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ORIGINAL ARTICLE

Dexpanthenol attenuates lipid peroxidation and testicular damage at experimental ischemia and reperfusion injury

Barlas Etensel · Sezen Özkısacık · Esra Özkara · Aslıhan Karul · Onur Öztan · Mesut Yazıcı · Harun Gürsoy

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Abstract Prevention of tissue damage after testicular torsion caused by I/R injury is still a clinical and experimental problem. There are many experimental studies made with several chemicals in the literature for decreasing the effect of reactive oxygen species after ischemia and reperfusion. Dexpanthenol (Dxp) is the biologically active alcohol of pantothenic acid. Pantothenic acid increases the content of reduced glutathione, Coenzyme A and ATP in cell. We studied the effect of Dxp on lipid peroxidation and testicular damage. Forty adult rats were separated randomly into five groups: group Sh, Sham-operation; group TD, torsion–detorsion; group NS, torsion–normal saline-detorsion; group D, torsion–Dxp 250 mg/kg detorsion; group D2, torsion–Dxp 500 mg/kg detorsion group. Serum MDA levels were taken before detorsion, after torsion at the first and fifth minute and at the first hour. Tissue sample was taken at the first hour. The alterations of I/R injury on testis were histological graded. Serum MDA levels were significantly lower in group D2 compared to all groups. The histopathology score of group D2 was significantly lower than groups TD, NS and D. Histopathological score and serum MDA levels are strikingly compatible. Dxp attenuated lipid peroxidation and tissue damage at I/R injury. This effect depends on its antioxidant effect with increasingly reduced glutathione, Coenzyme A and ATP. The effect of Dxp on I/R injury has been shown for the first time in the experimental testicular torsion.

Keywords Dexpanthenol · Pantothenic acid · Phospholipid hydroperoxide glutathione peroxidase · Ischemia reperfusion · Testis torsion

B. Etensel (✉) · S. Özkısacık · O. Öztan · M. Yazıcı · H. Gürsoy
Department of Pediatric Surgery, Adnan Menderes University, Aydin, Turkey
e-mail: betensel@adu.edu.tr

E. Özkara
Department of Pathology,
Adnan Menderes University, Aydin, Turkey

A. Karul
Department of Biochemistry,
Adnan Menderes University, Aydin, Turkey

Introduction

Testicular torsion (TT) is a urologic emergency. The severity of testicular damage is generally suggested to be related to the time and degree of TT. Despite the prompt diagnosis and treatment, testicular atrophy and fertility are major problems in the follow up [1, 2].

The main cause of tissue damage after TT is reactive oxygen species (ROS). ROS causes damage to DNA, impairment of protein function and peroxidation of lipids [3]. Malondialdehyde (MDA) is used widely as an indicator of oxidative stress in tissue [4, 5] and blood samples [6, 7] induced by ischemia-reperfusion (I/R). Creatine kinase (CK) catalyzes the transfer of high-energy phosphate from creatine phosphate to ADP, a reaction necessary for resynthesis of ATP [8].

Dexpanthenol (Dxp) (provitamin B5) is the biologically active alcohol of pantothenic acid (PA) (vitamin B5). Dxp given orally or parenterally is converted to PA in mammalian tissues [9, 10]. PA increases the content of reduced glutathione (GSH),

Coenzyme A (Co A) and ATP synthesis in cell [11–13]. GSH and glutathione-dependent peroxidases (GPX) are the major defense systems against lipid peroxidation and oxidative stress [14–16]. We conducted an experimental study to investigate the effect of Dxp on lipid peroxidation and testicular damage after TT.

Materials and methods

Ethical approval for the study was granted by the Laboratory Animal Ethical Committee of University. Principles of laboratory animal care (NIH publication No. 86–23, revised 1985) were followed. Forty male adult Wistar albino rats were maintained on a standard diet and water ad libitum in a temperature and light controlled room. Rats were separated randomly into five groups as follows. group Sh, Sham-operation (*n*: 8); group TD, torsion + detorsion (*n*: 8); group NS, torsion + normal saline + detorsion (*n*: 8); group D, torsion + Dxp 250 mg/kg + detorsion (*n*: 8); group D2, torsion + Dxp 500 mg/kg + detorsion (*n*: 8)

Rats were anesthetized with intramuscular injection of Ketamine (90 mg/kg) and Xylazine (10 mg/kg). Surgery was performed through a left scrotal incision. In groups TD, NS, D, D2 the left testis was rotated 1,080° in a counterclockwise direction and fixed to the scrotum with three 5/0 silk suture. The scrotum was closed with 3/0 silk suture. Torsion was maintained for 4 h. Then the testis was detorsed. Reperfusion time was 60 min.

About 30 min before detorsion, in group NS, normal saline; in group D, Dxp 250 mg/kg (Bepanthene amp. Roche); in group D2, Dxp 500 mg/kg, was injected intraperitonally.

After the anesthesia, at the fourth hour, just before detorsion, blood samples were taken from tail vein for MDA assay. Then, detorsion of testis was performed and testis was replaced into the scrotum. Following detorsion at the first and fifth minute, blood samples were taken from tail vein for MDA assays. In all groups at the end of 60th minute, left orhiectomy was performed. The testes were divided in to two pieces to determine the tissue levels of MDA and histopathologic examination. Intracardiac 3 ml blood sample was taken to measure the CK and MDA levels and then rats were sacrificed with high dose anesthesia.

In the sham operated group, main procedure included mobilization of testis under anesthesia at the beginning and at the end of ischemia period, avoiding either torsion or treatment. Blood and tissue samples after the fourth hour of ischemia were obtained as performed in the protocol of other groups.

Biochemical analysis

Tissue MDA

Homogenates were prepared in a ratio of 1 g of wet tissue to 9 ml, 0.13 mol/l KCl with a homogenizer. One volume of homogenate was mixed thoroughly with two volumes of a stock solution of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 mol/l hydrochloric acid (Sigma). The combination of sample and stock solution was heated for 30 min. After cooling, the precipitate was removed by centrifugation at 1,000×*g* for 15 min. The absorbance of the clear supernatant was determined at 535 nm and MDA concentration calculated using 1.56×10^5 mol^{-1}/cm^{-1} as molar absorbance coefficient [17]. Tissue MDA concentrations were calculated per gram tissue as nmol/g of wet tissue

Serum MDA

Sera were obtained by centrifugation at 1,000×*g* for 5 min at room temperature. The samples were stored at –70°C until analysis. MDA concentration was measured in terms of thiobarbituric acid reactive substances, spectrophotometrically. Samples (0.125 ml) were mixed with 20% trichloroacetic acid (1.25 ml) and 0.67% thiobarbituric acid (0.5 ml). Mixture was then boiled at 95°C for 30 min, followed by cooling on ice. Reaction mixture was then vortexed, following the addition of *n*-butanol (2 ml). All vials were then centrifuged at 1,000×*g* for 10 min. Absorbance of the supernatant was then measured at 535 nm. Concentration of lipid peroxidation products was calculated as MDA concentration using the extinction coefficient for MDA-thiobarbituric acid complex of 1.56×10^5 mol^{-1}/cm^{-1} [18].

CK assay

The catalytic activity of CK was determined at 37°C according to the IFCC method on an ARCHITECT C800 (Abbott, Illinois, USA) analyzer using CK reagents. The ATP produced in reaction is subsequently used to phosphorylate glucose to produce glucose-6-phosphate (G-6-P) in the presence of hexokinase. G-6-P is then oxidized by G-6-P dehydrogenase with the concomitant reaction of NADP to NADPH. The rate of formation of NADPH is monitored at 340 nm and is proportional to the activity of CK in the sample [19].

For quality control of biochemical analysis, internal quality control sera of Abbott and external quality

assurance services of Bio-Rad Laboratories (Bio-Rad Laboratories, CA, 92618, USA) were used in our laboratory.

Histopathologic evaluation

The specimens were fixed in Bouin's solution and then embedded in paraffin. The tissue samples were stained with H&E. Specimens were evaluated by a single pathologist in a blinded fashion. Testicular tissue injury was graded on a system described by Cosentino et al. [20] Grade 1 showed normal testicular architecture. Grade 2 injuries showed less orderly, noncohesive germinal cells and closely packed seminiferous tubules. Grade 3 injury exhibited disordered, sloughed germinal cells with shrunken, pyknotic nuclei and less distinct seminiferous tubule borders. Grade 4 injuries defined seminiferous tubules that were packed closely with coagulative necrosis of the germinal cells.

Statistical analysis

All results were expressed as mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA) with Newman–Keuls multiple comparisons test. Statistical analysis was performed with the statistic software GraphPad InStat 3.0 (San Diego, CA). A level of $P < 0.05$ was considered to be statistically significant.

Results

Serum and tissue MDA levels are given in Table 1. Serum MDA levels before the detorsion (b MDA) were significantly lower in group D2 in the dose of Dxp 500 mg/kg compared to other groups. This significant difference for serum MDA levels of group D2 from all other groups appeared in all sample times. There was no significant difference observed between groups at tissue MDA levels (Table 1). Neither 250 mg/kg nor 500 mg/kg of Dxp treatment had attenuated tissue lipid peroxidation.

In histopathologic evaluation of testicular tissue (Fig. 1), the mean Cosentino score of group D2 was significantly lower than groups TD, NS and D ($P < 0.05$). Groups TD, NS and D have at least two grade 4 injuries in histopathologic evaluation; however, there wasn't any grade 4 injury in D2 group. (Fig. 2) The findings of histopathologic Cosentino score and serum MDA levels are strikingly compatible.

There is no significant difference among groups in CK levels (Table 2).

Discussion

I/R injury following detorsion of TT are characterized by distinct temporal events with biphasic responses, start during ischemia and exacerbates during reperfusion. ROS production seems to possess two phases after TT. The first phase occurs immediately after reperfusion, extends for a few hours and is a typical oxidative stress situation, reversible in terms of cellular injury. In this phase, in which there is an increased mitochondrial production of ROS with mitochondrial dysfunction and failure of oxidative phosphorylation, the tissue is able to counteract the oxidative stress situation derived from a rapid ROS generation through the antioxidant defenses; the GPX system appears to constitute the first line of defense. The second phase extends for hours or days depending on the maintenance of the oxidative stress insult. This phase is associated with the appearance of irreversible tissue damage and inflammation, with

Table 1 Serum and tissue MDA levels of all groups

	Serum MDA (nmol/ml)				Tissue MDA (nmol/g)
	Before detorsion (b MDA)	After detorsion (1 min MDA)	After detorsion (5 min MDA)	After detorsion (1 h MDA)	After detorsion (1 h)
Group Sh	1.05 ± 0.22**	1.29 ± 0.40***	1.02 ± 0.2**	1.12 ± 0.10***	36 ± 84
Group TD	1.22 ± 0.20***	1.22 ± 0.29***	1.07 ± 0.32***	1.09 ± 0.10***	53 ± 21
Group NS	1.17 ± 0.11***	0.98 ± 0.11*	1.13 ± 0.17***	1.24 ± 0.17***	50 ± 13
Group D	1.04 ± 0.14***	1.11 ± 0.18***	1.13 ± 0.25***	1.05 ± 0.15***	50 ± 13
Group D2	0.68 ± 0.16	0.57 ± 0.17	0.57 ± 0.06	0.36 ± 0.06	59 ± 18

Results are expressed as means ± SD

*$P < 0.05$ compared with group D2, **$P < 0.01$ compared with group D2, ***$P < 0.001$ compared with group D2

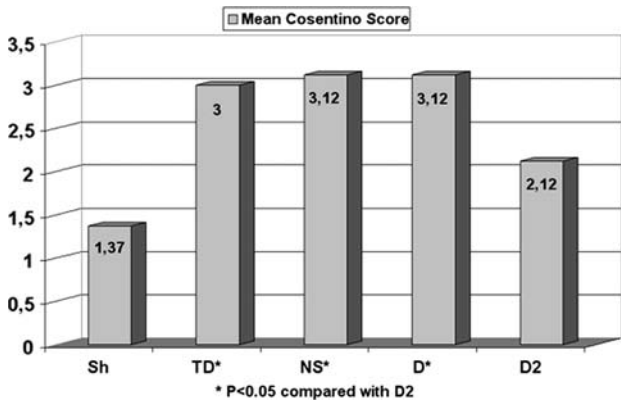

Fig. 1 Histopathologic evaluation of groups regarding to Cosentino classification

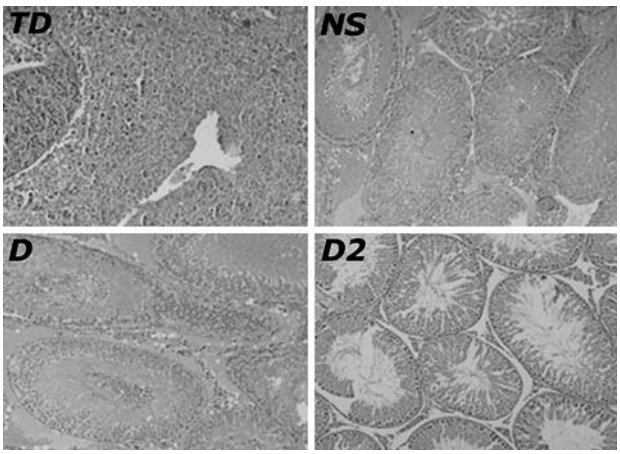

Fig. 2 Group TD: grade IV injury (H&E ×100), group NS: grade III injury (H&E ×100), group D: grade III injury (H&E ×100), group D2: grade II injury (H&E ×100)

Table 2 Serum CK level of groups

	CK
Group Sh	1,271 ± 582
Group TD	1,208 ± 613
Group NS	1,275 ± 872
Group D	1,263 ± 651
Group D2	2,284 ± 649

Results are expressed as means ± SD

neutrophils infiltration and macrophages recruitment [3, 21].

Dxp significantly attenuated serum lipid peroxidation after TT. This alleviating effect was present in the dose of 500 mg/kg of Dxp (Table 1). Serum MDA level was suggested as determinant of the extension of I/R injury in TT [6, 7]. Serum MDA levels are elevated in all groups except D2 group. There was no significant difference between these groups at MDA levels. The elevation of serum MDA on sham group indicates that mobilization of testicular vessels might not be an inert operation on serum MDA levels. These levels could bring up a new controversy so that serum MDA might be probably not a good marker for I/R injury after testicular torsion. Significantly reduced serum MDA levels of group D2 support the protective effect of Dxp on I/R injury. Lipid oxidation, the free radical process of oxidation of unsaturated lipids and cholesterol in membranes and lipoproteins, is considered as the most deleterious consequence of I/R situation [3, 21]. The protective effect of PA and its derivatives against cell damage produced by ROS were well documented [11–13, 16]. PA provides an increase in cell CoA content and also increases ATP and GSH synthesis in cells [11]. GSH and GPX are involved in the ultimate removal of detoxified oxidation products from the cell. Besides working as a scavenger of ROS, GSH is involved in a variety of metabolic functions such as DNA repair, activation of transcription factors, cell cycle regulation, modulation of calcium homeostasis and regulation of enzyme activities [3, 22].

In our study, we have especially chosen the postpubertal rats because the enzymatic activity of the phospholipid hydroperoxide GPX, the major antioxidant in testicular tissue in mammals appeared and increased only after puberty [23–26]. We suggest that postpubertal testicular torsion should have different antioxidative defense mechanisms than prepubertal torsion. The last discovered member of the GPX family is the sperm nucleus GPX. It protects especially the sperm DNA against oxidative damage after puberty [27].

Conservation of cell integrity was well confirmed by our histological results; it has been shown that Dxp had a significant alleviating effect on I/R injury of TT in the dose of 500 mg/kg. This result is in correlation with serum MDA levels. CoA and ATP are two major requirements for the synthesis of phospholipids and cholesterol and in this way participate in repair of cell membranes and tissue injury after TT [14].

In conclusion, as far as we know, the alleviating effect of Dxp on testicular damage after TT has been shown for the first time. To our suggestion, it depends on its antioxidant effect by increasing the reduced glutathione in cells. Our histopathological investigation has proven positive effect of Dxp on protection of testicular tissues in TT. This may be via positive effect of Dxp on cell CoA content. There are many experimental studies made with several chemicals in the literature for decreasing the effect of ROS in TT, but none of these or other medical methods of alleviating tissue damage after TT were adapted for routine clinical management.

Dxp has FDA approval in enteral and parenteral form. It is safe, cost effective and readily available [9]. Parenteral form of Dxp can be easily utilized in emergency conditions, when indicated. Therefore, Dxp deserves rapid attention and further experimental and clinical studies are needed for its effects.

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