Protective effect of pyrrolidine dithiocarbamate on kidney tissue in streptozotocin-induced diabetic rats

Streptozotosin ile diyabet oluşturulan sıçanlarda pirolidyum dithiyokarbamat'ın böbrek dokusu üzerine koruyucu etkisi

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Abstract

Objective: In this study, we investigated protective effects of pyrrolidine dithiocarbamate (PDTC), which is an antioxidant and nuclear factor kappa B (NF-κB) inhibitor, on nephropathy in diabetic rat model.

Materials and methods: Twenty-eight Sprague-Dawley rats were allocated into 3 groups. Control group (n=8) received no treatment; diabetes group (n=10) received single intraperitoneal (i.p.) injection of streptozotocin (STZ, 65 mg/kg) to induce experimental diabetes; and PDTC group (n=10) received i.p. 100 μL/day PDTC for a total of 10 weeks following diabetes induction with i.p. STZ injection. At the end of the study kidneys were excised from sacrificed rats, and glomerular and tubular changes were examined under light microscopy. Immunohistochemically, NF-κB (p65) and inducible nitric oxide synthase (iNOS) expression were evaluated in the renal cortex.

Results: In diabetes group, immunohistochemical NF-κB expression was higher than control and PDTC groups ($p<0.05$). In PDTC group, NF-κB expression level was very similar to control group, but less than diabetes group ($p<0.05$). Immunohistochemical iNOS expression was less in control group. In PDTC group, iNOS expression was more intense than control group ($p<0.05$), but less than that in diabetes group ($p<0.05$).

Conclusion: PDTC has a protective effect on renal injury secondary to diabetes and can be a treatment option for patients with diabetic nephropathy.

Key words: Diabetic nephropathy; NF-kappa B; oxidative stress.

Özet

Amaç: Bu çalışmada, nükleer faktör-kappa B (NF-κB) inhibitörü ve antioksidan özelliği olan pirolidyum dithiyokarbamat'ın (PDTC) diyabetik sıçan modelinde nefropati üzerine koruyucu etkisini araştırdık.

Gereç ve yöntem: Sprague-Dawley cinsi 28 sıçan üç gruba ayrıldı. Kontrol grubu (n=8) tedavi almadı; diyabet grubuna (n=10) intraperitoneal (i.p.) streptozotosin (STZ, 65 mg/kg) verildi; ve PDTC grubuna (n=10) i.p. STZ ile diyabet oluşturulduktan sonra 10 hafta süreyle i.p. 100 μL/gün PDTC verildi. Çalışma sonunda öldürülen tüm sıçanların böbrekleri alındı. Işık mikroskobu altında glomeruler ve tübüler değişiklikler incelendi. İmmunohistokimyasal olarak renal kortekste NF-kappa B (p65), indüklenebilir nitrik oksit sentaz (iNOS) ekspresyonu değerlendirildi.

Bulgular: Diyabet grubunda immunohistokimyasal NF-κB ekspresyonu kontrol ve PDTC grubuna göre artmış olarak izlendi ($p<0.05$). PDTC grubunda bu ekspresyon düzeyi kontrol grubuna yakın idi, diyabet grubuna göre ise daha düşüktü ($p<0.05$). İmmunohistokimyasal iNOS ekspresyonu kontrol grubunda daha azdı. PDTC grubunda ise kontrol grubuna göre boyanma daha yoğun ($p<0.05$) ve diyabet grubuna göre azalmış olarak izlendi ($p<0.05$).

Sonuç: Diyabete bağlı gelişen renal hasara karşı PDTC'nin koruyucu etkisi vardır ve diyabetik nefropatili hastalarda bir tedavi seçeneği olabilir.

Anahtar sözcükler: Diyabetik nefropati; NF-kappa B; oksidatif stres.

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Diabetes mellitus (DM) is a widely seen metabolic disorder which causes many structural and metabolic macro- and micro-angiopathic complications.[1] Diabetic nephropathy (DN), one of the micro-angiopathic complications, is seen in 20-40% and 5-10% of type 1 and type 2 DM, respectively.[2] DN occurs in only 30-40% of type 1 diabetic patients. The risk factors for DN have not been completely elucidated. Poor metabolic control is an important risk factor, although there are patients who do not develop renal disease despite having suboptimal glucose levels for long periods. Such susceptibility to DN could be explained by genetic predisposition.[3,4] Pathophysiology of DN is hyperglycemia-induced angiotensinogen gene expression, inducible nitric oxide synthetase (iNOS) expression, and oxidative stress in renal cells.[2] Oxidative stress and secondary to oxidative stress, overproduction of free radicals (FR) is reported in studies on diabetic patients and diabetic rat models.[5,6] FR lead to tissue damage by nuclear factor kappa B (NF-κB) and iNOS activation.

Transcription factor, NF-κB is a factor that regulates many gene expressions and is critical for development of acute inflammation. NF-κB is binded to I kappa B, an inhibitor protein in cytoplasm of cells which are not induced. TNF-alfa, IL-1 beta and reactive oxygen complexes are disintegrated and NF-κB is exposed secondary to inflammatory induction. Exposed NF-κB translocates into the nucleus and initiates gene transcription by binding to specific promoters.[7] NF-κB affects target genes for pro-inflammatory cytokines, chemokines and immunreceptors, cell adhesion molecules, acute phase proteins, and iNOS. Activation of NF-κB leads to coordination of increase in genetic transcription of many products affecting inflammatory response. NF-κB is the modulator of acute and chronic kidney inflammation.[8,9]

iNOS is one of the three NOS isoforms that is affected by NF-κB as a result of tissue damage. The process of iNOS expression involves different signal transduction pathways, including nuclear translocation of the transcription factor, NF-κB.[10] The contribution of NO to tissue injury can be secondary to a direct effect mediated by the NO molecule itself and an indirect effect mediated by reactive nitrogen species produced by the interaction of NO with superoxide anions or oxygen.[11] The iNOS-mediated NO production is significantly elevated with increased oxidative stres[11,12] and excessive NO production secondary to elevated expression of iNOS may impose cytotoxic effects on various organs, including the kidney.[13]

It is reported that NF-κB plays an important role in the formation of DN.[14] Antioxidants which decreases the effects of FR and are NF-κB inhibitors have been used in the treatment to prevent the damage caused by NF-κB.[1] Red wine, green tea, ginseng, vitamin C, vitamin E, taurin, pyrrolidine dithiocarbamate (PDTC), and pomegranate juice are examples of these antioxidants and antioxidant containing substances. It is reported that free radical inhibitory and antioxidant effects of PDTC are very strong, thus it is protective on kidney.[15-18]

In this experimental study, we aimed to investigate whether PDTC is protective over nephropathy by inhibition of NF-κB and iNOS formation and prevention of oxidative stress in streptozotocin-induced diabetic rats.

Materials and methods

We conducted the study after the approval of Ethics Review Committee for Animal Experimentation, Istanbul University Faculty of Medicine. Thirty-six adult male Sprague-Dawley rats (300-350 g) aged 12 weeks were acquired from the experimental Animal Laboratory of Medical Research Center of Istanbul Faculty of Medicine (DETAE), and maintained in a 14-hr light/10-hr dark cycle with free access to standard rat chow and water. Rats were kept in the laboratory at least for one week for adaptation. Rats were enrolled into the study after completion of adaptation period. Prior to study, body weights and blood glucose levels of rats were measured. Animals were placed in metabolic cages for 24 hr to collect urine and measure microalbumin levels. Diabetes group (n=10) received single intraperitoneal (i.p.) injection of streptozotocin (STZ) (65 mg/kg; Sigma, Diesenoffen, Germany) dissolved in 0.1 M sodium citrate buffer (pH 4.5) to form experimental diabetes. Untreated rats (control group, n=8) were housed under the same conditions and used as controls. Three days after STZ injection, blood samples were collected from the tail vein, and blood glucose levels were determined by a glucometer. Rats with blood glucose levels higher than 200 mg/dL were accepted as diabetic and enrolled into the study. In the follow-up, 8 rats were excluded from the study, because 5 rats with plasma glucose concentration >500 mg/dL died and 3 rats were not considered as diabetic with plasma glucose concentration <200 mg/dL. Finally, 3 groups were formed by dividing remaining 20 rats equally into two groups. During the study, plasma glucose concentration of all the rats were checked daily, body weights were measured weekly, and the day before sacrification all the rats were placed in metabolic cages for 24 hr. At the end of 10 weeks, the rats were sacrificed under nembutal anaesthesia (50 mg/kg; Ulagay, İstanbul, Turkey), intracardiac blood was aspirated and their kidneys were harvested. Tissue samples were fixed in 10% formalin and paraffin embedded for subsequent light microscopic and immunohistochemical examination.

Experimental groups

Group I (control group, n=8): On the 10th week following daily intraperitoneal saline injection rats were sacrificed, intracardiac blood aspirated, and their kidneys were harvested.

Group II (diabetes group, n=10): Rats with plasma glucose concentration >200 mg/dL were observed with no treatment. On the 10th week after the induction of diabetes, rats were sacrificed, intracardiac blood aspirated and their kidneys were harvested.

Group III (diabetes+PDTC group, n=10): Following diabetes formation, rats received intraperitoneal PDTC injection (100 µL/day) (Sigma-Aldrich Chemical Corp, MO, USA) for 10 weeks. At the end of 10 weeks, rats were sacrificed, intracardiac blood aspirated, and their kidneys were harvested.

Microscopic examination

For the histopathological examination, the tissues were prepared for routine examination by light microscopy after staining with haematoxylin and eosin (H&E). The kidney sections were analysed semi-quantitatively for glomerular necrosis, glomerular basement membrane thickening, enlargement of mesengial matrix, tubular hydropic degeneration, and tubular dilatation. The changes were graded as 0 for normal, +1 for focal, +2 for moderate, +3 for multifocal, and +4 for diffuse.[19]

Immunohistochemical evaluation

For the immunohistochemical evaluation, specimens were processed for light microscopy. Sections were incubated at 60°C overnight and then dewaxed in xylene for 30 min. After soaking in a decreasing series of ethanol, sections were washed with distilled water and phosphate-buffered saline (PBS) for 10 min. Sections were then treated with 2% trypsin in 50 mM Tris buffer (pH=7.5) at 37 °C for 15 min and washed with PBS. Sections were delineated with a Dako pen (Dako, Glostrup, Denmark) and incubated in a solution of 3% H_2O_2 for 15 min to inhibit endogenous peroxidase activity. Sections then were incubated with NF-κB/P65 (Rel A) antibody (Ab-1; Neomarkers R-B-1638-R7) and iNOS antibody (Ab-1; Neomarkers R-B-1605-R7). The Ultra-vision HRP-AEC staining protocol was used.[16] Sections prepared for each case were examined by light microscopy. Sections of rat lung were used for the control of immunohistochemical staining specificity according to the data provided by the antibody-producing company. According to the diffuseness of the staining, sections were graded as 0 for no staining, 1 for staining <25%, 2 for staining between 25% and 50%, 3 for staining between 50% and 75%, or 4 for staining >75%.[16] According to staining intensity, sections were graded as 0 for no staining, 1 for weak but detectable staining, 2 for distinct staining, or 3 for intense staining. Immunohistochemical values were obtained by adding the diffuseness and intensity scores.[16]

Statistical methods

Statistical analyses of the histopathological examination were performed with the Kruskal-Wallis and the Mann-Whitney U-tests and $p<0.05$ was considered to indicate statistical significance. Kolmogorov-Smirnov test was used to evaluate coherence of parameters to normal distribution. One-way Anova test and Tukey HSD as Post Hoc test were used for comparison of datas showing normal distribution.

Results

Plasma glucose levels

Hyperglycemia was recorded for both diabetes and PDTC groups during the study and difference between the groups for hyperglycemia was not statistically significant. The plasma glucose levels in the control group were significantly lower than the diabetes and PDTC groups ($p<0.01$) (Table 1).

Urinary microalbumin levels

In 24 hr collected urine, urinary microalbumin levels of rats at the beginning and at the end of the study were given in Table 2. Urinary microalbumin levels of all rats were similar at the beginning of the study. At the end of the study urinary microalbumin levels of rats in control group was not changed, whereas urinary microalbumin levels of diabetes and control groups have increased. Increase in microalbumin levels of PDTC group was less than diabetes group ($p<0.05$) and higher than control group ($p<0.05$) (Table 2).

Daily urine output

There was no significant difference in urine levels of groups on the first day of the study ($p>0.05$). In measurements performed through the study, there was a significant increase in urine levels in diabetes and PDTC groups compared to control group ($p<0.001$). At the end of the study, daily urinary output of PDTC group decreased compared to diabetes group ($p<0.05$) (Table 3).

Body weight

There was no significant difference in body weights of the groups at the beginning of the study ($p<0.05$). At the end of the study, body weights of diabetic group decreased compared with control group ($p<0.05$), but there was no significant difference between diabetes and PDTC groups ($p>0.05$) (Table 4).

Microscopic evaluation

Tubules and glomerular structures of the kidneys were normal in control group (Fig. 1A). Of diabetes group, 6 rats (60%) had diffuse, 2 rats (20%) had multifocal, and 2 rats (20%) had moderate focal pathological changes. Normal histological appearance was not observed in any of the rats in diabetes group. Of PDTC group, 3 rats (30%) had multifocal, 4 rats (40%) had moderate focal, and 3 rats (30%) had focal inflammation. In PDTC group, histological changes was less severe than diabetes group. Pathological changes were not diffuse in PDTC group.

Glomerular changes

In control group, glomerules were histologically in normal appearance (Fig. 1A). In diabetes group, glomerulosclerosis was seen in patches (Fig. 1B). Extracellular matrix accumulation was seen in glomerules in diabetes group (Fig. 1C). Some of the glomerules were sclerotic. Rate of glomerular degeneration in PDTC group was higher than control group, although it was less than diabetes group ($p<0.05$).

Tubular changes

Histologically tubular structures of the control group were normal (Fig. 1A). In diabetes group, patchy hydropic degeneration, tubular necrosis, vacuolization in some of the tubules, and marked paleness within tubular cell cytoplasm were observed (Fig. 1B). In PDTC group, all these pathological changes decreased and histological appearance was nearly similar to control group (Fig. 1C). In PDTC group, pathological changes like glomerulosclerosis, enlargement of extracellular matrix, basement membrane thickening, tubular necrosis, and hydropic degeneration were seen in lesser extent than diabetes group. Microscopic examination for evaluation of histological damage score revealed that histological damage score was higher in diabetes and PDTC group compared to control group ($p<0.05$). Histological damage score in diabetes group was higher than PDTC group ($p<0.05$). There was more histological damage in PDTC group compared to control group ($p<0.05$) (Table 5).

Immunohistochemical evaluation

iNOS expression was minimal in control group (Fig. 2A). In diabetes group, diffuse and intense iNOS expression was observed compared to control group (Fig. 2B). In PDTC group, iNOS expression was stronger than control group ($p<0.05$) and decreased compared to diabetes group ($p<0.05$) (Fig. 2C).

NF-κB/p65 staining was negative in control group and minimal NF-κB/p65 expression was seen in glomerular and tubular epithelial cells (Fig. 3A). Intracytoplasmic intense and diffuse NF-κB/p65 expression was seen in tubular cells of diabetic group (Fig. 3B). Significant NF-κB/p65 expression was observed in diabetic group compared to control group. Moderate intracytoplasmic NF-κB/p65 expression was seen in PDTC group and NF-κB/p65 expression levels were decreased in glomerular and tubular epithelial cells of PDTC group compared to diabetic group (Fig. 3C).

Discussion

Diabetic nephropathy is the most common single cause of renal insufficiency in the western world.

Table 1. Plasma glucose levels (mg/dL) of experimental groups (mean±standard deviation)

	Day 0	Week 1	Week 5	Week 10
Control group	92±4	94±6	92±4	93±5
STZ	94±3	398±10[a]	412±13[a]	431±15[a]
STZ+PDTC	91±7	381±11[a]	398±18[a]	409±12[a]

[a]$p<0.05$ vs. control group.
STZ: Streptozocin, PDTC: Pyrrolidine dithiocarbamate.

Table 2. Urinary microalbumin levels (mg/L) of experimental groups (mean±standard deviation)

	Day 0	Week 5	Week 10
Control group	1.10±0.01	1.10±0.01	1.12±0.01
STZ	0.99±0.10	2.10±0.42[a]	2.23±0.34[a]
STZ+PDTC	1.10±0.07	1.89±0.51[a.b]	1.35±39[a.b]

[a]$p< 0.05$ vs. control group. [b]$p< 0.05$ vs. STZ group.
STZ: Streptozocin, PDTC: Pyrrolidine dithiocarbamate.

Glomerular changes, such as capillary basement membrane thickening, mesangial proliferation, and nodular glomerulosclerosis, are pathogenomic for DN.[20] Tubulointerstitial fibrosis is a predictor of progressive renal failure.[21] Traditionally, DN has been considered a nonimmune, degenerative disease. However, Bohle et al.[22] described the presence of monocytes, macrophages, T cells, and fibroblasts associated with the tubulointerstitial changes seen in DN. More recent reports have suggested that inflammation may underlie disease progression in DN.[23-25] One of the most supported theories in DN pathogenesis is deteriorated peritubular microcirculation and sequential tubular damage.[26] Increases intratubular glucose level is observed following increase in plasma glucose levels.[27] Elevated levels of glucose in glomerular filtrate causes an increase in glucose absorption of proximal tubules and intracellular glucose deposition.[28,29] Glucose dependant metabolic pathways and vasoactive hormones may cause to non-glomerular renal dysfunction by affecting tubular and interstitial cells.[30] Increase in advanced glycation end-products (AGEs) such as carboxymethyllysine and consecutive NF-κB elevation is reported secondary to high intracellular glucose levels.[31,32] The activation of NF-κB linked regulatory pathways generally underlies inflammatory processes, and an increase in the nuclear translocation of NF-κB has been demonstrated in human DN.[33,34]

NF-κB proteins are activated in a variety of experimental models of renal inflammatory disease, including antiglomerular basement membrane nephritis, ureteral obstruction, endotoxemia, and immune complex nephritis.[35-39]

In STZ-induced diabetic rats, mesengial enlargement, GBM thickening, proteinuria and, tubular dilatation are observed.[40] It was reported that glycogen deposition in renal tubules leads to cellular damage, ending with cellular death and these changes can be reversed by insulin treatment.[41] Kang KS et al.[14] demonstrated protective effect of sun ginseng against

Table 3. Daily urinary output (mL/24 h) of experimental groups (mean±standard deviation)

	Day 1	Week 1	Week 5	Week 10
Control group	15.00±2.0	18.00±1.0	17.00±2.1	17.00±1.8
STZ	14.00±1.5	35.00±3.2[a]	43.00±1.8[a]	45.00±2.7[a]
STZ+PDTC	15.00±1.8	32.00±1.8[a]	28.00±2.0[a.b]	25.00±3.0[a.b]

[a]p< 0.001 vs. control group. [b]p< 0.05 vs. STZ group.
STZ: Streptozocin, PDTC: Pyrrolidine dithiocarbamate.

Table 4. Body weights (gr) of experimental groups (mean±standard deviation)

	Day 0	Week 1	Week 2	Week 4
Control group	307.00±15	312.00±17	325.00±22	342.00±21
STZ	308.00±18	291.00±20[a]	265.00±25[a]	242.00±18[a]
STZ+PDTC	311.00±13	293.00±14[a]	270.00±11[a]	245.00±31[a]

[a]p< 0.05 vs. control group.
STZ: Streptozocin, PDTC: Pyrrolidine dithiocarbamate.

Table 5. Distribution of experimental groups according to histopathological scores (as number of patients)

Histopathological score	Control group (n=8)	Diabetes group (n=10)	PDTC group (n=10)
0	8	0	0
1	0	0	3
2	0	2	4
3	0	2	3
4	0	6	0

PDTC: Pyrrolidine dithiocarbamate.

diabetic renal damage. Tugcu V et al.[15,16] demonstrated protective effect of PDTC on shockwave lithotripsy-induced renal injury and in the kidney of rats with nephrolithiasis induced by ethylene glycol. Cam M et al.[30] demonstrated protective effects of chronic melatonin treatment against renal injury in streptozotocin-induced diabetic rats. Aoki et al.[42] demonstrated that oxidative stress induced apoptosis in human endothelial cells through the down regulation of bcl-2, translocation of bax, and upregulation of p53, probably through NF-kappaB activation. Oxidative stress may play an important role in endothelial apoptosis mediated by hypoxia, through the activation of NF-kB. In this study antiapoptotic effect of PDTC is indicated by NF-kappaB inhibition on endothelial tissue.[42] In our study, basement membrane thickening, elevation in mesengial matrix, vacuolization in cortical tubules, and tubular dilatation were observed as a marker of glycogen deposition, in many glomerules of kidneys of diabetic rats. These changes were partially attenuated with PDTC treatment. Morphological findings seen in diabetic kidney tissues and microalbuminuria, marker of early DN, were increased in diabetic rats. In PDTC group, general kidney morphology was attenuated compared with diabetes group and elevation of microalbuminuria level was partially regressed compared to diabetes group.

Raij et al.[43] reported that most of the histological changes seen in diabetes were related with increased iNOS synthesis. Especially in proximal tubule of the kidney, iNOS elevation mediated by STZ and cytokines, indicates that these changes could be related with NO. Raij and Baylis[43] reported that increased NO production may be involved in the early pathogenic hemodynamic changes in diabetes. Recent experimental studies indicated that excessive NO excretion could be responsible for a part of glomerular hyperfiltration. NO plays an important role in capillary blood pressure, plasma flow, and physiological regulation of rate of filtration. NO could play a role in regulation of glomerular pressure and flow rate through the mesengium in macro- and micro-molecular level. Excessive amount of NO synthesized from glomerular cells or macrophages may cause injury, during glomerular inflammation.[44] In our study, we demonstrated elevated iNOS activity in diabetic rats immunohistochemically and we think that the changes in glomerular and tubular cells of the kidneys in diabetes group depend on these processes.

With administration of PDTC, NF-κB inhibitor, during 10 weeks following STZ injection, persistence of capillary irregularity in a part of glomerules and tubular damage seen in a lesser extent indicates that, PDTC partially inhibits the tubular damage. In

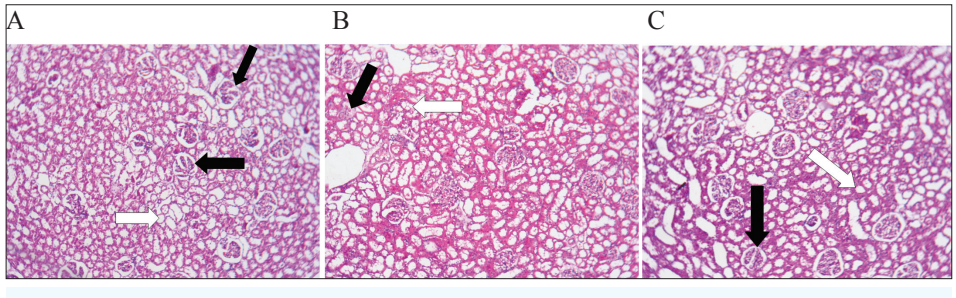

Figure 1 Glomerules (black arrows) and tubules (white arrow) of control group (A), glomerulosclerosis (black arrows) and hydropic degeneration (white arrow) in tubules of diabetes group (B), glomerules (black arrow) and tubules (white arrow) of PDTC group (C). (H&E x100).

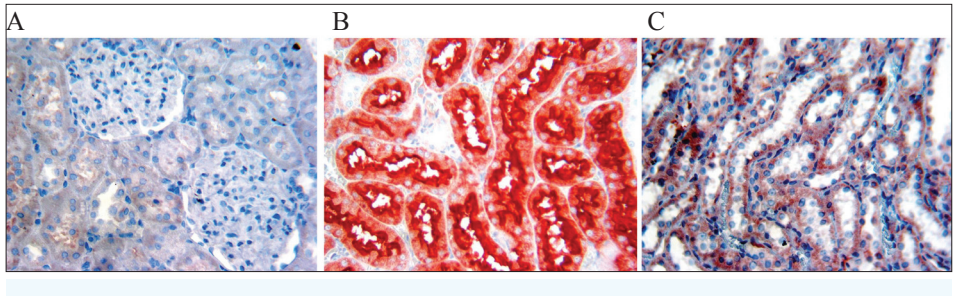

Figure 2 Immunohystochemical staining for iNOS expression. iNOS expression in control group (A, x400), diffuse and intense staining in diabetes group (B, x200) and in PDTC group (C, x200). Staining in PDTC group was stronger compared to control group ($p<0.05$) and decreased compared to diabetes group ($p<0.05$).

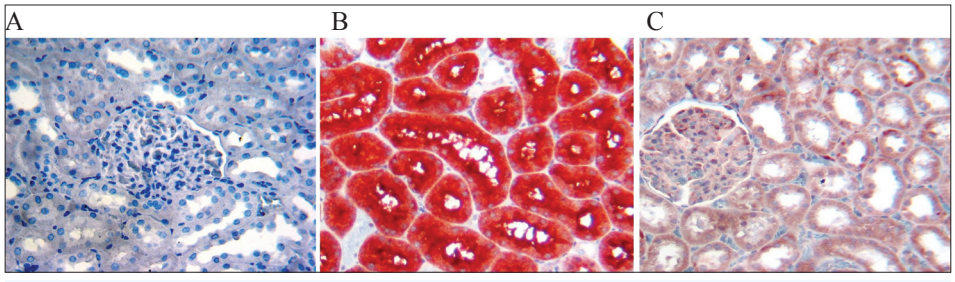

Figure 3 Immunohystochemical staining for NF-κB/p65 expression. Negative NF-κB/p65 staining and minimal NF-κB/p65 expression in glomerular and tubular epithelial cells in control group (A, x400), intracytoplasmic intense and diffuse NF-κB/p65 expression in tubular cells and significant NF-κB/p65 expression in diabetic group (B, x200) compared to control group, moderate intracytoplasmic NF-κB/p65 expression in PDTC group (C, x100). NF-κB/p65 expression was decreased in glomerular and tubular epithelial cells in PDTC group.

our study, in PDTC group, some of the glomerules were more regular compared to diabetes group. Additionally, inhibition of iNOS synthesis indicates that, PDTC may partially inhibit the pathophysiological events caused by diabetes, and the importance of iNOS synthesis is understood.

Many researchers thought that pathophysiological changes caused by diabetes can be removed by keeping iNOS synthesis in normal levels.[45] Sugimoto[46] observed the protective effect of aminoguanidine (AG), selective iNOS inhibitor, against kidney damage. Elevation of iNOS positive cell numbers in glomerules of diabetic rats is reported immunohistochemically and with in situ hybridization. This elevation is parallel to increase in intraglomerular nitrite and nitrate levels.[47] In diabetic rats, iNOS and intraglomerular production of nitrite and nitrate was decreased following AG administration.[48]

In previous studies, electron microscopic evaluation of renal cortex of PDTC, in combination with STZ treated animals' kidneys revealed that microvilli and basal foldings were similar to control group, and this confirms that cellular damage caused by iNOS may be partially suppressed by PDTC.[47,49] In present study, we recorded significant decrease in iNOS expression immunohistochemically in PDTC group compared with diabetes group.

In experimental diabetes the effect of antioxidant treatment on renal injury was well investigated. Investigated agents are vitamin E, vitamin C, lipoic acid and taurin. In most of the studies, kidney protective effects were shown due to decreased oxidative stress, reduction of renal lipid peroxidation index, and secondary to replacement of decreased renal cortical glutathione.[50-54] But, in some studies these traditional antioxidants were not reported to improve DN in experimental models.[52] The dose of the administered antioxidants may be important, agents like vitamin E may have both prooxidant and antioxidant effects when administered in vivo.[53] Vitamin E was even reported to ease renal damage in diabetes.[52]

In a nephrolithiasis model induced by ethylene glycol (EG), Tugcu et al.[16] tried to prevent oxidative stress by NF-κB inhibitor, PDTC. In this study, they observed increased iNOS and NF-κB activity in kidney caused by EG. Following PDTC treatment, declined calcium oxalate deposition in a kidney and decreased iNOS and NF-κB activity were shown immunohistochemically.[16] In our study, we aimed to decrease diabetes-induced oxidative stress by antioxidants like PDTC. PDTC almost completely blocked the diabetes-induced increase in NF-κB binding activity. PDTC also attenuated the diabetes-induced tubular injury and interstitial fibrosis. Thus, NF-κB activation and iNOS expression could play an important role in the progression of renal injury in diabetic nephropathy.[55]

Effects of antioxidants on DN were investigated in some studies, but these studies included a few number of subjects and had relatively short duration. Thus, even though antioxidants were proved to decrease the proteinuria in diabetic cases with short duration,[56,57] we do not have definitive data supporting permanent benefits of these agents on progression of DN.

As a conclusion, blockade of NF-κB activation by antioxidants could be an effective strategy for the prevention of changes of DN according to our model and PDTC might be useful clinically. However, further studies are needed on this issue before clinical application becomes possible.

Conflict of interest

No conflict of interest was declared by the authors.

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