Selective phosphodiesterase-5 (PDE-5) inhibitor vardenafil ameliorates renal damage in type 1 diabetic rats by restoring cyclic 3′,5′ guanosine monophosphate (cGMP) level in podocytes

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Conclusions. Our data suggest that a dysfunctional NO-cGMP pathway exacerbates podocyte damage in diabetes. In conclusion, vardenafil treatment preserves podocyte function and reduces glomerular damage, which indicates therapeutic potential in patients with DN.

INTRODUCTION

The incidence of diabetes mellitus and diabetic nephropathy (DN) is increasing exponentially worldwide [1, 2]. DN is characterized by oxidative stress [3], podocyte damage [4] and glomerulosclerosis [5]. Decreasing bioavailability of nitric oxide (NO) in diabetes might play a crucial role in the progression, but the detailed pathomechanism is not fully understood.

NO-cyclic 3′,5′ guanosine monophosphate (cGMP) axis is essential in the maintenance of renal perfusion and glomerular filtration [6]. NO is synthesized in the vascular endothelial cells by endothelial nitric oxide synthase (eNOS) from l-arginine. NO exerts many of its biological actions by cGMP, which is degraded rapidly by phosphodiesterases (PDE). In the kidney, the cGMP hydrolysing enzyme phosphodiesterase-5 (PDE-5) is expressed in proximal tubules, collecting ducts and the glomerulus [7]. Among haemodynamic actions [6, 8], NO-driven cGMP regulates glomerular filtration by modulating the slit membrane and cytoskeletal reorganization in podocytes.
podocytes [9, 10]. In diabetes, cGMP production was shown to decrease in the glomeruli [11]. Causes of NO–cGMP dysfunction in DN include scavenging of NO by reactive oxygen species (ROS) [12] and increased PDE-5 activity [13] which was reported to be the main cGMP hydrolysing enzyme in rat glomeruli [14].

The beneficial effect of elevating cGMP levels by selective PDE-5 inhibition has been proven in several renal disease models [15–18], including animal models of both type 1 and type 2 diabetes where reduced glomerulosclerosis and proteinuria was shown [19–21]. Although podocytes are postulated to play a pivotal role in the progression of DN [22, 23], and cGMP presumably plays an important role in the regulation of podocyte function [9, 10], the effect of PDE-5 inhibition on podocyte function in diabetes is still unknown.

In our study, we investigated whether the elevation of cGMP levels by pharmacological treatment with vardenafil, a highly selective PDE-5 inhibitor, could preserve podocyte function and reduce podocyte damage in the rat model of streptozotocin (STZ)-induced type 1 diabetes mellitus. We found that vardenafil treatment raised serum cGMP levels and intracellular cGMP levels in podocytes, decreased proteinuria, attenuated podocyte damage and restored nephrin and podocin expression.

**MATERIALS AND METHODS**

**Animals**

Male Sprague–Dawley rats (250–300 g, Charles River, Sulzfeld, Germany) were housed at a constant temperature of 22 ± 2°C with 12 h light/dark cycles, had access to standard rodent chow and water ad libitum.

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All procedures and handling of animals during the investigations were reviewed and approved by the local Ethical Committee for Animal Experimentation.

**Induction of diabetes mellitus**

Type 1 diabetes mellitus was induced in rats with a single intraperitoneal (i.p.) dose of STZ (60 mg/kg) in citrate buffer (0.1 mol/L). Control animals received buffer only. After 72 h, blood glucose level was determined (Accu-Chek®, Roche, Mannheim, Germany). Animals with a random blood glucose level >15 mmol/L were considered as diabetic and were included into the study.

**Experimental groups, treatment protocol**

Diabetic rats were randomized to diabetic control (STZ, n = 6) and vardenafil treatment (STZ-Vard, n = 8) groups. Rats injected with citrate buffer served as non-diabetic controls (Control, n = 7). Diabetic animals were treated for 8 weeks with the selective PDE-5 inhibitor, vardenafil (STZ-Vard group, 10 mg/kg/day dissolved in 0.01 mol/L citrate buffer) or with vehicle (Control and STZ groups) per os in drinking water. The daily water intake was registered and the dose of vardenafil was repeatedly adjusted.

Body and kidney weights were measured at harvest.

**Blood pressure measurements, blood and urine chemistries**

At the end of the treatment period, rats were anaesthetized with ketamine (100 mg/kg) and xylazine (3 mg/kg) i.p. and were placed on controlled heating pads. Arterial blood pressure was recorded by 2 F microtip pressure–volume catheter (SPR-838, Millar Instruments, Houston, TX), and the mean arterial pressure (MAP) was computed.

Blood samples were taken from the inferior caval vein. Urine samples were obtained by sterile puncture of the urinary bladder. Serum glucose and urea levels as well as urine creatinine concentration were determined on a Reflotron analyser (Roche). Urine protein concentration was measured using BCA Protein Assay (Pierce Thermo Scientific, Rockford), and urinary protein/creatinine ratios were calculated.

Serum cGMP levels were determined by enzyme immunoassay (EIA) using a commercial kit (Amersham cGMP EIA Biotrak System, GE Healthcare, Buckinghamshire, UK).

Serum thiobarbituric acid reactive substances (TBARS) as markers of lipid peroxidation were determined by fluorometric assay using a commercial kit (Oxylab TBARS Assay Kit, Zep- tomex, Inc., Buffalo, NY).

**Renal histology and immunohistochemistry**

Periodic-acid Schiff (PAS) staining was performed on formalin fixed, paraffin-embedded kidney samples. Glomerular damage was assessed according to el Nahas et al. [24]. The glomerular score of each animal was derived as the arithmetic mean of 60 glomeruli (×400 magnification). The tubular damage (dilatation, atrophy, hyalin in tubular lumen, infiltration of mononuclear cells and interstitial fibrosis) was assessed as previously described [25].

Immunohistochemistry was performed on paraffin sections, using the avidin–biotin method, as previously described [25]. After antigen retrieval with citric buffer, slides were incubated with primary antibodies [rabbit polyclonal anti-fibronectin, 1:1000, Sigma-Aldrich, Budapest, Hungary; rabbit polyclonal anti-transforming growth factor (TGF)-β1, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA; mouse monoclonal anti-desmin, 1:50, Dako, Frank Diagnostika, Budapest, Hungary; rabbit polyclonal anti-nephrin, Abcam, USA; rabbit polyclonal anti-nitrotyrosine, 1:200, Chemicon/Millipore, Billerica, MA; rabbit polyclonal anti-cGMP 1:2000, AbD Serotec, Düsseldorf, Germany] then with appropriate secondary antibodies (BioGenex, San Ramon, CA) and developed using Fast Red (Dako). Immunohistochemical reactivity (×400 magnification) was evaluated in a blinded manner as described elsewhere [25]: 0, no staining; 1, weak; 2, mild; 3, strong. Fibronectin staining and glomerular cGMP content were quantified as follows: intensity score (1, weak staining; 2, intermediate staining; 3, extensive staining) and area score (1, up to 10% positive cells; 2, 11–50% positive cells; 3, 51–80% positive cells; 4, >80% positive cells) were assessed, and an average
score was calculated for each field of view (intensity score multiplied by area score) [26].

Nephrin and cGMP double immunostaining was performed on frozen kidney sections. Briefly, acetone-fixed sections were rehydrated with phosphate-buffered saline, blocked with 5% serum, incubated with primary antibodies (anti-cGMP, 1:4000, AbD Serotec, and guinea pig polyclonal anti-nephrin, 1:500, Fitzgerald Industries, Acton, MA), then with secondary antibodies (Dylight-488 conjugated donkey anti-guinea pig and Cy3 conjugated donkey anti-rabbit, both at 1:500, Jackson Immunoresearch, West Grove, PA), then washed, mounted and analysed under a fluorescent microscope.

**Immunoblot**

Kidney samples (20 mg) were homogenized in RIPA lysis buffer containing complete protease inhibitor cocktail (Roche). Protein concentration was determined by the BCA assay. Samples were mixed with 2× Laemmli buffer and boiled. Equal amounts of protein (40 µg) were separated on 10% sodium dodecyl sulphate–polyacrylamide gel, transferred to nitrocellulose membranes and blocked with 5% skim milk in Tris-buffered saline, containing 0.1% Tween-20. Membranes were incubated overnight at 4°C with either rabbit polyclonal anti-PDE5a antibody (1:1000, Enzo, Plymouth Meeting, PA) or mouse monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:2000, Millipore, Billerica, MA), washed and incubated with peroxidase-conjugated secondary antibody (anti-mouse IgG or anti-rabbit IgG, 1:2000, Cell Signaling, Danvers, MA). Blots were visualized by ECL detection kit (Pierce Thermo).

**Quantitative RT–PCR**

One hundred milligrams of whole kidneys were homogenized and total RNA was isolated according to the manufacturer’s protocol (SV Total RNA kit, Promega, Madison, WI). Two micrograms of RNA were reverse transcribed (High Capacity cDNA Reverse Transcription kit, Applied Biosystems, Forster City, CA) using random primers. PCR reactions were performed on a BioRad CFX thermal cycler (BioRad, Hercules, CA) using the Power SYBR Green PCR Master Mix (Applied Biosystems). The specificity and efficiency of the PCR reaction was confirmed with the melting curve and standard curve analysis, respectively. Duplicate samples were normalized to GAPDH expression. The mean values are expressed with the formula \(2^{-\Delta\Delta Ct} \). Primer sequences were as follows: TGF-β1 forward: 5’-CACCATCCATGACCCATGAACC-3’; TGF-β1 reverse: 5’-TCATGTTGGACAACTGCTCC-3’; eNOS forward: 5’-TGACCCCTACCGATA-CAACA-3’; eNOS reverse: 5’-CTGGCCTTCTGCTCATTTTC-3’; neuronal nitric oxide synthase (nNOS) forward: 5’-TGACCCTCACCGATA-CAACA-3’; nNOS reverse: 5’-CTGGCCTTCTGCTCATTTTC-3’; nephrin forward: 5’-GCCCTTGGACCATGGTCAATG-3’; nephrin reverse: 5’-GACACCCCTACCGATA-CAACA-3’; podocin forward: 5’-TCCCTTTTTCATTGCTTTGCTG-3’; podocin reverse: 5’-CTTGTGATAGGT-GTCCAGGC-3’; GAPDH forward: 5’-CATGACCCCTTACCGATA-CAACA-3’; GAPDH reverse: 5’-CGCCAGTA-GACTCCACAA-3’.

**RESULTS**

**Vardenafil increased serum cGMP levels in diabetic rats**

Effectiveness of vardenafil treatment was evaluated by serum cGMP levels at harvest. Diabetes tended to reduce serum cGMP levels in non-treated STZ rats when compared with non-diabetic controls, but vardenafil treatment significantly restored, and actually elevated, serum cGMP levels above the range of non-diabetic controls (Table 1).

**Vardenafil did not affect metabolic changes, renal hypertrophy or blood pressure in diabetic rats**

Serum glucose levels and daily water intake rose, while body weight decreased in diabetic rats, regardless of treatment. Kidneys of both vardenafil-treated and non-treated diabetic rats developed significant hypertrophy as shown by increased kidney weight/body weight ratio at the time of harvest (Table 1).

**STZ rats tended to have slightly elevated serum urea levels, but the difference was statistically not significant. There was no difference in the MAP values among the groups at harvest (Table 1).**

**Vardenafil decreased the extent of glomerular remodelling, proteinuria, fibronectin and TGF-β1 expression in diabetic rats**

When compared with controls, glomerular hypertrophy, mild mesangial expansion and adhesions to Bowman’s capsule were observed in diabetic rats, which were ameliorated by vardenafil treatment (Figure 1A–E). The tubulointerstitial lesions of STZ rats were characterized by tubular dilatation and atrophy, which was significantly ameliorated by vardenafil treatment. Mononuclear cell infiltration was not seen in control kidneys, and only to a minimal extent in diabetic kidneys regardless of treatment protocol (data not shown).

Diabetes led to significantly elevated urine protein/creatinine ratio in STZ rats when compared with healthy controls. Vardenafil-treated rats, however, had lower urinary protein/creatinine ratio when compared with STZ rats (Figure 1F).

Fibronectin expression, evaluated by immunohistochemistry, was augmented in STZ rats, but significantly reduced by vardenafil treatment (Figure 2A–C, G and H).

Both glomeruli and tubulointerstitium of STZ kidneys showed strong TGF-β1 immunostaining (Figure 3A–C), which was significantly reduced after vardenafil treatment (Figure 3G and H).

According to immunostaining results, TGF-β1 mRNA expression in whole kidney homogenates was significantly

**Statistics**

All the data are presented as mean ± SD. In the case of the functional measurements and blood chemistries, differences between the groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. Histology, immunohistochemistry and PCR data were analysed using the Kruskal–Wallis test followed by Dunn’s test. The level of significance was set to P < 0.05.
higher in non-treated STZ rats when compared with non-diabetic controls. Vardenafil treatment normalized the mRNA expression to similar levels as seen in non-diabetic controls (Figure 4A).

**Vardenafil restored cGMP content in podocytes and decreased podocyte damage in diabetic kidneys**

Immunoblot analysis revealed renal PDE-5 overexpression in both non-treated and treated diabetic rats when compared with controls (P < 0.05, Figure 5).

Immunostaining revealed significant glomerular cGMP content in non-diabetic kidneys, mostly localized to podocytes (Figure 2D, see arrows and inset picture) and to a less extent to endothelial cells. Decreased glomerular cGMP staining in STZ rats was almost restored by vardenafil treatment (Figure 2E–F, and I). Tubular cGMP staining showed similar results, which indicates that vardenafil could preserve intracellular cGMP levels in diabetic kidneys (Figure 2J). Double immunostaining depicted remarkable glomerular co-localization of cGMP and nephrin in controls and STZ-Vard.

**Table 1. Serum cGMP levels, metabolic parameters, kidney weights, serum urea levels and MAP were measured at the time of harvest**

<table>
<thead>
<tr>
<th></th>
<th>Serum cGMP (pmol/mL)</th>
<th>Serum glucose (mmol/L)</th>
<th>Body weight (g)</th>
<th>Kidney weight/body weight (g/g)</th>
<th>Serum urea (mg/dL)</th>
<th>MAP (mmHg)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>14.3 ± 9.5</td>
<td>12.3 ± 4.0</td>
<td>349 ± 27</td>
<td>0.46 ± 0.05</td>
<td>53.1 ± 9.8</td>
<td>77.2 ± 8.1</td>
</tr>
<tr>
<td>STZ</td>
<td>7.5 ± 1.4</td>
<td>33.6 ± 5.3*</td>
<td>332 ± 35*</td>
<td>0.64 ± 0.04*</td>
<td>73.2 ± 16.7</td>
<td>72.8 ± 5.1</td>
</tr>
<tr>
<td>STZ-Vard</td>
<td>26.9 ± 13.3*</td>
<td>35.5 ± 8.5*</td>
<td>317 ± 71*</td>
<td>0.66 ± 0.13*</td>
<td>64.1 ± 13.9</td>
<td>77.6 ± 12.8</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
MAP, mean arterial pressure.
*P < 0.05 versus Control.
#P < 0.05 versus STZ (ANOVA).

**FIGURE 1:** Kidney histology and urinary protein/creatinine ratio at harvest. Representative photomicrographs of PAS-stained kidneys (×400 magnification) show normal glomerular structure in the control (A), mild mesangial expansion and attachment to Bowman’s capsule in the STZ (B) and normal structure in the STZ-Vard (C) groups (bar represents 50 μm). (D–E) Glomerular and tubulointerstitial damage index scores from each group are shown. (F) Urinary protein/creatirnine ratio (mg protein/mg creatinine) is shown. Data are presented as mean ± SD.
*P < 0.05 versus control; #P < 0.05 versus STZ (Kruskal–Wallis).
FIGURE 2: Immunostaining for fibronectin and intracellular cGMP was performed. Representative photomicrographs of fibronectin–stained glomeruli and tubulointerstitium (×400 magnification) show mild staining in the control (A), increased glomerular and tubulointerstitial fibronectin staining in the STZ (B) and mild fibronectin staining in the STZ-Vard (C) groups (bar represents 50 μm). Glomerular (G) and tubulointerstitial (H) fibronectin immunoreactivity scores from each group are shown. (D–F) Representative photomicrographs of glomerular cGMP staining (×630 magnification) where arrows point on podocytes (inset: podocytes are further magnified to ×1000). The intensive immunoreactivity in control, presumably localized to podocytes (arrows) and endothelial cells (D), was almost absent in the STZ (E) but restored in the STZ-Vard (F) groups (bar represents 50 μm). Evaluation of cGMP staining in glomeruli (I) and tubular cells (J). Data are presented as mean ± SD. *P < 0.05 versus Control; #P < 0.05 versus STZ (Kruskal–Wallis).
FIGURE 3: Immunostaining for TGF-β1, desmin and nephrin were performed in paraffin-embedded kidney sections. Representative photomicrographs of TGF-β1-stained glomeruli and tubulointerstitium (×400 magnification) show no staining in control (A), strong glomerular and mild tubulointerstitial TGF-β1 expression in the STZ (B) but only weak TGF-β1 expression in the STZ-Vard (C) groups (bar represents 50 μm). Glomerular (G) and tubulointerstitial (H) TGF-β1 staining scores from each group are shown. Desmin and nephrin immunostaining were performed to evaluate podocyte damage. Representative photomicrographs of desmin-stained glomeruli (×630 magnification) show no desmin staining in the control (D), strong desmin expression in podocytes of the STZ (E) and only mild desmin expression in podocytes of the STZ-Vard (F) groups (bar represents 50 μm). Desmin (I) and nephrin (J) staining scores from each group are shown. Data are presented as mean ± SD. *P < 0.05 versus control; #P < 0.05 versus STZ (Kruskal–Wallis).
Compared with non-diabetic controls, cGMP staining intensity of STZ rats was reduced, and cGMP was practically absent in nephrin-positive podocytes. In contrast, podocytes of vardenafil-treated rats depicted apparently elevated cGMP content when compared with non-diabetic controls, but restored after chronic vardenafil treatment (Figure 6). In addition, 2–5% of cGMP-positive glomerular area was not co-localized with nephrin in the capillary loops, representing presumably endothelial cGMP staining (Supplementary data, Figure S1A–D).

Desmin and nephrin immunostaining were performed to evaluate the extent of podocyte damage [27]. Desmin staining was stronger in the STZ group than in non-diabetic controls. Vardenafil significantly reduced desmin expression to an intermediate level between the STZ and the control levels (Figure 3D, E and I). In contrast, nephrin staining was reduced in STZ rats when compared with non-diabetic controls, but restored after chronic vardenafil treatment (Figure 3J). During progression, podocin and nephrin expressions usually become reduced in podocytes, and that is accompanied by increased proteinuria. In our study, both nephrin and podocin mRNA expression levels in diabetic rats were reduced by ~50% when compared with non-diabetic controls, but vardenafil treatment restored nephrin and podocin mRNA expression to normal levels (Figure 4B and C).

Vardenafil had no effect on oxidative stress markers of diabetic rats

Diabetic kidneys presented strong nitrotyrosine immunoreactivity both in glomeruli and in tubulointerstitium as a marker of local nitrosoxidative stress [28]. Vardenafil had no significant influence on the amount of renal nitrotyrosine formation (Table 2). Serum TBARS levels (which reflect the amount of systemic lipid peroxidation) did not show significant difference among the groups (Control: 1.18 ± 0.12; STZ: 1.00 ± 0.15; STZ-Vard: 0.89 ± 0.10 MDA unit/mL; n.s., ANOVA).

Expression of nNOS mRNA was significantly increased in diabetic groups regardless of treatment protocol (Table 2). Although non-treated diabetic kidneys expressed slightly higher levels of eNOS mRNA than non-diabetic controls, the differences were not statistically significant and vardenafil did not alter eNOS expression (Table 2). The mRNA expression of iNOS was similar in all groups (Table 2).

DISCUSSION

DN is characterized by oxidative stress, podocyte damage and glomerulosclerosis, accompanied by increased PDE-5 activity which exacerbates NO-cGMP pathway dysfunction. PDE-5 inhibition has been shown to ameliorate experimental DN, yet the role of podocytes in this mechanism remains unclear. The
present study provides the first in vivo evidence that pharmacological treatment with selective PDE-5 inhibitor vardenafil attenuates renal damage in type 1 diabetic rats by raising intracellular cGMP content of podocytes, and restoring nephrin and podocin expression. Based on the results, we suggest that disturbances of NO-cGMP pathway due to PDE-5 overactivity in diabetes might significantly account for pathological changes in podocytes.

Vardenafil is a highly selective inhibitor of PDE-5 that causes accumulation of NO-driven cGMP. Vardenafil has been successfully used in the treatment of erectile dysfunction of diabetic patients [29], yet its effects on podocytes have not been evaluated.

An increasing number of studies suggest that reduced NO production in diabetes results in glomerular damage, yet the molecular mechanisms remain unclear [30]. TGF-β1 plays a crucial role in the progression of glomerulosclerosis [31, 32], characterized by the accumulation of extracellular matrix (ECM) components such as fibronectin [25, 33]. In mesangial cells, hyperglycaemia induces the production of latent TGF-β1 [34] and thrombospondin-1 (TSP-1), an activator of latent TGF-β1 [35]. In our study, vardenafil attenuated mesangial expansion, tubular damage, TGF-β1, and fibronectin expression, corroborating the recent report of Kuno et al. [21] on sildenafil-treated type 2 diabetic rats.

Accumulating evidence suggests a major role for podocytes in the pathogenesis of DN [4, 36]. Podocytes stimulated by high glucose and proteinuria may induce AngII-dependent TGF-β1 expression of mesangial cells [4, 37]. Albumin directly stimulates TGF-β1 production in podocytes [38], while high

Table 2. Results of renal immunohistochemistry and quantitative RT–PCR (qPCR) studies

<table>
<thead>
<tr>
<th>Nitrotyrosine</th>
<th>qPCR of NO synthase isoforms</th>
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<tr>
<td>Glomerular</td>
<td>Tubular</td>
</tr>
<tr>
<td>Control</td>
<td>0.19 ± 0.08</td>
</tr>
<tr>
<td>STZ</td>
<td>0.64 ± 0.07*</td>
</tr>
<tr>
<td>STZ-Vard</td>
<td>0.56 ± 0.14*</td>
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Light microscopy was used at magnification of ×400 for semiquantitative scoring of immunostaining. qPCR results were normalized to GAPDH and expressed using the formula $2^{-\Delta\Delta CT}$. All data are presented as mean ± SD.

*P < 0.05 versus control.

#P < 0.05 versus STZ (Kruskal–Wallis).

FIGURE 6: Localization of nephrin and cGMP on podocytes with dual immunofluorescent labelling. Nephrin staining depicts podocytes in glomeruli of control, STZ and STZ-Vard rats (green colour, left pane). Glomerular cGMP is abundantly detected in control and STZ-Vard kidneys, while it is almost absent in glomeruli of non-treated STZ rats (red colour, middle pane). The dual-labelling (yellow colour, right pane) shows co-localization of cGMP and nephrin and indicates that diabetes and vardenafil treatment influence cGMP content of podocytes (×630 magnification, bar represents 50 μm).
glucose increases podocyte sensitivity to ambient TGF-β1 [39] and induces TSP-1 production in podocytes [40]. Thus, podocytes may contribute to the activation of latent TGF-β1 secreted by mesangial cells in diabetes. Finally, mesangial TGF-β1 overproduction in diabetes [34, 39, 41] may, in return, promote podocyte damage, effacement [42] and apoptosis [43].

In diabetic kidneys, podocytes show cytoskeletal redistribution and widening of foot processes along with reduced nephrin and podocin expression [4, 36]. Nephrin and podocin are major components of the slit diaphragm between foot processes, and their loss contributes to pathological filtration, leading to proteinuria [44, 45]. We found reduced nephrin and podocin expression and significant proteinuria in kidneys of non-treated diabetic rats, while vardenafil treatment restored nephrin and podocin expression and reduced proteinuria almost to the normal level.

We suggest that these beneficial effects of vardenafil on podocytes in vivo were due to an increase in intracellular cGMP level, as shown by immunostaining. Compared with podocytes, the minor changes in glomerular endothelial cGMP immunoreactivity suggest that it contributed marginally to the glomerular effects of vardenafil. In podocytes, cGMP regulates the filtration barrier by modulating the slit membrane and cytoskeletal protein reorganization [9, 10]. In mesangial cells, cGMP-dependent protein kinase (PKG) inhibits glucose-induced TSP-1 expression [46] and vardenafil was reported to decrease TSP-1 expression in anti-Thy1 nephritic rats [47]. Therefore, decreased cGMP levels in diabetes might account for restored nephrin and podocin expression and proteinuria. We suggest that the preservation of podocyte cGMP content significantly accounts for restored nephrin and podocin expression. In addition, restoring podocyte and circulating cGMP levels might reduce the glomerular activation of latent TGF-β1, as supported by reduced glomerular TGF-β1 immunostaining.

PDE-5 inhibition might also improve diabetic kidney disease by its potential anti-inflammatory, anti-oxidative or haemodynamic effects. Experimental renal mass reduction studies have shown that sildenafil, another selective PDE-5 inhibitor, may reduce inflammatory infiltration and oxidative stress [15]. In our study, however, vardenafil had no effect on renal mononuclear cell infiltration. One explanation may be that in our model, tubulointerstitial infiltration was not significant in non-treated diabetic animals when compared with non-diabetic controls. Secondly, different PDE-5 inhibitors may exert different effects on inflammation [48]. In fact, Hohenstein et al. [47] have recently reported that vardenafil administration did not affect renal inflammatory cell influx in experimental glomerulonephritis.

In diabetes, not only high glucose but also the increased generation of ROS may contribute to augmented AngII production in podocytes [4], leading to mesangial TGF-β1 overproduction. It has been reported that intracellular cGMP accumulation reduce oxidative injury of the tissues in diabetes [49] and sildenafil reduces superoxide generation [50]. We have therefore postulated that vardenafil would decrease oxidative stress in the kidneys. In contrast to our expectations, we could not confirm the potential antioxidative effect of vardenafil in our model. Neither serum TBARS concentration nor renal nitrotyrosine immunostaining showed significant differences in any diabetic groups studied.

Potential renal haemodynamic changes would also be expected due to PDE-5 inhibition. The elevated serum cGMP level presumably covered the biological effects of NO; nevertheless, the NO production remained unchanged. This may raise the question whether changes in cGMP levels could interfere with blood pressure and, consecutively, with renal damage. As the MAP did not differ considerably among treated and non-treated rats, the beneficial effects of vardenafil on renal pathology were independent of blood pressure.

Nitric oxide causes vasodilatation in the afferent arterioles [8] which leads to glomerular hypertension and hyperfiltration. Elevating the levels of cGMP, the effector of NO, by vardenafil administration would be therefore expected to aggravate glomerular hyperfiltration. Recent studies, however, do not confirm this hypothesis. In the study of Lau et al. [19], vardenafil administration did not induce hyperfiltration in diabetic rabbits, and sildenafil treatment of type 2 diabetic rats was reported to attenuate hyperfiltration [21] instead of aggravating it. In our study, relative kidney weights were not different in diabetic groups regardless of vardenafil treatment, suggesting that PDE-5 inhibition with vardenafil did not influence renal hyperfiltration.

These findings suggest that the observed beneficial effects of vardenafil on podocytes in vivo were mostly due to an increase in intracellular cGMP content.

We conclude that in the STZ model of diabetes, pharmacological inhibition of PDE-5 by vardenafil raised serum cGMP levels, preserved intracellular cGMP levels of podocytes and podocyte function by restoring nephrin and podocin expression. The beneficial effects of selective PDE-5 inhibition on the progression of DN were independent of blood pressure or the extent of nitrosative stress. Our findings pinpoint the podocytes as target cells that suffer NO-cGMP signalling disturbances in diabetes. The observation of the beneficial effect of restoring cGMP levels on podocyte and renal damage in type 1 diabetes might suggest the possible use of vardenafil as a new regimen in the treatment of DN.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. ERA-EDTA. ERA-EDTA Registry Annual Report 2008. Amsterdam, The Netherlands: Academic Medical Center, Department of Medical Informatics, 2010


42. Dessapt C, Baradez MO, Hayward A et al. Mechanical forces and TGFbeta1 reduce podocyte adhesion through alpha3beta1 integrin downregulation. Nephrol Dial Transplant 2009; 24: 2645–2655


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