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Endothelial Rehabilitation: The Impact of Chronic PDE5 Inhibitors on Erectile Function and Protein Alterations in Cavernous Tissue of Diabetic Rats

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Abstract

Background: Diabetic men generally have reduced efficacy with PDE-5 inhibitors (PDE5i) for the treatment of erectile dysfunction (ED).

Objective: To determine whether chronic vardenafil exposure alters cavernous protein expression predicting improved erectile function in diabetes.

Design: Forty-two adult male Sprague Dawley rats with streptozotocin-induced (50 mg/kg IP) diabetes for 4 wk, were exposed to either vehicle or vardenafil for 6 wk. Assessments compared the impact of vardenafil given at 1 h and 20 h to erectile function and cellular alterations and downstream translation of cavernous protein profiles were aimed.

Intervention: Vehicle or vardenafil 0.5 mg/kg/day by oral gavage for 6 wk.

Measurements: Erectile function, penile tissue morphology, protein expression and surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI) protein profiling were determined.

Results and Limitations: A significant increase of intracavernous pressure was seen in both treatment arms compared to diabetic rats not receiving vardenafil. Immunohistochemical staining showed improved endothelial and smooth muscle cell staining with chronic vardenafil use. Western blot analysis demonstrated increased endothelial cell eNOS and smooth muscle α-actin protein content. SELDI protein profiling showed enhanced proteins expression at molecular weights of 14.7, 20, 41.9, 66.2, and 83.9 kDa in the chronically treated vardenafil group.

Conclusions: Vardenafil was effective in treating diabetic-induced ED with the greatest effect achieved through chronic dosing, with no additive effect measured with the final acute dose. Changes noted in the histology and protein expression indicate that vardenafil may have a protective effect in this disease state. This finding may serve as a basis for further work evaluating the utility of chronic vardenafil dosing in diabetic men.

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1. Introduction

Erectile dysfunction (ED) is common in the diabetic population. Diabetic men suffer from ED at a younger age and with a greater frequency compared to nondiabetics, with prevalence rates of 20% to 75% [1]. Normal erectile function requires intact neural and vascular pathways, both of which are impaired in diabetes. Nitric oxide (NO) production, the key process of a normal erection, is primarily affected by diabetes-induced endothelial dysfunction [2]. Additionally, hyperglycemia leads to oxidation of low density lipoprotein and increased production of free-oxygen radical species, leading to smooth muscle dysfunction [3]. Direct inactivation of NO, largely by a superoxide anion believed to be present in higher than normal concentrations in diabetes, may also play a role in producing impaired cavernosal relaxation and ED [4].

Initiation of penile erection is dependent on release of NO from penile distal nerve terminals, which stimulates the production of cyclic GMP (cGMP) and results in smooth muscle relaxation. cGMP is normally metabolized by the cGMP-specific type 5 phosphodiesterase (PDE5), which is the major isozyme metabolizing cGMP in the corpus cavernosum. Inhibition of PDE5 potentiates the cGMP smooth muscle relaxing signal and is a proven pharmacologic agent for the treatment of ED across a broad range of etiologies and ages [5]. Although the efficacy of PDE5 inhibitors (PDE5i) in a general population is 70%–89%, only 50% of diabetic men with ED respond favorably [6]. This relative treatment resistance to PDE5i has sparked new research to develop strategies to treat diabetic men with ED. It is hypothesized that chronic daily dosing of PDE5i may act through enhancing endothelial rehabilitation in vascular causes of ED, as shown in human and animal models [7–9]. The efficacy and mechanism by which this strategy may impact diabetic patients with ED has yet to be elucidated. This study utilizes a diabetic rat model to gain further insight into the questions of whether chronic PDE5 inhibitor exposure alters cavernous protein expression and whether these changes lead to improved erectile function.

2. Materials and methods

2.1. Diabetic animal model

Forty-two adult male Sprague Dawley rats aged 20–24 wk received streptozotocin (50 mg/kg ip) in pH 4.5 citric acid buffer to induce diabetes. Rats that tested positive for hyperglycemia by tail vein sampling at 2 d were used in the study. After a 4-wk conditioning treatment-free period, the diabetic rats were assigned randomly to an experimental group [Fig. 1]. Control 1 (C1) rats were studied for ED following 4 wk of diabetes, with 7 rats receiving an acute dose of vardenafil (0.5 mg/kg) by oral gavage 1 h prior to erectile function testing (C1/VD). Control 2 (C2) rats were given an additional 6 wk of water gavage, with 7 rats receiving an acute dose of vardenafil (0.5 mg/kg) by oral gavage 1 h prior to erectile function testing (C2/VD). The treatment groups composed of 7 rats in each arm, with both arms receiving chronic vardenafil (0.5 mg/kg) by oral gavage daily for 6 wk but erectile function testing was carried out either at 1 h (T) or 20 h (T/VD) after the last dose of vardenafil.

2.2. Evaluation of erectile function

Erectile function was assessed via cavernous nerve electro-stimulation as described previously [10]. Intracavernosal pressure was evoked with 0.2 ms pulses of 2 mA at 20 Hz for a 40 s duration and recorded using LabVIEW 7 Express software (National Instruments, Austin, Texas). Three electro-stimulations were replicated at intervals of 10 m. All treatments were stopped at least 20 h before evaluation of erectile function. The final dose of vardenafil was given either 20 h preceding the electro-stimulation experiments for group T or 1 h for groups C1/Vd, C2/Vd and T/VD.

The rats were sacrificed using pentobarbital (200 mg/kg iv) and then penile tissue was harvested for analysis. The animal experimental protocol was approved by the University Council on Animal Care Animal Use Subcommittee, University of Western Ontario, London, Canada.

2.3. Immunohistologic analysis

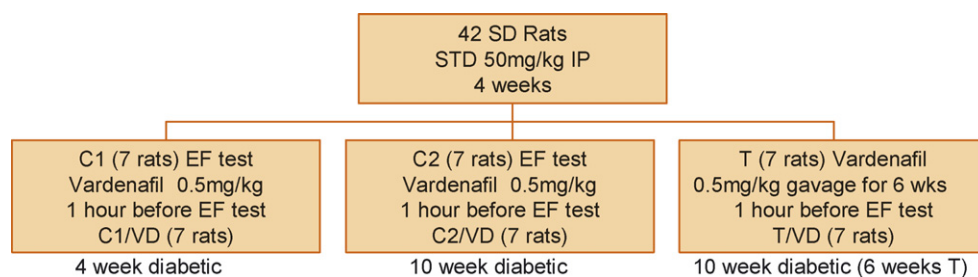Penile tissue was fixed in cold fresh 2% formaldehyde, cryo-protected in 30% sucrose, then embedded in OCT compound

Fig. 1 – Animal distribution.

(Tissue-Tek, Sakura,USA) and stored at −70 °C as described previously [10]. 5 μM sections were immunostained with primary antibody: neuronal nitric oxide synthase mouse anti-nNOS, endothelial cell marker mouse anti-CD31 (Transduction Lab Mississauga, Canada), mouse anti-α-actin (Lab Vision, Fremont, CA), secondary antibody biotin conjugated goat anti-mouse IgG (Sigma, St.Louis, MO, USA), and anti-biotin clone BN-34 peroxidase conjugate IgG fraction (Sigma)). Histologic examinations were performed using a Zeiss microscope with a computerised imaging system (Northern Eclipse, Empix, Canada) by a blinded reviewer. Positive staining of nNOS in dorsal nerve was counted. Positive staining of CD31 within the major penile sinusoidal lining was measured under 400× power. The area of positive staining of smooth muscle α- actin was calculated as the ratio of total sectional area under 25× power in 4 duplicate sections of each sample.

2.4. Protein preparation

The inner layer of penile crus was harvested under optical magnification and washed, frozen on dry ice, and stored at −70 °C. Frozen tissue was thawed and washed once with red blood cell lysis buffer and PBS. The pellet was homogenized in a small volume of tissue protein extracting buffer (pH 7.2, Pierce, Rockford, Illinois, USA) supplemented with protease inhibitor (Roche, Penzberg, Germany). All procedures were carried out on ice using an Ultra Turrax homogeniser at high speed. Insoluble material was pelleted by centrifugation (10,000 rpm, 5 m, 4 °C). The soluble fraction was collected and protein concentration was determined by using a BCA protein assay according to the manufacturer's protocol (Pierce, Rockford, Illinois, USA).

2.5. Protein expression profiling

Proteins were analyzed using surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS, Ciphergen Biosystems, Fremont, CA, USA). A CM10 (weak cation-exchange) protein chip 8-spot array was pre-washed with 50ul of pH 4 0.1 M sodium acetate binding buffer for 5 m on a shaker, 5 μg of protein was added to the designated spots of a CM10 chip with 95 μl binding buffer using a bio-processor and incubated at RT for 1 h with vigorous shaking on DPC MicroMix5 (DPC Los Angeles, CA). The protein-bound CM10 arrays were then washed and air-dried. Each spot was spotted with 1 μl of a saturated solution of sinapinic acid (50% acetonitrile, 0.5% trifluoroacetic acid) (Sigma) and repeated once. Dried protein chips were placed in the SELDI ProteinChip System Series 4000 Reader using an automated data collection protocol.

2.6. Western blotting

5 μg of protein was separated on 10% polyacrylamide gel electrophoresis and transferred to Hyboud-ECL nitrocellulose membrane (Amersham, Piscataway, NJ, USA). Mouse anti smooth muscle α-actin, eNOS, caveolin-1 and β-actin were used as primary antibodies. Goat anti-mouse horseradish peroxidase-conjugated IgG) (Santa Cruz, CA) was the second-ary antibody. Antibody specific bands were detected using enhanced chemiluminescence (ECL) reagents (Amersham Biosciences) visualized and analysed by using Chemi Genius² Gel Documentation System (PerkinElmer, Quebec, Canada). Each densitometric value was normalized against the loading control of β-actin densitometric value.

2.7. Statistical analysis

Values are expressed as the mean plus and minus standard error of 7 experiments for each group. Data were compared using one way ANOVA. SELDI-TOF-MS spectra were normalized on total ion current, with normalization coefficients 95% confidence interval. Subsequently, each spectrum was analyzed using the Ciphergen Express™ 3.0 software, Biomarker Wizard.

3. Results

3.1. Body weight, serum triglycerids, and cholesterol

The animals in all six groups showed persistent hyperphagia, polydipsia, and polyuria consistent with poorly controlled diabetes. No differences in body weight or degree of hyperglycemia were measured between control and treatment groups. Interestingly, in spite of similar elevations in glucose levels between C1 and C2, the serum triglyceride levels were significantly higher in C2, which may reflect the extended study period of this group. The vardenafil treatment group showed lower concentrations of serum triglycerides compared with C2, but did not reach statistical significance due to high internal variability among samples in the C2 group [Table 1].

3.2. Penile response

The area under the curve of penile intracavernous pressure (ICP) following electro-stimulation of the cavernous nerve is depicted in Fig. 2. Penile pressures (PP) at 4 wk (C1) and 10 wk (C2) were 2085 ± 143 and 2218 ± 1 64, compared with 3542 ± 231 (C1/Vd), 2942 ± 189 (C2/Vd) ($p < 0.05$) with a single dose of vardenafil 1 h before the erectile function test. Following 6 wk of daily vardenafil, PP were 3223 ± 193 (T) and 3321 ± 1 89 cmH2O second (T/Vd). There was no additional increase of ICP with an acute dose of vardenafil (T/VD). The 10-wk diabetic rats had a smaller increase in PP following an acute dose vardenafil.

3.3. Immunohistologic results

There was no difference of nNOS staining among the control and treatment groups, therefore the results

Table 1 – Body weight, serum triglycerides and cholesterol

	C1 (4 wk)	C2 (10 wk)	Treatment (10 wk)
Body weight (g)	429.5 ± 4.8	434.2 ± 3.9	424.9 ± 2.9
Serum triglycerides (mM/l)	$2.15 \pm 0.9^{*}$	$5.56 \pm 2.3^{*}$	2.99 ± 0.8
Serum cholesterol (mM/l)	1.34 ± 0.1	1.06 ± 0.9	1.24 ± 0.1

* $p < 0.05$, C1 compared with C2.
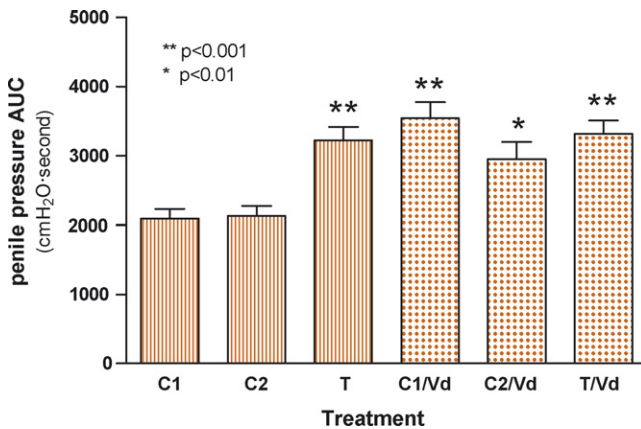
Fig. 2 – Penile pressure measured in response to electro-stimulation of the cavernous nerve. Bar graph shown the ICP area under curve obtained from seven animals in triplicate of mean ± SE. A significant difference was measured between diabetic animals (C1,C2) and diabetic animals exposed to an acute (C1/Vd, C2/Vd) or chronic (6 wk) dose of vardenafil.

Table 2 – Summary of immunohistological results

	C1	C2	Treatment
% Endothelial cell (CD31)	21.8 ± 6.4	22.8 ± 8.7	$50 \pm 9.3^{*}$
% smooth muscle α-actin	6.6 ± 1.0	$6.1 \pm 0.9^{*}$	$8.4 \pm 0.8^{*}$

The proportion of positive staining of endothelial cell marker CD31 within the major penile sinusoids (power 400×) was significantly increased in the treatment group. The proportion of positive staining of smooth muscle α-actin to the cross-sectional area (power 25×) was significantly increased in the treatment group compared with C2. $^{*}p < 0.05$.

are not reported.. The treatment group showed a significantly higher positive staining for α-actin compared to the 10-wk nontreated diabetic rats (C2) [Table 2].

Penile cavernosum sinusoidal endothelial cell staining was altered in all groups to varying degrees. Among the animals receiving vardenafil, greater intensity of endothelial marker CD31 was measured [Fig. 3]. The proportion of positive staining of CD31 within the major penile sinusoidal lining was significantly increased in the treatment group [Table 2].

3.4. Western blot results

Western blot analysis of smooth muscle α-actin, eNOS and caveolin-1 from penile tissue extracts demonstrated strong bands of 40 kDa (smooth muscle α-actin) and 140 kDa (eNOS) in treatment groups compared with C2 [Fig. 4]. There was a significant increase of densitometric value of both smooth muscle α-actin and eNOS in the treatment groups and this was supported by the immunohistologic results. These results indicate that PDE5i have a positive impact in preventing endothelial cell damage and preserving smooth muscle content, which is proportional to the preservation of erectile function.
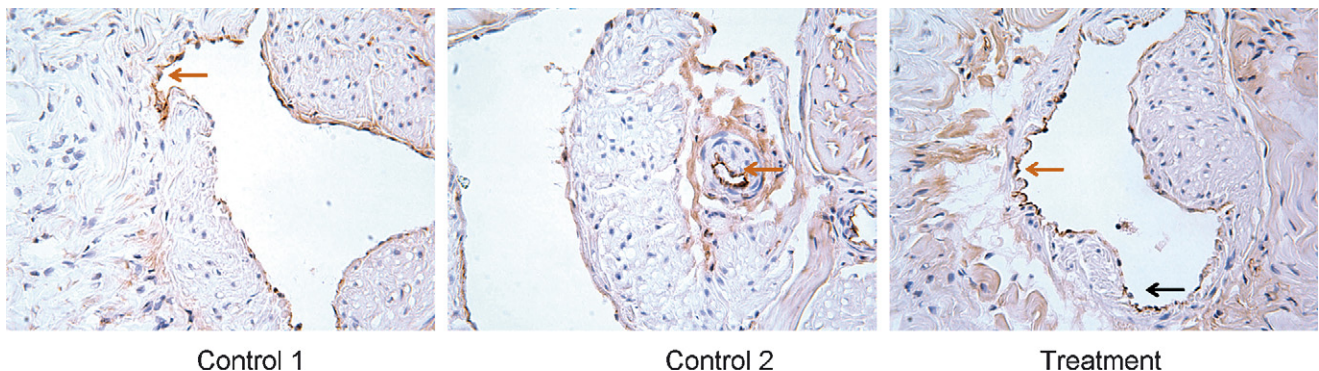

Fig. 3 – Representative slides of immunostaining of corpora cavernosa with endothelial cell marker CD31. Note the strong positive staining (dark brown) in the endothelium lining the cavernous sinusoid in the treatment group compared to control (power 400×).
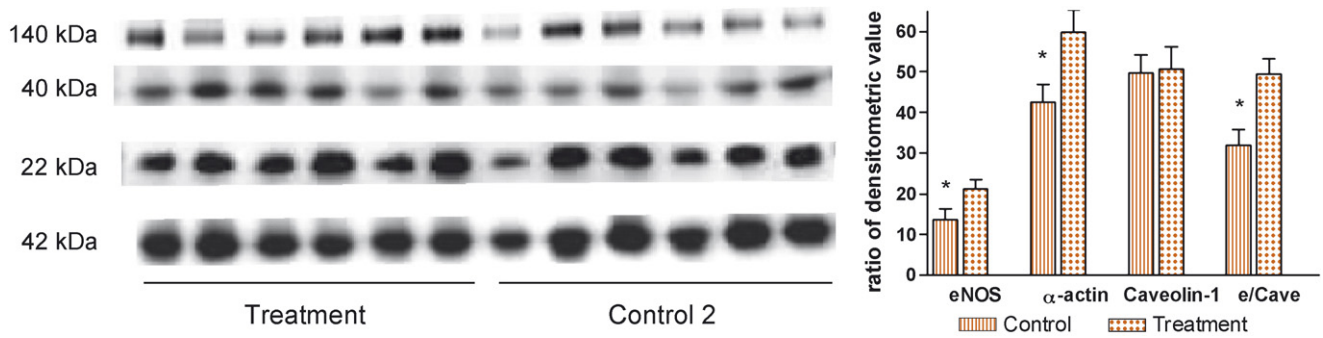
Fig. 4 – Western blot analysis of penile tissue extracts. Left blot shows 140 kDa eNOS, 40 kDa smooth muscle α-actin, 22 kDa Caveolin-1, and 42 kDa β-actin (protein loading control). Right panel shows the percentage of protein densitometric value over β-actin of 7 penile tissue extracts from each group. The treatment group demonstrated higher expression of α-actin, eNOS content and eNOS/Caveolin-1 (e/Cave) *: $p < 0.05$.

3.5. Protein expression profile

SELDI mass identification was performed by averaging a minimum of 10 laser shots at the laser intensity of 4000 to various regions of the protein chip surface. Fig. 5 compares the gel spectral view of proteins from C2 and the treatment group, with masses ranging from 35 to 75 kDa. Increased intensity of the 41.9 kDa protein peak in the treatment group was observed [Table 3].

4. Discussion

Diabetes is a systemic disorder resulting in metabolic derangements leading to eventual end organ damage, including ED. Prolonged periods of high circulating serum glucose increase the concentration of free oxygen radicals and advanced glycosylation end products, which may lead to vascular and neural impairmen [11]. Penile erection is dependent on intact vascular and neural pathways, which become deranged in diabetes, making diabetes a significant risk factor for development of erectile dysfunction. Unfortunately, the clinician treating diabetic men with erectile dysfunction has few evidence-based studies to support strategies designed to adequately prevent and treat diabetes-induced ED, apart from recommending tight blood sugar control.

PDE5i are first-line oral therapy for the management of ED across a wide range of aetiologies and
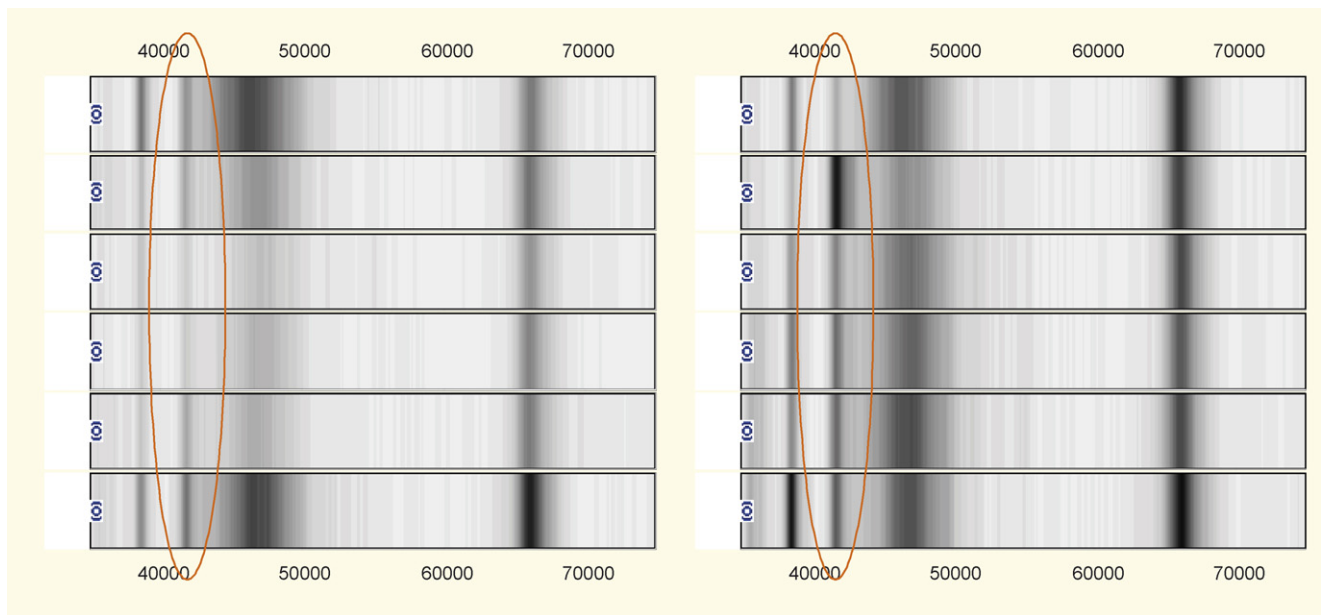

Fig. 5 – Gel spectral view of the SELDI penile tissue protein profile using the CM10 chip array. Six samples from C2 (left) and Treatment group (right). Spectra from 35 to 75 kDa demonstrates an increased intensity of molecular weight 41.9 kDa protein moiety in the Treatment group compared with C2 group.

Table 3 – SELDI protein profile

M/Z kDa	C2	Treatment	P-Value
14.7	167 ± 56	269 ± 64	0.013
20.0	6.6 ± 2	11.7 ± 8.7	0.034
41.9	3.4 ± 1.2	5.6 ± 1.9	0.025
66.2	5.8 ± 1.3	7.5 ± 1.3	0.034
83.9	0.4 ± 0.2	0.7 ± 0.2	0.013

The SELDI protein intensity profile of Control 2 (C2) vs. treatment (T) group. Each spectrum was analyzed using the Ciphergen Express™ 3.0 software, Biomarker Wizard.

ages, with some reports demonstrating that over 90% of ED patients choose PDE5i as their primary treatment [12,13]. Sildenafil, tadalafil, and vardenafil have all undergone extensive testing and have consistently demonstrated good response rates in most cohorts of patients. However, diabetic men with ED tend to have an impaired PDE5i response compared to other cohorts in clinical trials [14]. Epidemiologic studies have shown an increasing prevalence of obesity [15,16], making diabetes-associated ED a possible epidemic in North America. Therefore, there is a need to develop new treatment strategies for men suffering with diabetes-associated ED.

In this study, we explored the potential effect of a PDE5i, vardenafil, on the erectile mechanism utilizing a diabetic rat animal model. We wanted to answer the following question: Does acute versus chronic exposure to PDE5i in diabetes have measurable differences in erection quality, and if so, can protein analysis identify a biomarker responsible for this effect? We showed that vardenafil had affects on more than just erectile function. In this animal model, rats that were diabetic for 10 wk had higher triglyceride levels than those that were diabetic for 4 wk. Additionally, vardenafil-treated rats had lower serum triglyceride levels compared to rats not receiving the drug, which supports a previously reported observation [10]. There are also reports that another PDE inhibitor, the PDE3i (cilostazol), improves endothelial function and reduces serum triglyceride levels in animals and in human clinical trials [17,18] by increasing cAMP and PKA signaling. Epidemiologic studies demonstrate that men with ED are at a higher risk of having dyslipidemia, including hypertriglyceridemia [19] and animal study implicated hypertriglyceridemia as a potential etiology of ED [20]. The clinical significance of the PDE5i effect on lipid profile remains to be answered.

Diabetes-associated ED is a complicated pathological process that affects erectile function at a very early stage [10,21] There is abundant evidence to support the theory that endothelial cell damage is the primary cause of diabetes-associated ED [22,23].

This was supported in our study, where the diabetic control rats showed a significant loss of cavernous sinusoidal endothelial lining by immunohistologic staining and protein contents by western blot [Table 2, Figs. 3 and 4]. There was a protective effect demonstrated in rats receiving daily vardenafil, with improved endothelial and smooth muscle cell staining on immunohistochemistry [Table 2, Fig. 3] and increased endothelial cell eNOS and smooth muscle α-actin protein content on western blot analysis [Fig. 4]. Our findings are consistent with those of Schäfer et al, who reported that chronic treatment of diabetic rats with sildenafil resulted in a significant improvement of endothelium-dependent as well as -independent vasorelaxation, even more than 24 h after the last dosing [24]. Additionally, chronic usage of PDE5i may also benefit the host across a wide range of nonvascular therapeutic indications such as high altitude sickness to Peyronie's disease [25]. As expected, rats that received vardenafil, either acutely or chronically, had significantly improved ICP compared with control rats. Erectile and endothelial parameters were enhanced with chronic PDE5i administration by other study [26].

Interestingly, we were not able to demonstrate a further increase in ICP with chronic vardenafil treatment compared with a single acute dose. This finding was also reported by Behr-Ross's group using normal rats with daily Sildenafil for 8 wk [27]. However, we were able to demonstrate that rats with diabetes for only 4 wk had a better response (improved ICP with electro- stimulation) compared to rats with diabetes for 10 wk. This finding supports our current understanding of the pathophysiology of diabetes, where erectile function declines as diabetes progresses.

The eNOS pathway plays a critical role in the physiology of penile erection. Endothelium Nitric Oxide Synthase (eNOS) is negatively controlled by Caveolin-1, a 22 kDa protein that is ubiquitously expressed and abundant in endothelial cells [28]. Caveolin-1 inhibits eNOS through two mechanisms: First, it impedes the signaling of caveolae-targeted receptors that transfer eNOS-stimulatory signals, and second, it blocks the calmodulin-binding site of eNOS [29]. Caveolins play a dynamic role in the trafficking of proteins on membranes and regulating signaling molecules in disease conditions in which Caveolins have been implicated, including atherosclerosis, hypertension, cardiomyopathy, diabetes, and oncogenesis [30]. In this study, there was no difference of Caveolin-1 expression in both control and treatment groups. However, the rats receiving chronic vardenafil (treatment group) had a

statistically higher ratio of eNOS/Caveolin-1. This reflects a higher eNOS content in the treatment group and a higher capacity to overcome the negative inhibition of Caveolin-1.

One of the novel and exciting aspects of this study was the discovery of differential protein expression between the control rats and those exposed to Vardenafil chronically for 6 wk [Table 3, Fig. 5]. Although these are preliminary results, these proteins could represent biomarkers that may help to prognosticate treatment affect with PDE5i in patients with diabetes-associated ED. Further work is needed to identify these useful markers in the hope that they may give us insight on how to manage this difficult to treat subset of men with ED.

5. Conclusions

This is the first report describing the effects of chronic vardenafil exposure in a diabetic animal model evaluating physiologic, morphologic, and protein changes as they relate to erectile function. Chronic use of the PDE5 inhibitor vardenafil for 6 wk significantly improved erectile function in this diabetic rat model. These physiological changes were further supported by penile cavernosal tissue protein expression of increasing eNOS and smooth muscle α-actin protein content and the upregulation of protein species on a proteomic assay compared to untreated diabetic rats.

References

[1] Hakim LS, Goldstein I. Diabetic sexual dysfunction. Endocrinol Metab Clin North Am 1996;25:379–400.

[2] Sàenz de Tejada I, Goldstein I, Azadzoi K, Krane RJ, Cohen RA. Impaired neurogenic and endothelium mediated relaxation of penile smooth muscle from diabetic men with impotence. N Engl J Med 1989;320:1025–30.

[3] Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999;48:1–9.

[4] Bivalacqua TJ, Usta MF, Kendirci M, Pradhan L, Alvarez X, Champion HC, et al. Superoxide anion production in the rat penis impairs erectile function in diabetes: influence of in vivo extracellular superoxide dismutase gene therapy. J Sex Med 2005;2:187–97.

[5] Sadovsky R, Miller T, Moskowitz M, Hackett G. Three-year update of sildenafil citrate (Viagra) efficacy and safety. Int J Clin Pract 2001;55:115–28.

[6] Vickers MA, Satyanarayana R. Phosphodiesterase type 5 inhibitors for the treatment of erectile dysfunction in patients with diabetes mellitus. Int J Impot Res 2002;14:466–71.

[7] Musicki B, Champion HC, Becker RE, Liu T, Kramer MF, Burnett AL. Erection capability is potentiated by long-term sildenafil treatment: role of blood flow-induced endothelial nitric oxide synthase phosphorylation. Mol Pharmacol 2005;68:226–32.

[8] Rosano GM, Aversa A, Vitale C, Fabbri A, Fini M, Spera G. Chronic treatment with tadalafil improves endothelial function in men with increased cardiovascular risk. Eur Urol 2005;47:214–22.

[9] Sommer F, Klotz T, Engelmann U. Improved spontaneous erectile function in men with mild-to-moderate arteriogenic erectile dysfunction treated with a nightly dose of sildenafil for one year: a randomized trial. Asian J Androl 2007;9:134–41.

[10] De Young L, Yu D, Freeman D, Brock GB. Effect of PDE5 inhibition combined with free oxygen radical scavenger therapy on erectile function in a diabetic animal model. Int J Impot Res 2003;15:347–54.

[11] Vlassara H, Fuh H, Makita Z, Krungkrai S, Cerami A, Bucala R. Exogenous advanced glycosylation end products induce complex vascular dysfunction in normal animals: a model for diabetic and aging complications. Proc Nat Acad Sci U S A 1992;89:12043–7.

[12] Martin-Morales A, Haro JM, Beardsworth A, Bertsch J, Kontodimas S. Therapeutic effectiveness and patient satisfaction after 6 months of treatment with tadalafil, sildenafil, and vardenafil: results from the Erectile Dysfunction Observational Study (EDOS). Eur Urol 2007;51:541–50.

[13] Goldstein I, Kim E, Steers WD, Pryor JL, Wilde DW, Natanegara F, et al. Efficacy and safety of tadalafil in men with erectile dysfunction with a high prevalence of comorbid conditions: results from MOMENTUS: multiple observations in men with erectile dysfunction in National Tadalafil Study in the US. J Sex Med 2007;4:166–75.

[14] Tsujimura A, Yamanaka M, Takahashi T, Miura H, Nishimura K, Koga M, et al. The clinical studies of sildenafil for the ageing male. Int J Androl 2002;25:28–33.

[15] Lipscombe LL, Hux JE. Trends in diabetes prevalence, incidence, and mortality in Ontario, Canada 1995–2005: a population-based study. Lancet 2007;369:750–6.

[16] Luo W, Morrison H, de Groh M, Waters C, DesMeules M, Jones-McLean E, et al. The burden of adult obesity in Canada. Chronic Dis Can 2007;27:135–44.

[17] Weintraub WS. The vascular effects of cilostazol. Can J Cardiol 2006;22(Suppl B):56B–60B.

[18] Matsumoto T, Kobayashi T, Wakabayashi K, Kamata K. Cilostazol improves endothelium-derived hyperpolarizing factor-type relaxation in mesenteric arteries from diabetic rats. Am J Physiol Heart Circ Physiol 2005;289:H1933–40.

[19] Smith NJ, Sak SC, Baldo O, Eardley I. The prevalence of newly diagnosed hyperlipidaemia in men with erectile dysfunction. BJU Int 2007;100:357–61.

[20] Srilatha B, Adaikan PG. Characterization of hypertriglyceridemia-induced erectile dysfunction. Urology 2006;67:642–6.

[21] Milani E, Nikfar S, Khorasani R, Zamani MJ, Abdollahi M. Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats. Comp Biochem Physiol C Toxicol Pharmacol 2005;140:251–5.

[22] De Angelis L, Marfella MA, Siniscalchi M, Marino L, Nappo F, Giugliano F, et al. Erectile and endothelial dysfunction in Type II diabetes: a possible link. Diabetologia 2001;44:1155–60.

[23] Bivalacqua TJ, Champion HC, Usta MF, Cellek S, Chitaley K, Webb RC, et al. RhoA/Rho-kinase suppresses endothelial nitric oxide synthase in the penis: a mechanism for diabetes-associated erectile dysfunction. Proc Natl Acad Sci U S A 2004;101:9121–6.

[24] Schafer A, Fraccarollo D, Pfortsch S, Flierl U, Vogt C, Pfrang J, et al. Improvement of vascular function by acute and chronic treatment with the PDE-5 inhibitor sildenafil in experimental diabetes mellitus. Br J Pharmacol 2008;153:886–93.

[25] Bella AJ, DeYoung LX, al-Numi M, Brock GB. Daily administration of phosphodiesterase type 5 inhibitors for urological and nonurological indications. Eur Urol 2007;52:990–1005.

[26] Ahn GJ, Yu JY, Choi SM, Kang KK, Ahn BO, Kwon JW, et al. Chronic administration of phosphodiesterase 5 inhibitor improves erectile and endothelial function in a rat model of diabetes. Int J Androl 2005;28:260–6.

[27] Behr-Roussel D, Gorny D, Mevel K, Caisey S, Bernabé J, Burgess G, et al. Chronic sildenafil improves erectile function and endothelium-dependent cavernosal relaxations in rats: lack of tachyphylaxis. Eur Urol 2005;47:87–91.

[28] Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG. Caveolin, a protein component of caveolae membrane coats. Cell 1992;68:673–82.

[29] Michel JB, Feron O, Sacks D, Michel T. Reciprocal regulation of endothelial nitric-oxide synthase by Ca^{2+}-calmodulin and caveolin. J Biol Chem 1997;272:15583–6.

[30] Williams TM, Lisanti MP. The Caveolin genes: from cell biology to medicine. Ann Med 2004;36:584–95.