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Resveratrol and sildenafil synergistically improve diabetes-associated erectile dysfunction in streptozotocin-induced diabetic rats

Yufeng Bai, Ruihua An *

Department of Urology, The First Affiliated Hospital of Harbin Medical University, Harbin 150001, China

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ABSTRACT

Aims: Despite effective control of blood glucose levels in diabetic patients, complaints of diabetes-associated erectile dysfunction (ED) persist. Resveratrol has been indicated to possess anti-diabetic effects and therapeutic potential for ED. This study was conducted to observe the effect of resveratrol alone or in combination with sildenafil on ED in streptozotocin (STZ)-induced diabetic rats.

Main method: Among 58 adult male STZ-induced (60 mg/kg) diabetic Sprague–Dawley rats, 48 STZ-induced diabetic rats were randomized equally to four groups: untreated diabetic rats, resveratrol (25 mg/kg), sildenafil (5 mg/kg) or resveratrol (25 mg/kg) plus sildenafil (5 mg/kg) through oral gavage for 8 weeks. Additionally, 12 age-matched rats were chosen as controls. Intracavernous pressure (ICP) and mean arterial blood pressure (MAP) were used to measure erectile function. The cavernous level of cyclic guanosine monophosphate (cGMP), protein and mRNA of endothelial NO synthase (eNOS), neuronal NOS (nNOS), and phosphodiesterase-5 (PDE5) was measured.

Key findings: Treatment with either resveratrol or sildenafil improved ICP/MAP compared to the untreated diabetic rats ($P < 0.05$). Treatment with resveratrol increased nNOS and eNOS expression, inhibited PDE5 expression, and increased the cavernous cGMP level compared to the untreated diabetic rats. Resveratrol significantly decreased superoxide anion and ROS production. Two-way ANOVA indicated that resveratrol in combination with sildenafil therapy had a significant synergistic effect in improving ICP/MAP and cavernous cGMP levels.

Significance: Resveratrol improves diabetes-associated ED in rats. Combination therapies with resveratrol and sildenafil have a synergistic effect in improving ED. The mechanisms might be attributed to its anti-oxidative properties and NO–cGMP signaling pathway upregulation.

1. Introduction

Erectile dysfunction (ED) is one of the most frequent vascular complications of diabetes mellitus, with an estimated prevalence of more than 50% of diabetic men worldwide [15,24]. Despite effective control of blood glucose levels in diabetic patients, complaints of diabetes-associated ED persist. A fundamental breakthrough of drug treatment in ED drugs involves phosphodiesterase type 5 (PDE5) inhibitors with inspiring effects; however, not all patients with diabetes respond well to these drugs [17,19]. Therefore, exploration of new therapeutic strategies for the management of diabetic ED is urgent.

Oxidative stress is implicated in the pathophysiological mechanism of ED [31]. Excessive production of reactive oxygen species (ROS) in penile tissue plays a key role in the development of diabetic ED [18,20]. The superoxide anion rapidly inactivates nitric oxide (NO), limiting its ability to relax cavernosal smooth muscles [27]. There is growing evidence demonstrating that the NO-cyclic guanosine monophosphate (cGMP) pathway takes part in maintaining normal erectile function [1,3,9].

Resveratrol is a naturally occurring phytoalexin rich in grapes and numerous plant species. Resveratrol has been used in preventing and treating DM [8,11,29,30]. Resveratrol possesses anti-oxidative properties [12,22] and has potential therapeutic benefits in ED. Furthermore, resveratrol could upregulate the NO–cGMP signaling pathway. Recently, two studies have focused on improving erectile dysfunction using resveratrol [13,34]. These findings suggested that resveratrol could be identified as a potential therapeutic agent in diabetes-associated ED.

The aim of this study was to investigate the effect of resveratrol alone or in combination with sildenafil on ED in streptozotocin (STZ)-induced diabetic rats.

* Corresponding author at: Department of Urology, The First Affiliated Hospital of Harbin Medical University, 23rd Street, Nangang District, Harbin 150001, China. Tel.: +86 0451 855558821; fax: +86 0451 85556000.
E-mail address: anruihua62@163.com (R. An).

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2. Materials and methods

2.1. Materials

All the experimental protocols were approved by the laboratory animal center of the Academy of the Harbin University. Seventy healthy male Sprague–Dawley (SD) rats (200–250 g) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. The rats were maintained in a temperature-controlled room at 23 ± 1 °C and 40–50% humidity and a 12 h light/dark cycle. All animals were adapted to the new environment for 3 days before the experiment. Resveratrol (purity of >98%) was purchased from Sigma Chemical Co. STZ was obtained from Sigma Chemical Co. Sildenafil was purchased from Pfizer.

2.2. Experimental protocol

Seventy adult male SD rats were confirmed to be having normal erectile function by undergoing transsexual activity with female rats. Rats were rendered diabetic by a single intraperitoneal injection of 60 mg/kg body weight STZ dissolved in 1 ml sodium citrate buffer at 4 °C according to previous studies [32,33]. 72 h after the injection of STZ, random blood glucose levels were determined with a OneTouch Ultra Blood Glucose Meter (Hong Kong Enjoying Co., Ltd). Rats with blood glucose levels >16.6 mmol/l were judged as the eligible diabetic models. Forty-eight eligible diabetic rats were randomized equally to four groups as follows: untreated diabetic rats, resveratrol (25 mg/kg), sildenafil (5 mg/kg), or resveratrol (25 mg/kg) and sildenafil (5 mg/kg). Resveratrol and sildenafil were suspended in 3‰ hydroxyethyl starch solution and administered by daily oral gavage for 8 weeks. Twelve male rats with normal erectile function were chosen as the controls which received the same volume of 3‰ hydroxyethyl starch solution. Blood samples were collected by tail prick. Fasting blood glucose levels were assayed using the same blood glucose meter. At the end of the experiment, after evaluating erectile function, rats were sacrificed and the penis was stored at − 70 °C until further analysis.

2.3. Intracavernous pressure (ICP)/mean arterial pressure (MAP) measurements

After the 8-week treatment, rats were anesthetized by using 30 mg/kg sodium pentobarbital intraperitoneally, then the major pelvic ganglion, cavernous nerves, and pelvic organs were exposed, and a 23-gauge needle connected to PE-50 tubes containing 250 IU/ml heparinized saline was carefully inserted into the cavernous tissues. The other end of the PE-50 tube was connected to a pressure transducer (Statham P23 Gb, Waltham, Mass, USA) integrated into a computerized data acquisition system (BioPac system, Goleta, CA,USA) to measure ICP and MAP under 20 Hz, 5 V and 60 s electric stimulation. A butterfly needle was inserted into the aorta at the aortic bifurcation to determine the ICP/MAP ratio using the same equipments.

2.4. Determination of testosterone in the serum

Serum testosterone levels were measured by a lucigenin chemiluminescence assay (Bayer Diagnostics, East Walpole, MA, USA) according to the manufacturer's instructions.

2.5. Determination of cGMP levels in the corpora cavernosum

The harvested penis was homogenized in cold sodium acetate, and cGMP was extracted by the following method. Cavernous cGMP was detected using an enzyme-linked immunosorbent assay kit (R&D Systems China Co. Ltd., Shanghai, China) according to the manufacturer's instruction. The protein level was determined by a BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). Results were expressed as pmol/mg protein.

2.6. Determination of corpus cavernosum protein levels

Western blotting was used to detect PDE5, eNOS, and nNOS protein levels in the corpus cavernosum. Briefly, equal amounts of the rat cavernosum (60 mg) from the different groups were resolved on 10% SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 5% milk in Tris-buffered saline for 2 h, these membranes were incubated with the following polyclonal antibodies at 4 °C overnight: PDE5 (1:200 dilution), eNOS (1:200 dilution), nNOS (1:100 dilution), or β-actin (1:500 dilution). Then the membranes were washed and incubated with secondary antibodies at a dilution of 1:1000 for 2 h. Specific bands were detected by a chemiluminescent substrate. Band intensities of PDE5, eNOS, and nNOS were quantified by using an image analysis system (Quantity One, Bio-Rad) and expressed as relative to β-actin bands.

2.7. Determination of mRNA levels in the corpus cavernosum

Semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analyses were used to detect the mRNA expression level using the Gene Amplify PCR System. RNA was extracted from 50 mg of cavernosum using the Trizol reagent. The purity of RNA was quantified using 1.8% agarose gel electrophoresis at the 260/280 ratio. The primers for eNOS (261 bp), nNOS (210 bp), PDE5 (417 bp), and β-actin (271 bp) were as follows: eNOS (forward, 5′-AGGCTGCTGCCCGAGATATCTTCA-3′; reverse, 5′-TTGGGTGGGCACACACCTATGTGG-3′); nNOS (forward, 5′-CCGGCTACACTTCTCCTCAC-3′; reverse, 5′-CACGAAGCAGGGGACTACAT-3′); PDE5 (forward, 5′-GGACCAGTGCTCAAGACTCTT-3′; reverse, 5′-GATGGCCTGAGCTACACCA-3′); and β-actin (forward, 5′-TGTATGCCTCTGGTCGTACCAC-3′; reverse, 5′-ACAGAGTACTTGCGCTCAGGA-3′).

The protocols for amplification of eNOS, nNOS, and PDE5 were an initial incubation of 45 °C for 30 min, followed by 94 °C for 4 min, 35 cycles of 94 °C for 30 s, 55 °C for 60 s, and 72 °C for 10 min. The cycles of denaturation were 28 and annealing at 55.5 °C for β-actin. mRNA amplification was quantified by using an image analysis system (Quantity One, Bio-Rad) and expressed as relative to β-actin.

2.8. Determination of ROS and superoxide anion in the corpus cavernosum

The levels of superoxide in the corpus cavernosum were determined by dihydroethidium staining (DHE) as described by Davidson et al. [25]. In brief, frozen sections of cavernous tissues (10 μm) were incubated with 1 μmol/l of DHE (Sigma Chemical Co., USA) at 37 °C under a dark humidified chamber for 30 min. After being rinsed with the excessive reagent, a fluorescent image of ethidium bromide was detected by using an LSM 510 laser scanning confocal microscope (ZEISS, German) at the excitation and emission wavelengths of 488 nm and 610 nm, respectively, with detection at 585 nm. The number of nuclei labeled by DHE was automatically counted by using the image analysis software (Quantity One, Bio-Rad). ROS and superoxide anion levels were detected through a LumiMax detection kit and lucigenin chemiluminescence [25]. The ROS value was expressed as U/mg protein and the superoxide anion value was expressed as counts/min/mg protein.

2.9. Data analyses

Results were presented as mean ± standard deviation (SD). Two-way analysis of variance (ANOVA) was used to investigate the interaction between the effects of resveratrol and sildenafil. One-way ANOVA followed by Bonferroni's multiple comparison tests was performed for significant difference with the SPSS 16.0 software. P value <0.05 was considered statistically significant.