Ethyl pyruvate ameliorates albuminuria and glomerular injury in the animal model of diabetic nephropathy

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1Department of Internal Medicine and 2Clinical Research Institute, Seoul National University Hospital, 3Transplantation Research Institute, Seoul National University, and 4Eulji General Hospital, Eulji University College of Medicine, Seoul, Korea

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Ju KD, Shin EK, Cho EJ, Yoon HB, Kim HS, Kim H, Yang J, Hwang YH, Ahn C, Oh KH. Ethyl pyruvate ameliorates albuminuria and glomerular injury in the animal model of diabetic nephropathy. Am J Physiol Renal Physiol 302: F606–F613, 2012. First published November 30, 2011; doi:10.1152/ajprenal.00415.2011.—Pyruvate is an endogenous antioxidant and anti-inflammatory substance. The present study was implemented to investigate the protective effect of ethyl pyruvate (EP) against the development and progression of diabetic nephropathy in an in vivo and in vitro model. Diabetic rats were prepared by injecting streptozotocin (65 mg/kg). Those that developed diabetes after 72 h were treated with EP (40 mg/kg) intraperitoneally. Diabetic rats without pyruvate treatment and nontreated diabetic rats were used for control. As an in vitro experiment, rat mesangial cells cultured primarily from Sprague-Dawley rats were treated in high-glucose (HG; 50 mM) or normal-glucose (NG; 5 mM) conditions and with or without pyruvate. Pyruvate-treated diabetic rats exhibited decreased albuminuria and attenuated NADPH-dependent reactive oxygen species generation. Immunohistochemistry showed reduced laminin, type IV collagen, and fibronectin deposition in the glomeruli compared with nontreated diabetic rats. Parallel changes were shown in tissue mRNA and protein expression levels of monocyte chemoattractant protein-1, transforming growth factor-β1, laminin, fibronectin, and type IV collagen in the kidney. Concordantly, protective effects were also exhibited in the mesangial cell culture system. These findings suggest that pyruvate protects against kidney injury via NADPH oxidase inhibition. The present study established that activation of NADPH oxidase plays a crucial role in diabetes-induced oxidative stress, glomerular hypertrophy, and ECM molecule expression. Pyruvate exhibited a renoprotective effect in the progression of experimental diabetic nephropathy. Future research is warranted to investigate the protective mechanism of pyruvate more specifically in relation to NADPH oxidase in diabetic nephropathy.

Antioxidant; mesangial cells

Diabetic nephropathy (DN) is one of the most important complications of diabetic patients and is characterized histologically by an accumulation of extracellular matrix (ECM) protein in the glomeruli and the interstitia. Hyperglycemia triggers a series of intracellular events in glomerular and tubular cells such as reactive oxygen species (ROS) generation, protein kinase C and mitogen-activated protein kinase activation, and transcription factor induction (13, 15, 22, 23, 34). High glucose (HG) increases proinflammatory and profibrotic factors as well as induces cellular hypertrophy (4, 9, 27).

Recently, numerous studies suggested that oxidative stress is involved in the pathogenesis of diabetic complications (2, 14, 24, 26). Indeed, a number of experimental and clinical studies have shown that oxidative stress plays an important role in the development and progression of DN (10). In addition, patho-logical changes in the glomeruli and an increase in urinary albumin excretion in diabetic rats were ameliorated by treatment with antioxidants such as vitamin E, taurine, or lipoic acid, supporting the role of oxidative stress in the pathogenesis of DN (6, 20, 29). Renal hypertrophy and ECM accumulation are early features of DN (3, 37, 40, 41). Elevated ROS levels contribute to the development of diabetic vascular complications, such as atherosclerosis and DN. In experimental and human diabetes, ROS generation is known to be increased via multiple pathways, including glucose autooxidation, increased mitochondrial superoxide production, PKC-dependent activation of NADPH oxidase, uncoupled endothelial nitric oxide synthase (eNOS) activity, formation of advanced glycation end products (AGEs), and stimulation of cellular ROS production by extracellular AGEs through their receptors (5, 10). Among these, the activation of NADPH oxidase and uncoupling of eNOS mainly contribute to the increase in glomerular superoxide production in experimental diabetic rats (41). Recent studies indicate that a multicomponent phagocyte-like NADPH oxidase is a major source of ROS in many nonphagocytic cells, including renal cells such as tubular epithelial cells and glo-merular mesangial cells (8, 11, 17, 25). Under physiological conditions, NADPH oxidases have a very low constitutive activity that can be upregulated in response to various stimuli (1, 16, 18, 19).

Pyruvate, a key intermediate metabolite of glucose and a potent antioxidant and free radical scavenger, has antioxidant and anti-inflammatory effects in an in vivo and in vitro model (31). However, the instability of pyruvate limits its usefulness as a therapeutic agent (31). Ethyl pyruvate (EP), a simple aliphatic ester derived from pyruvic acid, is safer and more stable than pyruvate. In addition, in vitro EP was found to suppress the expression of several proinflammatory mediators (7, 28). Based on these observations, it was suggested that EP has a potential role as an anti-inflammatory agent, although its mode of action was not elucidated.

To date, however, the effect of pyruvate on diabetic kidney disease has not been explored yet. The following were the objectives of the present study: 1) to determine whether NADPH oxidase mediates ROS generation induced by experimental diabetes in vivo and by HG in cultured mesangial cells; 2) to explore whether pyruvate has a protective effect in the development and progression of
experimental DN; and 3) to investigate whether its protective effect is mediated by the inhibition of NADPH oxidase and downstream proinflammatory and profibrotic molecules.

MATERIALS AND METHODS

Animals and Treatments

All animal studies were carried out with the approval of the Institutional Animal Care and Use Committee (IACUC) of Seoul National University Hospital, Male Sprague-Dawley rats (Koatech), weighing between 200 and 225 g, were divided into 4 groups of 12 rats/group. Rats were injected intraperitoneally (ip) with either 65 mg/kg body wt streptozotocin (STZ, Sigma, St. Louis, MO) in sodium citrate buffer (0.01 M, pH 4.5, Sigma) to induce diabetes or injected with vehicle only as a nondiabetic control. Blood glucose concentration (LifeScan One Touch glucometer, Johnson&Johnson) was monitored periodically before and after the injection. Diabetic rats with blood glucose above 300 mg/dl were randomly divided into a pyruvate-treated group (DM+PY) and an untreated diabetes group (DM).

Seventy-two hours after STZ injection, rats in the DM+PY group were injected with EP (40 mg/kg ip, Sigma) every other day. All the diabetic rats were treated with 2 U of regular insulin (ip, Sigma) every other day. Rats injected with vehicle alone were further subdivided into a nondiabetic untreated control (Con) and nondiabetic pyruvate-treated control group (C+PY). All rats had unrestricted access to food and water. Body weights, serum glucose, blood urea nitrogen, serum creatinine, and 24-h urine albumin were measured every 4 wk. Glycated hemoglobin (HbA1c) was measured at the time of death. Rats were maintained in accordance with IACUC procedures. At week 12, all rats were euthanized, and the kidneys were weighed at the time of death. A slice of whole kidney at the pole was embedded in paraffin and sectioned, and their kidneys were removed. Renal cortical tissues were separated from the medulla and minced in D-Hanks’ balanced buffer (LifeScan One Touch glucometer, Johnson&Johnson) was monitored periodically before and after the injection. Diabetic rats with blood glucose above 300 mg/dl were randomly divided into a pyruvate-treated group (DM+PY) and an untreated diabetes group (DM).

Isolation and Culture of Primary Rat Mesangial Cells

Primary cultured mesangial cells were seeded in six wells or 100-mm plastic dishes in RPMI under normal glucose (NG; 5 mmol/l glucose) or HG (50 mmol/l glucose) conditions. The cultured mesangial cells at ~70–80% confluence were cultured in serum-free RPMI with NG for 24 h to synchronize cell growth. After that, the media was replaced with fresh serum-free media containing NG, HG alone, or NG/HG plus various levels of EP. Treatment with 5 mmol/l glucose plus 45 mmol/l mannitol was employed for osmotic control.

RT-PCR

Gene expressions of monocyte chemoattractant protein (MCP)-1, TGF-β1, laminin, fibronectin, and type IV collagen mRNA were assessed using RT-PCR standardized by coamplifying with the housekeeping gene GAPDH, which served as an internal control. Total RNA was isolated from the glomeruli and mesangial cells with TRIzol (Invitrogen, Japan, Tokyo, Japan) by the guanidine thiocyanate extraction method. Total RNA was reverse transcribed into DNA and used for PCR with rat-specific primers for MCP-1, TGF-β1, laminin, type IV collagen, and fibronectin. The promoter-specific primers used for 447-bp fragments of rat MCP-1 were 5'-ATGCCAGTCTCTGTCACG-3' (forward primer) and 5'-CTAGTTGCTGTCTTTTGACG-3' (reverse primer), and for 154-bp fragments of TGF-β1 primers were 5'-TGAGTGCGCTGTCTTTTGACG-3' (forward primer) and 5'-TGGGACTGATCCCACTTATG-3' (reverse primer). The primers for laminin corresponded to 5'-GGTCAGGTGACTCGCTTTG-3' (forward primer) and 5'-GCTCTTAACGTTGGCAGTCTGT-3' (reverse primer); the final PCR product was 275 bp in size. The primers for type IV collagen corresponded to 5'-CAGGGTCTGGCTTAAATCTCTG-3' (forward primer) and 5'-AGCTCCCCCTGCTCTTCAAGCTT-3' (reverse primer); the final PCR product was 328 bp in size. The primers for fibronectin corresponded to 5'-GTGGGTGCTCTCAAACTCTCTC-3' (forward primer) and 5'-AGCTCCCCCTGCTCTTCAAGCTT-3' (reverse primer); the final PCR product was 213 bp in size. Sequences of GAPDH primers were 5'-TTCTCAAGGTG-3', (forward primer) and 5'-AGCTCTTAAAAAAGGCCTGTAAT-3' (reverse primer); the final PCR product was 515 bp in size. Western Blot Analysis

The whole cell extracts (50 μg of protein/lane) were loaded, separated by 8–12% SDS-PAGE under reducing conditions, and transferred onto nitrocellulose membranes (Amersham, Arlington Heights, IL) by electroblotting. The transfer of the protein and the equality of the loading in the lanes were verified using reversible staining with Ponceau S. The membranes were blocked with 5% nonfat dry milk in TBS-T (Tris-buffered saline and 0.15% Tween 20) for 1 h at room temperature. The proteins were detected by antibodies for MCP-1, TGF-β1, laminin (Santa Cruz Biotechnology, Santa Cruz, CA), type IV collagen, and fibronectin (Abcam, Cambridge, UK) diluted in TBS-T containing 5% dry milk and incubated overnight at 4°C in a 1:1,000 dilution of polyclonal antibody. After washing in TBS-T, the immunoreactive proteins were visualized using horseradish peroxidase-linked goat anti-mouse, goat anti-rabbit, or donkey anti-goat IgG (Santa Cruz Biotechnology) at 1:1,000 dilution, which was followed by enhanced chemiluminescence (Amersham). Exactly equal amounts of protein, determined by the Bradford method, were loaded in each lane.

ELISA

Levels of MCP-1 (Invitrogen, Camarillo, CA) and TGF-β1 in the culture medium were determined by ELISA kits (R&D System, Minneapolis, MN) according to the manufacturer’s instructions.

NADPH Oxidase Assay

NADPH oxidase activity was measured by the lucigenin-enhanced chemiluminescence method.

Kidney cortex and glomeruli. Homogenates from the renal cortex or isolated glomeruli were prepared in 1 ml and 500 μl, respectively, of lysis buffer (20 mM KH2PO4, pH 7.0, 1 mM EGTA, 1 mM PMSF, 10 μg/ml aprotinin, and 0.5 μg/ml leupeptin) by using a Dounce homogenizer (100 strokes on ice). Homogenates were subjected to

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Table 1. Parameters of experimental animals at euthanasia

<table>
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<th>Con (n = 12)</th>
<th>DM (n = 12)</th>
<th>C+PY (n = 12)</th>
<th>DM+PY (n = 12)</th>
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<tr>
<td>Body weight, g</td>
<td>438 ± 12</td>
<td>239 ± 8*</td>
<td>439 ± 12</td>
<td>239 ± 12*</td>
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<tr>
<td>Kidney wt/body wt, %</td>
<td>0.62 ± 0.01</td>
<td>1.18 ± 0.08*</td>
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<td>Glucose, mg/dl</td>
<td>102.3 ± 1.6</td>
<td>608.6 ± 54.7*</td>
<td>104.2 ± 2.3</td>
<td>615.3 ± 29.3*</td>
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<td>BUN, mg/dl</td>
<td>14.0 ± 0.3</td>
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<td>13.9 ± 2.8</td>
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<tr>
<td>Serum creatinine, mg/dl</td>
<td>0.65 ± 0.02</td>
<td>0.99 ± 0.12*</td>
<td>0.74 ± 0.13</td>
<td>0.97 ± 0.04*</td>
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<tr>
<td>Systolic blood pressure, mmHg</td>
<td>108.3 ± 1.3</td>
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<td>105.8 ± 1.1</td>
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<tr>
<td>HbA1c, %</td>
<td>4.22 ± 0.05</td>
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<td>4.17 ± 0.02</td>
<td>8.18 ± 0.30*</td>
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<td>Albuminuria, mg/day</td>
<td>0.24 ± 0.01</td>
<td>5.13 ± 0.38*</td>
<td>0.29 ± 0.01</td>
<td>1.96 ± 0.13*</td>
</tr>
</tbody>
</table>

Values are means ± SE from 12 animals (n = 12/group). BUN, blood urea nitrogen; Con, untreated nondiabetic control group; DM, untreated diabetic group; C+PY, pyruvate-treated nondiabetic group; DM+PY, pyruvate-treated diabetic group. *P < 0.05 vs. Con. #P < 0.05 DM+PY vs. DM.

low-speed centrifugation at 800 g, 4°C, for 10 min to remove the unbroken cells and debris, and aliquots were used immediately. To start the assay, 100 μl of homogenates were added to 900 μl of 50 mM phosphate buffer, pH 7.0, containing 1 mM EGTA, 150 mM sucrose, 5 μM lucigenin, and 100 μM NADPH. Photon emission in terms of relative light units (RLU) was measured every 20 or 30 s for 10 min in a luminometer. There was no measurable activity in the absence of NADPH. A buffer blank (<5% of the cell signal) was subtracted from each reading. Superoxide production was expressed as RLU per milligram protein. Protein content was measured using the Bio-Rad protein assay reagent.

Cultured mesangial cells. NADPH oxidase activity in cells was measured as described previously (12). Briefly, mesangial cells grown in serum-free medium containing 5 or 50 mM glucose were washed three times in PBS-0.1% BSA and then incubated with biotinylated peroxidase in methanol to block nonspecific peroxidase activity and antibody was identified by immunoperoxidase ABC staining following the manufacturer’s instructions (Vector Laboratories, Burlingame, CA). The sections were then dehydrated and mounted with Permount (Sigma) and viewed by brightfield microscopy.

RESULTS

Animal Experiments

Briefly, male Sprague-Dawley rats were divided into four groups. At 12 wk, rats were euthanized. At the time of death, the ratios of kidney weight to body weight were significantly higher in DM and DM+PY rats than in Con rats (P < 0.05). The mean blood glucose levels at 12 wk were significantly elevated in both diabetic groups (DM and DM+PY). However, no differences were observed in blood glucose, HbA1c, and systolic blood pressure between the DM and DM+PY groups. The mean serum creatinine was higher in DM (0.99 ± 0.12 mg/dl) and DM+PY rats (0.97 ± 0.04 mg/dl) compared with Con rats (0.65 ± 0.02 mg/dl) (P < 0.05). However, the difference in serum creatinine concentration between DM and DM+PY rats did not reach statistical significance. The mean blood urea nitrogen was significantly higher in DM and DM+PY rats relative to Con rats but did not reach statistical significance between DM and DM+PY. Compared with the Con group (0.24 ± 0.01 mg/day), 24-h urinary albumin excretion was significantly higher in the DM group (5.13 ± 0.38 mg/day, P < 0.05), and pyruvate treatment remarkably re-

Statistical Analysis

All values are expressed as means ± SE. Statistical analyses were performed using the statistical package SPSS for Windows version 11.0 (SPSS, Chicago, IL). Results were analyzed using the Kruskal-Wallis nonparametric test for multiple comparisons. Significant differences by the Kruskal-Wallis test were confirmed post hoc by the Mann-Whitney U-test. P values <0.05 were considered statistically significant.

Fig. 1. Effects of pyruvate treatment on diabetes-induced reactive oxygen species (ROS) generation. A: NADPH oxidase activity in glomerular homogenates. NADPH oxidase activity was measured by lucigenin-enhanced chemiluminescence and expressed as relative light units (RLU)/mg protein. B: hydrogen peroxide generation was detected by STA-347. Values are means ± SE of the activities from the glomeruli of 6 animals/group. Con, untreated nondiabetic group; DM, untreated diabetic group; C+PY, pyruvate-treated nondiabetic group; DM+PY, pyruvate-treated diabetic group. *P < 0.05 vs. Con. #P < 0.05 DM+PY vs. DM.
Effect of Pyruvate on MCP-1 and TGF-β1 expression in glomeruli

The effect of pyruvate on mRNA and protein expression levels of MCP-1 and TGF-β1 were measured by RT-PCR and Western blot analysis in glomeruli. mRNA expression levels of MCP-1 and TGF-β1 were increased in glomeruli of diabetic rats (DM group), and these increases were significantly reduced by pyruvate treatment (Fig. 2A). As shown the Fig. 2B, pyruvate markedly ameliorated MCP-1 and TGF-β1 protein expression in glomeruli. Taken together, our data suggest that pyruvate led to a significant reduction of MCP-1 and TGF-β1 in glomeruli.

Effect of Pyruvate on Laminin, Type IV Collagen, and Fibronectin Deposition in Glomeruli

mRNA expressions of type IV collagen and fibronectin were increased in DM rats compared with Con rats, and these increases were attenuated by treatment with pyruvate (Fig. 2A). Similar to the mRNA results, glomerular type IV collagen and fibronectin protein expression determined by Western blot were significantly increased in DM compared with Con rats, and these increases were significantly ameliorated by pyruvate treatment (Fig. 2B). Immunohistochemistry revealed a strong expression of laminin, type IV collagen, and fibronectin in the renal cortex of the diabetic rats (DM group), and the expression of the above ECM proteins were remarkably reduced in DM+PY rats (Fig. 3A), which were consistent with the changes exhibited by RT-PCR and Western blotting. Collectively, these data indicated that pyruvate treatment attenuates the glomerular expression of ECM molecules in DN.

Effect of Pyruvate on Glomerular Diameter and Cross-Sectional Area

Glomerular diameter and glomerular cross-sectional area were quantified in the histological sections of the kidneys removed from the rats. Figure 3B shows that the glomeruli of diabetic rats (DM group) are significantly larger, in terms of both the diameter and cross-sectional area, compared with the controls (Con). Pyruvate treatment resulted in a decrease in glomerular size. Therefore, these results demonstrate that pyruvate treatment ameliorates glomerular hypertrophy in diabetic rats.

Fig. 3. Histological analysis of kidney sections. A: representative micrographs showing positive laminin, type IV collagen, and fibronectin immunostaining in glomeruli (magnification: ×400). B: quantitation of glomerular diameter. C: quantitation of glomerular cross-sectional area. Glomerular cross-sectional areas were measured in kidney sections by using Image-ProPlus 4.5 software. The histograms represent means ± SE from 20 individual glomeruli in sections from 5 individual rats/group. Groups are as defined in the legend for Fig. 1. *P < 0.05 vs. Con. #P < 0.05 DM+PY vs. DM.
**Effect of HG on NADPH Oxidase Activity and ROS Generation in Cultured Mesangial Cells**

NADPH oxidase activity was determined by the lucigenin-enhanced chemiluminescence method. Compared with NG-treated cells, NADPH oxidase activity in HG-treated mesangial cells was significantly increased by 8.3-fold and EP treatment significantly ameliorated NADPH oxidase activity to 2.2-fold in HG-stimulated mesangial cells ($P < 0.05$, HG vs. HG+EP; Fig. 4A). 

**Effect of EP on HG-Induced MCP-1 and TGF-β1 in Cultured Mesangial Cells**

mRNA and protein expression levels of MCP-1 and TGF-β1 were detected by RT-PCR and ELISA. mRNA expression levels of MCP-1 and TGF-β1 were increased in HG-treated mesangial cells in a time-dependent manner, and these increases were significantly attenuated by EP treatment in a dose-dependent manner (Figs. 5 and 6). In Figure 7, osmotic controls were included to determine whether the effects of HG might be due to hyperosmolarity. Thus treatment with 5 mmol/l glucose plus 45 mmol/l mannitol (NG+mannitol for osmotic control) had no effect on the expressions of MCP-1 and TGF-β1 compared with NG. 

**Effect of EP on HG-Induced Laminin, Type IV Collagen, and Fibronectin Expression in Cultured Mesangial Cells**

The effect of HG and EP on the mRNA and protein expression levels of the ECM molecules were determined by RT-PCR and Western blot analysis. Figure 8 shows increased expression of laminin, type IV collagen, and fibronectin in a time- and dose-dependent manner in both mRNA and protein expression levels (Fig. 8, A and B). EP treatment markedly suppressed HG-induced laminin, type IV collagen, and fibronectin expression (Fig. 8, C and D). Taken together, our data suggest that EP treatment led to the significant suppressions in the mesangial cells of MCP-1, TGF-β1, and ECM molecules.
NADPH oxidase was originally found in neutrophils and cations such as atherosclerosis and DN (2, 14, 16, 18, 19, 24, 26). NADPH-derived ROS mediates glomerular hypertrophy is one of the major sources of ROS overproduction in DN and understood. In this study, we demonstrated that NADPH oxidase mechanisms of ROS generation in diabetes are not fully understood. In this study, we showed increased expression of NADPH oxidase activity and ROS are upregulated both by experimental diabetes in vivo and by HG in vitro. It was evident in the present study that upregulations of NADPH oxidase activity and the ROS system induced by experimental diabetes in vivo and by HG were significantly ameliorated by pyruvate treatment. Pyruvate exhibited a protective effect on the development and progression of DN in the animal experiment. We proved the protective effects of pyruvate in a number of different ways: significant reduction of albuminuria and amelioration of tissue expression of inflammatory, profibrotic, and ECM molecules. Such protective effects of pyruvate were also demonstrated in a mesangial cell culture system under HG.

Oxidative stress has been implicated in the pathogenesis of diabetic complications (2, 14, 16, 19, 24, 26). However, the mechanisms of ROS generation in diabetes are not fully understood. In this study, we demonstrated that NADPH oxidase is one of the major sources of ROS overproduction in DN and that NADPH-derived ROS mediates glomerular hypertrophy and ECM expression. Several studies reported that elevated ROS levels contribute to the development of diabetic complications such as atherosclerosis and DN (2, 14, 16, 18, 19, 24, 26). NADPH oxidase was originally found in neutrophils and is composed of the catalytic subunit gp91phox together with the regulatory subunits p22phox, p47phox, and p67phox and the small GTPase Rac (11, 18, 32). Electrons from NADPH are transferred through the enzyme to molecular oxygen to generate superoxide and subsequently other ROS such as hydrogen peroxide. In this study, we showed increased expression of NADPH oxidase activity in the kidneys of diabetic rats that is associated with an increase in NADPH-dependent ROS generation in the renal cortex and isolated glomeruli. We have also shown that NADPH oxidase is involved in the upregulation of MCP-1, TGF-β1, and various ECM molecules in experimental DN. Effective inhibition of mRNA and protein upregulations of the above molecules in mesangial cells cultured under HG at pyruvate doses inhibiting NADPH oxidase activation are the supportive evidences for the role of NADPH oxidase in DN. In a future study, it is warranted to investigate the activation of each NADPH oxidase subunit using immunostaining and Western blot analysis. A more complete explanation will await a better understanding of the mechanism of NADPH oxidase activation and its precise subunit requirement.

Pyruvate is an intermediate metabolite in glucose metabolism and an effective scavenger of ROS. The redox imbalance induced by elevated glucose levels in the diabetic milieu results in increased oxidation of sorbitol to fructose, which is coupled to reduction of NAD(P)^+ to NAD(P)H. High intracellular concentration of glucose, both by glycolysis and activating polyl pathway, leads to the increased intracellular NAD(P)H/NAD(P)^+ ratio. Such an increase in the cytosolic NAD(P)H/NAD(P)^+ ratio, described as diabetic pseudohypoxia, plays an important role in mediating various complications associated with diabetes. It was shown that EP could be converted into lactate with coproduction of NAD(P)^+ from NAD(P)H, thus resulting in the normalization of the NAD(P)H/NAD(P)^+ ratio. Pyruvate could be an escape route to reduce the potential toxicity associated with HG in diabetes (21, 23, 35, 36). Strong antioxidant and anti-inflammatory effects of pyruvate have been implicated in an animal model of endotoxemia, wherein pyruvate treatment suppressed the production of TNF-α, the NF-κB signaling pathway, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and IL-6 mRNA (28, 30, 38, 39). However, no one has yet shown the beneficial effect of pyruvate on DN.

The present study is the first report elucidating the renoprotective effect of pyruvate in experimental DN. Based on our results, we evaluated the underlying mechanism of the renoprotective effect of pyruvate. Pyruvate-treated diabetic animals exhibited a significantly ameliorated albuminuria, glomerular hypertrophy, and tissue expression of MCP-1, TGF-β1, and ECM molecules. This protective effect of pyruvate was independent of blood glucose concentration and blood pressure, since the pyruvate-treated rats were similar to the untreated diabetic rats with respect to the above parameters. Pyruvate-treated rats exhibited significantly reduced glomerular NADPH oxidase activity and ROS generation, compared with diabetic control. The protective effect of pyruvate on oxidative stress and various inflammatory and profibrotic molecules were also shown in the mesangial cells cultured under HG. Unlike other exogenous antioxidants or anti-inflammatory agents that have been suggested to exhibit a protective effect on DN, pyruvate is an endogenous molecule and constitutes the physiological antioxidant system of the human body. Therefore, it is likely that pyruvate will be established as a safer therapeutic agent in DN.
In conclusion, the present study established that activation of NADPH oxidase plays a crucial role in diabetes-induced oxidative stress, glomerular hypertrophy, and ECM molecule expression. Pyruvate exhibited a renoprotective effect on the progression of experimental DN. Future research is warranted to investigate the protective mechanism of pyruvate more specifically in relation to NADPH oxidase in DN.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


