The Effect of Sildenafil and Udenafil on Testicular Damage Following Ischemia-Reperfusion Injury in Rats

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Abbreviations and Acronyms

I/R = ischemia and reperfusion

MDA = malondialdehyde

NO = nitric oxide

PDE5 = phosphodiesterase-5

T/D = testicular torsion and detorsion

T-SH = total sulfhydryl

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Study received animal ethics committee approval.

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Purpose: Ischemia-reperfusion injury can cause testicular damage and phosphodiesterase inhibitors are reported to regulate antioxidant activity. We investigated the prevention of ipsilateral and contralateral testicular damage using 2 phosphodiesterase inhibitors after testicular detorsion in rats.

Materials and Methods: A total of 28 adult male rats were randomly divided into 4 groups of 7 each, including group 1—sham operation, group 2—testicular torsion and detorsion, group 3— testicular torsion and detorsion with sildenafil administration before detorsion and group 4— testicular torsion and detorsion with udenafil administration before detorsion. Tissue levels of malondialdehyde, total sulfhydryl and nitrite were evaluated, and histopathological changes in the groups were examined.

Results: Compared to group 1 significantly increased tissue malondialdehyde ($p = 0.001$), significantly decreased total sulfhydryl ($p = 0.038$) and insignificantly increased nitrite were found in group 2. Compared to group 2 malondialdehyde decreased significantly and total sulfhydryl increased significantly in groups 3 and 4. The decrease in nitrite was insignificant in the latter 2 groups. Histopathology revealed increased hemorrhage, congestion and edema in group 2 rats. The testicular injury score was lower in groups 3 and 4. In group 2 grades II to IV injury was detected while most specimens in treated groups showed grade II injury.

Conclusions: This study indicates that intraperitoneal administration of sildenafil and udenafil efficiently suppresses radical production while decreasing histological changes after testicular ischemia-reperfusion injury.

Key Words: testis, spermatic cord torsion, reperfusion injury, sildenafil, udenafil

TESTICULAR torsion is a urological emergency that may occur at any age. To establish blood flow surgical detorsion is required in most cases while detorsion can sometimes be achieved by hand assistance. Regardless of the correction method 2 phases (I/R) negatively affect germ cells. The torsion phase decreases the oxygen supply and cell energy, and the detorsion phase affects reactive radical formation.[1] After reperfusion some oxidative stress parameters were noted in organs such as the liver, heart, brain and colon, in addition to the testis.[2,3]

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We tested the hypothesis that PDE5 inhibitors would have a protective effect on I/R injury in a rat model of bilateral T/D. We sought an easily achieved chemical that reduces organ damage.

MATERIALS AND METHODS

We calculated a required total sample size of 28 subjects by a priori analysis for a given effect size of 0.8, expected power of 0.90 and $\alpha = 0.05$, and divided the result into 4 groups. We used 28 Sprague Dawley® rats weighing 200 to 255 gm at ages 2 to 3 months, correlating with age 13 to 15 years in humans. All rats had free access to standard laboratory rat chow and water on a 12-hour light/dark cycle at 21C to 24C. After receiving animal ethics committee approval all rats were handled in compliance with the recommendations of the animal care committee of the university and the NIH (National Institutes of Health) Principles of Laboratory Animal Care, 1985 revision.

Rats were randomly divided into 4 groups of 7 each, including group 1—sham operation, group 2—T/D, group 3—T/D plus sildenafil and group 4—T/D plus udenafil. Assignment was random to avoid bias due to rat weight and age.

After administering 5 mg/kg ketamine and 2 mg/kg xylazine the right testes were exteriorized through a midline laparotomy incision. In group 1 the testes were removed and replaced through the incision, and a silk suture was placed through the tunica albuginea. Group 2 rats served as controls. Saline (2 ml 0.9% NaCl) was injected intraperitoneally 1 hour before detorsion. Group 3 rats were treated intraperitoneally with sildenafil citrate (Viagra® 100 mg) 2 ml from 1 mg/kg dissolved in 0.9% NaCl 1 hour before detorsion. Group 4 rats were treated intraperitoneally with udenafil citrate (Zydena, Zentiva, Prague, Czech Republic) 2 ml from 1 mg/kg dissolved in 0.9% NaCl 1 hour before detorsion.

Rats were maintained in an ambient temperature of 24C. Torsion was induced by twisting the testes 720 degrees clockwise. The testes were examined for an ischemic appearance before detorsion and torsion was repaired by rotating the right testes counterclockwise. After detorsion the testes were returned through the incision. Torsion lasted for 2 hours followed by a 4-hour detorsion period. Subsequently the rats were sacrificed and bilateral orchiectomy was performed.

Tissues were stored at −80C until assays were examined. MDA levels were calculated by the fluorometric method described by Wasowicz et al.[4] After thiobarbituric acid reaction with MDA the reaction product was extracted in butanol and measured by a spectrofluorometer at a wavelength of 525 nm for excitation and 547 nm for emission. The standard was 0 to 5 μmol/l 1,1′,3,3′-tetraethoxypropane solution.

T-SH in each group was measured by a spectrophotometer using the method of Sedlak and Lindsay.[5] Aliquots of 250 μl of the supernatant fraction of tissue homogenate were mixed in 5 ml test tubes with 750 μl 0.2 M tris buffer (pH 8.2) and 50 μl 0.01 M 5,5′-dithiobis (2-nitrobenzoic acid). The mixture was brought to 5 ml with 3,950 μl absolute methanol. Supernatant fraction absorbance was read in a spectrophotometer at 412 nm.

To measure nitrite levels 300 μl homogenate were added to 300 μl KH_2PO_4/K_2HPO_4 buffer (pH 7.5), 50 μl 2 mmol/l nicotinamide adenine dinucleotide phosphate, 50 μl 50 μmol/l flavin adenine dinucleotide and 50 μl 1 U/ml aspergillus nitrate reductase. This was incubated at room temperature for 1 hour followed by the addition of $ZnSO_4$ and NaOH to deproteinate the sample. After centrifugation the supernatant was added to sulfanilic acid in 4 mol/l HCl. The resultant color change was measured at 548 nm using a spectrophotometer. The nitrite concentration was calculated from 5, 12.5, 25 and 50 μmol/l sodium nitrite standards.[6]

The testis was fixed in Bouin fixative. Tissues were sectioned at 5 μm, stained with hematoxylin and eosin, and evaluated by an experienced genitourinary pathologist. Histopathological changes were scored according to the classification of Cosentino et al (supplementary Appendix, http://jurology.com/).[7]

Statistical analysis was done with SPSS®, version 16.0.1. Data with a normal distribution and homogenous variance were analyzed using 1-way ANOVA followed by the Tukey post hoc test. Data with a nonnormal distribution and nonhomogeneous variance were analyzed by the Kruskal-Wallis test followed by the Mann-Whitney U test with values shown as the median. All data are expressed as the mean ± SD with $p < 0.05$ considered significant. Power analysis was performed with G*Power 3.1.7.

RESULTS

In the right testes a significant difference was observed in MDA and T-SH levels in groups 1 and 2 ($p < 0.001$ and 0.038, respectively). The difference in nitrite levels in groups 1 and 2 was insignificant. MDA was significantly decreased and T-SH was significantly increased in groups 3 and 4 compared to group 2. There was also a decrease in tissue nitrite in groups 3 and 4 with an insignificant difference compared to group 2 (table 1).

We calculated achieved power by post hoc power analysis using the equations, power(1 − β err prob) right = 1.000 and left = 0.92 for MDA, power(1 − β err prob) right = 1.000 and left = 1.000 for T-SH, and power(1 − β err prob) right = 0.050 and left = 0.052 for nitrite.

In contralateral testes there was an insignificant difference between MDA and nitrite levels in all groups. T-SH significantly decreased in group 2 compared to group 1. T-SH increased insignificantly while MDA and nitrite decreased insignificantly in groups 3 and 4 compared to group 2. Table 1 lists MDA, T-SH and nitrite levels in each group. Results did not significantly differ in groups 3 vs 4 ($p > 0.05$).

More disorderly, noncohesive, sloughed germinal cells as well as more congestion, edema and necrotic areas were found in the right testes of group 2 with