Effect of Antioxidants on Outcome of Testicular Torsion in Rats of Different Ages

Carina T. Ribeiro, Raquel Milhomem, Diogo B. De Souza,* Waldemar S. Costa, Francisco J. B. Sampaio and Marco A. Pereira-Sampaio

From the Urogenital Research Unit, State University of Rio de Janeiro (CTR, RM, DBDS, WSC, FJBS, MAP-S), Rio de Janeiro and Department of Morphology, Fluminense Federal University (MAP-S), Niteroi, Brazil

Abbreviations and Acronyms

ARG = TT and arginine
ARG4 = 4-week-old ARG
ARG6 = 6-week-old ARG
ARG9 = 9-week-old ARG
Av = absolute volume
R = right testicle
RES = TT and resveratrol
RES4 = 4-week-old RES
RES6 = 6-week-old RES
RES9 = 9-week-old RES
SH = sham operation
SH4 = 4-week-old SH
SH6 = 6-week-old SH
SH9 = 9-week-old SH
TT = testicular torsion
TT4 = 4-week-old TT
TT6 = 6-week-old TT
TT9 = 9-week-old TT
Vv = volumetric density

Accepted for publication September 30, 2013.
Study received local ethics committee approval.
Supported by grants from the National Council of Scientific and Technological Development, Coordination for Improvement of Post-Graduated Students and Foundation for Research Support of Rio de Janeiro, Brazil.
* Correspondence: Urogenital Research Unit, State University of Rio de Janeiro, Av. 28 de Setembro, 87-Fundos, Rio de Janeiro, RJ, Brazil, 20551-030 (telephone and FAX: + 55 21 2868-8399; e-mail: diogobenchimol@gmail.com).

Purpose: We assessed reproductive and testicular function in adult rats after testicular torsion created before, during and after puberty, and with vs without resveratrol or arginine treatment.

Materials and Methods: Age matched rats were divided into groups, including simulated surgery without testicular torsion, 720-degree testicular torsion for 4 hours, testicular torsion with resveratrol treatment and testicular torsion with arginine treatment. To study reproductive function at age 12 weeks each rat mated with 3 females. The males were sacrificed at age 14 weeks. Spermatozoids were collected from the epididymal tail and evaluated for concentration, motility and viability. Testicular samples were collected for morphological analysis.

Results: Reproductive function was not altered by testicular torsion but antioxidants improved potency. Compared to sham operated and contralateral samples all spermatozoid parameters from testicular torsion samples were inferior. Resveratrol and arginine did not improve spermatozoid quality or quantity in torsed testes but contralateral samples were improved by each drug. The seminiferous epithelium of rats submitted to testicular torsion during puberty was least affected. Each antioxidant partially to totally prevented the morphological alterations found in rats with untreated testicular torsion. Rats submitted to testicular torsion before puberty that were treated with antioxidants showed the fewest changes.

Conclusions: Testicular morphology was altered less in rats when torsion occurred earlier in life, that is during puberty. Treatment with antioxidants improved contralateral spermatozoid production and some fertility parameters. Each antioxidant also prevented testicular morphology alterations after testicular torsion. Prepubertal rats benefited most from antioxidant treatment.

Key Words: testis, spermatic cord torsion, puberty, resveratrol, arginine

TESTICULAR torsion is a urological emergency that induces biochemical and morphological changes.[1] TT can affect males of any age but it occurs more often in neonates, boys and young men.[2] To our knowledge the impact on prognosis of age at TT is unknown.

The prognosis of TT is related to the duration and degree of torsion, resulting in different levels of parenchymal injury by oxidative stress.[3] Therefore, beyond rapid diagnosis and treatment several methods have been investigated to minimize the injury caused by TT.[4,5] Although rat

0022-5347/14/1915-1578/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2013.09.066
Vol. 191, 1578-1585, May 2014
Printed in U.S.A.

testes differ somewhat from human testes, rats have been widely used as experimental models in TT studies because lesions in rat testes are comparable to those in human testes after torsion.[6]

Several antioxidants have been investigated with promising results in rats submitted to TT. Of these antioxidants resveratrol and arginine have shown good results when used in testicular ischemia and reperfusion situations.[7,8] Arginine, an amino acid with antioxidant properties, is important for nitric oxide synthesis.[9] Resveratrol is a potent antioxidant present in many food sources that has inhibitory activity against reactive oxygen species and also enhances nitric oxide bioavailability.[10]

Although some groups reported beneficial results using these drugs, no quantitative evaluation of testicular parenchyma was performed. Also, to our knowledge no study has addressed morphological damage to the torsed testis, spermatozoid production or reproductive function after resveratrol and arginine treatment in rats submitted to TT at different ages.

We quantitatively assessed testicular morphology, spermatozoid parameters and reproductive function in adult rats that underwent TT before, during and after puberty. We also evaluated the effect of resveratrol and arginine treatments.

MATERIALS AND METHODS

We used 106 male Wistar rats for TT experiments. Three unrelated females per male (total 318) were used for the fertility tests described. The rats remained with the mother until week 3 of life. They were then kept in a room with a controlled temperature (mean $\pm$ SD 25C $\pm$ 1C) and artificial dark-light cycle (lights on from 7:00 am to 7:00 pm), and had free access to standard rat chow and water. All experiments were done by blinded observers according to the Brazilian law for scientific use of animals and they were approved by the local ethics committee.

Male rats were randomly assigned to 12 groups. The 3 age groups were prepuberty (4 weeks), puberty (6 weeks) and adulthood (9 weeks). The treatment groups were SH, TT without antioxidant therapy, RES and ARG. The SH group included 10 prepubertal, pubertal and adult rats each. The TT group included 10 prepubertal, 9 pubertal and 9 adult rats. The RES and ARG groups included 8 prepubertal, pubertal and adult rats each.

After general anesthesia induction TT was induced by opening the scrotum and the lamina parietalis of the tunica vaginalis, and twisting the right testis 720 degrees clockwise. The torsed testicle was fixed in position by sutures and torsion was maintained for 4 hours with the rat under general anesthesia. Rotation duration and degree were based on a previous study showing that they produced significant damage in the rat testis.[11] After this period the organs were untwisted and fixed in anatomical position. In SH rats the same surgical approach was used to open the tunica vaginalis. The testicle was sutured in anatomical position for the same period but not twisted.

RES4, RES6 and RES9 rats received resveratrol (30 mg/kg) intraperitoneally 30 minutes before testicle detorsion. For 7 days postoperatively resveratrol was administered daily by gavage at the same dose.[12] ARG4, ARG6 and ARG9 rats received arginine (650 mg/kg) by gavage for 7 days postoperatively.[13]

At age 12 weeks all male rats were mated with 3 estrous females to determine fertility parameters.[14] Females were sacrificed on day 20 of gestation. The uterus was opened, pregnancy was confirmed and the number of fetuses and implantation sites was recorded. The ovaries were observed under magnification and the number of corpora lutea was counted. Potency was calculated as the percent of female rats with confirmed copulation divided by the number exposed for mating. The fertility index was calculated as the percent of implantation sites divided by the number of corpora lutea. The fecundity of each group was considered the percent of male rats that generated at least 1 fetus divided by the total number of male rats in the same group. We also calculated preimplantation and postimplantation losses.

All males were sacrificed at age 14 weeks by anesthetic overdose. Just after sacrifice spermatozoids were collected from the epididymal tail to determine concentration and motility in a Neubauer chamber.[15] Spermatozoid viability was assessed by the hypo-osmotic test.[16] In this analysis 200 spermatozoids were evaluated per rat. Samples were collected and analyzed from the right torsed and the contralateral epididymides.

After sacrifice each testicle was dissected from the appendix and weighed. Volume was measured using the Scherle method.[17] The organ was then fixed and processed for paraffin embedding to obtain 5 μm histological sections. Morphometric analysis was performed on hematoxylin and eosin stained slices and captured on a BX51 microscope with a coupled DP70 digital camera (Olympus, Tokyo, Japan).

Testicular structure Vv was assessed by the point counting method.[17] Using ImageJ (http://rsb.info.nih.gov/ij/) we superimposed a test grid with 100 points over the testicular photomicrographs. Each structure touched by a point was counted and density was determined as a percent of the analyzed field.[18] For each testicle 25 fields were evaluated under 400× magnification. We recorded the Vv of the tunica propria, seminiferous epithelium, tubular lumen, seminiferous tubule (the sum of these 3 structures), the vessels and the intertubular compartment, including vessels.

We calculated the Av of each mentioned structure by dividing testicular volume by structure Vv, expressed in ml.[19] Total tubular length was calculated as previously described.[20]

The diameter of 125 seminiferous tubules per rat was measured in each testis by applying a straight line that crossed the tubule. For this purpose we used ImageJ, which was previously calibrated to 100× magnification. The line was applied in such a manner that it always passed through the center of the tubule. For this analysis we excluded tubules with an irregular shape.[21] Also, using this software the seminiferous epithelium height of randomly selected tubules was measured in each testis.