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ORIGINAL ARTICLE

The protective effect of dexpanthenol on testicular atrophy at 60th day following experimental testicular torsion

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Abstract Despite the prompt diagnosis and treatment of testicular torsion (TT), there are problems with fertility and atrophy after testicular salvage. Dexpanthenol (Dxp) is the biologically active alcohol of pantothenic acid (PA). Dxp is converted to PA in tissues. PA increases the content of reduced glutathione (GSH), Coenzyme A and ATP synthesis in cells. GSH and glutathione-dependent peroxidases (GPX) are the major defense systems against oxidative stress. GPX-4 is the major antioxidant in testicular tissue. However, the activity of GPX-4 appeared and increased only after puberty. We investigated the effect of Dxp on testicular atrophy after TT at the 60th day. Rats were separated randomly into four groups. Group C: control group, group Td: torsion + detorsion, group Sal: torsion + saline + detorsion, group Dxp: torsion + Dxp + detorsion. The left testis was rotated 720° for 2 h. In group Sal, normal saline and in group Dxp, Dexpanthenol were injected intraperitonally, 30 min before detorsion. After 60 days, the testicular weights and volumes were measured. Histopathology of the left testis was evaluated with mean seminiferous tubular diameter (MSTD) and mean testicular biopsy score (MTBS). The left (torsed) testicular weight and volume of groups Td and Sal were significantly lower compared to group Dxp. The MSTD and MTBS of group Td and Sal were significantly lower than group Dxp. Contralateral testicular weight and volume of groups Td, Sal and Dxp had no significant difference compared to the control group. Dxp significantly prevented testicular atrophy after 60 days of TT. Dxp has FDA approval, is safe, cost effective and readily available. Its relevance for clinical trials may especially be for the problem of testicular atrophy catastrophe, seen very frequently following testicular salvage.

Keywords Testis torsion · Testis atrophy · Dexpanthenol · Glutathione peroxidase · Ischemia–reperfusion

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Introduction

Torsion of the testis (TT) is a true urologic emergency and despite the prompt diagnosis and treatment, there are major problems with testicular atrophy and fertility in the follow-up period of testicular salvage [1].

Dexpanthenol (Dxp) (provitamin B5) is the biologically active alcohol of pantothenic acid (PA) (vitamin B5) and when given orally or parenterally, it is converted to PA in rat and mammalian tissues [2, 3]. PA increases the content of reduced glutathione (GSH), Coenzyme A (CoA) and ATP synthesis in cells [4–6]. GSH and glutathione-dependent peroxidases (GPX) are the major defense systems against lipid peroxidation and oxidative stress [7, 8]. Phospholipid hydroperoxide (GPX-4) is the major antioxidant in testicular tissue against lipid peroxidation [9–12]. Protection by PA against heart and liver injury caused by ischemia–reperfusion (I/R) has also been reported [6, 7, 13].

We planned an experimental study to investigate the protective effect of Dxp on testicular atrophy at the 60th day following TT. Our study is the first experimental study on the use of Dxp using rat TT model on a long-term period.

Materials and methods

Ethical Committee on Animal Research of our university approved the animal experiments protocol for our study. The principles of laboratory animal care (NIH publication no. 86–23, revised 1985) were followed. Male, postpubertal (9 weeks) Wistar albino rats (220–255 g) were maintained on a standard diet and water, ad libitum, in a temperature- and light-controlled room. The rats were separated randomly into four groups of eight rats each. Group C: control group, sham-operation. Group Td: torsion + detorsion. Group Sal: torsion + saline treatment + detorsion. Group Dxp: torsion + Dxp 500 mg/kg + detorsion.

Before all surgical procedures, the 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, Sal, Dxp, the left testis was rotated 720° in a counterclockwise direction and fixed to the scrotum with three 5/0 silk sutures placed through the testis. The scrotum was closed with 3/0 silk suture. Torsion was maintained for 2 h.

In group Sal, normal saline and in group Dxp, Dexpanthenol 500 mg/kg (Bephanthene amp, Roche) were injected intraperitonally, 30 min before detorsion. In the sham-operated group, the left testis was brought through the incision and then replaced, with fixation to the scrotum.

At the end of second hour of torsion, detorsion of testis was performed. The testis was fixed to the scrotum with three 5/0 silk sutures and the scrotum was closed. The rats were maintained on a standard diet and water, ad libitum, in a temperature- and light-controlled room for 60 days.

On the 60th day (18 weeks, 415–448 g), bilateral orhiectomy was performed in all groups. Then the rats were sacrificed with an overdose of anesthesia. Both testes were weighed and measured three dimensionally for volume calculation (elipsoid volume: length × width × thickness × 0.523). Histopathologic examination of the left (ipsilateral) testis was performed.

Histopathologic evaluation

The testes were fixed in Bouin's solution, processed routinely into paraffin wax and stained with haematoxylin and eosin. The testicular tissue was evaluated by a pathologist in a random order with light microscopy. The mean seminiferous tubular diameter (MSTD) and the mean testicular biopsy score (MTBS) were used to evaluate the histology of the testes in 20 seminiferous tubules of each section. The MSTD was calculated using a microscope-adaptable micrometer. The MSTD of each testis was determined in microns. The MTBS was graded using the Johnsen score. The Johnsen score [14], a well-recognized histological index of spermatogenesis, is based on the premise that with testicular damage there is successive disappearance of the most mature cell type, with progressive degeneration of the germinal epithelium, with the disappearance of spermatozoa and spermatids, then spermatocytes and finally Sertoli cells, in that order (Table 1).

Statistical analysis

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

Table 1 Johnsen testicular biopsy score system

Score	Description of scoring system
10	Complete spermatogenesis with many spermatozoa (determined by head form). Germinal epithelium organized in regular thickness leaving an open lumen
9	Many spermatozoa present, but germinal epithelium disorganized with marked sloughing or obliteration of lumen
8	Only a few spermatozoa present (<5–10)
7	No spermatozoa, but many spermatids present
6	No spermatozoa and only a few spermatids present (<5–10)
5	No spermatozoa and no spermatids, but several or many spermatocytes present
4	Only a few spermatocytes (<5), but no spermatids or spermatozoa present
3	Spermatogonia are the only germ cells present
2	No germ cells, but Sertoli cells are present
1	No cells in tubular section

Results

During the 60-day period, a total of three rats died (one rat of groups C, Td and Sal each). At the end of the follow-up period, the number of rats in the groups were group C: 7, group Td: 7, group Sal: 7 and group Dxp: 8.

The testicular weight and volume of the ipsilateral and contralateral testes are given in Table 2. Extremely significant differences in testicular weight and volume were observed in groups Td and Sal compared to groups Dxp and C ($P < 0.001$). After the 60-day period, two ipsilateral testes (one in group Td and other in group Sal) were totally atrophied. There was no significant difference in the testicular weight and volume between group Dxp and group C.

Neither contralateral testicular weight nor contralateral testicular volume of groups Td, Sal and Dxp were significantly different from group C.

The histopathological results of the ipsilateral testicular tissue are given in Table 3 and Fig. 1. The MSTD of group Dxp was significantly higher than groups Td and Sal ($P < 0.05$). There was no significant difference in the MSTD between group Dxp and group C. The MTBS of group Dxp was significantly higher than groups Td and Sal ($P < 0.001$). There was no significant difference in the MTBS of group Dxp compared with group C.

Table 3 MSTD and MTBS (Johnsen score) of the groups after 60 days of TT

	Ipsilateral testis	
	MSTD	MTBS
Group C	254 ± 27	8.27 ± 0.99
Group Td	164 ± 80*	2.91 ± 1.94**
Group Sal	167 ± 91*	2.91 ± 1.78**
Group Dxp	267 ± 48	7.61 ± 1.65

Results are expressed as mean ± SD

*$P < 0.05$ compared with group Dxp and C; **$P < 0.001$ compared with group Dxp and C

Discussion

Torsion of the testis occurs frequently during the pubertal period. After treatment and testicular salvage, a testicular atrophy rate of 68% still remains [1]. In the literature, there are many experimental studies made with several chemicals for decreasing the effect of the reactive oxygen species (ROS) in TT. However, none of these or other medical methods of alleviating tissue damage after TT were was adopted for routine clinical management [1, 15, 16].

The main cause of tissue damage after TT is ROS. ROS causes damage to DNA, impairment of protein function and peroxidation of lipids [16, 17]. Lipid oxidation is considered to be the most deleterious consequence of the I/R situation. Lipid peroxides rupture the membranes and cause structural and functional alterations in the testis [8, 17].

In our study, treatment with Dxp, 30 min before detorsion of the testis, prevented testicular atrophy. Our histological results of the MSTD and MTBS of group Dxp were significantly higher than those of groups Td and Sal. It has been shown that Dxp had a significant preventive effect on testicular atrophy of TT at a dose of 500 mg/kg. Dxp is effective after conversion to PA in the tissues [2, 3]. The protective effect of PA and its derivatives against cell damage produced by ROS are well documented [4–6, 13]. PA provides an increase in cell CoA content and also increases ATP and GSH synthesis in cells [4, 5]. GSH and GPX play central roles in intracellular antioxidant metabolic processes, as they are involved in not only the first and second lines of defense against ROS but also the ultimate removal of detoxified oxidation products from the cell [17]. GPX constitute a family of antioxidative enzymes that are capable of reducing organic and inorganic hydroperoxides to the corresponding hydroxyl compounds, utilizing GSH as a reducing equivalent [12, 18, 19]. The human GPX family includes cytosolic (GPX-1), gastrointestinal (GPX-2), plasma (GPX-3), phospholipid hydroperoxide (GPX-4), epididymal (GPX-5) and selenoprotein P [17]. The most recently discovered member of the GPX family is the sperm nucleus GPX. It protects especially the sperm DNA against oxidative damage [20].

GPX-4 is the major antioxidant in testicular tissue against lipid peroxidation and widely expressed in the

Table 2 Weights and volumes of testes of groups after 60 days of TT

	Ipsilateral testis		Contrlateral testis	
	Weight (g)	Volume (cm^3)	Weight (g)	Volume (cm^3)
Group C	1.482 ± 0.18	1.389 ± 0.15	1.555 ± 0.19	1.408 ± 0.15
Group Td	0.555 ± 0.26*	0.447 ± 0.21*	1.557 ± 0.26	1.266 ± 0.18
Group Sal	0.752 ± 0.36*	0.708 ± 0.33*	1.621 ± 0.10	1.544 ± 0.15
Group Dxp	1.617 ± 0.26	1.502 ± 0.25	1.672 ± 0.15	1.535 ± 0.12

Results are expressed as mean ± SD

*$P < 0.001$ compared with group Dxp and C

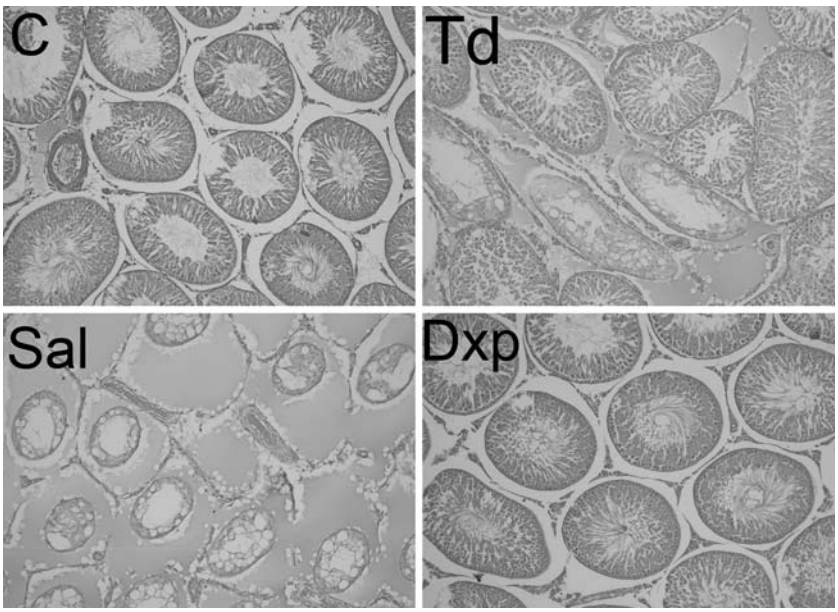

Fig. 1 Histopathology of group C, group Td, group Sal and group Dxp (H&E, ×100)

testes of mammals. However, the activity of GPX-4 appears and increases only after puberty [11, 12]. Roveri et al. [10] reported that GPX-4 activity depends on the stimulation of gonadotropin in hypophysectomized rats. GPX-4 has been shown to work as an active peroxidase in spermatogenic cells by protecting the rapidly dividing cells against oxidative injury and as a structural protein by building up the mitochondrial capsule [21]. Since both GPX-4 and sperm nucleus GPX are mainly active following puberty, we suggest that postpubertal testicular torsion should have different antioxidative defense mechanisms than prepubertal torsion.

Testicular volume is a trustful marker of fertility because approximately 90% of testicular volume is represented by seminiferous tubules [15]. In our study, after 60 days of TT, group Dxp had very significantly higher testicular weight and volume than groups Td and Sal ($P < 0.001$). There was no significant difference in the contralateral testicular volume and weight of the groups compared to the contralateral testes of the control group. Besides working as a scavenger of reactive species, GSH is involved in a variety of other metabolic functions such as DNA repair, activation of transcription factors, cell cycle regulation, modulation of calcium homeostasis and regulation of enzyme activities [8]. PA and GSH are also known to play a major role in the control of apoptosis [6, 13]. CoA and ATP are two major requirements for the synthesis of phospholipids and cholesterol. In this way, CoA and ATP participate in the repair of cell membranes and tissue injury [7, 17].

In conclusion, Dxp when administered intraperitonally after 90 min of torsion, 30 min before detorsion, significantly prevented testicular atrophy after 60 days of TT in rats. Dxp is provitamin B5 and has FDA approval in the enteral and parenteral form. It is safe, cost effective and readily available [2]. Parenteral form of Dxp can be easily utilized in emergency conditions, when indicated. Its relevance for clinical trials may especially be for the problem of testicular atrophy catastrophe, seen very frequently following testicular salvage.

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