Reactive oxygen species mediate high glucose–induced plasminogen activator inhibitor-1 up-regulation in mesangial cells and in diabetic kidney

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Reactive oxygen species mediate high glucose–induced plasminogen activator inhibitor-1 up-regulation in mesangial cells and in diabetic kidney.

Background. Plasminogen activator inhibitor-1 (PAI-1) plays an important role in remodeling of extracellular matrix (ECM) in the glomeruli. PAI-1 is up-regulated by high glucose and is overexpressed in diabetic kidney. Since reactive oxygen species (ROS) mediate ECM accumulation in diabetic glomeruli and was recently found to mediate transforming growth factor-β1 (TGF-β1)-induced PAI-1 up-regulation in glomerular mesangial cells, we examined the role of ROS in high glucose–induced PAI-1 expression in cultured glomerular mesangial cells and in streptozotocin-induced diabetic rat glomeruli.

Methods. Growth arrested and synchronized primary rat mesangial cells were treated with different concentrations of glucose in the presence or absence of N-acetylcysteine (NAC) or trolox, or after cellular reduced form of glutathione (GSH) depleted with DL-buthionine-(S,R)-sulfoximine (BSO). Taurine was administered to diabetic rats from 2 days to 4 weeks after streptozotocin injection. Urinary protein excretion, glomerular volume, and fractional mesangial area were measured as markers of renal injury and lipid peroxide (LPO) as an oxidative stress marker. PAI-1 mRNA expression was measured by Northern blot analysis in mesangial cells and reverse transcription-polymerase chain reaction (RT-PCR) in glomeruli, PAI-1 protein by Western blot analysis and enzyme-linked immunosorbent assay (ELISA), and plasmin activity by fluorometry.

Results. High glucose significantly increased PAI-1 mRNA and protein expression and decreased plasmin activity in mesangial cells. Equimolar concentrations of L-glucose or mannitol did not affect PAI-1 expression. BSO pretreatment significantly increased basal PAI-1 expression and amplified the response to high glucose. NAC effectively inhibited high glucose–induced, but not basal, PAI-1 expression. Reduced plasmin activity in mesangial cells by high glucose was rescued by antioxidants.

Conclusion. These results demonstrate that ROS mediate high glucose–induced up-regulation of PAI-1 expression in cultured mesangial cells and in diabetic glomeruli. Since both high glucose and TGF-β1 induce cellular ROS and ROS mediate both high glucose– and TGF-β1–induced PAI-1, ROS appear to amplify TGF-β1 signaling in high glucose–induced PAI-1 up-regulation. Antioxidants can prevent accumulation of ECM protein in diabetic glomeruli partly by abrogating up-regulation of PAI-1 and suppression of plasmin activity.

Diabetic nephropathy is characterized by glomerulosclerosis and tubulointerstitial fibrosis as a result of overproduction and/or decreased degradation of extracellular matrix (ECM) in the kidney. This process had been considered irreversible until Fioretto et al [1] showed the regression of diabetic nephropathy by normoglycemia of more than 10 years after pancreas transplantation in type 1 diabetes. This suggests that the glomerular and tubulointerstitial fibrosis is reversible and that degradation of ECM plays an important role in ECM remodeling in diabetic kidney.

Two main enzyme systems, plasminogen activator (PA)/plasmin/PA inhibitor (PAI) system and matrix metalloproteinase (MMP)/tissue inhibitor of metalloproteinase (TIMP) system, are involved in ECM degradation [2–6]. Plasmin, a key ECM degrading enzyme, is generated from plasminogen by tissue type and urokinase type PA (tPA and uPA). MMPs, another ECM degrading proteases, require plasmin for the activation from their latent forms. Plasminogen activator inhibitor-1 (PAI-1) interferes with the generation of plasmin and activation of MMP by blocking PA. PAI-1 may thus play a critical role in ECM remodeling in the kidney [7, 8].
PAI-1 is a 50 kD single chain glycoprotein originating from various cells, including glomerular mesangial cells, vascular endothelial cells, fibroblasts, hepatocytes, adipocytes, and inflammatory cells [9]. PAI-1 is secreted into the plasma immediately after synthesis and is activated by binding vitronectin. PAI-1 exists mostly (about 80%) as an active form and the remainder in latent form bound to plasma proteins or complex with tPA. While normal human kidneys do not express PAI-1 [7], PAI-1 is overexpressed in pathologic conditions associated with renal fibrosis, including thrombotic microangiopathy [10], hypertensive nephrosclerosis [11], renal vasculitis [12], proliferative glomerulonephritis [13–15], aging kidney [16], obstructive uropathy [17], and diabetic nephropathy [11, 18–20]. PAI-1 expression is upregulated by high glucose in glomerular mesangial cells [21, 22]. However, the signals and pathways involved in high glucose–induced PAI-1 up-regulation have not been clearly elucidated.

Cellular reactive oxygen species (ROS) are increased in diabetes and contribute to pathogenesis of chronic vascular complications including nephropathy [23–31]. Hyperglycemia-induced mitochondrial superoxide overproduction was shown to induce PAI-1 expression in the vascular endothelial cells [25]. We recently demonstrated that TGF-β–induced PAI-1 up-regulation in mesangial cells is mediated by ROS [32]. ROS have also been shown to mediate cyclic strain-induced PAI-1 release from endothelial cells [33] and radiation-induced PAI-1 up-regulation in renal tubular epithelial cells [34]. The present study, therefore, examined the role of ROS in high glucose–induced PAI-1 expression by utilizing cultured glomerular mesangial cells and streptozotocin-induced diabetic rat glomeruli.

**METHODS**

All chemicals and tissue culture plates were obtained from Sigma-Aldrich Company (St. Louis, MO, USA) and Becton Dickinson Labware (Lincoln Park, NJ, USA), respectively, unless otherwise stated.

**Mesangial cell culture**

Primary rat mesangial cells were isolated from kidneys of 100 to 150 g male Sprague-Dawley rats by a conventional sieving method and characterized as described previously [29]. Cells (between the eighth and twelfth passages) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco BRL, Gaithersberg, MD, USA) containing 20% fetal bovine serum (FBS) (Gibco BRL), 100 U/mL penicillin, 100 μg/mL streptomycin, 44 mmol/L NaHCO₃, and 14 mmol/L N-hydroxyethylpiperazine-N’-2-ethane sulfonic acid (Hepes). Cells were cultured in 100 mm culture dish for Northern blot analysis, in 6-well plates for Western blot analysis and plasmin activity. Near confluent mesangial cells were incubated with serum-free media for 24 hours to arrest and synchronize the cell growth. After this time period, the media were changed to fresh serum-free and phenol red-free DMEM containing different concentrations of d-glucose, 25 mmol/L t-glucose, or 25 mmol/L mannitol for up to 48 hours. In some experiments, cells were pretreated with 1 mmol/L DL-buthionine-(S,R)-sulfoximine (BSO) for 24 hours to deplete intracellular glutathione or with antioxidants [5 mmol/L N-acetylcysteine (NAC) or 500 μmol/L trolox (Aldrich Chemical Co., Milwaukee, WI, USA)], 1 μg/mL anti-transforming growth factor-β1 (TGF-β1) antibody (Genzyme Diagnostics, Cambridge, MA, USA), or control IgG for 1 hour before the addition of glucose. BSO pretreatment decreased intracellular reduced form of glutathione (GSH) from 10.96 ± 0.43 to 4.82 ± 0.49 nmol/mg of cell protein in mesangial cells under the present experimental condition. The effective concentrations of antioxidants were decided based on our published data [24, 29, 32]. Cells were processed for measurement of PAI-1 mRNA and media for protein expression and plasmin activity at the end of incubation period as described below.

**Induction of experimental diabetes mellitus**

Male Sprague-Dawley rats weighing 200 to 250 g were obtained from Biogenomics, Seoul, Korea. The animals were housed two to three per cage in an animal room controlled at 23 ± 2°C and 55 ± 5% room humidity, under a 12-hour light and 12-hour dark cycle. All rats were maintained on standard rat chow (Samyang Rat Chow, Seoul, Korea) and tap water ad libitum.

Diabetes was induced by administration of 50 mg/kg of streptozotocin dissolved in pH 4.5 citrate buffer into the tail vein, as described previously [24]. Control rats received the same volume of citrate buffer (1 mL/kg). Induction of diabetes was confirmed by determining plasma glucose 2 days after streptozotocin injection. Rats with plasma glucose level above 300 mg/dL were used as diabetic rats. A group of diabetic rats was treated with taurine (Yakuri Pure Chemical Co., Tokyo, Japan) 1% solution in drinking water starting 2 days after streptozotocin. Diabetic rats receiving no taurine served as diabetic control. All diabetic rats survived without insulin treatment in the present study. We did not administer insulin to avoid nonmetabolic effects of insulin.

At the end of given experimental periods, 1 and 4 weeks after streptozotocin injection, blood samples were drawn from each animal for measurement of plasma glucose, and lipid peroxide (LPO). Urine samples were collected over a 24-hour period for 2 consecutive days for protein and LPO level. After sacrifice, both kidneys were...
removed and weighed. Glomeruli from the left kidney were isolated by conventional graded sieving method to assess PAI-1 mRNA (1 week after streptozotocin) and PAI-1 protein (4 weeks after streptozotocin). The right kidney was fixed with 10% formalin for assessment of glomerular morphology.

**Measurement of LPO in plasma and urine**

A modification of the thiobarbituric acid method of Ohkawa, Ohishi, and Yagi [35] was used to measure LPO, as previously described [24]. In brief, after mixing diluted plasma or urine specimen with 8% sodium dodecyl sulfate (SDS), reaction solution composed of 0.8% 2-thiobarbituric acid and 20% acetic acid was added and incubated at a 95°C water bath for 60 minutes. After stopping the reaction by cooling with tap water, the mixture (Beckman Coulter DU® 530; Beckman Coulter, Fullerton, CA, USA) at wavelength 535 nm and calculated using a tetraethoxypropane standard curve.

**Measurement of urinary protein**

Urine samples were centrifuged at 3000g for 10 minutes, and urinary protein in the supernatants was determined by Bradford method [36]. All samples were assayed in triplicate and the mean value from a given rat was calculated.

**Measurement of glomerular volume and fractional mesangial area**

In each rat, at the end of 4 weeks after streptozotocin, quantitative morphometric analysis of different glomeruli were performed as described previously [30] by a blinded observer. A 5 μm thick section was stained with periodic acid-Schiff (PAS) reagent. On this section, 30 different superficial glomeruli were randomly sampled for morphometric analysis. The microscopic slide was scanned clockwise along the superficial cortex and one glomerulus out of three was analyzed. For every investigated glomerulus, total glomerular area and glomerular tuft area were determined by tracing the outline of the Bowman’s capsule and the tuft, respectively, using Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA). Fractional mesangial area was calculated as the percentage of area positive for PAS occupying glomerular tuft area. Glomerular volume was determined by the following formula.

\[
\text{Glomerular volume} = \frac{B}{k} \left(\text{cross-sectional area of a glomerulus}\right)^{3/2}
\]

Where B (=1.38) is the shape coefficient for spheres, and k (=1.1) is a size distribution coefficient.

**Northern blot analysis for PAI-1 mRNA expression by cultured mesangial cells**

Total RNA from mesangial cells was isolated by the single step method using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer’s instructions. Twenty micrograms of total RNA was denatured and separated by electrophoresis through a 1.2% agarose gel with 2.2 mol/L formaldehyde, transferred onto nylon membranes (Amersham Pharmacia Biotech, Buckinghamshire, UK) using a capillary transfer, and covalently cross-linked to the membrane with ultraviolet light using a gene-linker (Bio-Rad, Richmond, CA, USA). The membranes were hybridized with 32P-labeled cDNA probe for PAI-1 overnight at 65°C in buffer containing 50% formaldehyde and then rehybridized with a probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control to assess RNA quantity and integrity. Quantification of mRNA signals was performed by densitometry and normalized with GAPDH mRNA signals.

**Reverse transcriptase-polymerase chain reaction (RT-PCR) for glomerular PAI-1 mRNA expression**

Total RNA was extracted from lysate of isolated glomeruli using TRIzol reagent as in mesangial cells. One microgram of RNA/20 μL reaction volume was combined with random hexamer primers (2.5 μmol/L) and reaction components for 30 minutes at 42°C and then heated to 95°C for 4 minutes to inactivate the enzyme and to denature RNA-cDNA hybrids. cDNA amplification was performed at a final concentration of 1 × PCR buffer, 1.5 mmol/L MgCl2, 20 μmol/L deoxynucleoside triphosphate (dNTP), 10 pmol/L of PAI-1 primers or GAPDH primers, and 1.25 U of AmpliTaq DNA polymerase (Promega Corp., Madison, WI, USA) in a total volume of 40 μL. cDNA probes for rat PAI-1 and GAPDH were constructed based on the previously published sequences using the PCR: GAPDH sense 5’-ATG GTC TAC ATG TTT CAG TA-3’, antisense 5’-TCA GAT CCA CAA CGG ATA CA-3’; and PAI-1 sense 5’-ATG GAA CAA GAA TGA GAT CA-3’, antisense 5’-TCA GTC TCC AGA GAG AAC TT-3’. The amplification profile involved denaturation at 95°C for 1 minute, primer annealing at 61°C for PAI-1 or at 55°C for GAPDH for 1 minute, and extension at 72°C for 1 minute. Final PCR products were electrophoresed in 1% agarose gel containing 0.2 μg/mL ethidium bromide. The expected size of the amplified fragments was 471 and 590 bp for PAI-1 and GAPDH, respectively. Quantification of mRNA bands was performed by densitometry using TINA 2.0 Image program and normalized with GAPDH mRNA signals.
Immunoblot analysis of PAI-1 protein

Immunoblot analysis was performed to determine PAI-1 protein in the cell culture media and lysate of isolated rat glomeruli. After measuring the concentration of cellular protein using Bio-Rad assay, aliquots of media corresponding to the same cellular protein were mixed with sample buffer containing SDS and β-mercaptoethanol and heated at 95°C for 15 minutes. Samples were applied to 10% polyacrylamide gel and electrophoresed. After electrophoresis, the proteins were transferred onto nitrocellulose membranes using a transblot chamber with Tris buffer. Western blots were incubated with rabbit antirat PAI-1 (1:1000) (American Diagnostica, Greenwich, CT, USA) overnight at 4°C, washed with phosphate-buffered saline (PBS)-Tween 20 for 1 hour, and incubated with peroxidase-conjugated secondary antibody, rabbit anti-IgG for 2 hours at room temperature. After washing, the membranes were incubated with enhanced chemiluminescence (ECL) detection reagent (Amersham Life Science) according to the manufacturer's instructions. Positive immunoreactive bands were quantitated densitometrically and normalized by β-actin, and were compared with those of control.

Enzyme-linked immunosorbent assay (ELISA) of PAI-1 protein

To quantify the level of PAI-1 protein secreted into the media under different experimental conditions, PAI-1 was measured in mesangial cell supernatant using a commercial solid phase quantitative sandwich ELISA kit for PAI-1 (American Diagnostica, Inc., Greenwich, CT, USA) according to the manufacturer's instructions [32]. The assay reacts with latent, active, and inactive PAI-1 as well as tPA/PAI-1 complexes. ELISA kits for PAI-1 are specific for rat PAI-1 according to the manufacturer's instructions.

Measurement of plasmin activity

Plasmin activity in the culture media was determined as previously described [32] using a synthetic fluorometric plasmin substrate methoxy succinyl-L-Ala-L-Phe-L-Lys-7-amido-4-methyl-coumarin. Twenty-five microliter of fivefold concentrated media were mixed with 31.2 µL of dH2O and 112.5 µL of 0.2 mol/L Tris-HCl, pH 7.4, containing 0.2 mol/L NaCl. Each reaction was started by adding 36.3 µL of the substrate in water (final concentration 5.0 µmol/L). Immediately after the addition of substrate, each tube was mixed well, transferred to a 37°C water bath, and incubated for 40 minutes. After the incubation period, each reaction was stopped by the addition of 25 µL of soya bean trypsin inhibitor. The fluorescence of each sample was measured in a fluorometer (Wallac Victor³ 1420 Multilabel Counter) (Turku, Finland) at an emission wave length at 450 nm at an excitation wave length of 360 nm and calculated using a plasmin standard curve.

Statistical analysis

All results are expressed as means ± standard error (SE). Analysis of variance (ANOVA) was used to assess the differences between multiple groups. If the F statistics was significant, the mean values obtained from each group were then compared by Fisher's least significant difference method. P value < 0.05 was used as the criterion for a statistically significant difference.

RESULTS

High glucose up-regulates PAI-1 mRNA and protein expression and suppresses plasmin activity in mesangial cells

Thiry millimoles per liter glucose up-regulated PAI-1 mRNA expression by 1.3-fold that of control at 6 hours and 1.6-fold at 12 hours. The up-regulation remained significant up to 24 hours (Fig. 1A). High glucose also increased PAI-1 protein secretion by mesangial cells by 1.5-fold that of control (Fig. 2) at 48 hours and suppressed plasmin activity to 70% of control (Fig. 3) at 48 hours. Basal PAI-1 secretion was 15.6 ± 7.7 ng/µg of cellular protein as measured by ELISA in mesangial cells maintained in serum-free DMEM for 48 hours and basal plasmin activity 2.8 ± 0.8 mU/µg cellular protein. High glucose increased PAI-1 protein to 24.9 ± 12.7 ng/µg of cellular protein and decreased plasmin activity to 2.1 ± 0.5 mU/µg cellular protein. Addition of 25 mmol/L concentration of L-glucose or mannitol to media containing 5.6 mmol/L D-glucose did not affect PAI-1 expression (Fig. 2A).

Effects of depletion of cellular reduced GSH on PAI-1 expression in mesangial cells

Depletion of intracellular GSH in mesangial cells by pretreatment with BSO significantly up-regulated high glucose–induced as well as basal PAI-1 mRNA expression at 12 hours (Fig. 1B). Qualitatively similar effect of BSO on both basal and high glucose–induced PAI-1 protein secretion at 48 hours was observed (Fig. 2B).

Effects of antioxidants on high glucose–induced PAI-1 expression and plasmin activity in mesangial cells

NAC at 5 mmol/L effectively inhibited high glucose–induced PAI-1 mRNA (Fig. 1C) and protein (Fig. 2C) expression without significant effect on basal PAI-1 expression. Trolox at 500 mmol/L also inhibited high glucose–induced, but not basal, PAI-1 protein secretion (Fig. 2C). Catalase at 500 U/mL also effectively inhibited high glucose–induced, but not basal, PAI-1 protein secretion (data not shown). NAC and trolox rescued high
Fig. 1. Effect of high glucose (A), DL-buthionine-(S,R)-sulfoximine (BSO) (B), and N-acetylcysteine NAC (C) on plasminogen activator inhibitor-1 (PAI-1) mRNA expression in mesangial cells. Synchronized quiescent mesangial cells were stimulated with 5.6 mmol/L (control) or 30 mmol/L glucose (high glucose) up to 48 hours. BSO was added 24 hours before the addition of high glucose and NAC 1 hour before. The upper panel shows a representative Northern blot analysis and the lower panel represents fold increases relative to control in mean ± SE of four to six experiments. *P < 0.05 compared to control glucose; †P < 0.05 compared to high glucose. Glc is glucose; GADPH is glyceraldehyde-3-phosphate dehydrogenase.

Fig. 2. Effect of L-glucose and mannitol on plasminogen activator protein-1 (PAI-1) protein secretion (A) and effect of DL-buthionine-(S,R)-sulfoximine (BSO) (B) and antioxidants (C) on high glucose–induced PAI-1 protein secretion by mesangial cells. Synchronized quiescent mesangial cells were stimulated with 30 mmol/L of D-glucose, 25 mmol/L of L-glucose or mannitol added to media containing 5.6 mmol/L D-glucose (A). In separate experiments, synchronized quiescent mesangial cells were stimulated with control or high glucose in the presence and absence of BSO (B) or antioxidants (C) for 48 hours. The upper panel shows a representative Western blot analysis and the lower panel represents fold increases relative to control in mean ± SE from six experiments. *P < 0.05 compared to control glucose; †P < 0.05 compared to high glucose. Glc is glucose; NAC is N-acetylcysteine.

glucose–induced decrease in plasmin activity to control level (Fig. 3).

Role of TGF-β1 in high glucose–induced PAI-1 expression in mesangial cells

Since TGF-β1 increases PAI-1 expression through ROS [32] and ROS up-regulate TGF-β1 expression in mesangial cells [37], we determined the role of TGF-β1 in high glucose–induced PAI-1 up-regulation using anti-TGF-β antibody. High glucose–induced PAI-1 protein secretion was effectively inhibited by anti-TGF-β antibody but not by IgG (Fig. 4A). Anti-TGF-β1 antibody did not affect basal PAI-1 secretion (data not shown). H_2O_2 increased PAI-1 secretion as in our previous study [32] and anti-TGF-β antibody effectively inhibited H_2O_2-induced PAI-1 up-regulation (Fig. 4B).
Glomerular PAI-1 mRNA expression was significantly up-regulated in diabetic rats compared to control rats at 1 week after streptozotocin. Taurine effectively inhibited glomerular PAI-1 mRNA expression in diabetic rats (Fig. 5A). Glomerular PAI-1 protein expression also increased in diabetic rats at 4 weeks, but the difference did not reach statistical significance. Taurine, however, significantly reduced glomerular PAI-1 secretion (Fig. 5B).

Evidence of oxidative stress in diabetic rats and effect of taurine

Plasma LPO level increased significantly in diabetic rats compared to control rats at 1 week after streptozotocin. Taurine reduced urinary LPO excretion but the difference did not reach statistical significance.

Renal injury in diabetic rats and effect of taurine

Diabetic rats failed to gain body weight compared to control rats. Taurine did not affect kidney weight (Table 1) but significantly reduced glomerular volume (Fig. 7A), fractional mesangial area (Fig. 7B), and protein excretion (Fig. 7C) in diabetic rats at 4 weeks after streptozotocin.

DISCUSSION

We [24, 29, 31] and others [27, 28] have shown that ROS play a major role in hyperglycemia-induced ECM up-regulation in both cultured mesangial cells and in diabetic glomeruli. We previously reported that high glucose, but not media hyperosmolality, increased dichlorofluorescin (DCF)-sensitive cellular ROS [29] and that H2O2 continuously generated by glucose oxidase up-regulated PAI-1 mRNA and protein expression and suppressed plasmin activity in mesangial cells [32]. PAI-1 is overexpressed in diabetic kidneys [11, 18–20] and mesangial cells. PAI-1 expression is up-regulated by high glucose [21, 22]. The present study confirms that high glucose, but not hyperosmolality, up-regulates PAI-1 expression in cultured mesangial cells and in streptozotocin-induced diabetic rat glomeruli and suppresses plasmin activity in mesangial cells. The observation that antioxidants effectively inhibit high glucose–induced up-regulation of PAI-1 and suppression of plasmin activity provides evidence that hyperglycemia-induced ROS also play a role in ECM degradation, independent of their well recognized role in ECM synthesis and secretion, and suggests the importance of ROS in ECM remodeling in diabetic glomeruli.

ROS have been shown to mediate PAI-1 expression in different cells. Hyperglycemia- [25] and cyclic strain [33]-induced PAI-1 expression in endothelial cells and radiation-induced PAI-1 expression in rat renal tubular epithelial cells [34] are mediated by ROS. Uchida et al [38] recently reported that high glucose, advanced glycated end products (AGEs), and 4-hydroxy-2-nonenal up-regulate PAI-1 mRNA and protein expression in rat visceral adipocytes through ROS. In mesangial cells, we [32] showed that ROS mediate TGF-β1–induced PAI-1 mRNA and protein expression. Streptozotocin-induced diabetic rats with superimposed deoxycorticosterone acetate (DOCA)/salt hypertension had upregulation of PAI-1, vascular endothelial growth factor (VEGF), and TGF-β1 in the kidney and this was effectively inhibited by treatment with a cyclooxygenase-2 inhibitor [11]. On the other hand, Tada et al [39] reported decreased PAI-1 expression in mesangial cells cultured under high glucose. PAI-1 release was not altered in human peritoneal mesothelial cells cultured under high glucose [40]. Although the reasons for this discrepancy are not clear, regulation of PAI-1 expression may vary in different cells and under different experimental conditions.
We observed in the present study that depletion of cellular GSH by BSO increased basal as well as high glucose–induced PAI-1 secretion. We also observed in a previous study that dehydroisoandrosterone-3-acetate (DHEA), an uncompetitive inhibitor of G6PD and a rate-limiting enzyme for regeneration of principal intracellular reductant nicotinamide adenine dinucleotide phosphate (NADPH), increased basal as well as high...
Table 1. Characteristics of study animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Body weight g</th>
<th>Kidney weight g</th>
<th>Kidney weight/body weight mg/g</th>
<th>Plasma glucose mg/dL</th>
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<td>1 week</td>
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<td>321.0 ± 6.1</td>
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<td>12.7 ± 0.8a</td>
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<tr>
<td>4 week</td>
<td>CR</td>
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<td>466.7 ± 17.2</td>
<td>1.8 ± 0.1</td>
<td>7.6 ± 0.4</td>
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<tr>
<td></td>
<td>DR</td>
<td>7</td>
<td>282.9 ± 22.1a</td>
<td>1.8 ± 0.1</td>
<td>12.9 ± 0.7a</td>
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<tr>
<td></td>
<td>DR + T</td>
<td>6</td>
<td>265.8 ± 21.1a</td>
<td>1.8 ± 0.1</td>
<td>12.0 ± 1.0a</td>
</tr>
</tbody>
</table>

Abreviations are: CR, citrate buffer–treated control rats; DR, streptozotocin-induced diabetic rats; DR + T, streptozotocin-induced diabetic rats treated with taurine.

Data are presented as mean ± SE.

*P < 0.05 compared to citrate buffer–treated control rats.

The in vivo data in the present study suggest that ROS mediate hyperglycemia-induced PAI-1 up-regulation in streptozotocin-induced diabetic glomeruli. Taurine significantly inhibited PAI-1 mRNA expression in diabetic glomeruli. Up-regulation of PAI-1 mRNA in the glomeruli of diabetic rats at 1 week after streptozotocin agrees with a recent study [20] which demonstrated 60% to 80% increase in renal PAI-1 mRNA in diabetic rats at 10 days and 3 weeks after streptozotocin
injection and 2.5-fold increase in 38-week-old Otsuka Long-Evans Tokushima Fatty (OLETF) rats compared to age-matched control Long-Evans Tokushima Otsuka (LETO) rats. PAI-1 mRNA was observed in intraglomerular cells and tubular epithelial cells [19]. Glomerular expression of PAI-1 protein also increased at 4 weeks after streptozotocin, although statistically insignificant, but taurine significantly inhibited glomerular expression of PAI-1 protein. A recent study [20] reported increased PAI-1 protein expression at 3 weeks after streptozotocin treatment by immunohistochemistry. Taurine also significantly inhibited plasma LPO, glomerular volume, fractional mesangial area, and urinary protein excretion in diabetic rats without effect on plasma glucose.

It still remains unclear whether hyperglycemia-induced PAI-1 expression and/or suppression of plasmin is causally related to diabetic glomerulosclerosis. In this regard, our preliminary study [abstract; Lee HB et al, J Am Soc Nephrol 13:169A, 2002] showed that PAI-1 antisenese oligonucleotides, but not sense oligonucleotides, effectively inhibited basal as well as high glucose- and TGF-β1-induced fibronectin protein secretion. Other studies also suggest an important role of PAI-1 in glomerulosclerosis and tubulointerstitial fibrosis. Administration of tPA accelerates ECM degradation in experimental glomerulonephritis [42], crescentic nephritis model of PAI-1 knockout mice exhibit reduced renal collagen content and proteinuria compared to wild type mice [15], unilateral ureteral obstruction in PAI-1 knockout mice produces less tubulointerstitial fibrosis compared to wild type [43], and a mutant, non-inhibitory PAI-1 decreases matrix accumulation, proteinuria, and macrophage infiltration in experimental glomerulonephritis [44].

CONCLUSION

Our present data demonstrate that ROS mediate high glucose–induced up-regulation of PAI-1 expression in both mesangial cells and in diabetic glomeruli. Since both high glucose and TGF-β1 induce cellular ROS and ROS mediate both high glucose– and TGF-β1–induced PAI-1, ROS appear to amplify TGF-β1 signaling in high glucose–induced PAI-1 up-regulation. Increase in PAI-1 expression may lead to diabetic glomerulosclerosis through inhibition of ECM degradation. Antioxidants can prevent accumulation of ECM protein in diabetic glomeruli partly by abrogating up-regulation of PAI-1 and suppression of plasmin activity.

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