Effects of 3-aminobenzamide on unilateral testicular ischemia–reperfusion injury: What is the role of PARP inhibition?

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Abstract: The therapeutic effects of poly(adenosine diphosphate-ribose) polymerase inhibition by 3-aminobenzamide (3-AB) were investigated in testicular ischemia–reperfusion (I/R) injury, using sperm analysis and histopathological and biochemical examinations, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities and reduced glutathione (GSH) levels. Male rats were divided into 3 groups: sham (n = 12), I/R (n = 12), and I/R with 3-AB (I/R–3-AB) (n = 12). The left testicular artery was occluded for 1 h, followed by 24 h (for biochemical and histopathological examinations) and 30 days (for sperm analysis) of reperfusion. 3-AB treatment intraperitoneally 10 min prior to and 1 h after reperfusion increased the I/R-induced decrease in sperm motility in both testes and reduced the increased abnormal sperm rates in the ipsilateral testis. However, 3-AB treatment failed to prevent the I/R-induced decrease in sperm concentration in both testes. SOD and CAT activities did not change in any group. GSH-Px activity and GSH levels were increased by I/R. 3-AB treatment reversed the I/R-induced increase in GSH-Px activity, similar to the level in sham rats, but did not alter GSH levels. 3-AB treatment significantly increased the I/R-induced decrease in histopathologic score. In conclusion, 3-AB treatment has potential biochemical and histopathological benefits beyond improving sperm quality and may have the potential to decrease damage from testicular torsion.

Key words: 3-aminobenzamide, testis, ischemia–reperfusion, sperm analysis.

Résumé : On a examiné les effets thérapeutiques de l’inhibition de la poly(adénosine diphosphate-ribose) polymérase par le 3-aminobenzamide (3-AB) dans une lésion d’ischémie-reperfusion (I/R) des testicules en utilisant une analyse de sperme. On a effectué des examens histopathologiques et biochimiques pour mesurer les activités de la superoxyde dismutase (SOD), de la catalase (CAT), de la glutathion peroxydase (GSH-Px), et les taux de glutathion (GSH) réduit. On a divisé des rats mâles en 3 groupes : ayant subi une opération factice (*sham*) (n = 12), I/R (n = 12) et I/R avec 3AB (I/R–3-AB) (n = 12). On a occlus l’artère testiculaire gauche pendant 1 h, puis on l’a soumis à une reperfusion pendant 24 h (pour les examens biochimiques et histopathologiques) et 30 jours (pour l’analyse de sperme). L’administration de 3-AB par voie intrapéritonéale 10 min avant et 1 h après la reperfusion a augmenté la diminution induite par l’I/R de la motilité du sperme dans les 2 testicules, et elle a diminué l’augmentation anormale des taux de sperme dans le testicule ipsilatéral. Toutefois, le traitement par 3-AB n’a pu prévenir la diminution induite par l’I/R du taux de sperme dans les 2 testicules. Les activités de SOD et de CAT sont demeurées stables chez tous les groupes. L’I/R a augmenté l’activité de la GSH-Px et les taux de GSH. Le traitement par 3-AB a renversé l’augmentation induite par l’I/R de l’activité de la GSH-Px comme chez les *sham*, mais il n’a pas modifié les taux de GSH. Le traitement par 3-AB a augmenté de manière significative la diminution induite par l’I/R du score histopathologique. En conclusion, un traitement par 3-AB offre des avantages biochimiques et histopathologiques potentiels au-delà de la qualité du sperme et pourrait diminuer l’altération de la torsion testiculaire.

Mots-clés : 3-aminobenzamide, testicule, ischémie–reperfusion, analyse de sperme.

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Introduction

Testicular torsion occurs frequently during the peripubertal period. The duration of ischemia and severity of torsion cause diverse levels of testicular damage (Anderson and Williamson 1986; Saba et al. 1997). Although detorsion is mandatory for the survival of ischemic tissue, the main pathophysiology of testicular torsion is ischemia–reperfusion (I/R) injury of the testis caused by reperfusion (Makgür et al. 1994). A pathophysiologic cascade, including an inflammatory response with liberation of cytokines and reactive oxygen species (ROS) generation triggered by I/R injury, may be one of the possible causes of injury (Ambrosio et al. 1991; Cuzzocrea et al. 2001). The poly(adenosine diphosphate-ribose) polymerase (PARP) pathway is involved in the pathogenesis of various forms of I/R injury (Szabó and Dawson 1998; Shall and de Murcia 2000; Tentori et al. 2002). ROS produced during I/R are powerful triggers of DNA single-strand breakage and the consequent activation of the nuclear enzyme PARP (Zingarelli et al. 1997).

Inhibition of PARP activation exerts beneficial effects that ameliorate the metabolic changes but not the development of DNA damage in inflammation during I/R processes (Zingarelli et al. 1996). Overactivation of PARP may lead to cell death due to energy depletion (Wintersberger and Wintersberger 1985; Cochrane 1991; Banasik et al. 1992). Because of a rapid depletion of intracellular nicotinamide adenine dinucleotide (NAD^+) and adenosine triphosphate (ATP) energy pools, glycolysis and the mitochondrial respiration rate decelerate, leading to cellular dysfunction and death (Ueda and Hayaishi 1985). Overall, this process is termed “the poly(ADP-ribose) polymerase suicide hypothesis” (Berger 1985). This cell suicide phenomenon is driven by PARP activation and has been demonstrated in several cell types (Zingarelli et al. 1996).

PARP plays another critical role in the regulation of the expression of endothelial adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and P-selectin. The infiltration of inflammatory cells, such as neutrophils, into the injured area initiates an inflammatory cascade leading to oxidative damage. Neutrophil infiltration becomes a source for ROS and reactive nitrogen species (RNS), such as peroxynitrite, and the erythrocyte antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), are inactivated by peroxynitrite (Zingarelli et al. 1998, 1999; Stern et al. 1999).

PARP inhibition exerts beneficial effects against free radical-mediated cell injury (Pacher et al. 2006). In several organ systems, the PARP inhibitor 3-aminobenzamide (3-AB) has been used successfully to decrease I/R injury (Thiemermann et al. 1997). We investigated whether 3-AB prevents testicular I/R injury in a rat model. We evaluated epididymal sperm concentration, motility, and morphology. Biochemical and histopathological alterations were also determined.

Materials and methods

Animals

Thirty-six male Wistar albino rats (250–350 g) were placed in a temperature- (21 ± 2 °C) and humidity- (60 ± 5%) controlled room with a 12 h light : 12 h dark cycle. The animals were obtained from the University of Inonu, Faculty of Medicine, Experimental Research Center (Malatya, Turkey). All experiments were performed in accordance with the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1984) and were approved by the Committee on Animal Research at Firat University, Elazig. These studies were carried out in the Experimental Research Unit of Firat University.

Chemicals

Ketalar (ketamine hydrochloride, Eczacıbasi Inc., Turkey), Rompun (xylazine 2%, Bayer Turk Chemical Inc., Turkey), 3-aminobenzamide, and other chemicals (Sigma–Aldrich, St. Louis, Mo.) were used in the current studies.

Experimental groups and sample collection

Thirty-six male Wistar albino rats were randomly divided into 3 experimental groups: sham (n = 12), I/R (n = 12), and I/R with 3-AB (I/R–3-AB) (n = 12). The sham group received an intraperitoneal (i.p.) injection of vehicle (0.5 mL double-distilled water). The left testicular artery and vein were occluded for 1 h, followed by 24 h and 30 days of reperfusion. Either vehicle (double-distilled water) or 3-AB (10 mg/kg) was injected intraperitoneally into I/R or I/R–3-AB animals 10 min prior to and 1 h after reperfusion. Sham groups (sham 24 h (n = 6) and sham 30 days (n = 6)) received an i.p. injection of vehicle (0.5 mL). I/R groups received 1 h ischemia and vehicle administration and were reperfused for 24 h and 30 days. I/R–3-AB groups similarly received 1 h ischemia and 3-AB and were reperfused for 24 h and 30 days. Epididymides and testes were collected after 24 h for the histopathological analysis and evaluation of sperm characteristics.

Surgery and experimental protocol

All surgical procedures were performed under anesthesia induced by an i.p. injection of 75 mg/kg ketamine hydrochloride and 8 mg/kg xylazine. An abdominal incision was made and the testicular artery and vein of the left testis were occluded with a vascular clamp for 1 h. The clamp was removed, and the organ was reperfused for 24 h and 30 days in different groups. Sham operations were performed in a similar fashion, but the vessels were not clamped. The rats were treated with either 3-AB or vehicle at 10 min prior to and 1 h after reperfusion. 3-AB was dissolved in mildly warmed double-distilled water.

The rats were sacrificed by cardiac extirpation following anesthetization at the end of the treatment period. Testes and epididymides were removed, cleared of adhering connective tissue, and weighed. Testis samples were fixed in a 10% neutral buffered formalin solution for histopathological examinations in the 24 h reperfusion group. The remaining testis samples in the 24 h reperfusion groups were stored at –80 °C for the biochemical analysis. The 30 day reperfusion groups were used for evaluation of sperm characteristics.

Epididymal sperm concentration, motility, and morphology

The epididymis was finely minced with anatomical scissors in a Petri dish containing 5 mL physiological saline

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and sperm were released from epididymal tissue at room temperature for 2 min. Epididymal sperm were counted with a hemocytometer using a modification of the method described by Yokoi et al. (2003). A sperm suspension of 5 μL was diluted with 95 μL PBS (pH 7.4) solution containing 10% formalin and 10% sucrose. Approximately 10 μL of the diluted sperm suspension was transferred to each counting chamber of the hemocytometer and was allowed to stand for 5 min. A light microscope (magnification 200×) was used to count settled cells. Motile sperm percentage was evaluated visually in each sample according to previously described methods (Sönmez et al. 2005). Briefly, a drop of sperm suspension diluted with Tris buffer solution (Tris 3.63 g, glucose 0.50 g, citric acid 1.99 g, and distilled water 100 mL) on a prewarmed (37 °C) slide under a prewarmed (37 °C) cover slip was viewed with a light microscope at 400× magnification. Motility estimations were performed from 4 different fields in each sample, and the mean of the 4 estimations was used as the final motility score.

Sperm morphological examination was performed as follows. A smear was prepared on clean slides after the sperm was stained with 1% eosin Y. The slides were air dried and coded for subsequent examination. Sperm morphological evaluation was carried out using a light microscope at 400× magnification.

Histopathological analysis

The rats were sacrificed after 24 h of reperfusion for histopathological evaluation. For light microscope evaluation, testes were fixed in 10% neutral buffered formalin, processed routinely by an automatic tissue processor, and embedded in paraffin wax. Four micrometre sections were stained with hematoxylin-eosin (H+E) before investigation under a light microscope (Olympus BX-51 TF, Tokyo, Japan). Histological findings in seminiferous tubuli were evaluated according to Johnsen's scoring system (Johnsen 1970). Tubuli in 10 consecutive 400× field areas were scored and mean values were determined. The Johnsen score is based on the premise that with testicular damage, there is progressive degeneration of germinal epithelium, with the disappearance of sperm and spermatids, then spermatocytes, then Sertoli cells, in that order. A score of 1 indicates no seminiferous epithelial cells and tuberous sclerosis; score 2, no germ cells, only Sertoli cells; score 3, spermatogonia only; score 4, no spermatids, since spermatocytes and arrest of spermatogenesis at the primary spermatocyte stage; score 5, no spermatids and many spermatocytes; score 6, no late spermatids with few early spermatids, arrest of spermatogenesis at the spermatid stage, and disturbance of spermatid differentation; score 7, no late spermatids and many early spermatids; score 8, few late spermatids; score 9, late spermatids and disorganized tubular epithelium; and score 10, full spermatogenesis.

Biochemical measurements

To evaluate biochemical parameters, rats in the 24 h reperfusion group were used. The ipsilateral (IL) testis tissue samples were used for the measurement of SOD, CAT, and glutathione peroxidase (GSH-Px) activities and glutathione (GSH) levels.

Weighed testes were homogenized for 3 min in a Teflon–glass homogenizer in glass tubes with ice-cold buffer containing 1.5% potassium chloride to obtain 1:10 (*w*/*v*) whole homogenate. The homogenates were centrifuged at 25 000*g* (4 °C) for 45 min to obtain supernatants for the determination of GSH concentrations and GSH-Px and CAT activities. The obtained supernatants were centrifuged again at 25 000*g* (4 °C) for 45 min to determine SOD activities.

Determination of SOD activity

Tissue SOD activity determination was based on the formation of color by the reduction of nitroblue tetrazolium by superoxide radicals produced by the xanthine–xanthine oxidase system. In this method, the supernatant was mixed with phosphate buffer (50 mmol/L, pH 7.8), EDTA (5 mmol/L), BSA (40 mg/L), nitroblue tetrazolium (0.2 g/L), xanthine oxidase, and xanthine (0.1 mmol/L). Reduction of nitroblue tetrazolium by superoxide radicals results in the formation of blue formazan with a maximum absorbance at 560 nm spectrophotometrically. Formation of colored formazone occurs in inverse proportion to enzyme concentration. Results are expressed as U/mg protein (Sun et al. 1988).

Determination of CAT activity

Testicular tissue CAT activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (1984), and was expressed as *k* U/g protein, where *k* is the first-order rate constant. The principle of the assay was based on the determination of the rate constant of hydrogen peroxide decomposition by CAT. In the experiment, 2 mL of the sample was added to 1 mL of 40 mmol/L H_2O_2 in phosphate buffer (50 mmol/L, pH 7.0, prepared by mixing the solutions 0.681 g KH_2PO_4 in 100 mL water and 1.335 g $Na_2HPO_4 \cdot 2H_2O$ in 150 mL water) and the decrease in H_2O_2 was measured spectrophotometrically at 240 nm for 3 min.

Determination of GSH-Px activity

GSH-Px activity in testicular tissue was determined according to the method of Lawrence and Burk (1976). The reaction mixture consisted of 50 mmol/L potassium phosphate buffer (pH 7.0), 1 mmol/L EDTA, 1 mmol/L sodium azide (NaN_3), 0.2 mmol/L NADPH, 1 U/mL oxidized glutathione (GSSG)-reductase, 1 mmol/L GSH, and 0.25 mmol/L H_2O_2. The enzyme source (0.1 mL) was added to 0.8 mL of the above mixture and incubated at 25 °C for 5 min before initiation of the reaction with the addition of 0.1 mL of the peroxide solution. The absorbance at 340 nm was recorded for 5 min on a spectrophotometer. The activity was calculated from the slope of the line as micromoles of NADPH oxidized per minute. The blank value, in which the enzyme was replaced with distilled water, was subtracted from each value. The protein concentration was measured by the method of Lowry et al. (1951). The GSH-Px activity was expressed as U/g protein.

Determination of reduced GSH levels

The reduced GSH levels of testicular tissue were measured at 412 nm using the method of Sedlak and Lindsay (1968). The samples were precipitated with 50% TCA and centrifuged at 1000*g* for 5 min. The reaction mixture contained 0.5 mL supernatant, 2.0 mL Tris–EDTA buffer (0.2 mol/L; pH 8.9), and 0.1 mL of 0.01 mol/L 5,5′-dithio-

Table 1. Results of sperm analysis of ipsilateral (IL) and contralateral (CL) testes 30 days after reperfusion.

	Motility (%)		Abnormal sperm rate (%)		Sperm count ($\times 10^{-6}$)	
Group	IL	CL	IL	CL	IL	CL
Sham	64.2±1.88^{b}	65.2±1.87^{b}	8.6±1.11^{b}	6.4±1.23	228.6±49.34^{b}	281.4±40.1^{b}
I/R	24.9±3.86^{a}**	40.4±5.95^{a}	22.0±3.12^{a}**	8.0±2.06	26.7±11.36^{a}**	140±20.52^{a}
3-AB	49.5±5.74^{c}**	57.8±6.15^{b}	12.3±2.75^{b}	12.0±2.32	62.3±20.18^{a}*	83±25.51^{a}
p	< 0.001	< 0.01	< 0.05		< 0.001	0.01

Note: Data shown are mean values ± SEM. Asterisks indicate that ipsilateral testes (IL) are statistically significantly different from the contralateral testes (CL) (*, $p < 0.05$; **, $p < 0.01$). I/R, ischemia–reperfusion; 3-AB, I/R–3-AB.

[a]Different from sham ($p < 0.05$).

[b]Different from I/R ($p < 0.05$).

[c]Different from sham and I/R ($p < 0.05$).

bis(2-nitrobenzoic acid). The solution was kept at room temperature for 5 min and read at 412 nm on the spectrophotometer. The level of GSH was expressed as nmol/mL.

Statistical analysis

All data are expressed as the arithmetic mean ± SEM. $p < 0.05$ was considered to be statistically significant. The sperm concentration and motility data were analyzed by one-way analysis of variance (ANOVA) and post hoc Tukey's HSD. The paired t test and the Wilcoxon rank sum test were used to assess the differences between the IL and contralateral (CL) testes. Biochemical results were statistically analyzed by ANOVA. The histological results and abnormal sperm rate were statistically analyzed by the Kruskal–Wallis H test. The differences between the groups were evaluated by the Mann–Whitney U test.

Results

Sperm analysis

Sperm motility was significantly ($p < 0.01$) different between IL and CL testes in the I/R and 3-AB groups. I/R decreased sperm motility significantly ($p < 0.01$) compared with the sham and 3-AB groups in both testes. 3-AB treatment prevented the I/R-induced decrease in sperm motility significantly in both testes and reversed it to levels similar to the sham group in the CL testis ($p < 0.01$) (Table 1).

The abnormal sperm rate was significantly ($p < 0.01$) different between IL and CL testes in the I/R group. The abnormal sperm rate in IL testes was significantly ($p < 0.05$) higher in the I/R group. 3-AB treatment significantly prevented the I/R-induced increase in total abnormal sperm rate ($p < 0.05$) and reversed it to levels similar to the sham group (Table 1).

I/R decreased the sperm concentration in both IL and CL testes ($p < 0.001$). There were significant differences in sperm concentration between IL and CL testes in the I/R ($p < 0.01$) and 3-AB ($p < 0.05$) groups. 3-AB treatment failed to prevent the I/R-induced decrease in sperm concentration in both testes (Table 1).

Histopathological results

Results of the mean testicular biopsy scores are shown in Table 2. Mean testicular biopsy scores were decreased by I/R in both the IL and CL testes ($p < 0.05$). Orderly maturation was observed in the sham group (Fig. 1A). Complete maturation arrest, disappearance of mature sperms, disorganization, and sloughing of the cellular elements into the lumina of seminiferous tubuli were observed (Fig. 1B). 3-AB treatment decreased the I/R injury in both testes (Fig. 1C) but failed to reverse biopsy scores to sham levels ($p < 0.05$) (Fig. 1A). The 3-AB treated group showed some improvement when compared with the I/R group. This improvement is observed as the beginning of spermatogenesis with a few mature spermatids at the lumen and considerably improved orderly lining of germ cells. There were no differences between IL and CL testes in any group ($p > 0.05$).

Table 2. Semiquantitative estimates of testicular injury 24 h after reperfusion.

	Testis score	
Group	IL	CL
Sham	9.0±0.18^{b}	8.9±0.23^{b}
I/R	6.1±0.11^{a}	6.5±0.15^{a}
3-AB	7.6±0.13^{ab}	7.3±0.11^{a}

Note: Data shown are mean values ± SEM.

[a]$p < 0.05$ vs. sham.

[b]$p < 0.05$ vs. I/R.

Biochemical results

The results of biochemical analysis in the sham, I/R 24 h, and 3-AB 24 h groups are shown in Table 3. SOD and CAT activities did not change significantly in any group. GSH-Px activity was increased by I/R treatment ($p < 0.05$). 3-AB treatment decreased GSH-Px activity to levels seen in the sham group ($p > 0.05$). I/R-induced increases in GSH levels were not affected by 3-AB treatment ($p > 0.05$).

Discussion

Although increasing the duration of ischemia from minutes to hours results in progressive damage to the testes, the critical time of vascular-occluding torsion after which severe testicular damage occurs in the rat model is between 30 min and 1 h (Turner and Brown 1993). Ischemia produces significant effects on spermatogenesis and biochemical and histological parameters during this period (Beheshtian et al. 2008*a*, 2008*b*). We selected a 1 h ischemia model as the minimum effective time of induction for significant testicular damage.

In previous studies, sham occlusion had no effect on the IL testis, but a progressive loss from mild to severe, reach-

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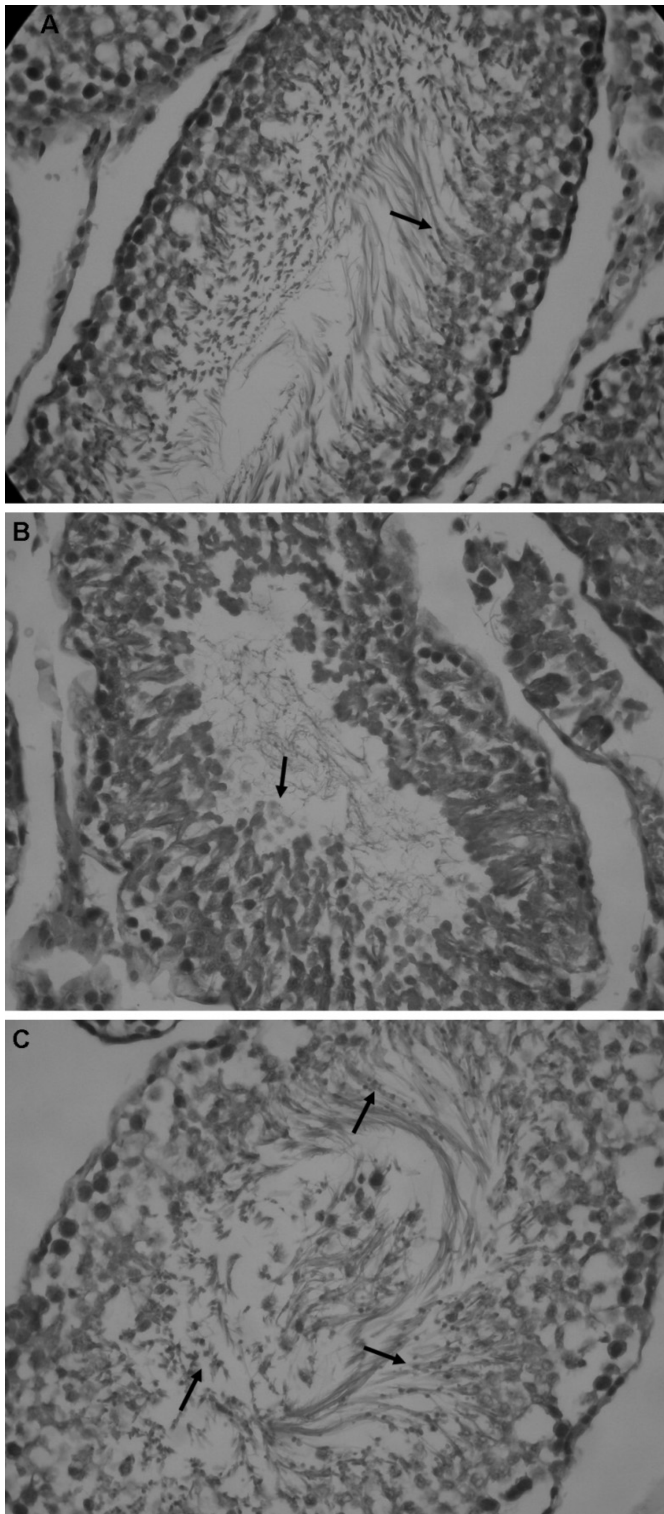
Fig. 1. Histopathological changes of testicular ischemia–reperfusion (I/R) injury and the effects of 3-aminobenzamide (3-AB) on these parameters. (A) Normal maturation of germ cells (arrow) in the sham group (hematoxylin-eosin (H+E), 400×). (B) Nearly complete maturation arrest with no mature spermatids (arrow) at the lumen in the I/R group of ipsilateral testis (H+E, 400×). (C) Reversal of maturation back to normal with sloughed germ cells (arrows) at the lumen of the ipsilateral testis after 3-AB treatment (H+E, 400×).



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ing an almost total loss of spermatogenesis, was observed by increasing the time of torsion to 1, 2, and 4 h measured 30–60 days after repair of torsion (Turner 1984, 1987). Similarly, 1 h ischemia decreased sperm concentration significantly in both IL and CL testes in our study. I/R had a detrimental effect on spermatogenesis and the epididymal sperm maturation process, although inducing necrotic degeneration of testicular tissue may contribute to spermatogenesis (Visser and Heyns 2003). Although oxidative stress plays a crucial role in the etiology of defective testes functions via mechanisms involving the induction of peroxidative damage to the plasma membrane, it is also associated with DNA damage and produces a higher frequency of abnormal sperm with significant effects on male fertility (Agarwal and Saleh 2002; Rajesh Kumar et al. 2002).

I/R decreased motility significantly in both testes, but the decrease in the IL testis was lower than that in the CL testis. 3-AB treatment significantly prevented the I/R-induced decrease in sperm motility in both testes and reversed the levels to similar to the sham group in the CL testis. I/R also increased abnormal sperm rate in the IL testis but did not affect the rate in the CL testis. The increase in morphologically abnormal sperm rate is a clear indication of testicular damage, as demonstrated by histological analysis. 3-AB treatment reversed the I/R-induced abnormal sperm rate to levels seen in sham animals. In the present context, the decrease in abnormal sperm population after 3-AB treatment indicates a reduction of cytotoxic and pathological alterations in germ cells. Nonetheless, 3-AB treatment failed to prevent the I/R-induced reduction in sperm concentration in both testes. Previous studies have reported that 3-AB treatment prevents I/R injury in experimental testicular torsion models (Bozlu et al. 2003, 2004). However, we could not reach a similar conclusion. To our knowledge, this is the first time that the effects of 3-AB have been observed on spermatogenesis.

Bozlu et al. (2004) showed that I/R caused a significant decrease in the mean testicular biopsy score in IL testes. The animals treated with 3-AB had a statistically significant increase in these histological parameters compared with the I/R group (Bozlu et al. 2004). We also investigated the changes in histopathological score in both IL and CL testes. Similarly, our results indicate that testicular I/R significantly decreased the mean testicular histopathological score in both testes. We viewed a decrease of histopathological score as evidence of the damage at 24 h after ischemia in both testes. 3-AB treatment significantly increased the I/R-induced decrease in histopathological score.

Stern et al. (1999) showed that treatment with a PARP inhibitor suppresses the tissue damage in laryngeal injury by inhibiting the recruitment of neutrophils and the subsequent generation of nitrogen-centered oxidants. Neutrophils are a potential source of ROS, such as superoxide and hydrogen peroxide, and RNS, such as nitric oxide and peroxynitrite (Felley-Bosco 1998). Peroxynitrite is a reactive oxidant produced from the reaction of nitric oxide with superoxide anion that impairs several functions through multiple mechanisms, including the activation of the nuclear enzyme PARP (Pacher et al. 2005). Peroxynitrite inactivates erythrocyte antioxidant enzymes with a potency of GSH-Px > SOD > CAT (Grzelak et al. 2000). PARP inhibition by 3-

Table 3. The biochemical analysis results of IL testes in the 24 h reperfusion group.

Group	SOD (U/mg protein)	CAT (*k* U/g protein)	GSH-Px (IU/g protein)	GSH (nmol/mL)
Sham	85.2±8.08	3.6±0.49	63.2±2.67[b]	2.7±0.28[b]
I/R	114.8±27.71	5.7±1.44	85.2±5.72[a]	5.3±0.80[a]
3-AB	67.9±18.1	4±0.72	71.1±6.01[b]	5.3±0.55[a]

Note: Data shown are mean values ± SEM. SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; GSH, glutathione.

[a]$p < 0.05$ vs. sham.

[b]$p < 0.05$ vs. I/R.

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AB reduces neutrophil infiltration and prevents tissue injury (Cuzzocrea et al. 1997; Zingarelli et al. 1997). Bozlu et al. (2003) showed that 3-AB improves some of the biochemical parameters that had increased, including the lipid peroxidation indicator malondialdehyde (MDA), nitric oxide content (NOx), and myeloperoxidase activity, which indicates neutrophil infiltration by testicular I/R injury. In our study, after 1 h ischemia and 24 h reperfusion, SOD and CAT levels were not changed in any group, while GSH and GSH-Px levels increased in the I/R group. 3-AB reduced the increased GSH-Px levels by I/R injury. Similarly, previous studies also show that the protective effects of 3-AB on testicular I/R may depend on reducing the I/R-induced ROS generation by decreasing nitric oxide production and (or) neutrophil infiltration. I/R increased GSH-Px activity and GSH levels, but 3-AB treatment reversed only GSH-Px activity to levels similar to those in the sham group. To our knowledge, these parameters have not been studied.

Under these conditions, we found that PARP activation may have a pathologic role, because treatment with 3-AB markedly suppressed tissue injury. From the in vivo data, it is difficult to establish the prevalent pathological pathway of testicular I/R injury and the protective action of 3-AB because several mechanisms may contribute. Our data suggest that testicular injury is a result of I/R injury driven by PARP activation. This characterization of the mechanism is controversial, because our results do not confirm previous data (Grzelak et al. 2000; Bozlu et al. 2003). However, we did not evaluate inflammatory cell infiltration, the generation of nitrogen-centered oxidants, or the DNA of the rescued germ cells. Our results provide a basis for other researchers to evaluate these factors in future studies.

In conclusion, the results of the current investigation indicate that inhibition of PARP with 3-AB produces beneficial effects on sperm characteristics by increasing sperm motility and decreasing abnormal sperm rate in testicular I/R injury. 3-AB also ameliorated biochemical parameters and histological findings in testicular I/R injury. In light of these findings, we propose that PARP inhibition may be a novel therapeutic approach for I/R injury of the testes.

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References

Aebi, H. 1984. Catalase in vitro. Methods Enzymol. **105**: 121–126. doi:10.1016/S0076-6879(84)05016-3. PMID:6727660.

Agarwal, A., and Saleh, R.A. 2002. Role of oxidants in male infertility: rationale, significance, and treatment. Urol. Clin. North Am. **29**(4): 817–827. doi:10.1016/S0094-0143(02)00081-2. PMID:12516754.

Akgür, F.M., Kilinç, K., Tanyel, F.C., Büyükpamukçu, N., and Hicsmez, A. 1994. Ipsilateral and contralateral testicular biochemical acute changes after unilateral testicular torsion and detorsion. Urology, **44**(3): 413–418. doi:10.1016/S0090-4295(94)80105-3. PMID:8073557.

Ambrosio, G., Flaherty, J.T., Duilio, C., Tritto, I., Santoro, G., Elia, P.P., et al. 1991. Oxygen radicals generated at reflow induce peroxidation of membrane lipids in reperfused hearts. J. Clin. Invest. **87**(6): 2056–2066. doi:10.1172/JCI115236. PMID:1645750.

Anderson, J.B., and Williamson, R.C. 1986. The fate of the human testes following unilateral torsion of the spermatic cord. Br. J. Urol. **58**(6): 698–704. doi:10.1111/j.1464-410X.1986.tb05916.x. PMID:3099882.

Banasik, M., Komura, H., Shimoyama, M., and Ueda, K. 1992. Specific inhibitors of poly(ADP-ribose) synthetase and mono(ADP-ribosyl)transferase. J. Biol. Chem. **267**(3): 1569–1575. PMID:1530940.

Beheshtian, A., Salmasi, A.H., Payabvash, S., Kiumehr, S., Nezami, B.G., Rahimpour, S., et al. 2008*a*. Role of endogenous cannabinoids in ischemia/reperfusion injury following testicular torsion in rats. Int. J. Urol. **15**(5): 449–454. doi:10.1111/j.1442-2042.2008.02018.x. PMID:18452464.

Beheshtian, A., Salmasi, A.H., Payabvash, S., Kiumehr, S., Ghazinezami, B., Rahimpour, S., et al. 2008*b*. Protective effects of sildenafil administration on testicular torsion/detorsion damage in rats. World J. Urol. **26**(2): 197–202. doi:10.1007/s00345-008-0243-6. PMID:18265987.

Berger, N.A. 1985. Poly(ADP-ribose) in the cellular response to DNA damage. Radiat. Res. **101**(1): 4–15. doi:10.2307/3576299. PMID:3155867.

Bozlu, M., Eskandari, G., Cayan, S., Canpolat, B., Akbay, E., and Atik, U. 2003. The effect of poly(adenosine diphosphate-ribose) polymerase inhibitors on biochemical changes in testicular ischemia–reperfusion injury. J. Urol. **169**(5): 1870–1873. doi:10.1097/01.ju.0000049228.37887.4d. PMID:12686864.

Bozlu, M., Coşkun, B., Cayan, S., Acar, D., Aktaş, S., Ulusoy, E., and Akbay, E. 2004. Inhibition of poly(adenosine diphosphate-ribose) polymerase decreases long-term histologic damage in testicular ischemia–reperfusion injury. Urology, **63**(4): 791–795. doi:10.1016/j.urology.2003.10.062. PMID:15072913.

Cochrane, C.G. 1991. Mechanisms of oxidant injury of cells. Mol. Aspects Med. **12**(2): 137–147. doi:10.1016/0098-2997(91)90009-B. PMID:2072822.

Cuzzocrea, S., Zingarelli, B., Costantino, G., Szabó, A., Salzman, A.L., Caputi, A.P., and Szabó, C. 1997. Beneficial effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase in a rat model of splanchnic artery occlusion and reperfusion. Br. J. Pharmacol. **121**(6): 1065–1074. doi:10.1038/sj.bjp.0701234. PMID:9249240.

Cuzzocrea, S., Riley, D.P., Caputi, A.P., and Salvemini, D. 2001. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia–reperfusion injury. Pharmacol. Rev. **53**(1): 135–159. PMID:11171943.

Felley-Bosco, E. 1998. Role of nitric oxide in genotoxicity: implication for carcinogenesis. Cancer Metastasis Rev. **17**(1): 25–37. doi:10.1023/A:1005948420548. PMID:9544421.

Grzelak, A., Soszyński, M., and Bartosz, G. 2000. Inactivation of antioxidant enzymes by peroxynitrite. Scand. J. Clin. Lab. Invest. **60**(4): 253–258. doi:10.1080/003655100750046413. PMID:10943595.

Johnsen, S.G. 1970. Testicular biopsy score count—a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones, **1**(1): 2–25. doi:10.1159/000178170. PMID:5527187.

Lawrence, R.A., and Burk, R.F. 1976. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem. Biophys. Res. Commun. **71**(4): 952–958. doi:10.1016/0006-291X(76)90747-6. PMID:971321.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**(1): 265–275. PMID:14907713.

Pacher, P., Schulz, R., Liaudet, L., and Szabó, C. 2005. Nitrosative stress and pharmacological modulation of heart failure. Trends Pharmacol. Sci. **26**(6): 302–310. doi:10.1016/j.tips.2005.04.003. PMID:15925705.

Pacher, P., Liaudet, L., Mabley, J.G., Cziráki, A., Haskó, G., and Szabó, C. 2006. Beneficial effects of a novel ultrapotent poly(ADP-ribose) polymerase inhibitor in murine models of heart failure. Int. J. Mol. Med. **17**(2): 369–375. PMID:16391839.

Rajesh Kumar, T., Doreswamy, K., Shrilatha, B., and Muralidhara. 2002. Oxidative stress associated DNA damage in testis of mice: induction of abnormal sperms and effects on fertility. Mutat. Res. **513**(1-2): 103–111. PMID:11719095.

Saba, M., Morales, C.R., de Lamirande, E., and Gagnon, C. 1997. Morphological and biochemical changes following acute unilateral testicular torsion in prepubertal rats. J. Urol. **157**(3): 1149–1154. doi:10.1016/S0022-5347(01)65158-9. PMID:9072560.

Sedlak, J., and Lindsay, R.H. 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. **25**(1): 192–205. doi:10.1016/0003-2697(68)90092-4. PMID:4973948.

Shall, S., and de Murcia, G. 2000. Poly(ADP-ribose) polymerase-1: What have we learned from the deficient mouse model? Mutat. Res. **460**(1): 1–15. PMID:10856830.

Sönmez, M., Türk, G., and Yüce, A. 2005. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology, **63**(7): 2063–2072. doi:10.1016/j.theriogenology.2004.10.003. PMID:15823361.

Stern, Y., Salzman, A., Cotton, R.T., and Zingarelli, B. 1999. Protective effect of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase, against laryngeal injury in rats. Am. J. Respir. Crit. Care Med. **160**(5 Pt 1): 1743–1749. PMID:10556150.

Sun, Y., Oberley, L.W., and Li, Y. 1988. A simple method for clinical assay of superoxide dismutase. Clin. Chem. **34**(3): 497–500. PMID:3349599.

Szabó, C., and Dawson, V.L. 1998. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia–reperfusion. Trends Pharmacol. Sci. **19**(7): 287–298. doi:10.1016/S0165-6147(98)01193-6. PMID:9703762.

Tentori, L., Portarena, I., and Graziani, G. 2002. Potential clinical applications of poly(ADP-ribose) polymerase (PARP) inhibitors. Pharmacol. Res. **45**(2): 73–85. doi:10.1006/phrs.2001.0935. PMID:11846617.

Thiemermann, C., Bowes, J., Myint, F.P., and Vane, J.R. 1997. Inhibition of the activity of poly(ADP ribose) synthetase reduces ischemia–reperfusion injury in the heart and skeletal muscle. Proc. Natl. Acad. Sci. U.S.A. **94**(2): 679–683. doi:10.1073/pnas.94.2.679. PMID:9012844.

Turner, T.T. 1984. Acute experimental torsion: no effect on the contralateral testis. J. Androl. **6**: 65–72.

Turner, T.T. 1987. On unilateral testicular and epididymal torsion: no effect on the contralateral testis. J. Urol. **138**(5): 1285–1290. PMID:3499519.

Turner, T.T., and Brown, K.J. 1993. Spermatic cord torsion: loss of spermatogenesis despite return of blood flow. Biol. Reprod. **49**(2): 401–407. doi:10.1095/biolreprod49.2.401. PMID:8373967.

Ueda, K., and Hayaishi, O. 1985. ADP-ribosylation. Annu. Rev. Biochem. **54**(1): 73–100. doi:10.1146/annurev.bi.54.070185.000445. PMID:3927821.

Visser, A.J., and Heyns, C.F. 2003. Testicular function after torsion of the spermatic cord. BJU Int. **92**(3): 200–203. doi:10.1046/j.1464-410X.2003.04307.x. PMID:12887467.

Wintersberger, U., and Wintersberger, E. 1985. Poly ADP-ribosylation — a cellular emergency reaction? FEBS Lett. **188**(2): 189–191. doi:10.1016/0014-5793(85)80369-0. PMID:3928401.

Yokoi, K., Uthus, E.O., and Nielsen, F.H. 2003. Nickel deficiency diminishes sperm quantity and movement in rats. Biol. Trace Elem. Res. **93**(1-3): 141–154. doi:10.1385/BTER:93:1-3:141. PMID:12835498.

Zingarelli, B., O'Connor, M., Wong, H., Salzman, A.L., and Szabó, C. 1996. Peroxynitrite-mediated DNA strand breakage activates poly-adenosine diphosphate ribosyl synthetase and causes cellular energy depletion in macrophages stimulated with bacterial lipopolysaccharide. J. Immunol. **156**(1): 350–358. PMID:8598485.

Zingarelli, B., Cuzzocrea, S., Zsengellér, Z., Salzman, A.L., and Szabó, C. 1997. Protection against myocardial ischemia and reperfusion injury by 3-aminobenzamide, an inhibitor of poly(-ADP-ribose) synthetase. Cardiovasc. Res. **36**(2): 205–215. doi:10.1016/S0008-6363(97)00137-5. PMID:9463632.

Zingarelli, B., Salzman, A.L., and Szabó, C. 1998. Genetic disruption of poly(ADP-ribose) synthetase inhibits the expression of P-selectin and intercellular adhesion molecule-1 in myocardial ischemia/reperfusion injury. Circ. Res. **83**(1): 85–94. PMID:9670921.

Zingarelli, B., Szabó, C., and Salzman, A.L. 1999. Blockade of poly(ADP-ribose) synthetase inhibits neutrophil recruitment, oxidant generation, and mucosal injury in murine colitis. Gastroenterology, **116**(2): 335–345. doi:10.1016/S0016-5085(99)70130-7. PMID:9922314.

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