PHARMACOLOGICAL INTERVENTION BY EXOGENOUS SUPEROXIDE DISMUTASE ATTENUATES DIABETES-INDUCED OXIDATIVE AND NITROSATIVE STRESS MEDIATED RENAL DAMAGE


Aims and hypothesis: This study evaluated the rescue effect of pharmacological SOD administration on diabetic renal injury in an animal model. The effect of antioxidant intervention by SOD-PEG on the indices of oxidative stress, nitrosative stress, fibrosis and apoptosis was assessed.

Methods: The STZ-induced diabetic rats were randomly assigned to two groups: diabetes with SOD-PEG treatment and diabetes with vehicle treatment. Measurement of superoxide, NO, IL-1β and TGF-β1 production were carried out. Kidneys were harvested for immunohistochemical and immunoblotting analysis.

Results: This study demonstrated diabetes significantly increased urinary albumin excretion coinciding with increased systemic levels of superoxide, IL-1β and TGF-β1. Interestingly, exogenous SOD-PEG treatment significantly reduced the promotion effect of diabetes. In a diabetic animal model, SOD treatment effectively attenuated 8-hydroxy-guandine expression associated with reduced TUNEL and PCNA staining in the kidney of diabetic rats. Further studies showed higher ONOO• expression in diabetic renal immunostaining. Exogenous SOD administration successfully lessened OONO• over-expression in diabetic renal injury. Immunohistochemical observations showed exogenous SOD treatment attenuated TGF-β1, and fibronectin expression coincided with decreased phospho-ERK and phospho-p38 expression in diabetic renal glomeruli. Immunoblotting showed exogenous SOD alleviated nitrotyrosine, 8-hydroxy-guandine, fibronectin, and caspase-3 protein expression of kidney in diabetic rats.

Conclusions: An alternative redox modulation strategy with exogenous SOD may provide a promising regimen for rescuing diabetes-promoted nitrosative and oxidative damages-related renal apoptosis and fibrosis. (Acta Nephrologica 2008; 22: 20-30)

Key words: diabetes, oxidative stress, nitrosative stress, superoxide dismutase, renal fibrosis, and apoptosis

INTRODUCTION

Diabetic nephropathy is characterized by excessive deposition of extracellular matrix (ECM) in the kidney, leading to glomerular mesangial expansion.1,2 Compared with other chronic renal diseases, diabetic nephropathy more rapidly results in functional deterioration eventually progressing to renal failure requiring hemodialysis [3]. Thus, in diabetic nephropathy, detecting renal dysfunction early and prompt treatment before renal progression is critical to patient outcome. Previous studies pointed out high glucose and Advanced Glycation Endproducts (AGEs) through induction of ROS have also been found to induce cell apoptosis, vascular injury and renal fibrosis.4,5,6 These findings imply the kidney, a major organ involved in diabetes, is an organ susceptible to ROS.

Oxidative stress induced by superoxide has been implicated in inducting certain cell injuries.7,8 In contrast, superoxide plays an important role in regulating cell proliferation and metabolism.9,10,11 Local overproduction of NO, which is seen in early stage diabetes, can react with O2• to form peroxynitrite (ONOO•).12 Further,
ONOO$^-$ is a potent oxidant that can modify proteins. Nitrated tyrosine epitopes provide a marker for oxidative and nitrosative stress induced by the interaction of O$_2$ and NO and may become pathogenic by changing protein function.$^{12,13,14}$

Recently, superoxide mediated high glucose or AGEs-induced TGF-β1 and fibronectin expression of renal mesangial cells has been demonstrated.$^{15}$ Pretreatment with DPI reduced high glucose or AGEs augmentation of superoxide TGF-β1 and fibronectin expression in vitro. Further studies also showed scavenging of superoxide by SOD or DPI, or inhibiting ERK by PD98059 reduced the promotion effect of high glucose or AGEs on ERK and AP-1 expression.$^{15}$ It has been elucidated in vitro that an appropriate modulation of redox reaction by SOD may provide a promising regimen for regulating diabetes-induced fibrosis in diabetic nephropathy. To support this theory, this study evaluated the rescue effect of pharmacological SOD administration on diabetic renal injury in a diabetic animal model. The effect of anti-oxidant intervention by SOD-PEG on the indices of oxidative stress, nitrosative stress and apoptosis was assessed for the diabetic kidney. To clarify the role of activated ERK and p38 in SOD-PEG-rescued diabetic nephropathy, the expression of cellular proliferation, extracellular matrix (ECM) accumulation and mitogen-activated protein (MAP) kinase in immunohistochemistry were also examined.

**MATERIALS AND METHODS**

**Streptozotocin-induced diabetes**

All studies were approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital, Taiwan. Four-month-old male Wistar rats were caged in pairs and maintained on rodent chow and water ad libitum. Rats with diabetes were induced as previously described.$^{16}$ Briefly, diabetes was induced by a single intraperitoneal injection of streptozotocin (50 mg/kg body weight; Sigma Chemical Inc. St Louis, MO, USA). Streptozotocin was dissolved in 50 mM citric acid and sterile filtered through a 0.22 µm filter. One week after injection, whole blood glucose was measured in a sample obtained from tails. Rats with whole blood glucose > 300 mg/dl, defined as successful induction of diabetes, were used for successive experiments. To equalize blood glucose levels in all diabetic rats, intermediate-acting insulin (NPH insulin) was given subcutaneously once a day until the animals were sacrificed. Whole blood glucose levels were measured every day just before the insulin injections. The dose of insulin was adjusted to reach the target glucose level of 200-250 mg/dL. At the end of the treatment (4th week), rats were anesthetized with ether, blood samples taken from the heart were collected into heparinized tubes, and HbA1c levels were measured by an automatic analyzer (Primus Corp-CLC 385).

**SOD treatment**

Sixteen rats with diabetes were randomly divided into two groups. Eight rats without streptozotocin injections were used as normal controls. Diabetic rats were given intraperitoneal SOD-PEG at doses of 10, 20, 40, 50, and 100 U/kg/day for 28 consecutive days. Since 50 U/kg/day SOD-PEG treatments significantly reduced the promotion effect of diabetes on urinary albumin excretion and systemic superoxide levels compared with those treated with 10, 20, 40, and 100 U/kg/day. 50 U/kg/day SOD-PEG treatments were applied to the following studies. Rats (n=8) in each group were given intraperitoneal SOD-PEG (50 U/kg/day) or a vehicle for 28 consecutive days, respectively. The SOD-PEG was dissolved in normal saline and sterile filtered through a 0.22 µm filter. At day 28, urinary albumin and creatinine levels were determined with respective assay kits (Sigma Chemical Inc. St. Louis, MO, USA) according to the manufacturer’s instructions. Rats were sacrificed with an overdose of pentobarbital sodium and the kidneys were harvested for immunohistochemical analysis. After perfusion with PBS, fresh kidney tissues were ground with a mortar and pestle under liquid nitrogen, lysed with ice-cold PBS containing 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, 100 µg/ml PMSF and 30 µg/ml aprotinin, and homogenized by ultrasonication. Aliquots of kidney tissue homogenate (50 µg) were subject to assessment of fibronectin (marker for fibrosis), caspase-3 (marker for apoptosis), nitrotyrosine (marker for nitrosative stress) and 8-hydroxy-guandine (8-OH-Gn) (marker for oxidative stress) expression using immunoblotting.$^{15}$

**Measurement of superoxide, NO, IL-1β and TGF-β1 production**

Mononuclear cells (1×10$^6$ cells/well, in a 96-well plate) from the peripheral blood of diabetic rats with or without SOD treatment were assessed for superoxide. Superoxide synthesis was determined using a horse heart cytochrome c reduction assay in the absence and presence of SOD and calculated from the molar extinction coefficient of 0.0282 µM$^{-1}$ cm$^{-1}$ as described previously.$^{17}$ Nitrite and nitrate levels from the urine were measured to reflect NO production as described previously.$^{18}$ Results were normalized with protein concentration in each sample and were expressed as µM NO/mg protein/min.$^{18,19}$ Production of the IL-1β and TGF-β1 was determined by enzyme-linked immunosorbent assay (Quantikine®, R & D Systems Inc.) according to the
manufacturer’s instructions.

**Immunohistochemistry**

Kidneys were fixed in 4% PBS-buffered paraformaldehyde, embedded in paraffin and then sliced longitudinally into 5-µm thick sections. Monoclonal antibodies against proliferating cell nuclear antigen (PCNA), nitrotyrosine, phosphorylated ERK, phosphorylated P38, TGF-β1, fibronectin, and 8-OH-Gn (Chemicon International Inc, Temecula, USA) were used for immunohistochemistry. Immunoreactivity in sections was demonstrated using a horseradish peroxidase (HRP)-3’-, 3’-diaminobenzidine (DAB) kit (R & D Systems, Inc. Minneapolis, MN, USA) according to the manufacturer’s instructions, followed by counterstaining with hematoxylin, dehydration and mounting. Sections without primary antibodies were enrolled as negative controls for the immunostaining.

**Terminal deoxynucleotidyl transferase (TdT-mediated) deoxyuridine triphosphate-biotin end-labelling (TUNEL)**

Specimens using in situ cell death detection kits (Roche Diagnostics GmbH, Mannheim, Germany) pretreated with 50U/ml DNase I (Sigma Chemical Inc.) or incubated in reaction buffer without TdT were used as positive or negative controls. TUNEL stained cells were recognized using fast red as substrates.

**Histomorphometry**

Six regions within glomeruli from three sections obtained from eight rats were studied. For immunostaining quantification, sections were analyzed using a Zeiss Axioskop 2 plus microscope (Carl Zeiss, Gottingen, Germany). Areas (3 mm²) containing positive immunostained cells were examined. Ten random images of 0.75 mm² from each selected area (3 mm²) were then taken under 200-400 X magnifications. All images for each specimen were captured using a Cool CCD camera (SNAP-Pro c.f. Digital kit; Media Cybernetics, Sliver Spring, MD, USA). Images were analyzed using Image-Pro ® Plus image-analysis software (Media Cybernetics, Sliver Spring, MD, USA). The percentages of positive immunolabeled cells and total cells in each area were counted. Renal mesangial cells were identified morphologically.

**Statistical analysis**

All values were expressed as means ± standard errors. The Wilcoxon rank sum test was used to evaluate differences between the sample of interest and its respective control. For analysis of time course, a multiple range of repeated-measures one-way ANOVA and Bonferroni post hoc tests were used. A P-value of < 0.05 was considered statistically significant.

**RESULTS**

**Metabolic characteristics of the animals**

Blood glucose concentrations, HbA1c levels and the kidney weight of rats with diabetes were much higher than the control but were not affected by SOD treatment (Table 1). Interestingly, the mean values of urinary albumin excretion in the diabetic group were significantly higher than in those without diabetes. Further, the amount of urinary albumin excretion in diabetic rats with SOD treatments was dramatically lower compared with non-treatments group but still higher than the control group. In table 1, we found diabetes induces superoxide, plasma IL-1β and plasma TGF-β1 production. To our interest, SOD treatment reduced the superoxide and nitrite levels.

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose (mg/dL)</th>
<th>HbA1c (%)</th>
<th>Kidney weight / BW (mg/g)</th>
<th>Plasma Cr (mg/dL)</th>
<th>Urine (albumin/ Cr: mg/mg)</th>
<th>Plasma Superoxide (RLU)</th>
<th>Plasma IL-1β (pg/mL)</th>
<th>Plasma TGF-β1 (ng/mL)</th>
<th>Urinary NO₂⁺NO₃ (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>89.45± 7.62</td>
<td>3.14±0.06</td>
<td>0.412±0.063</td>
<td>0.50±0.06</td>
<td>0.32±0.14</td>
<td>113.42±27.63</td>
<td>82.14±11.32</td>
<td>1.12±0.21</td>
<td>15.96±3.91</td>
</tr>
<tr>
<td>Diabetic (n=8)</td>
<td>263.21±42.11*</td>
<td>5.49±0.21</td>
<td>0.614±0.079*</td>
<td>0.49±0.12</td>
<td>3.41±0.63*</td>
<td>496.41±73.42*</td>
<td>367.21±56.31*</td>
<td>2.12±0.32*</td>
<td>5.21±1.19*</td>
</tr>
<tr>
<td>Diabetic with SOD (n=8)</td>
<td>274.48±49.12#</td>
<td>5.51±0.19</td>
<td>0.541±0.041</td>
<td>0.49±0.21</td>
<td>1.12±0.36*#</td>
<td>119.49±32.61#</td>
<td>169.46±26.12*#</td>
<td>1.28±0.31*</td>
<td>14.43±3.11*</td>
</tr>
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</table>

* and # respectively show differences from the normal and DM-vehicle group, p < 0.05
TGF-β1 level to the baseline. As for IL-1 β level, there is a significantly decrement in diabetic rats treated with SOD compared to those without SOD but still higher than the baseline. Moreover, urinary nitrate and nitrite levels were significantly lower in the diabetic group compared to the control group. The SOD administration clearly restored diabetes attenuation of urinary NOx levels.

**Exogenous SOD treatment attenuated DNA oxidative damage, apoptosis, and DNA replication in diabetic nephropathy.**

We explored whether diabetes could stimulate superoxide in renal tissue. Superoxide easily reacts with nucleotide resulting in DNA damage. We used monoclonal antibodies against 8-OH-Gn to detect superoxide-induced oxidative stress. As shown in fig. 1, immunohistochemical observation revealed glomerular mesangium, from diabetic renal cortex of rats displayed evident DNA oxidative damage compared with a healthy kidney. To our interest, SOD treatment significantly decreased tissue 8-OH-Gn levels in the diabetic renal cortex. Thus, these results suggested exogenous SOD treatment could rescue DNA oxidative stress in diabetic renal injury.

To examine the degree of apoptosis and cell proliferation in diabetic renal cortical sections, TUNEL and immunohistochemical stainings for PCNA were used. The TUNEL-positive cells including glomeruli were significantly increased in diabetic cortical sections compared to the healthy groups (Fig. 2). The TUNEL staining for apoptosis was markedly reduced in diabetic rats treated with SOD treatment compared to those without SOD treatment. To evaluate whether cell replication and damage repair factor play an important role in diabetic rats under SOD treatment, we use PCNA staining. As shown in fig. 3, expression levels of PCNA remained relatively higher in diabetic nephropathy. Four weeks after exogenous SOD treatment, mesangial cells in glomeruli display relatively weak PCNA expression when compared to the diabetic group without SOD treatment.

**Effect of exogenous SOD treatment on immunohistochemical stainings of nitrotyrosine in diabetic renal injuries.**

Compared to the renal cortical section from the healthy group, immunohistochemical analysis of renal sections from diabetic rats revealed significant positive staining for nitrotyrosine. In contrast, substantially reduced staining was observed in the renal sections from diabetic rats that were given exogenous SOD (Fig. 4).

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**Fig. 1.** Immunohistochemical photograph of glomeruli with or without SOD treatment. In comparison with the normal group, cells over glomerular areas in diabetic kidneys express strong 8-hydroxy-guandine immunostaining. Decreased 8-hydroxy-guandine was observed in diabetic rats after SOD-PEG treatment. Positive 8-hydroxy-guandine immunostained cells were brown in color. * and # respectively show differences from the normal and DM-vehicle group, *p < 0.05
Fig. 2. Immunohistochemical photograph of glomeruli with or without SOD treatment. In comparison with the normal group, cells over glomerular areas in diabetic kidneys express strong TUNEL. Decreased TUNEL was observed in diabetic rats after SOD-PEG treatment. Positive TUNEL cells were red in color. * and # respectively show differences from the normal and DM-vehicle group, \( p < 0.05 \)

Fig. 3. Immunohistochemical photograph of glomeruli with or without SOD treatment. In comparison with the normal group, cells over glomerular areas in diabetic kidneys express strong PCNA immunostaining. Decreased PCNA was noted in diabetic rats after SOD-PEG treatment. Positive PCNA immunostained cells were brown in color. * and # respectively show differences from the normal and DM-vehicle group, \( p < 0.05 \)
Fig. 4. Immunohistochemical photograph of glomeruli with or without SOD treatment. In comparison with the normal group, cells over glomerular areas in the diabetic kidneys express strong nitrotyrosine immunostaining. Decreased nitrotyrosine was identified in diabetic rats after SOD-PEG treatment. Positive nitrotyrosine immunostained cells were brown in color. * and # respectively show differences from the normal and DM-vehicle group, \( p < 0.05 \)

**Activated ERK and p38 expression in diabetic kidney reversed by exogenous SOD treatment.**

Without the antibody, no immunostaining of ERK (Fig. 5) and p38 (Fig. 6) was visible. The cytoplasm of cells expressing positive ERK and p38 was visualized as brown. The nuclei of renal cells were positively stained for phosphorylated ERK and phosphorylated p38 in diabetic nephropathy while those of glomeruli cells expressed limited positive phosphorylated ERK and p38 in the control section. Four weeks after the SOD treatments, cells in the mesangial area adjacent to endothelial cell and tubular cells displayed relatively weak phosphorylated ERK and phosphorylated p38 expression compared to the diabetic group without SOD treatment.

**SOD treatment effectively reduced diabetes promotion of renal fibrosis.**

In the control group, cells located at the glomeruli in the renal cortex expressed weak TGF-β1 and fibronectin expression. In the diabetes group, cells displayed strong TGF-β1 (Fig. 7) and fibronectin (Fig. 8) immunohistochemical stainings. Interestingly, SOD-PEG administration clearly rescued oxidative stress mediated ERK and p38 activation associated with down-regulated diabetes-induced TGF-β1 and fibronectin expression in renal tissue (Fig. 4, 7, 8), suggesting exogenous SOD-PEG treatment should be a potent regimen for rescuing diabetes-induced renal injury.

**Exogenous SOD alleviated nitrotyrosine, 8-hydroxy-2’-deoxyguanosine, fibronectin, and caspase-3 protein expression of kidney in diabetic rats**

Immunoblotting showed diabetes increased caspase-3 and fibronectin levels in kidney tissue. We found diabetes promoted nitrotyrosine and 8OH-Gn expression in kidney tissue. SOD treatment lessened diabetes promotion of nitrotyrosine, 8-OH-Gn, fibronectin, and caspase-3 protein expression (Fig. 9).

**DISCUSSION**

For the streptozotocin-induced diabetic rat model, we demonstrated an ideal SOD treatment could effectively protect the diabetic glomeruli from early diabetic glomerulopathy such as microalbuminuria, oxidative damage to DNA, apoptosis and progression of glomerulopathy to ECM accumulation. These experimental results thereby provide in vivo evidence that superoxide being involved in the pathogenesis of diabetic renal injury and anti-oxidant therapy is feasible. While previous studies have shown over expression of SOD can protect early diabetic glomerular injury like microalbuminuria
Fig. 5. Phospho-ERK expression of glomeruli in diabetic rats with or without SOD treatment. Compared with the normal group, nuclei of mesangial area in diabetic kidneys expressed strong phospho-ERK. Decreased phospho-ERK expression was noted in diabetic rats after SOD-PEG treatment. The positive phospho-ERK immunostained cells were brown in color in nucleus. * and # respectively show differences from the normal and DM-vehicle group, \( p < 0.05 \).

Fig. 6. Phospho-p38 expression of glomeruli in diabetic rats with or without SOD treatment. Compared with the normal group, cells over the glomerular and tubular areas in diabetic kidney express strong phospho- p38. Decreased phospho- p38 expression was noted in diabetic rats after SOD-PEG treatment. The positive phospho-p38 immunostained cells were brown in color in nuclei. * and # respectively show differences from the normal and DM-vehicle group, \( p < 0.05 \).
Fig. 7. Immunohistochemical photograph of glomeruli with or without SOD treatment. Compared with the normal group, cells over glomerular and tubular areas in diabetic kidneys express strong TGF-β1 immunostaining. Decreased TGF-β1 was observed in diabetic rats after SOD-PEG treatment. Positive TGF-β1 immunostained cells were brown in color. * and # respectively show differences from the normal and DM-vehicle group, p < 0.05.

Fig. 8. Immunohistochemical photograph of glomeruli with or without SOD treatment. Compared with the normal group, cells over glomerular and tubular areas in diabetic kidneys express strong fibronectin immunostaining. Decreased fibronectin was observed in diabetic rats after SOD-PEG treatment. Positive fibronectin immunostained cells were brown in color. * and # respectively show differences from the normal and DM-vehicle group, p < 0.05.
in transgenic mice, little research has been carried out to define the role of pharmacological modulation of SOD in diabetes promotion of nephropathy. Results in this study suggest exogenous SOD treatment can significantly reduce diabetic glomerular injury. In addition, in diabetic nephropathy, the ECM surrounding mesangial cells expand and the loss of glomerular permselectivity results in microalbuminuria which is correlated with mesangial matrix accumulation.21 Our findings support this implication showing obvious immunostaining for TGF-β1 and fibronectin correlated with microalbuminuria in streptozotocin-induced diabetic nephropathy were markedly reduced by exogenous SOD treatment. Further, the 8-OH-Gn staining increased in the diabetic renal cortex. This increased staining in the kidney provides evidence of the involvement of oxidative stress in the disease process. More recent direct evidence has shown guanine residue was found to be oxidized to form 8-OH-Gn when DNA is exposed to oxygen radicals.22 Many studies have proven this modified purine residue in DNA to be an excellent marker measuring oxidative tissue damage.23,24 This in vivo study proved oxidative DNA damage in diabetic kidney displaying microalbuminuria was reversed by exogenous SOD intervention.

Experimental results clearly showed TUNEL in diabetic kidney becomes very prominent. Exogenous SOD administration was able to protect the kidneys from diabetes-induced apoptosis, strongly suggesting the role of oxidative stress in this process. Interestingly, the expression levels of PCNA remained relatively high in diabetic nephropathy indicating the diabetic milieu promotes cell replication coincided with increased 8-OH-Gn, cell oxidative stress factors. Recent evidence demonstrated PCNA was one of DNA damage repair factors.25 Therefore, the possibility that high levels of PCNA in diabetic kidney functioned as cell repair processes cannot be excluded. This also suggests diabetic kidneys were replicating in the presence of DNA damage under hyperglycemia for prolonged periods, possibly inducing secondary lesions, which contribute to genomic instability and activation of apoptosis. Other studies have also shown apoptosis induced by hyperglycemia were significantly paralleled with expression of PCNA and TGF-β by immunohistochemistry in diabetes.26,27 For exogenous SOD treatment for diabetic nephropathy, its protective effect on diabetic-induced apoptosis was associated with a significant inhibition of PCNA and 8-OH-Gn accumulation, which could then lead to fibrosis. These results suggest superoxide plays an important role in diabetes-induced renal cell damage, replication, apoptosis and fibrosis.

SOD has been reported to play a crucial role in protecting NO produced by endothelium cells.28,29 It is well established that authentic SOD induces endothelium-dependent vasodilatation by protecting basal nitric oxide from the destructive action of endogenously produced superoxide anion.30 Interestingly, this study found the urinary Nox level was decreased in diabetic nephropathy. Heightened ONOO⁻ expression in diabetic renal immunostatining was noted. Exogenous SOD treatment significantly restored the urinary Nox level coinciding with attenuated ONOO⁻ immunoreactivities in renal glomeruli of diabetic rats. Therefore, we suggest diabetic nephropathy actively responds to the superoxide scavenger by reducing nitrostative signaling and subsequently attenuate renal injuries.

Immunohistochemical observation of the current diabetic animal model clearly demonstrated intensive nuclear phosphorylated ERK and phosphorylated p38...
activity, which play, at least in part, an important role in diabetes-induced apoptosis and ECM accumulation. A growing body of evidence has shown phosphorylated ERK and phosphorylated p38 are detected in progressive diabetic nephropathy. Few in vivo studies have focused on the involvement of MAPK in diabetic nephropathy under exogenous SOD treatment. This study provides the first immunohistochemical evidence that exogenous SOD treatment reduced diabetes-enhanced phospho-ERK and phospho-P38 expression coincided with decreased TGF-β1 and fibronectin immunoreactivities. Inactivation of these signals by exogenous SOD treatment in turn leads to decreased apoptosis and ECM accumulation in diabetic nephropathy. Further, previous studies have demonstrated in an in vitro model that diabetic milieu-induced NADPH oxidase-dependent superoxide can activate cytosolic ERK in hyperglycemia and a high AGEs level. This in vitro study is in line with our findings of SOD-alleviated diabetic promotion of ERK and P38 phosphorylation in vivo.

For the defense system to oxidative stress, antioxidant enzymes such as SOD and catalase were also found to be enhanced in the kidneys of streptozotocin-induced diabetic rats, whereas other reports demonstrated totally different results in diabetic milieu [33]. A previous study showed glucose-induced increase in gene expression for SOD in vascular cells is associated with increased SOD protein synthesis and no increase in enzyme activity. Further, it is interesting to note in another report that changes in SOD gene expression fail to be translated into changes in enzyme activity. It has been also reported SOD activity is inhibited due to glycation of the enzyme. Therefore, it still remains unknown whether changes of diabetic nephropathy are normalized by the treatment with anti-oxidants such as SOD. Further, the failure of anti-oxidant therapy to prevent end-organ injury in the diabetic cohort of the Heart Outcome Prevention Evaluation (HOPE) study casts some doubt on the pathologic significance of oxidative stress in progressive diabetic injury. Thus, whether exogenous anti-oxidant treatments such as SOD are effective in delaying progression of diabetic nephropathy is a crucial question. Therefore, this and the previous studies provide practical and available therapeutic strategies by manipulation of the redox pathway to ameliorate the outcome of progressive diabetic nephropathy.

In summary, this study provided significant evidence that regulation of redox reactions by exogenous SOD might provide a promising regimen for regulating ERK or p38 signal transduction and alleviating DNA damages and apoptosis, resulting in a significant decrease in TGF-β production and ECM accumulation in diabetic nephropathy. By using the exogenous SOD-attenuated signal transduction pathway for diabetic renal fibrogenesis, the biopharmacological modulation of improvement of diabetic nephropathy may be possible.

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