Tranilast attenuates the up-regulation of thioredoxin-interacting protein and oxidative stress in an experimental model of diabetic nephropathy

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Abstract

Background. Diabetic nephropathy is the leading cause of kidney failure in the developed world. Tranilast has been reported to not only act as an anti-inflammatory and anti-fibrotic compound, but it also exerts anti-oxidative stress effects in diabetic nephropathy. Thioredoxin-interacting protein (Txnip) is the endogenous inhibitor of the anti-oxidant thioredoxin and is highly up-regulated in

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diabetic nephropathy, leading to oxidative stress and fibrosis. In this study, we aimed to investigate whether tranilast exerts its anti-oxidant properties through the inhibition of Txnip.

**Methods.** Heterozygous Ren-2 rats were rendered diabetic with streptozotocin. Another group of rats were injected with citrate buffer alone and treated as non-diabetic controls. After 6 weeks of diabetes, diabetic rats were divided into two groups: one group gavaged with tranilast at 200 mg/kg/day and another group with vehicle.

**Results.** Diabetic rats had a significant increase in albuminuria, tubulointerstitial fibrosis, peritubular collagen IV accumulation, reactive oxygen species (ROS) and macrophage infiltration (all P < 0.05). These changes were associated with an increase in Txnip mRNA and protein expression in the tubules and glomeruli of diabetic kidney. Treatment with tranilast for 4 weeks significantly attenuated Txnip up-regulation in diabetic rats and this was associated with a reduction in ROS, fibrosis and macrophage infiltration (all P < 0.05).

**Conclusions.** This is the first study to demonstrate that tranilast not only has anti-inflammatory and anti-fibrotic effects as previously reported but also attenuates the up-regulation of Txnip and oxidative stress in diabetic nephropathy.

**Keywords:** diabetic nephropathy; fibrosis; oxidative stress; thioredoxin-interacting protein

### Introduction

Diabetic nephropathy has become a worldwide epidemic, accounting for approximately one-third of all cases of end-stage renal disease [1]. Conventional therapies for the management and treatment of diabetic complications include tight glycaemic control and anti-hypertensive therapies such as agents that block the renin-angiotensin system (RAS) [2,3]. Nonetheless diabetic nephropathy continues to progress in a significant proportion of patients and often leads to organ failure and the need for dialysis and/or kidney transplant.

The role of oxidative stress in the pathogenesis of diabetic microvascular as well as macrovascular complications has gained considerable attention since the emergence of the proposed ‘unifying mechanism’ for the pathogenesis of diabetic complications: the overproduction of superoxide by the mitochondrial electron transport chain [4]. Reactive oxygen species (ROS) activate a number of signalling pathways including protein kinase C, p38 mitogen-activated protein kinase (MAPK), p42/p44 MAPK and transcription factor NF-κB, which leads to the increased activation of growth factors such as transforming growth factor-β (TGF-β), and contribute to the pathogenesis of diabetic nephropathy. In the diabetic kidney, enhanced glucose uptake has been identified in many of the cell populations including glomerular epithelial cells, mesangial cells and proximal tubular epithelial cells, leading to the excessive production of intracellular ROS making these cells particularly susceptible to the changing milieu of diabetes [5].

The ubiquitously expressed thioredoxin (Trx) system is a major antioxidant in cells along with glutaredoxin and glutathione [6,7]. The activity of Trx is regulated by its endogenous inhibitor, thioredoxin-interacting protein (Txnip). In diabetes, Txnip was reported to be significantly up-regulated in human islet and kidney tubular cell microarray studies [8,9]. It was thus suggested that increased ROS in diabetes is not solely due to the increased production of ROS but also the impaired thiol capacity to reduce ROS as a result of the changes in Txnip expression [10]. More recently, the role of Txnip in the pathogenesis of diabetic nephropathy has been examined in rat and human biopsy studies [10,11]. It is believed that Txnip not only induces oxidative stress but also increases extracellular matrix production leading to the progression of diabetic nephropathy [10]. Thus, inhibition of Txnip may be beneficial in the management of diabetic nephropathy.

Tranilast (N-[3,4-dimethoxycinnamoyl] anthranilic acid) has been extensively studied in experimental models of diabetic nephropathy and was reported to attenuate tubulointerstitial pathology [12] and glomeruli injury while improving renal function [13]. Furthermore, in a small human pilot study, tranilast was reported to slow the progression rate of advanced diabetic nephropathy [14]. Although the precise mechanism of action for tranilast is not known, its beneficial effects are believed to be largely attributed to the inhibition of TGF-β expression and its activity [12,13,15,16]. Recently, Akahori and colleagues reported that tranilast also attenuated oxidative stress in a diabetic spontaneous hypertensive rat model [13]. Moreover, tranilast has been reported to act as an ROS scavenger [17].

Since tranilast reduces ROS and Txnip plays an important role in inducing oxidative stress in diabetes, we aimed to examine whether tranilast exerts its anti-oxidant properties through the inhibition of Txnip in an experimental model of diabetic nephropathy.

### Table 1. Animal characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic + Tranilast</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>145 ± 6</td>
<td>182 ± 11*</td>
<td>195 ± 13*</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>6.25 ± 0.3</td>
<td>30.9 ± 1.2*</td>
<td>32.1 ± 0.7*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>259 ± 4</td>
<td>248 ± 9</td>
<td>274 ± 7**</td>
</tr>
<tr>
<td>Left kidney weight: BW</td>
<td>0.38 ± 0.01</td>
<td>0.67 ± 0.03*</td>
<td>0.73 ± 0.04*</td>
</tr>
<tr>
<td>Right kidney weight: BW</td>
<td>0.36 ± 0.01</td>
<td>0.72 ± 0.03*</td>
<td>0.76 ± 0.04*</td>
</tr>
<tr>
<td>AER (mg/24 h)</td>
<td>0.14 ×/÷ 1.1</td>
<td>6.16 ×/÷ 1.3*</td>
<td>3.27 ×/÷ 1.5*</td>
</tr>
</tbody>
</table>

BW, body weight; AER, albumin excretion rate. All data are presented as mean ± SEM except AER which are expressed as geometric means ×/÷ tolerance factors. *P < 0.05 versus control; **P < 0.05 versus diabetic.
Materials and methods

Animals

Twenty-nine female heterozygous Ren-2 rats, aged 6 weeks, were randomized to receive either 55 mg/kg of streptozotocin to induce experimental type 1 diabetes, or citrate buffer alone (non-diabetic control) by tail-vein injection, and studied for a further 10 weeks. Diabetic animals received 2–4 units of isophane insulin intraperitoneally (Humulin NPH Isophane, Eli Lilly and Co., USA) to promote weight gain and reduce mortality. A separate group of diabetic Ren-2 rats were given tranilast (200 mg/kg/day by

![Fig. 1](image1)

![Fig. 2](image2)

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![Fig. 1](image1)

![Fig. 2](image2)
gavage in 1% NaHCO3 administered as 100 mg/kg twice daily, Kissei Pharmaceutical Co., Japan) from week 6 to week 10, while untreated rats were gavaged with vehicle. Each week the rats were weighed and blood glucose was measured using a glucometer (Accu-check Advantage II Blood Glucose Monitor, Roche Diagnostics, USA). Every 4 weeks, systolic blood pressure (SBP) was recorded in preheated conscious rats by tail-cuff plethysmography [18]. An average SBP reading was taken from three consecutive measurements to reduce variability. Experimental procedures adhered to guidelines of the National Health and Medical Research Council of Australia's Code for the Care and Use of Animals for Scientific Purposes and was approved by the Bioethics Committee of St. Vincent's Hospital, Melbourne, Australia.

Albuminuria

At 10 weeks, 2 days prior to sacrifice, rats were individually housed in metabolic cages, habituated for 2–3 h and urine was collected over 24 h. Animals continued to have free access to tap water and standard laboratory chow during this period. After 24 h in the metabolic cages, an aliquot of urine (5 mL) was collected from the 24-h urine sample and stored at −70°C for subsequent analysis of albumin. Albuminuria was determined by a double antibody radioimmunoassay as previously performed in our laboratory [19].

Tissue preparation

At the end of the study, rats were anaesthetized (Nembutal 60 mg/kg body weight i.p., Boehringer-Ingelheim, Australia). The renal artery was clamped and the kidneys were excised, decapsulated, sliced transversely and fixed in 10% neutral-buffered formalin overnight. Tissues were routinely processed, paraffin-embedded and sectioned at 4 μm.

33P in situ hybridization

Localization of Txnip transcript was determined in rat kidneys. Two rat Txnip probes were generated by RT–PCR, using rat kidney cDNA as template, to span the regions 1481–2004 (540 bp) and 2008–2384 (377 bp) of the rat Txnip sequence (accession number NM_001008767). The primers were designed using Oligo 6 primer design software (Molecular Biology Insight, Cascade, CO). Synthesis of riboprobes and in situ hybridization were performed as previously described [10]. Briefly, 33P-labelled anti-sense RNA probe for Txnip was generated by in vitro transcription (Promega, Madison, WI) from linearized templates. Purified riboprobe length was adjusted to ~150 bases by alkaline hydrolysis. In situ hybridization was performed on 4 μm thick sections of formaldehyde-fixed, paraffin-embedded kidney tissue. Briefly, tissue sections were dewaxed in histolene, rehydrated in graded ethanol and microwaved in 10 mM citrate buffer, pH 6.0 on medium–high (600–700 W) for 6 + 5 min [20]. Sections were washed in 0.1 M sodium phosphate buffer (pH 7.2), fixed in 4% paraformaldehyde for 10 min and washed again in phosphate buffer and milliQ water. After equilibration in P buffer (50 mM Tris–HCl pH 7.2, 5 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0), slides were incubated with 125 μg/mL Pronase E (Sigma) in P buffer (pH 7.2), refixed in 4% paraformaldehyde for 10 min, rinsed in milliQ water, dehydrated in 70% ethanol and air dried. Hybridization of the riboprobe to the pretreated tissue was performed overnight at 60°C in 50% formamide-humidified chambers, as previously described [21]. Sense probes were used on a further set of tissue sections as controls for non-specific binding. After

Fig. 3. Immunohistochemistry of osteopontin which acts as a chemotactic factor for macrophage in control (A), diabetic (B) and diabetic tranilast-treated animals (C). Diabetic animals were associated with a significant increase in the expression of osteopontin (brown), especially in the damaged tubules and adjacent to infiltrating cells. Treatment with tranilast reduced the expression of osteopontin in diabetic rats. Magnification ×100. Quantitation of osteopontin immunostaining is shown (D). Values are represented as mean ± SEM. *P < 0.05 versus control; †P < 0.05 versus diabetic.
hybridization, slides were washed, incubated with RNase A, dehydrated in graded ethanol, air dried and exposed to Kodak Biomax MR autoradiographic film (Kodak, Rochester, NY) for 3 days.

Quantitative autoradiography

Densitometry of autoradiographic images obtained by in situ hybridization was performed by computer-assisted image analysis using Micro Computing Imaging Device (MCID; Imaging Research, St. Catharine's, Ontario, Canada) as previously described [22]. In brief, in situ autoradiographic images were placed on a uniformly illuminating fluorescent light box (Northern Light Precision Luminator model C60, Imaging Research) and captured using a video camera (Dage MTI CCD72) connected to an IBM AT computer with a 512 × 512 pixel array imaging board with 256 grey levels. After appropriate calibration by constructing a curve of optical density versus radioactivity density using Amersham 14C microscale autoradiography standards, which were co-exposed with the hybridized sections, quantitation of digitalized autoradiographic images was performed with the MCID software and expressed as nCi/g.

Histopathology and immunohistochemistry

Changes in matrix deposition within the interstitium were assessed using a Masson's modified trichrome stain as previously described [23]. In addition, deposition of peritubular type-IV collagen was examined by immunostaining using a polyclonal goat anti-bovine/anti-human type-IV collagen antibody (Southern Biotechnology, Birmingham, AL, USA).

For immunohistochemistry, tissue sections were placed in histosol to remove paraffin wax, rehydrated in graded ethanol and washed in distilled water before being incubated for 15 min with 3% hydrogen peroxide to block endogenous peroxidase. Sections were then rinsed thoroughly with 0.1 M PBS at pH 7.4 before being incubated with 1:10 diluted normal swine serum. Sections were then incubated with primary antibodies at 4°C overnight. The following day, slides were washed thoroughly with PBS and treated with biotinylated goat anti-mouse (Dako Corp., CA, USA) for 1 h or rabbit anti-goat (1:200) biotinylated IgG (Dako Corp., CA, USA) for 1 h, followed by avidin–biotin peroxidase complex for another hour (Vector, CA, USA). Localization of the peroxidase conjugates was achieved using 3,3′-diaminobenzidine tetrahydrochloride (Dako Corp., CA, USA) as a chromagen.

Tissue expression of Txnip and nitrotyrosine, which served as markers for nitric oxide (NO)-dependent oxidative stress, was also assessed using immunostaining as described above except sections were incubated with rabbit anti-nitrotyrosine (Upstate Technology, Lake Placid, NY) and rabbit anti-Txnip antibody (Zymed Laboratories, South San Francisco, CA) overnight and incubated with biotinylated swine anti-rabbit IgG antibody the next day [9]. Furthermore, the infiltration of macrophages was examined using the mouse monoclonal rat macrophage marker (CD68/ED1, Serotec, Raleigh, NC, USA) and specific primary polyclonal antisera to osteopontin which is a macrophage chemotactic factor.

Quantitation of histological parameters

The accumulation of matrix within the tubulointerstitium was estimated on Masson's trichrome and type-IV collagen-stained sections using computer-assisted image analysis as previously reported [24,25]. Similarly, tissue expression of Txnip in the dilated tubules and glomeruli, tubular nitrotyrosine and the expression of osteopontin were also investigated on immunostained sections. In brief, 10 random non-overlapping fields from each stained section around the cortex were captured and digitized using an AxioImager.A1 microscope (Carl Zeiss AxioVision, Germany).

![Fig. 4. ED1 positive macrophages from control (A), diabetic (B) and diabetic tranilast-treated animals (C). Minimal number of macrophages was observed in shams, whereas diabetic kidney was associated with a significant increase of macrophages (arrow). Treatment with tranilast was associated with a reduction in macrophage numbers. Magnification ×400. Quantitation of the number of macrophages per field is shown (D). Values are represented as mean ± SEM. *P < 0.001 versus control; †P < 0.001 versus diabetic.](image-url)
attached to an AxioCam MRc5 digital camera (Carl Zeiss AxioVision, Germany) at ×100 magnification unless otherwise stated. Digital images were then loaded onto a Pentium D Dell computer. Areas of blue on Masson’s trichrome-stained sections (for matrix) or brown on immunostained sections (for collagen IV, Txnip, nitrotyrosine and osteopontin) were highlighted using a selective colour tool for their colour ranges, and the proportional area of the tissue with their respective ranges of colour was then quantified. Calculation of the proportional area stained blue or brown was then determined using image analysis software AIS (Analytic Imaging Station version 6.0, Imaging Research Inc., Ontario, Canada).

Quantification for ED1/CD68 positive cells

Macrophage numbers were estimated by manually counting the CD68/ED1 positive cells under a standard light microscope under ×200 magnification in 10–20 randomized non-overlapping fields in the interstitium of kidney cortex.

Lucigenin-enhanced chemiluminescence assay

The superoxide levels in the kidney tissues were measured using a lucigenin-enhanced chemiluminescence assay as previously described [26]. In brief, equal size kidney tissues were homogenized using a glass homogenizer in a buffer containing 250 mM sucrose and 10 mM HEPES with protease inhibitors. Lysate was collected by centrifugation at 12,000 rpm for 10 min at 4°C. Four microliters of lysate was transferred into a 96-well OptiPlate (Packard) with 200 μL Krebs-HEPES buffer comprising (mM): NaCl 98.0, KCl 4.7, NaHCO3 25.0, MgSO4 1.2, KH2PO4 1.2, CaCl2 2.5, D-glucose 11.1 and HEPES-Na 20.0 in each well. Added into each well were 5 μM lucigenin and 100 μM NADPH, and the chemiluminescence was detected with POLARstar OP-TIMA (BMG Labtech). Protein was quantitated using Bradford reagent (Bio-Rad, Hercules, CA), and all the chemiluminescence data were normalized to protein level.

Statistical analysis

Data are expressed as means ± SEM unless otherwise stated. Statistical significance was determined by a one-way ANOVA with a Fisher post hoc comparison. Albuminuria was skew distributed and was analysed following log transformation and presented as geometric means ±/÷ tolerance factors. All analyses were performed using Statview II + Graphics package (Abacus Concepts, Berkeley, CA). P < 0.05 was considered as statistically significant.

Results

Clinical parameters and albuminuria

In comparison with control animals, diabetic rats had elevated SBP and blood glucose that were unaffected by the treatment with tranilast (Table 1). After 10 weeks, diabetes was associated with increased kidney to body weight ratios (both left and right kidneys) and treatment with tranilast had no effect (Table 1). Diabetic rats also demonstrated a significant increase in albuminuria compared to control animals and this was not significantly reduced by the treatment with tranilast (P = 0.11, Table 1).
Tranilast inhibits the deposition of interstitial matrix and peritubular collagen IV

Consistent with our previous study [12], diabetic animals had increased tubulointerstitial matrix (P < 0.001, Figure 1) and increased immunostainable collagen IV in the peritubular basement membrane of the kidney (P < 0.001, Figure 1). Tranilast significantly attenuated the deposition of tubulointerstitial matrix and peritubular collagen IV accumulation in diabetic rats (both P < 0.001, Figure 1).

Tranilast attenuates oxidative stress

Nitrotyrosine is a marker of NO-dependent oxidative stress and has been implicated in the pathogenesis of diabetic complications [27]. Immunostaining of nitrotyrosine demonstrated a significant increase of nitrotyrosine expression in the tubules in diabetic animals and this increase of nitrotyrosine expression was significantly attenuated by tranilast in diabetic rats (P < 0.05, Figure 2). Measurement of superoxide levels using lucigenin-enhanced chemiluminescence assay also demonstrated that tranilast reduced oxidative stress in diabetic animals (P < 0.05, Figure 2E).

Tranilast attenuates the infiltration of inflammatory cells

Osteopontin has been reported to be a critical mediator of inflammation by promoting the accumulation of macrophages [28]. Minimal expression of osteopontin was observed in the tubules, and only a small number of macrophages were found in the interstitium and glomeruli of control animals. Osteopontin was abundantly expressed in the diabetic animals (Figure 3) and this was associated with a 4-fold increase in macrophages within the interstitium of the kidney cortex (Figure 4). Treatment with tranilast significantly reduced the expression of osteopontin (P < 0.05, Figure 3) and the number of macrophages (P < 0.001, Figure 4).

Tranilast attenuates the increase of Txnip mRNA and protein expression in vivo model

Quantitation of in situ hybridization suggested that Txnip mRNA expression was increased in diabetic animals compared to controls and that this increase of Txnip expression was attenuated by the treatment with tranilast (P < 0.05, Figure 5).

Similarly, Txnip protein expression as examined by immunohistochemistry was up-regulated in diabetic animals with expression most abundant in the glomeruli and the tubules in the kidney (Figure 6). Quantitative analysis demonstrated that the increase of Txnip protein expression in both the tubules and glomeruli were attenuated by tranilast (both P < 0.001, Figure 6).

Discussion

Tranilast has been reported to exert anti-fibrotic and anti-inflammatory effects in various diseases such as diabetic nephropathy and cardiomyopathy [12,29]. This is the first study to report that tranilast also inhibited the expression of Txnip in both the tubules and glomeruli of diabetic kidneys, in association with the attenuation of ROS production, a decrease in matrix and collagen IV accumulation and a reduction in macrophage infiltration.

The mechanism of action of tranilast is yet to be elucidated; however, its inhibitory effects on TGF-β expression and activity have been shown in a range of cells including skin fibroblasts [30], cardiac fibroblasts [31], vascular smooth muscle cells [15], hepatic stellate cells [32] and human renal cortical fibroblasts [12]. More recently, it was demonstrated that tranilast may also inhibit TGF-β-induced connective tissue growth factor and the phosphorylation of Smad2 in both proximal tubule cells and cortical fibroblasts [33]. Tranilast is also known to suppress the production and release of platelet-derived growth factor from monocytes and/or macrophages leading to an inhibition on proliferation, migration and collagen synthesis in smooth muscle cells [15,34,35]. Furthermore, tranilast was reported to inhibit interleukin-1β-induced monocyte chemoattractant protein-1 expression in mesangial cells, at least in part, by suppressing NF-κB activation [36]. The present study is the first to demonstrate that tranilast may also inhibit the expression of Txnip, which could lead to a reduction in oxidative stress and fibrosis.

Thioredoxin-interacting protein (Txnip), also known as vitamin D3 up-regulated protein 1 or Trx-binding protein-2, was first isolated and characterized in HL-60 cells as a protein that is up-regulated by 1α,25-dihydroxyvitamin D3, an active form of vitamin D3 [37]. It is induced by various stresses, including H2O2, irradiation, heat shock, serum starvation and treatment with TGF-β [38,39]. Using a yeast two-hybrid system, Txnip has been characterized as a Trx-binding protein [40] and was demonstrated to have an inhibitory effect on Trx-dependent reducing activity [39,40].

We have previously shown that Txnip mRNA and protein expressions were significantly increased in the glomeruli and distal nephron of diabetic kidneys in both the rat and human models of diabetic nephropathy [10]. Moreover, in vitro studies show that by silencing Txnip with siRNA technology, high glucose-induced oxidative stress and collagen production in both rat mesangial and normal rat kidney (NRK) (proximal tubule) cells were attenuated [10]. Emerging evidence has highlighted the role of Txnip in the pathogenesis of diabetic nephropathy [9,11,41], nonetheless there is currently no specific inhibitor of Txnip. A study by Xiang and colleagues utilizing a sequence-specific DNA enzyme for Txnip to down-regulate the mRNA expression showed that myocardial collagen gene expression, the deposition of collagen and scar formation were attenuated in the infarcted heart [42]. However, local administration of the DNA enzyme is only favourable to diseases with localized pathogenesis such as myocardial infarction.

In association with the inhibition of Txnip expression in both the tubules and glomeruli in diabetic animals treated with tranilast, the current study also shows that similar to our previous study, when given orally to rats with established diabetes, tranilast attenuated the accumu-
Fig. 6. Immunohistochemistry of Txnip in the tubules (A–C) and glomeruli (E–G) of sham (A, E), diabetic (B, F) and diabetic tranilast-treated animals (C, G). There was a significant increase in Txnip in both the tubules and glomeruli of diabetic animals, and treatment with tranilast was associated with a reduction in both tubular and glomerular Txnip expressions in diabetic rats. Magnification ×100 (A–C) and ×400 (E–G).

Quantitation of Txnip in the tubules (D) and glomeruli (H) is shown. Values are represented as mean ± SEM. *P < 0.001 versus control; †P < 0.001 versus diabetic.
Tranilast attenuates up-regulation of Txnip and oxidative stress.

lation of interstitial matrix and peritubular collagen IV [12]. Moreover, superoxide production and nitrotyrosine expression were reduced in diabetic rats treated with tranilast compared to untreated diabetic animals. This is in accordance with our previous study that demonstrated by silencing Txnip, high glucose-induced collagen and ROS production were attenuated in rat kidney cells [10].

The effect of tranilast on the generation of ROS was first published by Miyachi et al. using the cell-free, xanthine–xanthine oxidase system, where tranilast inhibited superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH$^-$) levels at concentrations comparable to therapeutic blood levels. It was also reported that tranilast suppressed the production of superoxide anion from human neutrophils [43]. Furthermore, it was shown that tranilast may also suppress the generation of ROS from murine macrophages and the development of lung fibrosis [44]. Thus far, tranilast has been shown to inhibit ROS production from inflammatory cells such as monocytes, macrophages and neutrophils. We report in this study that, in addition to inhibiting the expression of the macrophage chemokine, osteopontin and the infiltration of macrophage, tranilast may also inhibit the expression of Txnip, an endogenous inhibitor of cellular anti-oxidant Trx, thus relieving the inhibition of Txnip on Trx allowing the system to scavenge for ROS.

The transgenic Ren-2 rat has the mouse Ren-2 gene transfected into the genome and displays an amplified tissue RAS [45]. It is a well-characterized rodent model of diabetic nephropathy that develops severe renal impairment [46]. In contrast to conventional therapies, such as the angiotensin-converting enzyme inhibitors (ACEI) or angiotensin type I receptor blockers, tranilast attenuates fibrosis in the diabetic animals despite persistent hypertension [47,48]. Furthermore, a previous study has demonstrated that ‘add-on’ therapy with tranilast with ACEI led to further incremental benefits on kidney function beyond that with ACEI alone [49]. Angiotensin II is known to promote renal fibrosis through the stimulation of pro-fibrotic cytokines such as TGF-β [50]. Nonetheless, it was reported that high glucose induced Txnip in an angiotensin II and TGF-β-independent fashion [9]. Hence, tranilast may also inhibit oxidative stress and fibrosis through the inhibition of Txnip expression independent of angiotensin II or TGF-β.

The mechanism of how tranilast inhibits Txnip expression in the current model of diabetic nephropathy is not known. Several studies have demonstrated that high glucose may induce the up-regulation of Txnip at the transcriptional levels. Minn et al. was the first to report that glucose regulated Txnip at the transcription level through a distinct carbohydrate response element in the human Txnip promoter in INS-1 beta cells by recruiting the carbohydrate response element-binding protein [51,52]. Another study has reported that transcription factor Krüpel-like factor 6 (KLF6) may increase Txnip expression and promoter activity in diabetes [53]. It is therefore possible that tranilast may affect high glucose-induced Txnip expression at the transcriptional level.

Based on our previous study, we have demonstrated that the optimal dose for tranilast to exert beneficial effects on kidney function in the Ren-2 rat model of advanced diabetic nephropathy is 400 mg/kg/day [12]. In the current study, we investigated whether tranilast would attenuate the pathological changes in diabetic nephropathy at a lower dose. We show that in this study the attenuation of structural abnormalities and oxidative stress preceded the improvement in kidney function with tranilast treatment. It should also be noted that in the current study, the animals were given tranilast for a period of only 4 weeks compared to the previous study of 8 weeks. A previous study has reported the time-dependent effects of tranilast in the attenuation of albuminuria in a hypertensive rat model of diabetic nephropathy [13]. Hence, the lack of improvement in kidney function in the current study may also be attributed to the shorter treatment period.

In conclusion, in addition to its anti-fibrotic and anti-inflammatory effects as previously reported, tranilast may also attenuate the up-regulation of Txnip expression and oxidative stress in an animal model of diabetic nephropathy.

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Conflict of interest statement. None declared.

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