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Original Article

Effect of histological prostate inflammation on erectile function and its possible mechanism: a preliminary study

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Abstract: Objective: To investigate the effect of histological prostate inflammation on erectile function and its possible mechanism. Methods: After the induction of histological prostate inflammation in Sprague-Dawley (SD) rats, the erectile function, ratio of maximum intracavernosal pressure (ICP)/mean arterial pressure (MAP), enzyme activity of endothelial nitric oxide synthase (eNOS), cyclic guanosine monophosphate (cGMP) level and eNOS mRNA expression and protein levels of the corpus cavernosum were evaluated and compared between the experimental and control groups. Results: Haematoxylin-eosin staining showed a mild-to-moderate inflammation in the prostate tissue. The ICP_{max}/MAP × 100 was significantly decreased in the experimental group than in the control group (32.06 ± 7.21 vs. 51.96 ± 11.49, P = 0.01). The eNOS enzyme activity and cGMP level of the corpus cavernous tissue were significantly lower in the experimental group than in the control group (1.02 ± 0.40 vs. 1.97 ± 0.45 U/mg prot, P < 0.01; 27.33 ± 7.12 vs. 43.33 ± 5.47 pmol/ml, P < 0.01, respectively). The expression levels of eNOS mRNA and eNOS protein were significantly decreased in the experimental group than in the control group. Conclusion: Histological prostate inflammation caused a severe impairment in the erectile function of SD rats, indicated by the low eNOS enzyme activity and cGMP level and the decreased expression of eNOS mRNA and eNOS protein.

Keywords: Prostate inflammation, erectile function, animal model

Introduction

Erectile dysfunction (ED), defined as the persistent inability to attain and maintain an erection sufficient to permit satisfactory sexual performance, is a primary complaint in sexual medicine among male patients [1, 2]. Although ED is considered as a benign disorder, it seriously affects the quality of life in male patients and their female partners. Vasculogenic, hormonal, neurogenic, anatomical/structural and psychogenic factors are the widely accepted risk factors of ED [3, 4]. In addition, drugs or trauma can contribute to ED.

Prostate inflammation is characterised by chronic inflammatory cell infiltration, including the lymphocytes and mononuclear cells, at a histological level. Although the infiltration of inflammatory cells into the prostate does not always cause significant symptoms in patients, the pathological changes may influence the individual unconsciously. Inflammatory cell infiltration contributes to tissue injury and activates cytokine release, which could promote the proliferation of prostate tissues [5-8].

Patients with histological prostate inflammation are commonly detected in the clinic. Most of these patients are identified through biopsy when a prostate cancer is suspected or through prostate resection due to benign prostatic hyperplasia. However, only few patients realise the potential impact caused by this condition. Prostate inflammation may have an impact on sexual dysfunction in male patients [9, 10]. In a study on 46 patients with sexual dysfunction, more than half of them (56.5%) were found to have prostate inflammation [11]. Another study also confirmed the impact of prostate inflam-

mation on sexual dysfunction and reported that the condition could be detectable in almost two-thirds of 153 patients with sexual dysfunction [12].

The above account on prostate inflammation depicts its potential impact on sexual function. However, the exact relationship between prostate inflammation and sexual function remain unknown. Therefore, we investigated the effect of histological prostate inflammation on erectile function and its possible mechanism in an animal model, which could be useful for developing potential treatment strategies for such patients.

Materials and methods

Animals

A total of 20 male Sprague-Dawley (SD) rats provided by the Animal Laboratory Center of Sichuan University were used in this study. All the SD rats were housed in an environmentally controlled room according to the regulations of our Animal Laboratory Center and were provided food and water ad libitum. The experimental procedures complied with the requirements of the Provision and General Recommendation of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of West China Hospital of Sichuan University.

Establishment of histological prostate inflammation and animal grouping

An experimental autoimmune prostate inflammation rat model was created as previously described [13, 14] with some modifications. In total, eight male SD rats were sacrificed by decapitation for preparing prostate tissue homogenate supernatant (PTHS). The prostate tissue was homogenised in 0.5% Triton X-100 saline maintained in an icewater bath, and the homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was separated and collected. The protein concentration of PTHS was measured by the biuret method and then protein dilutions at a concentration of 30 mg/ml were prepared using phosphate-buffered saline and stored at -80°C. In experimental group, six male SD rats were intradermally immunised twice on days 0 and 7 with a mixture of PTHS (20 mg/ml) and complete Freund's adjuvant (CFA; 0.5 ml and 0.5 ml, respectively) followed by intraperitoneal injection with 0.5 ml of pertussis-diphtheria-tetanus (PDT). On day 21, all the rats were again intradermally injected with 1.0 ml of PTHS and CFA mixture. In the control group, another six male age-matched SD rats were treated with 0.9% NaCl instead of PTHS, CFA and PDT.

Assessment of mean arterial pressure (MAP) and intracavernosal pressure (ICP)

Erectile function was measured by ICP_{max}/MAP according to the method described by Jiang et al [15], with some modifications. In brief, the SD rats were anaesthetised with isoflurane anaesthesia (5% induction, 2% maintenance with oxygen) on day 45 after the first injection. The right carotid artery was exposed and cannulated with a polyethylene-50 catheter filled with 50 IU/ml heparinised saline to measure MAP. The penis was denuded of skin and a 25-gauge needle (heparinised with 50 IU/ml heparin) connected to a pressure transducer (BL-420S biological function experiment system; Chengdu TME Technology Co., Ltd., China) for recording was inserted into the right side of the penile crus. A midline abdominal incision was made at the lateral side of the prostate for isolating the cavernous nerve. A bipolar platinum electrode attached to an electrical stimulator (BL-420S biological function experiment system, Chengdu TME Technology Co., Ltd) was placed around the nerve for electrical stimulation. The right cavernous nerve of each rat was stimulated with monophasic rectangular pulses with stimulus parameters of 1.5 mA, 20.0 Hz, pulse width of 5.0 ms and duration of 50 s. The ICP responses were evaluated by calculating the ratio of the ICP_{max}/MAP.

Histological analysis

Prostate glands were collected and their wet weight was measured after the assessment of ICP_{max}/MAP. The specimens were processed using conventional histology. In brief, the prostate glands were fixed in 4% formalin solution, dehydrated in alcohol, cleared in xylol and embedded using paraffin. The specimens were then sectioned at 4-5 μm thickness and stained with haematoxylin-eosin (HE). The degree of inflammation in the prostate glands was assessed by two experienced pathologists using a scale of 0-4+, as previously described

Table 1. Body weights and plasma glucose levels of SD rats (Mean ± Standard Deviation)

Group	N	Body weight (g)	Plasma glucose (mmol/L)
Experimental	6	336.17 ± 14.49	5.48 ± 0.76
Control	6	346.50 ± 13.65	5.70 ± 0.58
P value		0.232	.575

[16]: 0, no inflammation; 1+, mild, but definite perivascular cuffing with mononuclear or mast cells; 2+, moderate perivascular cuffing; 3+, marked perivascular cuffing with some parenchymal inflammatory cells and 4+, marked perivascular cuffing and numerous mononuclear and mast cells in the parenchyma.

Detection of endothelial nitric oxide synthase (eNOS) mRNA expression by quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Penile cavernous tissues were quickly and carefully removed after the assessment of ICP and were frozen in liquid nitrogen. Trizol reagent (Sangon Biotech Co., Ltd, Shanghai, China) was used for the extraction of total RNA from frozen penile corpus cavernous tissues. Reverse transcription of eNOS RNA was performed according to the manufacturer's instructions (AMV First Strand cDNA Synthesis Kit, Invitrogen, CA, USA). The primers of eNOS and β-actin were synthesised by Sangon Biotech Co., Ltd; their sequences are shown in **Table 1**. qRT-PCR was performed on ABI step one plus™ real-time RT-PCR system using the SYBR Green PCR master mix (Applied Biosystems). The reaction conditions for PCR were as follows: 3 min at 95°C, followed by 40 cycles of 15 s at 95°C and 40 s at 60°C. The $2^{-\Delta\Delta CT}$ method was used for calculating the eNOS expression.

Western blotting of eNOS expression

Tissue proteins were extracted by homogenisation of penile corpus cavernosum using a radioimmunoprecipitation assay buffer (Beyotime Biotech, Shanghai, China), and the lysate was centrifuged at 14,000 rpm for 5 min at 4°C. Protein concentrations in the supernatants were estimated by the bicinchoninic acid protein assay kit (Beyotime Biotech). The proteins were separated using previously prepared 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and then transferred onto nitrocellulose membranes. After blocking with 5% non-fat milk, the membrane was incubated with anti-eNOS antibody (1:1000; Abcam) overnight at 4°C and then incubated with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit IgG; Beyotime). The protein expression of eNOS was normalised to β-tubulin expression, and protein bands were quantified using Quantity One software (Bio-Rad, USA). The ratio of eNOS/area vs. β-tubulin IOD/area was used to analyse the expression of eNOS protein.

eNOS enzyme activity and cyclic guanosine monophosphate (cGMP) assay in the cavernous tissue

The eNOS activity of the cavernous tissue was determined using a total NOS detection kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) by measuring the production of NO biosynthesised by NOS (substrate: L-arginine). The eNOS enzyme activity was expressed as unit activity per milligram protein in the sample (U/mgprot). Following the method of Zuo et al [17], the cavernous tissue samples were homogenised in an icewater bath and centrifuged at 6000 × g for 10 min (at 4°C). The cGMP level of the cavernous tissue was measured by a cGMP ELISA kit (Nanjing Jiancheng Bioengineering Institute).

Statistical analysis

Data are expressed as mean ± standard deviation (M ± SD). Independent samples Kruskal-Wallis test was performed to evaluate the differences between the groups. $P < 0.05$ was considered statistically significant. IBM® SPSS Statistics software, version 21.0, was used for all statistical analyses.

Results

No obvious signs of systemic illness were observed in both the rat groups. The activities, feeding and other general conditions of the rats were similar between the experimental and control groups. Plasma glucose concentration and body weight remained constant between the groups until the end of the study (**Table 1**). In the experimental group, four cavernous tissue specimens were considered as mild inflammation (1+) and other two were considered as

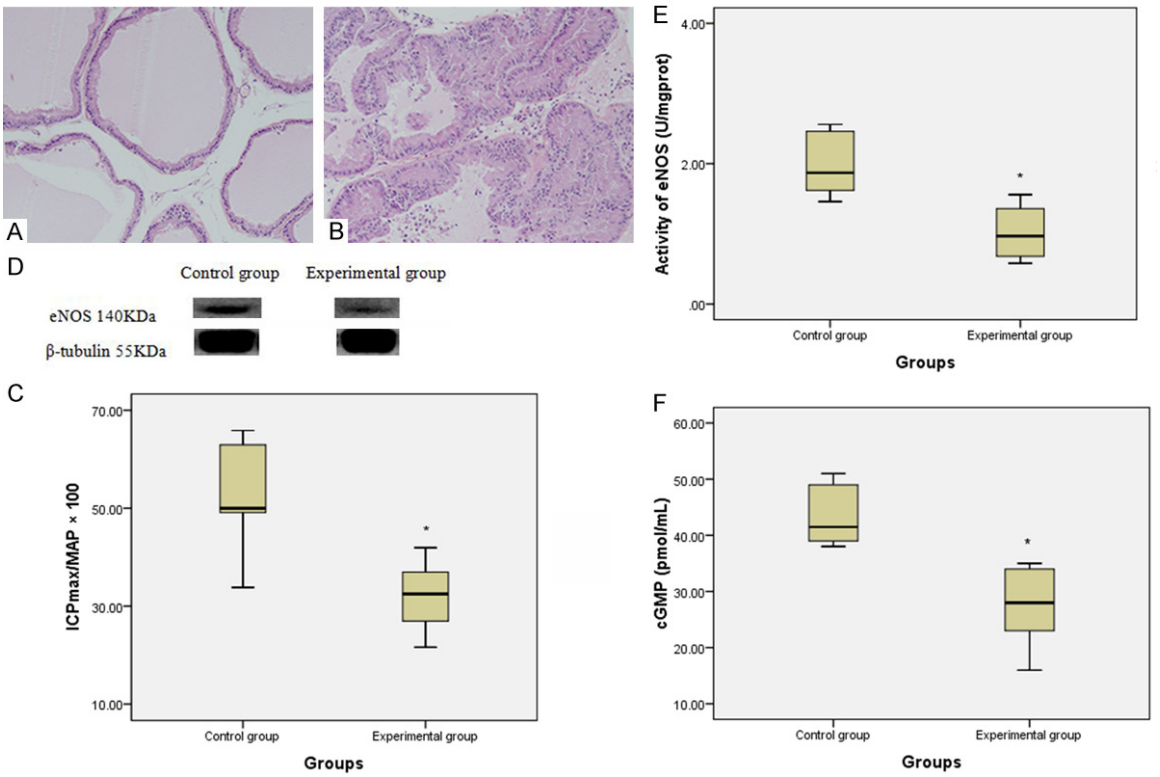

Figure 1. A: Histopathologic findings of prostate lateral lobe in control group (Hematoxylin and eosin stain, × 200); B: Histopathologic findings of prostate lateral lobe in experimental group (Hematoxylin and eosin stain, × 200); C: Comparison of intracavernosal pressure maximum/mean arterial pressure (ICPmax/MAP) in 2 groups; D: The protein levels of eNOS and β-tubulin in corpus cavernous in 2 groups; E: Comparison of activity of eNOS (U/mgprot) of corpus cavernous in 2 groups; F: Comparison of levels of cGMP (pmol/ml) in corpus cavernous in 2 groups.

Table 2. Expression of eNOS mRNA in corpus cavernosum of SD rats

Group	N	$2^{-\Delta\Delta CT}$
Experimental	6	0.485 ± 0.262
Control	6	1.078 ± 0.361
P value		.009

Table 3. Expression of eNOS protein in corpus cavernosum of SD rats

Group	N	eNOS/β-tubulin (IOD/area)
Experimental	6	0.621 ± 0.055
Control	6	0.748 ± 0.048
P value		.002

moderate inflammation (2+). No inflammation was detected in the control group. The results of HE staining are shown in **Figure 1**.

Erectile function was expressed as ICP_{max}/MAP × 100. After electrical stimulation, the ICP_{max}/MAP × 100 values in the experimental group were significantly decreased compared with those in the control group (32.06 ± 7.21 vs. 51.96 ± 11.49, P = 0.01) (**Figure 1C**). The eNOS mRNA expression showed a significant decrease in the experimental group compared to that in the control group (**Table 2**; **Figure 1D**). **Table 3** and **Figure 1D** showed the expression of eNOS protein.

The eNOS enzyme activity of the cavernous tissue in the experimental rats showed a significantly lower level than that in the control rats (1.02 ± 0.40 vs. 1.97 ± 0.45 U/mgprot, P < 0.01) (**Figure 1E**). Similarly, the cGMP level was significantly decreased in the experimental group than in the control group (27.33 ± 7.12 vs. 43.33 ± 5.47 pmol/ml, P < 0.01) (**Figure 1F**).

Discussion

ED is common in elderly men and shows an increasing pattern. Accumulating evidence

shows that chronic diseases have a close relationship with erectile function [18]. Vasculogenic factors such as cardiovascular diseases, hypertension, diabetes mellitus and hyperlipidaemia; neurogenic factors such as multiple sclerosis, Parkinson's disease, chronic renal failure and polyneuropathy and hormonal factors such as hypogonadism, hyperprolactinaemia and hyper/hypothyroidism are associated with ED [19]. In other words, ED is a complex condition associated with other physiological systems.

The prostate gland is an important male accessory gland surrounding the neck of the urinary bladder and urethra. The term histological prostate inflammation refers to the infiltration of inflammatory cells in the prostate gland. The latest National Institutes of Health consensus classification has classified prostatitis in humans into I, II, III and IV categories [20]. According to this classification, the diagnosis of prostatitis, particularly prostatitis III, in human beings is primarily based on the patients' symptoms. However, histological prostate inflammation could be commonly detected in the clinic. Histological prostate inflammation does not cause a prostatitis-like symptom, and no significant relationship between them has been detected. Clinical studies have shown that histological prostate inflammation may affect erectile function [3]. However, only few research studies have assessed whether histological prostate inflammation could lead to ED. Patients with this condition and even clinicians seldom pay attention to the potential harmful influence caused by histological prostate inflammation. Thus, there is a need for additional research to assess whether histological prostate inflammation could affect erectile function.

In this study, PTHS, CFA and PDT were used to create a histological prostate inflammation animal model. In the experimental group, HE staining revealed chronic inflammatory cells such as mononuclear cells and lymphocytes in the stroma and lumen of the gland. This autoimmune-induced inflammation is similar to the histological inflammation in human beings and is an effective animal model for histological prostate inflammation.

The erectile function showed a significant decrease in the experimental group, which indicated that the autoimmune-induced histological prostate inflammation in SD rats could lead to ED. The cGMP levels were also significantly lower than those in the control group, implying that prostate inflammation may influence erectile function by regulating the cGMP levels in the NO-cGMP-PKG signalling pathway. eNOS is the primary enzyme in the NO-cGMP-PKG signalling pathway. In the present study, both the eNOS mRNA and protein levels in the corpus cavernosum were significantly decreased in the experimental rats compared with those in the control rats. In addition, a significant low enzyme activity of eNOS was observed in the experimental rats. These findings demonstrate that histological prostate inflammation could decrease the expression of eNOS protein through the down-regulation of eNOS mRNA and cGMP levels and by decreasing the enzyme activity of eNOS in the corpus cavernosum. The reduced activity of eNOS would in turn decrease the NO level catalysed by L-arginine, which could ultimately affect erectile function.

Although this is the first study on histological prostate inflammation and ED in animal model, there are some limitations. First, the sample size was quite small, with only 12 SD rats, 6 each in the experimental and control groups. Second, we did not distinguish the different levels of inflammation, due to which their differential impacts on erectile function were not analysed. Third, because of the limited sample tissue from each rat, we could not assess other important signalling molecules such as iNOS and nNOS in the corpus cavernosum. Therefore, further research is needed to determine the detailed and exact correlation between histological prostate inflammation and erectile function.

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Disclosure of conflict of interest

None.

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