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Lazaroid U-74389G Decreases Testis Tissue Injury Induced by Testicular Torsion Detorsion: An Experimental Study

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Abstract

Research Article

It was mapped out to search the potential useful features of Lazaroid U-74389G (Laz) on bilateral testicular torsion detorsion injury in rats. 24 Wistar male rats were allocated to 3 groups. Groups of this research were designed as sham, torsion detorsion (T/D), and T/D+Laz (T/D+10 mg/kg dose of Laz) groups. TOS, MDA and OSI levels, MPO activity raised significantly in testicular torsion detorsion group compared to sham group in testes tissues ($p<0.05$). However, SOD and TAS values reduced in T/D group. On the contrary, SOD level increased while MPO activity, TOS, OSI and MDA levels decreased meaningfully due to Laz treatment ($p<0.05$). As a conclusion, Laz demonstrated an effective protection against testicular T/D-induced testis injury in experimental rats.

INTRODUCTION

Testicular torsion results in ischemia reperfusion (I/R) injury and an urologic emergency among young male population [1]. It may cause decrease in fertility if not treated [2]. It must be treated quickly to protect testicular functions. Detorsion of rotated testes composes the main treatment. I/R injury constitutes the pathophysiologic mechanism of testicular T/D [3,4]. Detorsion increased the testicular ischemic injury level due to increased ROS amounts [5-7], leading to germ cell injury and impaired spermatogenesis [8]. The torsion of spermatic cord leads to oxidative stress, inflammation and apoptosis which result in irreversible suppression of spermatogenesis [9]. Antioxidants prevented ROS related testicular tissue injuries in previous studies [2,6,7,10-13]. Various molecules were examined on testicular torsion but there is still no certain treatment for T/D [14].

Lazaroid U-74389G (Laz) is a member of 21-aminosteroids (lazaroids) family and it suppresses the peroxidation of lipid membrane [15,16]. Laz administration performed protective effects in a I/R induced small bowel injury model in rats [17]. In an endotoxin-induced liver model by Fukuma et al, Laz decreased lipid peroxidation [18].

Different agents performing anti-inflammatory, antioxidant and radical scavenging properties have been reported in alleviation or elimination of I/R injury on testis tissue [6,7,13]. In the current study, Laz eased testicular T/D damage with antioxidant effects. Therefore, current study was planned to determine the protective effects of Laz against testicular oxidative damage induced by T/D.

MATERIAL and METHODS

Ethical approval and experimental animals

Experimental animals were acquired from Atatürk University Experimental Animal Research and Application Center and the study was performed at the same place with the permission of Atatürk University Experimental Animal Ethics Committee (07.11.2019/206). Rats were housed in regular rat cages with appropriate laboratory conditions such as light/darkness, temperature and humidity. They were given standard rat feed and tap water. No food was given 12 hours before the experiment, but water was allowed to drink.

Groups and experimental design

All experimental steps were established under anesthesia. 24 Wistar Albino male rats were divided into 3 groups (n=8): Group I (sham group); for each rat, 2 cm of vertical scrotal incision was performed along the midline area of bilateral testes. The incisions were repaired with 3/0 silk suture without any application. In groups II (T/D) and III (T/D+Laz),

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following the incision procedure as described in group I, bilateral testes were twisted 720° clockwise with the spermatic cord and tunica vaginalis. The torsion was induced by applying a vascular clamp below the testis in rats. 2 hours of torsion and 2 hours of detorsion was performed as described in previous studies [7,13]. In group III, following the torsion, 10 mg/kg Laz (Sigma-Aldrich Co, USA) [19] was administered as intraperitoneal (i.p.) and then 2 hours detorsion was carried out. Ketamine-xylazine (ketamine, 50 mg/kg and xylazine 10 mg/kg i.p.) was preferred for the anesthesia as described [20]. The samples were cleaned up with cold saline and then kept at -80 °C for biochemical measurements. Laz was prepared in phosphate buffered saline (PBS) solution and administered as i.p. to the rats just before detorsion.

Biochemical assessments

Total antioxidant status (TAS) and total oxidant status (TOS) values were detected with appropriate kits (Rel Assay Diagnostics). TOS to TAS rate was admitted as the oxidative stress index (OSI). OSI was gauged as: OSI = [(TOS, µmol H_2O_2 equivalent L)/(TAS, mmol Trolox equivalent/L)×10] [21]. Superoxide dismutase (SOD) determination was based on the production of superoxide radicals [22]. Lipid peroxidation tissue was determined by assessing malondialdehyde (MDA) as described previously [23]. Myeloperoxidase (MPO) activity was gauged with the methods described by Bradley et al. [24].

Statistical analysis

Results were examined via One-way ANOVA. Tukey test was used for pairwise comparisons of groups. All the results were shown as Mean ± standard deviation (SD). The differences were admitted as significant if $p<0.05$.

RESULTS

The changes in biochemical parameters between the groups were demonstrated in table 1. While the TOS and MDA levels increased in T/D group ($p<0.001$), they decreased in Laz administered group ($p<0.001$). TAS and SOD levels decreased in T/D group ($p<0.001$) but they increased in T/D+Laz group ($p<0.001$). OSI level increased in T/D group ($p<0.001$) and decreased in the T/D+Laz group ($p<0.001$). MPO activity increased in T/D group ($p<0.001$) and decreased in T/D+Laz group ($p<0.001$).

Table 1 Comparisons of biochemical parameters among the experimental groups.

Experimental Groups n=6	TAS (mmol/L)	TOS (µmol/L)	OSI (arbitrary unit)	SOD (U/mg protein)	MPO (U/g protein)	MDA (µmol/g protein)
Sham (1)	1,49 ± 0,13	6,22 ± 0,94	0,41 ± 0,06	474,21 ± 20,47	35134,56 ± 4130,48	204,60 ± 16,64
T/D (2)	0,71 ± 0,09[a]	11,86 ± 0,85[a]	1,68 ± 0,29[a]	201,24 ± 19,63[a]	71602,35 ± 30311,90[a]	484,21 ± 20,70[a]
T/D+Laz 10 mg/kg (3)	1,38 ± 0,08[b]	6,99 ± 0,39[b]	0,50 ± 0,03[b]	409,70 ± 23,56[b]	37664,88 ± 4542,20[b]	220,16 ± 12,17[b]

[a]$p < 0.001$ compared to sham group. [b]$p< 0.001$ compared to T/D group.

DISCUSSION

Testicular torsion is a urological emergency and frequently occurs among children [25]. It results in hypoxia of the entire testicle due to the rotation of spermatic cord and the vascular structures around its axis [26]. Testicular torsion has a prevalence of in every 4000 men under the age of 25 (1/4000) [27] and a quick diagnosis with urgent intervention are necessary [28]. Delayed deformation after testicular torsion results in testicular atrophy, germ cell damage and infertility [2]. Recirculation following reperfusion causes much more damage contrary to expectations [26]. Reperfusion results in ROS generation including hydroxyl radicals, superoxide anions, peroxynitrite anions and hydrogen peroxide which cause damage in testicular cell membrane lipids, proteins, and even DNA [29,30]. Although various searches have been done on antioxidant molecules, there has not been found any definitive medical drug used in treatment. Studies with antioxidant molecules have focused on eliminating the effects of reactive oxygen radicals [31]. Therefore, in the current study, the potential protective effects of Laz in T/D induced testicular injury were examined.

Various steps play role in the formation of testicular injury following the T/D process [32]. ROS and oxidative stress play role in T/D damage. ROS directly damages the cytoskeleton and triggers many signals related with cell death

[33,34]. ROS interact with lipids, proteins and nucleic acids resulting in cell damage. ROS ends up with lipid peroxidation and formation of MDA. MDA occurs due to lipid peroxidation and indicates tissue damage [35,36]. MDA level raises in I/R injury [37] and it is a meaningful factor to determine I/R related oxidative injury [38]. MDA, a biomarker of oxidative stress, injures the mitochondrial membrane and induces cell death [39]. MPO, similar to MDA, is another enzyme to determine the oxidant stress in the cell (34). MPO is produced by neutrophils and acts as a indicator of neutrophil activation [35,36]. It causes tissue damage through reacting easily via various biological molecules [40]. Laz has been shown to reduce the increased MDA levels in liver [41], intestinal [42], and pancreas [43] I/R models.

Antioxidants protect target molecules such as proteins, nucleic acids, carbohydrates and membrane lipids against oxidation [44]. SOD is one of the important antioxidant enzymes. There are several studies demonstrating decline in SOD activity due to I/R procedure [45]. SOD enzyme performs antioxidant activity through converting superoxide to hydrogen peroxide and oxygen. Thus, it inactivates free radical and prevent tissues as the other antioxidants [46]. TAS and TOS show the balance between oxidation and antioxidation. TAS demonstrates all antioxidants while TOS is limited with ROS [47]. Oxidative stress shows that the oxidant activity is greater than the antioxidant system [48]. OSI reflects the TOS to TAS ratio and it indicates severity of oxidative stress [47,49]. In a study, Laz has been shown to increase the level of SOD in rat type II cells exposed to hyperoxia [50]. Laz reduced oxidative damage and protected cells in rat pancreas [51]. Oxidative stress in endrin-induced neurotoxicity and hepatotoxicity could be reduced via Laz administration [52]. In the current study, we demonstrated the preservative effects of Laz against oxidative stress induced testicular T/D related oxidative damage.

Laz suppressed lipid membrane peroxidation in previous studies [15,16]. Laz has been investigated in various I/R injury experiments such as renal [53], pancreatic [54] and intestinal I/R injuries [55]. Laz application also protected small intestine against I/R injury by suppressing lipid peroxidation in another rat model [56]. In an endotoxin-induced liver model by Fukuma et al, Laz decreased lipid peroxidation [18].

Anti-inflammatory and antioxidant molecule therapy may prevent I/R injury [57]. Any study about the effects of Laz on testicular I/R injury has not been found in the literature and the effects of Laz on oxidant-antioxidant parameters in testicular T/D injury model were examined in current study.

Laz demonstrated a protective performance against testis tissue injury related with testicular T/D via its antioxidant effects. Treatment with Laz at single dose (10 mg/kg) reduced testicular injury enhanced via testis T/D in rats.

Conflicts of Interest

The authors declare that they have no conflict of interests.

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