Preventive Effects of Cyclosporine A Combined With Prednisolone and Melatonin on Contralateral Testicular Damage After Ipsilateral Torsion-Detorsion in Pubertal and Adult Rats

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Abbreviations and Acronyms

BTB = blood-testis barrier
CsA = cyclosporine A
GCLT = germ cell layer thickness
I/R = ischemia/reperfusion
Pd = prednisolone
SD = Sprague-Dawley
ST = seminiferous tubule
TT = testicular torsion

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Purpose: We compared the preventive effects of cyclosporine A combined with prednisolone and melatonin (Sigma-Aldrich®) on damage to the contralateral testis after ipsilateral testicular torsion-detorsion between pubertal and adult rats.
Materials and Methods: We divided pubertal and adult Sprague-Dawley® rats into groups 1—sham operation, 2—detorsion, 3—detorsion plus cyclosporine A with prednisolone and 4—detorsion plus melatonin. After 4 hours of ipsilateral testicular torsion we treated the rats with detorsion only or with detorsion plus drug depending on the group.
Results: Seminiferous tubule diameter and germ cell layer thickness were greater in pubertal group 3 and adult group 4 than at each age in group 2 (each $p < 0.05$). The number of spermatids per tubule was greater in pubertal groups 3 and 4, and in adult group 4 than at each age in group 2 (each $p < 0.05$). Of pubertal rats those in groups 3 and 4 had fewer TUNEL positive cells than group 2 ($p = 0.061$ and 0.057, respectively). Of adult rats the number of TUNEL positive cells was greater in group 3 and significantly lower in group 4 vs that in group 2 ($p < 0.05$).
Conclusions: The preventive effects of cyclosporine A combined with prednisolone on contralateral testicular damage were noted only in pubertal rats while the preventive effects of melatonin were noted in pubertal and adult rats. Results suggest that damage to the contralateral testis induced by an immunological mechanism may be more significant during puberty than during adulthood.

Key Words: testis; spermatic cord torsion; cyclosporine; melatonin; rats, Sprague-Dawley

TESTICULAR torsion is a urological emergency. It may occur during the perinatal period or infancy but it develops most commonly during puberty.[1] Previously it was presumed that only the affected testis was functionally impaired. However, some reports indicate that infertility develops even with immediate management for TT during the pubertal stage[2] and abnormal sperm quality is observed in 40% to 60% of patients after TT.[3] Thus, contralateral testicular function may also be damaged by unilateral TT. Despite several etiological explanations for contralateral testicular damage after TT[4–11] the associated mechanism is not completely understood.

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Prior experimental studies of contralateral testicular damage were primarily done in adult animals without including pubertal animals. Since immunological, humoral and neural differentiation and maturation vary by age, a study of specific groups by age may provide important information. Generally BTB, which isolates the germinal epithelium from the immune system, begins to form at age 3 weeks in the rat and at age approximately 45 days rat BTB contains completely matured Sertoli's cells.[12] Thus, immunological protection for contralateral testicular damage after TT may decrease at puberty (ages 5 to 6 weeks) in the rat due to immature Sertoli's cells and the severity of contralateral damage caused by immunological activity may be different than that in adult rats. We previously reported that during puberty rats had more severe contralateral testicular damage than adult rats after TT.[13]

Most prior experimental studies of the prevention of testicular damage after TT used agents that prevent immunological[5,7,8,14] or I/R injury.[6,15] However, the degree of injury to the contralateral testis induced by these 2 mechanisms may be different during puberty and adulthood since BTB formation is incomplete during puberty. Also, the effects of an immunosuppressant or antioxidant on preventing contralateral damage may vary by age. However, to our knowledge the effects of these 2 agents have not been compared in rats during puberty vs adulthood.

We evaluated whether an immunosuppressant (CsA combined with Pd) and an antioxidant (melatonin) have different effects on the prevention of contralateral testicular damage after ipsilateral TT in pubertal vs adult rats.

MATERIALS AND METHODS

Animals

Male SD rats were allowed to acclimatize at 21C under a 12/12-hour dark/light cycle with free access to water and standard feed pellets. After 1-week acclimation we randomly separated 27, 6-week-old pubertal and 27, 10-week-old adult SD rats separated into each experimental group. The Clinical Research Institute institutional animal care and use committee at Seoul National University Hospital approved the protocol of this study. National Research Council guidelines for the care and use of laboratory animals were observed.

Experimental Groups

Male SD rats in each age group were divided into 4 study groups, including group 1—5 sham operated controls, group 2—6 with torsion-detorsion only, group 3—8 with torsion-detorsion plus CsA combined with Pd and group 4—8 with torsion-detorsion plus melatonin. Group 1 underwent a paramedian scrotal incision and physical manipulation of the left testis, after which the testis was replaced in the scrotum. After 4 hours the left testis was again manipulated without treatment. Group 2 underwent counterclockwise 720-degree TT of the left testis and testicular fixation with a 4-zero silk suture. After 4-hour torsion the testis was detorsed to its original place and the scrotal wound was repaired. In group 3 detorsion was done after 4-hour torsion. Immediately after detorsion the rats were injected subcutaneously with CsA (25 mg/kg per day) for 4 days combined with Pd (1-dehydrohydrocortisone) (3 mg/kg per day) for 2 days. In group 4 detorsion was done after 4-hour torsion and melatonin (N-acetyl-5-methoxytryptamine) (50 mg/kg) was injected intraperitoneally 15 minutes before detorsion.

Histological Procedure and Evaluation

After surgery all rats were maintained in cages until they were the same age, at which time the contralateral (right) testes of experimental animals were obtained. All contralateral testes were harvested at age 13 weeks. For light microscopy the testes were fixed in Bouin's solution, embedded in paraffin wax, sectioned at 4 μm and stained with hematoxylin and eosin. ST diameter, GCLT and number of spermatids per tubule were measured using an Olympus® BX-51TF light microscope. Two pathologists independently evaluated histological changes in blinded fashion without knowledge of study groups. Mean ST diameter was calculated under a 100× field with an ocular micrometer by averaging the diameter of the 10 most circular tubules identified in each tissue section. To measure mean GCLT the distance from the basement membrane to the lumen of the most circular tubule was calculated at 90, 180, 270 and 360 degrees under a 200× field and averaged. Ten tubules were evaluated per tissue section. The mean number of spermatids per tubule was calculated under a 400× field by averaging the number of spermatids of the 5 most circular tubules identified in each tissue section. Germinal cells were considered spermatids when they matured beyond metamorphosis (Sb_2 stage).

Apoptosis Evaluation

The TUNEL method with the ApopTag® S7100 Peroxidase kit was used to identify nuclei with DNA strand breaks at the cellular level in each tissue section from an area adjacent to hematoxylin and eosin stained areas. After deparaffinization and rehydration the sections were digested with proteinase K (20 μg/ml) for 15 minutes at room temperature. Endogenous peroxidase was inactivated by 3% H_2O_2 for 5 minutes at room temperature. The section was then incubated with equilibration buffer (75 μl) for 10 seconds at room temperature and incubated with working strength terminal deoxynucleotidyl transferase enzyme (55 μl/5 cm^2) at 37C for 1 hour in wet chamber. The section was then treated with working strength stop/wash buffer, agitated for 15 seconds and incubated for 10 minutes at room temperature. After washing the section was incubated with anti-digoxigenin peroxidase conjugate (65 μl/5 cm^2) for 30 minutes. The section was reacted in diaminobenzidine solution for 3 to 6 minutes at room temperature. Counterstaining for nuclei was done with 0.5% methyl green dye for 10 minutes at room temperature. A negative and a positive control were obtained. Nuclei with intense brown staining were considered positive for apoptosis. The 30 most circular STs were assessed and the apoptotic cells in each tubule were re-