Protective Effects of N-Acetylcysteine on Experimentally Undescended Testis

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Abbreviations and Acronyms

ACC = apoptotic cell count
FM = flutamide
GPx = glutathione peroxidase
MDA = malonyl dialdehyde
NAC = N-acetylcysteine
ROS = reactive oxygen species
STD = seminiferous tubule diameter
UT = undescended testis

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Purpose: We evaluated the efficacy of N-acetylcysteine for testicular damage induced by undescended testes in rats.

Materials and Methods: Flutamide was injected in the abdomen of pregnant rats daily from days 14 to 20 of gestation. Male offspring with cryptorchidism were randomly divided into 2 groups. Healthy male rats without undescended testes comprised the control group (group 1). Group 2 (undescended testes without N-acetylcysteine) received no treatment. Group 3 (undescended testes plus N-acetylcysteine) received intraperitoneal N-acetylcysteine daily. At 70 days after experiment initiation the testes were removed for histopathological and biochemical analysis.

Results: Mean malonyl dialdehyde values were lowest in group 1 and highest in group 2. In group 3 malonyl dialdehyde levels were significantly lower than in group 2 ($p < 0.001$). Conversely, mean glutathione peroxidase was highest in group 1 and lowest in group 2. Glutathione peroxidase levels in group 3 were significantly higher than in group 2 ($p < 0.001$). Histopathological differences between groups 1 and 3 in the modified Johnsen score were not significant ($p = 0.041$). However, the differences between these groups and group 2 were significant ($p < 0.001$). The median apoptotic cell count did not differ between groups 1 and 3 but it was significantly higher in group 2 than in the other groups ($p = 0.03$ and < 0.001, respectively).

Conclusions: N-acetylcysteine may alleviate undescended testis induced damage to testes through its antioxidant effects. The underlying mechanism of these effects merits further investigation. Long-term studies are also needed as well as comparative animal and human studies.

Key Words: testis, cryptorchidism, acetylcysteine, abnormalities, antioxidants

Cryptorchidism is a common anomaly in male newborns. Its frequency is 2% to 5% in full-term newborns but it may be as high as 15% to 30% in those with low birth weight and in premature neonates. In the first 3 months of life the testes descend into the scrotum in two-thirds of cases. Thus, the incidence of cryptorchidism decreases to 1% around age 1 year.[1] Hormonal or surgical treatment can be elected for cryptorchidism. However, apoptosis in the testes is an adverse effect of medical treatment[2] and testicular descent into the scrotum by surgical means should be

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the preferred treatment. Although surgery is advised before age 1 year, about 13% of patients undergo orchiopexy in the first year of life.[3]

As in cryptorchid cases, conditions that increase the temperature in the testes induce oxidative stress in testicular tissue. Oxidative stress in turn increases ROS and decreases the levels of antioxidants that comprise the defense system.[4] Apoptosis increases secondary to high temperature, resulting in germ cell dysmaturation. As a result, infertility frequently ensues in patients with bilateral cryptorchidism.[5]

NAC is the N-acetylated derivative of the amino acid L-cysteine. It increases glutathione synthesis by giving off a cysteine that is produced after deacetylation during glutathione synthesis. Glutathione is a fundamental antioxidant that scavenges ROS.[6] NAC can be used from birth and thereafter[7] as a mucolytic, or as the antidote to acetaminophen intoxication. It is efficacious for preventing contrast medium nephropathy, pulmonary fibrosis and infertility secondary to polycystic ovarian syndrome, and for cancer chemoprevention.[8]

A literature review yielded no studies that assessed NAC efficacy for preventing the tissue damage observed in UTs. We determined whether NAC has beneficial effects on tissue damage due to UTs.

MATERIALS AND METHODS

The current study was done at the Experimental Animals Application and Investigation Centre, Abant Izzet Baysal University. Local ethics committee approval was obtained. Eight 10-week-old pregnant Wistar albino rats were used in the study. During the investigation the rats were housed at a mean ± SD ambient temperature of 22 ± 2C, a 12-hour light/12-hour dark light cycle and 40% to 70% humidity. They were fed pelleted rat feed and received tap water.

As defined in an earlier cryptorchid model, 6 pregnant rats were given 7.5 mg FM for 7 days between days 14 and 20 of gestation.[9,10] We divided the rats into 3 study groups. Group 1 (controls) included a total of 8 rats with normal testes that were born to 2 mothers that did not receive FM. Group 2 (UT without NAC) included a total of 12 rats with cryptorchidism that did not receive NAC and were born to 3 mothers that received FM. Group 3 (UT plus NAC) included a total of 11 rats with cryptorchidism that received 100 mg/kg NAC intraperitoneally daily from birth and were born to 3 mothers that received FM.

On study day 70 all rats were sacrificed by intraperitoneal administration of 90 mg/kg ketamine and 10 mg/kg xylazine. Testicular tissues were removed for histopathological and biochemical analysis.

Histological Evaluation

Histopathological examination of testicular tissues was performed in all groups after fixing specimens in Bouin solution and embedding in paraffin. Hematoxylin and eosin staining was done to assess spermatogenesis. Based on a modified Johnsen score we assessed the germinal epithelium of at least 50 tubules.[9,11] TUNEL assay was performed to evaluate apoptosis in spermatogenic cells in seminiferous tubules. Under light microscopy at 40× magnification we recorded the number of apoptotic cells in 50 seminiferous tubules that stained positive in each section. STD was measured using a micrometer eyepiece. Mean STD was calculated from 50 round, randomly selected seminiferous tubules per tissue section.[9,11] All histopathological assessments were done by an experienced pathologist blinded to the groups.

Biochemical Evaluation

Testicular tissues weighing 100 mg each were placed in labeled glass tubes. Samples were washed with phosphate buffered saline once and stored at −80C until the day of biochemical analysis. Samples were thawed before study. Each tissue was homogenized once in 1 ml phosphate buffered saline. Samples were frozen and thawed twice to facilitate cell membrane disintegration. The homogenate was centrifuged at 2C to 8C at 5,000 × gravity for 5 minutes and the supernatant was removed. MDA and GPx activity was measured using commercially available enzyme-linked immunosorbent assay kits (Cusabio Biotech, Wuhan, People's Republic of China) according to manufacturer instructions. MDA and GPx linearity ranges were 31.25 to 2,000 pmol/ml and 12.5 to 800 mIU/ml, respectively.

Statistical Analysis

Data analysis was done with SPSS®, version 17 for Windows®. We assessed whether the distribution of continuous variables was normal using the Shapiro-Wilk test. The Levene test was used to evaluate variance homogeneity. Data are shown as the mean ± SD or median and IQR, as appropriate. Mean differences among groups were compared by 1-way or Welch ANOVA. The Kruskal-Wallis test was otherwise used to compare medians. When 1-way or Welch ANOVA, or Kruskal-Wallis test p values were statistically significant, we used the post hoc Tukey HSD or Tamhan T2 test for parametric data and the Mann-Whitney U test for nonparametric data to determine the significance of differences among groups with $p < 0.05$ considered statistically significant. The Bonferroni adjustment ($p < 0.017$) was used for all multiple comparisons to control type I errors.

RESULTS

When biochemical parameters were analyzed, MDA was lowest in controls and highest in UT group without NAC. MDA was significantly lower in UT group with NAC than in the UT group without NAC ($p < 0.001$). On the other hand, GPx was highest in controls. GPx in the cryptorchid groups was lower than in controls, although in the UT plus NAC group GPx was significantly greater than in the UT group without NAC ($p < 0.001$).