Pyridoxal phosphate prevents progression of diabetic nephropathy

Sakurako Nakamura1, Hongyan Li1,2, Ayinuer Adijiang1, Monika Pischetsrieder3 and Toshimitsu Niwa1

1Department of Clinical Preventive Medicine, Nagoya University Hospital, Nagoya, Japan, 2Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China and 3Institute of Pharmacy and Food Chemistry, University of Erlangen-Nürnberg, Erlangen, Germany

Abstract

Background. We have demonstrated that pyridoxal 5′-phosphate (PLP), an active form of vitamin B6, inhibits formation of advanced glycation end-products (AGEs) by trapping 3-deoxyglucosone. The present study aimed to clarify if PLP could exert beneficial effects on nephropathy in diabetic rats.

Methods. Streptozotocin (STZ)-induced diabetic rats were treated by oral administration of PLP or pyridoxamine (PM), another active form of vitamin B6, at a dose of 600 mg/kg/day for 16 weeks. AGEs [imidazolone, Nε-(carboxymethyl)lysine (CML) and N2-carboxyethyl-2′-deoxyguanosine (CEdG)], transforming growth factor-β1 (TGF-β1), type 1 collagen and fibronectin were detected in the kidneys using immunohistochemistry. Gene expression of TGF-β1 and receptor for AGEs (RAGEs) in the kidneys was determined using real-time quantitative polymerase chain reaction.

Results. Administration of PLP significantly inhibited albuminuria, glomerular hypertrophy, mesangial expansion, and interstitial fibrosis as compared with diabetic rats. PLP markedly inhibited accumulation of AGEs such as imidazolone, CML and CEdG, a DNA-linked AGE, in glomeruli. PLP significantly inhibited expression of TGF-β1, type 1 collagen, fibronectin and RAGE in the kidneys. PLP was superior to PM in inhibiting accumulation of AGEs, expression of TGF-β1, type 1 collagen, and fibronectin, and the development of diabetic nephropathy.

Conclusions. PLP prevented progression of nephropathy in STZ-induced diabetic rats by inhibiting formation of AGEs. PLP is considered a promising active form of vitamin B6 for the treatment of AGE-linked disorders such as diabetic nephropathy.

Keywords: advanced glycation end-products (AGEs); diabetic nephropathy; pyridoxal 5′-phosphate (PLP); receptor for advanced glycation end-products (RAGE); transforming growth factor-β1 (TGF-β1)

Introduction

Advanced glycation end-products (AGEs) play an important role in the development of diabetic [1,2] and uraemic complications [3,4], aging [5] and atherosclerosis [6,7]. There are different techniques to prevent or to treat AGE-linked pathologies such as normal glycaemia, AGE inhibitor, AGE crosslink breaker and receptor for advanced glycation end-products (RAGE) interaction blockade. AGE inhibitors such as aminoguanidine and OPB-9195 (2-isopropylidene-hydrazono-4-oxo-thiazolidin-5-yl-acetanilide) were used for diabetic rats, and their therapeutic effects were suggested [8,9]. However, because such inhibitors are hydrazine compounds, they trap necessary carbonyl species, causing their deficiency. Further, both aminoguanidine and OPB-9195 are strong inhibitors of nitric oxide synthase (NOS). Clinical trials of both compounds were discontinued, because of these side effects for both the compounds and apparent lack of efficiency for aminoguanidine [10].

Recently, it has been demonstrated that a group of vitamin B exerts an inhibitory effect on AGE formation [11,12]. Especially, pyridoxamine (PM), an active form of vitamin B6, is drawing attention due to its therapeutic effects on diabetic complications [13,14]. PM is converted to pyridoxamine 5′-phosphate and pyridoxal 5′-phosphate (PLP) in the liver and intestine [15,16]. Hence, PM may be exerting its beneficial effects, at least in part, by supplementation of PLP. PLP, a naturally occurring metabolite of vitamin B6, also inhibits AGE formation [17], but has a quite different chemical structure from PM as shown in Figure 1. PLP does not have any amino group, and instead has an aldehyde group and a phosphate group.
blood glucose levels were still 200 mg/dl even 3 days later, all rats were divided into four groups (eight rats per group): (i) non-diabetic normal group (C) \((n = 8)\), given intraperitoneal injection of 0.1 mol/l sodium citrate only; (ii) diabetes mellitus group (DM) \((n = 8)\), given intraperitoneal injection of STZ in 0.1 mol/l sodium citrate; (iii) PM-treated diabetes mellitus group (PM-DM) \((n = 8)\) treated with oral administration of PM (600 mg/kg/day) for 16 weeks; and (iv) PLP-treated diabetes mellitus group (PLP-DM) \((n = 8)\) treated with oral administration of PLP (600 mg/kg/day) for 16 weeks. PM and PLP were diluted in pure water, and pH of each solution was adjusted to 6.3–6.6 with 5 mol/l NaOH. PM and PLP were orally administered using gavage once a day everyday except Sunday for 16 weeks. The dose (600 mg/kg/day) of PM and PLP was set almost at the same as used in the study of Degenhardt et al. [13].

All rats were allowed free access to standard rat chow and water. There were no significant differences in the diet chow (30–40 g/day) between the four groups. After 16 weeks of PLP or PM treatment period, the rats were anaesthetized and their kidneys were excised. These procedures were in conformity with national and international laws for the care and use of laboratory animals, and the experimental protocols were approved by the Animal Research Committee of Nagoya University.

**Measurement of parameters**

Creatinine clearance (Ccr) and urinary albumin excretion were measured using urine samples collected for 24 h. Serum and urine creatinine concentrations were determined by Jaffe’s method. Serum total cholesterol was measured by a standard enzymatic method. Albumin concentration was measured by a specific enzyme-linked immunosassay for rat albumin (Nephrat\(^\text{®}\), Exocell Inc., Philadelphia, PA, USA). These parameters were checked every 4 weeks. Three days before sacrifice, blood pressure was measured using BP-98A\(^\text{®}\) (Softron Co., Ltd., Tokyo, Japan).

**Staining of kidney tissues**

After 16 weeks of PLP or PM treatment, all rats were anaesthetized by ether, and were killed by dislocation of cervical vertebrae. After drawing blood directly from the heart, the kidneys were removed and weighed. Three days after fixation of kidneys in 4% phosphate-buffered formalin, they were embedded in a paraffin block. Thin sections (3–4 μm) from paraffin-embedded kidneys were deparaffinized, and then stained by periodic acid-Schiff (PAS) and Masson’s trichrome, and by antibodies to AGEs, TGF-β1, collagen type 1 and fibronectin. All immunostaining was processed according to an avidin–biotin complex method. Prior to immunostaining of AGEs, deparaffinized sections underwent a microwave treatment in citrate buffer (10 mmol/l, pH6.0) for 10 min. The microwave treatment of the tissue sections likely generates AGEs such as N\(^\text{ε}\)-(carboxymethyl) lysine (CML) from fructosamines [19]. As there are increased fructosamines in the diabetic rats, this may give rise to artifactual high levels of CML.

For immunohistochemistry of imidazoline and CML, a monoclonal anti-imidazoline antibody (1:100, 18 μg/ml) and a monoclonal anti-CML antibody (1:200, 6 μg/ml) that were produced in our laboratory [6], were used.

A monoclonal anti-CEdG antibody (MAb M-5.1.6) that was produced by Pischetsrieder, and recognizes two diastereomers of CEdGA,B with high affinity and specificity [20] was used (1:1000, 1.8 μg/ml). CEdG was identified as a major product found in the reaction mixtures of guanosine and glucose in vitro, and can be used as a marker of advanced glycation of DNA [20].

For immunostaining of TGF-β1, the sections were treated with diluted proteinase K solution (0.01 mg/ml) at 37°C for 10 min before staining, and then stained using a polyclonal anti-TGF-β1 antibody (1:20, Santa Cruz Biotechnology, CA, USA). TGF-β1 is important in the development of renal hypertrophy and accumulation of extracellular matrix components in diabetic nephropathy. AGEs interact with specific receptors such as RAGE to influence the expression of growth factors including TGF-β1.

For immunostaining of collagen type 1, a polyclonal rabbit anti-rat collagen type 1 antibody (1:20, 5 μg/ml, Monosan, Uden, Netherlands) was used. Prior to immunostaining, deparaffinized sections were treated with diluted...
For immunostaining of fibronectin, a monoclonal anti-fibronectin antibody (no dilution, 200 μg/ml, NeoMarkers, Fremont, CA, USA) was used. Prior to immunostaining, deparaffinized sections underwent a microwave treatment in citrate buffer (10 mM/l, pH 6.0) for 10 min.

**Histological assessment**

For measurement of mean glomerular area and mean mesangial area, the pictures of PAS-staining were taken on 20 different glomeruli per section by a digital camera (DN100, Nikon, Tokyo, Japan) and displayed on a computer board using the software of Adobe Photoshop® 6.0. The glomerular area and PAS-positive area (identical with kidney cortex and the average area was obtained. Area, 10 glomeruli per section were randomly selected as a representative for each rat. The glomeruli selected for area measurement were localized within a 1 mm-depth from the surface of the kidney cortex, and were cut in half from a glomerular stalk with open capillary loops to distinguish mesangial structure.

To determine the extent of interstitial fibrosis and expression of type I collagen, the pictures of Masson's trichrome-staining and type 1 collagen-positive area were taken on 20 different sections in the renal cortex by the digital camera, and displayed on a computer board using the software of Adobe Photoshop® 6.0. The Masson's trichrome-positive area (identical with interstitial fibrosis) and type 1 collagen-positive areas in the tubulointerstitium were determined by Adobe Photoshop® and measured as percent of the tubulointerstitial area using NIH Image 1.62. The average value of 20 measurements was adopted as a representative of each rat. The glomeruli selected for area measurement were localized within a 1 mm-depth from the surface of the kidney cortex, and were cut in half from a glomerular stalk with open capillary loops to distinguish mesangial structure.

To clarify the number of CEdG-positive cells, CEdG-positive nuclei were counted on randomly selected 10 glomeruli. The average number on 10 glomeruli was adopted as a representative for each rat.

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**Total RNA preparation and quantification of mRNA levels**

A part of fresh kidney tissues taken at the time of sacrifice was cut into small pieces (5 x 5 x 2 mm) and Sunk in RNA later® (Ambion Inc., Austin, TX, USA) at 4°C to prevent RNA degradation. Total RNA was extracted using a kit (High Pure RNA Tissue Kit®, Roche Diagnostics GmbH, Mannheim, Germany). In brief, the tissue (<25 mg) was homogenized in 40 μl lysis/binding buffer, followed by lysis centrifugation for 2 min at maximum speed (13 000 x g) to collect supernatant. Next, ethanol (200 μl) was added to the lysate supernatant, and they were mixed well. The sample was put into a reservoir with glass fibre that has a character of binding RNA. After centrifugation at maximum speed (13 000 x g), residual contaminating DNA was digested with DNase I for 15 min. Bound RNA was washed three times, and eluted by nucleic-free, sterile and double distilled water. Immediately after RNA extraction, cDNA was prepared using Oligo(dT)15 primer and AMV reverse transcriptase [1st strand cDNA Synthesis Kit for RT-PCR (AMV), Roche Diagnostics GmbH, Mannheim, Germany]. The reaction was processed as follows: incubation of samples at 25°C for 10 min and then at 42°C for 60 min, followed by 95°C for 5 min and cooling to 4°C for 5 min.

The thermal cycling step was as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 62°C for 15 s and extension at 72°C for 12 s. Temperature transition rate was set at 20°C/s. Quantitative values were obtained from a standard regression line. The value of TGF-β1 mRNA was corrected by the expression of an internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

RAGE mRNA expression was also quantified using LightCycler. One set of primers were designed as follows: forward primer, 5'-CATCCTGTGGCGAATACTTATG-3' and reverse primer, 5'-CCTGTATTCCGTCTCCTT-3'. The product size was 176 bp. The reaction mixture consisted of 2 μl LightCycler-FastStart DNA Master Hybrization Probes® (FastStart Taq DNA polymerase, reaction buffer, dNTP mix, 10 mM MgCl2; Roche Diagnostics GmbH, Mannheim, Germany), 1.6 μl of 25 mM MgCl2, 1 μl of each primer and probe was added to the mixture, followed by addition of 5 μl of cDNA solution and PCR-grade water to make a final volume of 20 μl. PCR product of rat TGF-β1 was amplified as a standard cDNA. The thermal cycling step was performed as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 62°C for 15 s and extension at 72°C for 12 s. Temperature transition rate was set at 20°C/s. Quantitative values were obtained from a standard regression line. The value of TGF-β1 mRNA was corrected by the expression of an internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

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After separating PCR products of RAGE on a 2% agarose gel, the product of 189 bp was excised using a clean scalpel, and the targeting DNA fragment was extracted using the kit (QIAquick Gel Extraction Kit®, QIAGEN GmbH, Hilden, Germany). In brief, the gel slice was weighed, and dissolved at 50°C for 10 min. After complete dissolution of the gel, one gel volume of isopropanol was added to the sample. To bind DNA, the sample was applied to a
silica-membrane and centrifuged. After washing, DNA was eluted by addition of 10 mmol/l Tris–HCl (pH 8.5). Eluted DNA concentrations were immediately measured with the DNA calculation instrument (GeneQuant II, Pharmacia Biotech Ltd, Cambridge, UK), and the sample was serially diluted to make it standard. Serially diluted standard and cDNA samples were simultaneously amplified by PCR, as described earlier. Quantitative value was obtained from the standard regression line, and then corrected by GAPDH.

Statistics

Results are expressed as mean ± SD. One-way analysis of variance (ANOVA) was performed to determine whether parameters differed among the four groups. When there were significant differences by ANOVA, Fisher’s protected least significant difference (PLSD) test was used for further analysis between groups. Spearman rank correlation test was performed to determine whether a significant correlation existed between parameters. P < 0.05 was considered statistically significant.

Results

Figure 2 shows the time course of blood glucose concentration. The levels of blood glucose did not differ between three diabetic groups (DM, PM-DM, PLP-DM), and were maintained around 400 mg/dl in the diabetic groups. The three diabetic groups showed much higher glucose levels than the normal group throughout the experiment period.

Table 1 shows the parameters and the histological indices at the end of the experiment (16th week). Diabetic groups showed much lower body weight than normal. Mean blood pressure did not show any significant differences among the four groups. Urinary albumin excretion was increased in diabetes. The development of albuminuria in diabetes was significantly inhibited by PM and PLP treatment, whereas the PLP-treated diabetic group tended to show a lower urinary albumin level as compared with the PM-treated diabetic group. Serum creatinine level did not show any significant differences among the four groups. Creatinine clearance was significantly increased in diabetic and PM-treated diabetic groups, but not in PLP-treated diabetic group. Total cholesterol level did not show any significant differences among the four groups. Kidney weight index was increased in three diabetic groups. However, an increase in kidney weight index was significantly inhibited by PLP but not by PM.

Figure 3 shows PAS staining of the kidneys. Glomerular area and mesangial area were increased in the diabetic group as compared with the normal group. The PLP-treated diabetic group showed a significant decrease in glomerular area and mesangial area as compared with the diabetic group, whereas the PM-treated diabetic group did not show any significant decrease in glomerular area or mesangial area (Table 1). Thus, glomerular hypertrophy and mesangial expansion were prevented by PLP but not by PM.

Figure 4 shows immunostaining of TGF-β1 in the kidneys. TGF-β1 was hardly recognized in the normal group. TGF-β1 was markedly expressed in the diabetic group. In the PM-treated diabetic group,

Table 1. Parameters and histological indices at the end of experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>FBG (mmol/l)</th>
<th>Tcho (mg/dl)</th>
<th>Cr (µmol/l)</th>
<th>Ccr (ml/min)</th>
<th>UAE (mg/day)</th>
<th>MBP (mmHg)</th>
<th>KI (g/100g BW)</th>
<th>MGA (µm²/glomerulus)</th>
<th>MMA (µm²/glomerulus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>318 ± 11</td>
<td>6.2 ± 0.5</td>
<td>80 ± 20</td>
<td>22 ± 5</td>
<td>1.79 ± 0.29</td>
<td>3.1 ± 1.6</td>
<td>110 ± 6</td>
<td>0.65 ± 0.06</td>
<td>8860 ± 1352</td>
<td>1002 ± 427</td>
</tr>
<tr>
<td>DM</td>
<td>268 ± 9**</td>
<td>25.9 ± 3.1***</td>
<td>88 ± 11</td>
<td>21 ± 6</td>
<td>4.29 ± 1.95**</td>
<td>20.1 ± 4.7***</td>
<td>111 ± 12</td>
<td>1.12 ± 0.18***</td>
<td>10562 ± 1646</td>
<td>2133 ± 620***</td>
</tr>
<tr>
<td>PM-DM</td>
<td>266 ± 18**</td>
<td>25.3 ± 6.5***</td>
<td>88 ± 28</td>
<td>26 ± 8</td>
<td>4.29 ± 1.95**</td>
<td>15.5 ± 3.0***</td>
<td>105 ± 11</td>
<td>1.00 ± 0.13***</td>
<td>11391 ± 1134**</td>
<td>1597 ± 768**</td>
</tr>
<tr>
<td>PLP-DM</td>
<td>270 ± 14**</td>
<td>25.6 ± 2.9***</td>
<td>74 ± 10</td>
<td>21 ± 4</td>
<td>2.40 ± 0.50</td>
<td>13.7 ± 2.7***</td>
<td>114 ± 8</td>
<td>0.96 ± 0.08***</td>
<td>9402 ± 1685</td>
<td>973 ± 253***</td>
</tr>
</tbody>
</table>

C, non-diabetic normal group; DM, non-treated diabetic group; PM-DM, pyridoxamine-treated diabetic group; PLP-DM, pyridoxal phosphate-treated diabetic group; BW, body weight; FBG, fasting blood glucose; Tcho, total cholesterol; Cr, serum creatinine; Ccr, creatinine clearance; UAE, urinary albumin excretion; MBP, mean blood pressure; KI, kidney weight index; MGA, mean glomerular area; MMA, mean mesangial area.

*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 vs C; 1P < 0.05, 11P < 0.001 vs DM; 2P < 0.05 vs DM and PM-DM; 3P < 0.05 vs PM-DM.

All data are expressed as mean ± SD.
TGF-β1 was less prominent as compared with the diabetic group. More notably, in the PLP-treated diabetic group, TGF-β1 was hardly recognized, as in the normal group. The positive area for TGF-β1 in glomeruli is shown in Figure 5D. The diabetic group showed markedly increased TGF-β1-positive area in the glomeruli. PM significantly decreased TGF-β1-positive area as compared with the diabetic group. More markedly, PLP decreased TGF-β1-positive area more than PM.

Figure 5 shows immunostaining positive area for imidazolone (A), CML (B), and CEdG (C) in the glomeruli. Imidazolone and CML were hardly recognized in the glomeruli of the normal group. However, the diabetes group showed markedly increased imidazolone- and CML-positive areas in the glomeruli. PM significantly decreased imidazolone-positive area, but did not significantly decrease CML-positive area in the glomeruli as compared with the diabetic group. PLP significantly decreased both imidazolone- and CML-positive areas as compared with the diabetic group. CEdG was localized in the nuclei of various intrinsic glomerular cells, especially in the mesangial cells and podocytes. The number of CEdG-positive cells in the glomeruli was increased in the diabetic group as compared with the normal group. There were no statistical differences in the number of CEdG-positive cells between diabetic and PM-treated diabetic groups. However, PLP significantly decreased the number of CEdG-positive cells as compared with the diabetic group.

Figure 6 shows interstitial fibrosis area (A), immunostaining positive area for type 1 collagen in the tubulointerstitium (B) and fibronectin in the glomeruli (C). The extent of interstitial fibrosis area was increased in the diabetic group as compared with the normal group. The PLP-treated diabetic group showed a significant decrease in interstitial fibrosis area as compared with the diabetic group, whereas the PM-treated diabetic group did not show any significant decrease in interstitial fibrosis area. The expression of type 1 collagen in the tubulointerstitium was increased in the diabetic as compared with the normal group. Both PM- and PLP-treated diabetic groups showed a significant decrease in the expression of type 1 collagen in the tubulointerstitium. The expression of fibronectin in the glomeruli was increased in diabetic groups as compared with normal. Both PM- and PLP-treated
diabetic groups showed a significant decrease in the expression of fibronectin in the glomeruli. PLP was superior to PM in the reduction of interstitial fibrosis (\(P < 0.0001\)), type 1 collagen expression (\(P < 0.05\)) and fibronectin expression (\(P < 0.05\)).

Figure 7 shows the expression of TGF-\(\beta\)1 mRNA (A) and RAGE mRNA (B) in the kidneys. The expression of TGF-\(\beta\)1 mRNA was markedly increased in diabetic group. PM reduced the expression of TGF-\(\beta\)1 mRNA. More intensely, PLP reduced the expression of TGF-\(\beta\)1 mRNA than PM. The expression of RAGE mRNA was markedly increased in diabetic group. Both PM and PLP reduced the expression of RAGE mRNA.

**Discussion**

Because PM and PLP did not affect body weight, blood glucose and blood pressure in diabetic rats, the influences of these parameters can be neglected when comparing the efficacy between PM and PLP. Some effects of PM and PLP may be due to improvement of PLP deficiency in experimental diabetes [21,22]. Although an increase in urinary albumin excretion at the 16th week was significantly prevented by PM as well as PLP treatment, PLP more intensely decreased urinary albumin than PM. PLP prevented glomerular hypertrophy and mesangial expansion in diabetic rats, whereas PM did not. Degenhardt *et al.* [13] also reported that STZ-induced rats treated with PM at almost the same dosage as we used in this study, did not show any significant decrease in mesangial volume. Persistent hyperfiltration causes a mechanical stretch on glomerular cells, and such circumstances may result in the up-regulation of growth factors [23,24] to promote the progression of nephropathy. PLP prevented glomerular injury. Further, PLP prevented interstitial fibrosis, and inhibited the expression of type 1 collagen in the tubulointerstitium and fibronectin in the glomeruli.

It is widely known that not only hyperlipidaemia but also dyslipidaemia is partially responsible for the progression of glomerular changes in the pathogenesis of diabetic nephropathy. However, serum cholesterol levels did not differ between the four groups. Because hypercholesterolaemia associated with diabetes was not observed in our study despite sufficient
hyperglycaemia, it is difficult to evaluate the effect of PM as well as PLP on serum cholesterol levels. Recently, we have reported that intraperitoneal administration of PLP reduced accumulation of CML and imidazolone in the peritoneum, and prevented the progression of peritoneal sclerosis induced by glucose-based peritoneal dialysis fluids [17]. The present study demonstrated that PLP reduced immunostaining area of CML and imidazolone more intensely than PM. Thus, PLP exerts a more inhibitory effect on accumulation of AGEs than PM. AGEs cause an increase in the expressions of growth factors, which leads to histological damages and tissue fibrosis [17,25,26]. In fact, in parallel with a decrease in imidazolone and CML, a significant reduction in TGF-β1 expression was observed not only in protein level but also in mRNA level, especially in the PLP-treated group. Thus, PLP is useful in the treatment of diabetic nephropathy by inhibiting formation of AGEs and expression of TGF-β1.

AGE modification also takes place in nucleosides [27–30]. CEdG was identified as an AGE derived from 2'-deoxyguanosine, and is a major glycation product of DNA under physiological conditions [30]. We demonstrated that CEdG was significantly increased in atherosclerotic lesions of haemodialysis patients as well as in kidneys of diabetic nephropathy [31]. Nucleoside glycation leads to DNA damages including DNA strand breaks and mutations [30,32,33]. In the present study, we found that PLP significantly decreased the number of CEdG-positive cells as compared with the diabetic group, but PM did not. Inhibition of DNA glycation may contribute to normalization of genetic integrity in diabetic kidney cells.

Both PM and PLP attenuated expression of RAGE mRNA levels in diabetes. RAGE is a well-characterized AGE receptor, which is involved in intracellular signal transduction pathway rather than in AGE turnover [34]. CML adducts are ligands for RAGE [35], and RAGE expression is up-regulated in diabetes [36,37]. CML is taken up into the kidneys to induce the over-expression of RAGE, which results in the development of diabetic nephropathy [38,39]. Although inhibition of CML formation by PM was already demonstrated in
**in vitro** studies [40] and STZ-induced diabetic rats [13], we have demonstrated more effective inhibition of CML by PLP in the present study. In our study, CML was prominent in podocytes and mesangium, whereas imidazolone was almost exclusively localized in mesangium. Activation of podocytes by the deposition of AGEs such as CML may trigger the progression of diabetic nephropathy. Prevention of kidney cells from up-regulation of RAGE by PLP and PM may have caused a decrease in albuminuria.

In conclusion, PLP prevents progression of diabetic nephropathy by inhibiting formation of AGE. PLP is considered a promising active form of vitamin B6 for

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Fig. 6. Interstitial fibrosis area (A), and immunostaining positive area for type 1 collagen in the tubulointerstitium (B) and fibronectin in the glomeruli (C). C, normal group; DM, diabetic group; PM-DM, pyridoxamine-treated diabetic group; PLP-DM, pyridoxal phosphate-treated diabetic group. All data are expressed as mean ± SD (n = 8). **P < 0.0001 vs C, †††† P < 0.0001 vs DM.

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Fig. 7. Expression of TGF-β1 mRNA (A) and RAGE mRNA (B) in kidneys. C, normal group; DM, diabetic group; PM-DM, pyridoxamine-treated diabetic group; PLP-DM, pyridoxal phosphate-treated diabetic group. Gene expression was corrected by GAPDH mRNA. All data are expressed as mean ± SD (n = 8). **P < 0.01 vs C, † P < 0.05, †† P < 0.01 vs DM.
the treatment of AGEs-related disorders such as diabetic complications.

Conflict of interest statement. None declared.

References


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Conflict of interest statement. None declared.

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