day treatment group confirmed the previous studies in vivo. Degenhardt et al [18] reported that pyridoxamine inhibited the development of diabetic nephropathy and dyslipidemia while preventing AGE formation in type 1 diabetic rats. This result is not in conflict with our findings. Indeed, the fasting serum TG levels at 20 weeks of age in both the 200 and 400 mg/kg per day treatment groups were slightly decreased compared with those in the control group (Fig. 2A).

Oxidative stress is defined as tissue injury induced by an increase in reactive oxygen species, such as the hydroxyl radical, superoxide anion, and hydrogen peroxide, and is one of the proposed mechanisms underlying diabetic complications [28,29]. Oxidation influences AGE formation before and after the formation of Amadori products [10]. Peroxynitrite nitrosylates tyrosine moieties on proteins and produces nitrotyrosine in diabetic nephropathy. A previous

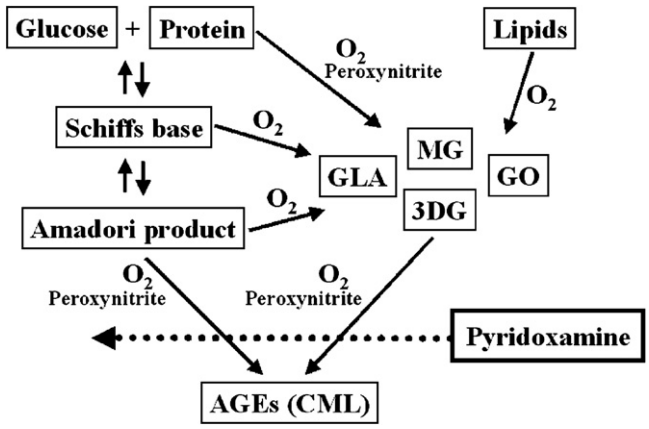

Fig. 5. Schematic representation of the formation of advanced AGEs and inhibition of AGEs by pyridoxamine. Pyridoxamine may prevent conversion of Amadori product to AGEs, trap dicarbonyl intermediates, and react directly with Maillard reaction intermediates. Pyridoxamine may also inhibit superoxide radical production and lipid peroxidation. GO indicates glyoxal; MG, methylglyoxal; GLA, glycolaldehyde; O_2, oxidation state.

report showed that peroxynitrite can induce CML formation through oxidative cleavage of Amadori products and through generation of reactive α-oxoaldehydes including glyoxal, methylglyoxal, and 3DG from glucose [11]. Jain and Lim [30] reported that pyridoxamine inhibits superoxide radical production and prevents lipid peroxidation and protein glycation. Therefore, it is postulated that the effect of 200 vs 400 mg/kg per day treatment on some parameters might be related to antioxidant effects by pyridoxamine. AGEs and oxidative stress might activate autocrine angiotensin II signaling and subsequently induce TGF-β1-Smad signaling in mesangial cells [5,31]. Our findings suggested that the amelioration of urinary ACR was related to the improvement of TGF-β1 and laminin-β1 expressions in kidney because CML and nitrotyrosine accumulations were improved and the levels of serum 3DG were reduced by anti-AGE and/or antioxidant effects of pyridoxamine (Fig. 5).

To our surprise, we observed that pyridoxamine prevented not only urinary ACR but also increases of BW, casual blood glucose, and HbA_{1c} in the 400 mg/kg per day treatment group compared with those in the 200 mg/kg per day treatment and control groups. Our data also showed that the fasting serum insulin level at 20 weeks of age in the 400 mg/kg per day treatment group was significantly lower than that in the control group. Chronic hyperglycemia leads to impaired insulin responsiveness in the hormone target tissues and is associated with diabetic nephropathy [32]. One of the mechanisms by which insulin sensitivity appears is mitochondrial membrane potential change. Kannan and Jain [28,33] reported that pyridoxamine inhibits superoxide radical generation and lipid peroxidation and prevents damage to the mitochondrial membrane potential in U937 monocytes. Miele et al [34] reported that human glycated albumin induces resistance to insulin at the insulin receptor substrate level by activating the protein kinase C (PKC)-α isoform. They noted that AGEs may participate in the development of insulin resistance. Kuniyasu et al [35] reported that AGE-modified bovine serum albumin is endocytosed by adipocytes via CD36. Their results suggested that CD36-mediated interaction of AGE-modified proteins with adipocytes might play a pathologic role in obesity or insulin resistance. In an in vivo study, Hofmann et al [36] demonstrated that reduced AGE intake leads to lower levels of circulating AGEs and to improved insulin sensitivity in diabetic db/db mice. Therefore, it is postulated that reduction of BW, casual blood glucose, and HbA_{1c} in the 400 mg/kg per day treatment group might be related to improvement of insulin resistance by pyridoxamine. Additional studies are needed to determine the mechanisms of these effects.

Although the water-soluble B vitamins are often considered to be nontoxic, pyridoxine is especially toxic to the peripheral nervous system [37]. Levine and Saltzman [38] reported that reduced renal excretion of pyridoxine might contribute to the development of neurotoxicity in rats. Pyridoxine is interconvertible in vivo with the vitamers pyridoxal and pyridoxamine [39]. Pyridoxamine was significantly less toxic, which made it possible to study effects after doses that matched or exceeded the doses of pyridoxine [40]. The better bioavailability of pyridoxamine and its low toxicity (5000-7500 mg/kg oral LD_{50} in rodents) [18,41], combined with its more pronounced effects on renal disease, hyperlipidemia, and metabolic changes suggest that pyridoxamine may be useful for clinical treatment of a wide range of diabetic complications including vascular disease and nephropathy [18]. It was suggested that the doses of pyridoxamine in this study were less toxic. Unlike aminoguanidine and OPB-9195, pyridoxamine does not trap pyridoxal. Its preclinical efficacy has been proven in animal models of diabetes and nephropathy [18,24,42,43]. Therefore, pyridoxamine is safer than aminoguanidine and OPB-9195.

In conclusion, it appears that pyridoxamine (K-163) improved urinary ACR in KK-A^y/Ta mice, a spontaneous animal model for type 2 diabetic nephropathy. This effect of pyridoxamine was related to improvement of CML and nitrotyrosine accumulation in kidneys by the anti-AGE and/or antioxidant effects. Furthermore, large doses of pyridoxamine prevented not only urinary ACR but also increases of BW, casual blood glucose, and HbA_{1c}. It is postulated that large doses of pyridoxamine might have other effects such as improvement of insulin resistance. Further studies are also needed to determine the mechanisms of such effects.

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