Reduced VEGF Signaling in Corpus Cavernosum of Rat with Alloxan Induced Type I Diabetes Mellitus

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Abstract: Aim: Vascular endothelial growth factor (VEGF) is an angiogenic growth factor that plays a critical role in controlling survival and apoptosis. It is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase. PTEN (phosphatase and tensin homologue deleted on chromosome ten) is a major negative regulator of the PI3K/Akt signaling pathway. We sought to investigate the changes in VEGF signaling in type I diabetes mellitus (DM) induced erectile dysfunction (ED) in rats. Material/Methods: In total, 16 were divided into 2 groups (n=8/group). Eight of these animals (Group 1) had no treatment. The remaining 8 of them (Group 2) were injected with alloxan (100mg/kg body weight) to induce DM. All rats were sacrificed 8 weeks after alloxan induced DM in Group 2. Corporal tissues were harvested and studied for level of VEGF and cyclic guanosine monophosphate (cGMP) by enzyme immunoassay assay (ELISA); Levels of VEGF receptor (VEGFR)-1, VEGFR-2, Akt, phosphorylated Akt, eNOS, and phosphorylated eNOS were assessed by western blot analysis. Results: VEGF, VEGFR-1, VEGFR-2, Akt phosphorylation, eNOS phosphorylation, and cGMP were significantly decreased in the corporal tissue of diabetic rats. Conclusions: The accompanying decrease in cGMP may be a result of VEGF signaling dysregulation and this may have an effect on erectile function.

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1. Introduction

Diabetes mellitus (DM) is one of the major risk factors for erectile dysfunction. It has been estimated that 50%–75% of diabetic men have some degree of erectile dysfunction and the incidence of erectile dysfunction is higher in diabetic men than in age-matched nondiabetic men [1-2]. Diabetic rat model provide excellent opportunities to explore potential mechanisms and therapeutic approaches for human erectile dysfunction (ED) [3-11]. The purpose of our current study is to test for changes in VEGF (vascular endothelial growth factor) signaling in corporal tissue of the diabetic rat.

One of the downstream effects of VEGF includes VEGF receptor (VEGFR) dimerization and autophosporylation, the phosphorylation and activation of Akt and endothelial nitric oxide synthase (eNOS), which has been shown to mediate VEGF-induced penile erection by further mediating activation of Guanosine 3', 5' -cyclic monophosphate (cGMP) [12, 13]. Therapeutic angiogenesis using vascular growth factors has recently been demonstrated to be feasible in animal models with diabetic ED [8-10]. However, the expression profile of VEGF signaling in corporal tissue of diabetic rat has not been studied. We sought to determine the potential changes.

2. Material and Methods

Animal model.

In total, 16 male Sprague-Dawley rats weighing 200–250g were obtained from Shanghai Slac Laboratory Animal Co Ltd (Shanghai, China). Eight of these animals were selected randomly and treated with vehicle (0.1 mol/L citrate-phosphate buffer, pH 4.5) as normal controls (Group 1). The remainders were injected with alloxan (100mg/kg body weight) to induce DM (Group 2). All rats were kept in a temperature-controlled, air-conditioned animal house with a 12-h light-dark cycle and were given free access to food and water. Seventy-two hours after the rats were injected with alloxan, the blood glucose level in each rat was monitored at regular intervals throughout the study and immediately before sacrifice. Of the 8 Alloxan-induced rats, those having blood glucose levels higher than 300 mg/dL (16.6 mmol/L) were selected for the study. Procedures were performed according to the recommendations of the institutional animal care committee.

Tissue procurement, histological section preparation, protein isolation.

At study termination all rats were deeply anesthetized with ketamine and xylazine and penectomy was performed with careful dissection of the corpora cavernosa from the tunica albuginea before sacrifice, as previously described. Tissue was cryoprotected and frozen sections (5μm) were prepared. Protein lysates were prepared and concentrations determined by Bradford assay, as previously described (14, 15).

Measurement of VEGF protein.

VEGF was determined using a solid-state ELISA system with a Quantikine VEGF ELISA Kit (R&D Systems China Co. Ltd.), as previously described (14). At the final step, the optical density of samples and standards were measured. The amount of the VEGF protein in each sample was calculated based on a standard curve.

Assessments of VEGFR-1, VEGFR-2, Akt, p-Akt, eNOS and p-eNOS.

Western analysis for VEGFR-1, VEGFR-2, Akt, phosphorylated Akt (p-Akt), eNOS, and phosphorylated eNOS (p-eNOS) expression was performed using methods previously described (15). Alpha-Tubulin was used as a protein loading control. The intensity for each band was quantified using NIH image software based on pixel values. The p-Akt or p-eNOS density was calculated relative to total Akt or eNOS giving the p-eNOS/eNOS fraction. The primary antibodies were purchased from Beijing GBI Biotechnology Co. Ltd (Beijing, China); all secondary antibodies were purchased from Beijing Chief-East Tech Co. Ltd (Beijing, China). The working concentration for all the first antibodies was 1:1000, and for all the secondary antibodies the concentration was 1:5000.

Quantitative Determination of cGMP Nucleotides.

Determination of the cyclic nucleotide concentration was done by using a commercial kit: Parameter cyclic GMP Assay kit (R&D Systems China Co. Ltd.). This method is based on the ELISA, a competitive immunoassay for the quantitative determination of the relevant nucleotide in samples. At the final step, the optical density of samples and standards were measured. The amount of the nucleotide in each sample was calculated based on a standard curve.

Statistical analysis.

Results are expressed as the mean ± SD. The mean values were compared using Student's t test. P<0.05 was considered statistically significant.

3. Results

General Data

Of 8 alloxan-induced rats, all had blood glucose higher than 300 mg/dL with significantly increased food and water intakes, hyperuresis, and weight loss compared with the rats in the control group. Body weight and blood glucose level of the rats are shown in Table 1.

TABLE 1. Results of Body Weight, Blood Glucose, VEGF, and cGMP (Group 2 vs. Group 1)

	Initial Body Weight (g)	*8 Weeks Body Weight (g)*	*Initial Blood Glucose (mg/dL)*	*8 Weeks Blood Glucose (mg/dL)*	*VEGF (pg/mg)*	*cGMP* (pmol/mg)
Group 1	225.88	380.88	75.75±	76.25±	413.67	0.01853
	±16.88	±21.87	6.61	6.48	±84.53	±0.00445
Group 2	225.13	189.38	377.75±	494.50±	292.61	0.00947
	±16.50	±16.76	31.23	80.07	±11.28	±0.00450
p value	p=NS	p<0.001	p<0.001	p<0.001	p<0.05	P<0.01

Table 1. 8 weeks: 8 weeks after inducing diabetes in Group 2; VEGF, vascular endothelial growth factor; cGMP, cyclic guanosine monophosphate

Diabetes mellitus was associated with decreased vasoreactivities in corporal tissue

The levels of VEGF protein was significant different between 2 groups (See Table 1, Figure 1).

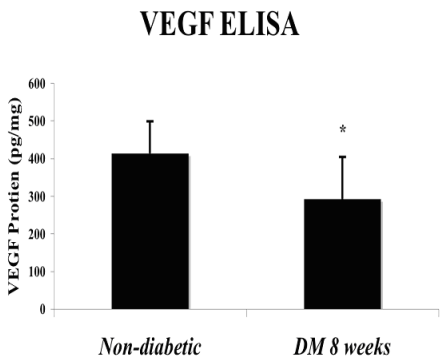

Figure 1. VEGF ELISA. There was significant decrease VEGF expression in diabetic rat. VEGF= vascular endothelial growth factor; DM=diabetes mellitus; p<0.05*

Diet Induced diabetes mellitus is associated with a reduction in VEGF downstream protein expression.

There was significant difference in VEGFR-1 (180 KD) or VEGFR-2 (200 KD) protein expression between 2 groups as detected by western blot. With respect to VEGFR-1 or VEGFR-2 expression, a 1.54-fold or 1.43-fold decrease was observed (Group 2 vs. Group 1; p<0.05 each) (See Figure 2). There was no difference in total Akt (60 KD) and total eNOS (135 KD) protein expression between 2 groups. However, the level of Akt phosphorylation at Ser 473 or eNOS phosphorylation at Ser 1177 was significantly

decreased. With respect to phospho-Akt or phospho-eNOS expression, a 1.75-fold or 1.72-fold decrease was observed (Group 2 vs. Group 1; $p<0.05$ each) (See Figure 2).

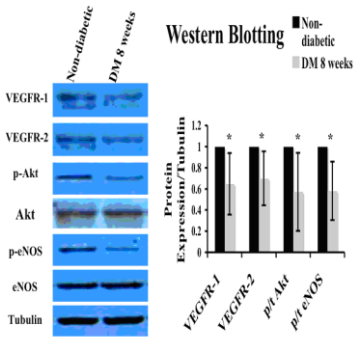

Figure 2. Western blotting for VEGFR-1, VEGFR-2, Akt and eNOS expression. There was significant decrease in Akt and eNOS phosphorylation. VEGF= vascular endothelial growth factor receptor; DM= diabetes mellitus; eNOS= endothelial nitric oxide synthase; p/t = phosphorylated/total; Tubulin =α-tubulin; $p<0.05$*

Diabetes mellitus is associated with abnormalities in cGMP expression

The levels of cGMP was significant different between 2 groups (See Table 1, Figure 3).

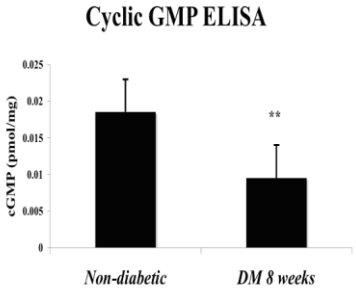

Figure 3. Cyclic GMP ELISA. There was significant decrease cGMP expression in diabetic rat. cGMP, cyclic guanosine monophosphate; DM=diabetes mellitus; $p<0.01$**

4. Discussions

Diabetes mellitus is a major cause of erectile dysfunction. The purpose of this study was to determine whether diabetes mellitus would result in any abnormalities in VEGF signaling in corporal tissues that is relevant to erectile dysfunction. We were able to demonstrate changes in Akt and eNOS phosphrylation, and cGMP level. Many of these changes are known to be part of pathophysiology of ED in human.

VEGF binds to one of three VEGF receptors that lead to receptor dimeration and autophosphorylation. This activates the enzyme phoshitidylinosityl 3 kinase (PI3K). PI3K in turn converts 4, 5-inositylphophate (PI) to 3, 4, 5 PI and this phosphorylates and thereby activates the protein kinase Akt. One of the downstream effects of Akt activation includes the phosphorylation and activation of eNOS [13].

Decreased VEGF or VEGF receptor protein expression in corporal tissue was shown in rats with diabetes or arteriogenic erectile dysfunction in limited number of studies [5, 16, 17]; however, it is not yet well clarified. This study has shown us clear decrease in the protein levels of VEGF and its receptors in rats with 8 weeks diabetes. Also, there follows a decrease in Akt and eNOS activation. However, we found the changes of Akt and eNOS were at phosphorylation which is similar to another study on Type I diabetic rats [7] but different from 3 studies on Type Ⅱ diabetic rats or Type I diabetic rats where they found that changes were at total Akt or total eNOS [5, 6, 17]. So different types or durations of diabetes may have different effects on Akt and eNOS changes.

Nitric oxide (NO) is the principal mediator of arterial dilation and smooth muscle relaxation within the sinusoids, which bring about tumescence of penile erectile tissue [1]. The NO-dependent signal transduction system contains several molecular targets available for pharmacologic manipulation to treat ED. The most prominent target identified thus far is phosphodiesterase 5 (PDE5), which enzymatically converts the intracellular second messenger molecule cGMP to its inactive form [18].

Endothelial NOS is an important source to generate NO and plays an important role in modulating intracorporal blood flow to maintain tumescence [3, 4, 7, 12]. In this study, decreased VEGF led to decreased Akt then eNOS phosphorylation and finally decreased cGMP expression. This observation advances our understanding of the mechanisms of diabetes mellitus induced ED and provides the theoretical basis for the application of VEGF gene therapy in vasculogenic ED.

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References

1. Lue TF. Erectile dysfunction. N Engl J Med, 2000; 342:1802-1813
2. Siu SC, Lo SK, Wong KW, Ip KM, Wong YS. Prevalence of and risk factors for erectile dysfunction in Hong Kong diabetic patients. Diabet Med 2001; 18:732-738

3. Musicki B, Burnett AL. eNOS function and dysfunction in the penis. Exp Biol Med (Maywood) 2006; 231:154-165
4. Musicki B, Burnett AL. Endothelial dysfunction in diabetic erectile dysfunction. Int J Impot Res 2007; 19:129-138
5. Jesmin S, Sakuma I, Salah-Eldin A, Nonomura K, Hattori Y, Kitabatake A. Diminished penile expression of vascular endothelial growth factor and its receptors at the insulin-resistant stage of a type II diabetic rat model: a possible cause for erectile dysfunction in diabetes. J Mol Endocrinol 2003; 31:401-418
6. Vernet D, Cai L, Garban H, Babbitt ML, Murray FT, Rajfer J, Gonzalez-Cadavid NF. Reduction of penile nitric oxide synthase in diabetic BB/WORdp (type I) and BBZ/WORdp (type II) rats with erectile dysfunction. Endocrinology 1995; 136:5709-5717
7. Musicki B, Kramer MF, Becker RE, Burnett AL. Inactivation of phosphorylated endothelial nitric oxide synthase (Ser-1177) by O-GlcNAc in diabetes-associated erectile dysfunction. Proc Natl Acad Sci U S A. 2005; 102:11870-11875.
8. Dall'Era JE, Meacham RB, Mills JN, Koul S, Carlsen SN, Myers JB, Koul HK. Vascular endothelial growth factor (VEGF) gene therapy using a nonviral gene delivery system improves erectile function in a diabetic rat model. Int J Impot Res. 2008; 20:307-314
9. Shirai M, Yamanaka M, Shiina H, Igawa M, Kawakami T, Ishii N, Lue TF, Fujime M, Dahiya R. Vascular endothelial growth factor restores erectile function through modulation of the insulin-like growth factor system and sex hormone receptors in diabetic rat. Biochem Biophys Res Commun. 2006; 341:755-762.
10. Yamanaka M, Shirai M, Shiina H, Tanaka Y, Enokida H, Tsujimura A, Matsumiya K, Okuyama A, Dahiya R. Vascular endothelial growth factor restores erectile function through inhibition of apoptosis in diabetic rat penile crura. J Urol. 2005; 173:318-323.
11. Yang R, Wang J, Chen Y, Sun Z, Wang R, Dai Y. Effect of caffeine on erectile function via up-regulating cavernous cyclic guanosine monophosphate in diabetic rats. J Androl. 2008; 29:586-591.
12. Musicki B, Palese MA, Crone JK, Burnett AL. Phosphorylated endothelial nitric oxide synthase mediates vascular endothelial growth factor-induced penile erection. Biol Reprod. 2004; 70: 282-289
13. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signaling - in control of vascular function. Nat Rev Mol Cell Biol. 2006; 7:359-371
14. Xie D, Hazarika S, Andrich AJ, Padgett ME, Kontos CD, Donatucci CF, Annex BH. High cholesterol feeding in C57/Blc6 mice alters expression within the VEGF receptor-ligand family in corporal tissue. J Sex Med. 2008; 5:1137-1148.
15. Xie D, Odronic SI, Wu F, Pippen AM, Donatucci CF, Annex BH. A mouse model of hypercholesterolemia-induced erectile dysfunction. J Sex Med. 2007; 4:898-907.
16. De Young L, Bella A, Howard J, Brock G. Arteriogenic erectile dysfunction alters protein expression within the cavernosal tissue in an animal model. J Sex Med. 2005; 2:199-206.
17. Liu G, Sun X, Dai Y, Zheng F, Wang D, Huang Y, Bian J, Deng C. Chronic administration of sildenafil modified the impaired VEGF system and improved the erectile function in rats with diabetic erectile dysfunction. J Sex Med. 2010;7:3868-3878.
18. Burnett AL. Phosphodiesterase 5 mechanism and therapeutic applications. Am J Cardiol 2005; 96: 29M-31M

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