The Possible Beneficial Effects of Lazaroid U-74389G on Ovarian Torsion Detorsion Injury

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Abstract

It was planned to search the possible beneficial effects of Lazaroid U-74389G (Laz) on ovarian tissue injury caused by bilateral ovarian torsion detorsion (T/D) in the experimental rat model. Wistar type female rats were randomly allocated to 3 groups. Groups of this research were designed as sham, T/D, and T/D+Laz groups. In sham group, the abdomen was incised and sutured but no intervention was performed. In T/D group, following the incision, ovarian T/D model was carried out and the incision was sutured. In treatment group, Laz was administered intraperitoneally at 20 mg/kg dose just before detorsion. After the detorsion period, rats were sacrificed and ovarian tissues were excised. Oxidant parameters elevated and antioxidant activity declined significantly in T/D group compared to sham group in ovarian tissues. Laz treatment reversed the oxidant and antioxidant parameters. Thereby, Laz protected against T/D-induced ovarian tissue injury in experimental rats.

Keywords: Lazaroid U-74389G, Ovarian Torsion Detorsion, Ovary, Rat..

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1. Introduction

Ovarian torsion detorsion (T/D) is among gynecological emergencies and it is widely observed in reproductive age (1). Ischemia reperfusion (I/R) injury induces oxidative stress and inflammation leading to tissue injury (2; 3). I/R injury enhances the generation of reactive oxygen species (ROS) and malondialdehyde (MDA) production (4). ROS acts on membrane lipids and elevates MDA level (5). MDA is a lipid peroxidation metabolite and used for oxidative stress determination (6). During detorsion, ROS production increases and plays role in ovarian injury (7). Antioxidant enzymes including superoxide dismutase (SOD) prevent oxidative injury (8). The levels of ROS and antioxidant activity determine the rate of oxidative stress (9). Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) exist in the beginning of inflammatory respond and induce the release of free radicals (10; 11).

Different agents have been studied to alleviate or eliminate I/R injuries in various organs (12-16). Lazaroid U-74389G (Laz), a lazaroid family member, blocks lipid peroxidation through removing free radicals (17; 18). Laz has been examined in various I/R injury models including renal I/R injury (19). Laz eased I/R-induced intestinal injury in a previous study (20). Laz declined lipid peroxidation through reduction in MDA level (21).

Current study was planned to investigate the protective effect of Laz against ovarian oxidative damage induced by T/D.

2. Materials and Methods

2.1. Experimental Animals and Ethical Approval

Animals were procured from Atatürk University Experimental Animal Research and Application Center and also experimental steps were carried out at same place. The rats were housed in laboratory conditions such as polypropylene cages, appropriate humidity, temperature, etc. They could reach both food

and water but fasted 12 hours prior to experiment. Atatürk University Experimental Animals Local Ethics Committee permitted the study (07.11.2019/203).

2.2. Groups and Torsion/Detorsion Model

Prior to experiment, animals were fixed in supine position. Abdominal region was shaved, cleaned. Anesthesia was applied to animals before surgical process. Povidone-iodine was used for disinfection. 10 mg/kg i.p. xylazine hydrochloride (Rompun®, Bayer, Istanbul) and 60 mg/kg intraperitoneally (i.p.) ketamine (Ketalar®, Pfizer, Istanbul) were preferred for anesthesia as described before (22). Laz purchased from Sigma Aldrich Co.

18 Wistar Albino female rats, weighing 230-240 g, were randomly divided into 3 groups. Group I (sham group), the abdominal area applied 1-2 cm incision and repaired with 3/0 silk suture and no additional intervention was done. Group II (T/D group), following incision as described in group I, the ovaries, fallopian tubes, ovarian veins and arteria were rotated in clockwise 720 degrees and fixed with clamps for 3 hours. Then, clamps were removed and blood flow restarted for 3 hours (23; 24). Group III (T/D+Laz group), same steps in group II were done and 10 mg/kg Laz was administered i.p. to the rats just before detorsion. Finally, at the end of the experiment, the ovarian tissues were removed, cleaned and held frozen the analysis.

3. Biochemical assessments

Total antioxidant status (TAS) and total oxidant status (TOS) values were evaluated via appropriate kits (Rel Assay Diagnostics). TOS to TAS ratio, the oxidative stress index (OSI), was gauged as: OSI=[(TOS, μmol H_2O_2 equivalent L)/(TAS, mmol Trolox equivalent/L)×10]. Evaluation of SOD activity depends on formazan dye level (25). Lipid peroxidation was measured by determining MDA level through thiobarbituric acid test (26). Myeloperoxidase (MPO) activity was gauged according to method described previously (27). IL-1β and TNF-α levels were evaluated via appropriate kits (Elabscience, Wuhan, China).

4. Statistical analysis

Data were analyzed with One-Way ANOVA and Tukey test using statistical package program, SPSS. All results were presented in table 1 and figure 1 as Mean±SD. P value was considered significant when $p<0.05$.

5. Results

TNF-α and IL-1β values, MDA, TAS, TOS, OSI levels, MPO and SOD activities in ovarian tissues were demonstrated in table 1 and figure 1. Oxidative parameters (MDA, TOS, OSI, MPO) and inflammatory mediators (TNF-α, IL-1β) elevated significantly in T/D group compared to sham group. In Laz treatment group, these parameters declined significantly. Moreover, antioxidant activity (TAS and SOD) diminished in T/D group, whereas Laz treatment elevated TAS and SOD levels (Table 1; Figure 1, $p<0.001$).

Table 1: Results of biochemical parameters among all 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 tissue)
Sham	0.86 ± 0.04	6.62 ± 0.89	0.76 ± 0.10	437.02 ± 16.57	259471.94 ± 302466.65	64.64 ± 5.71
T/D	0.22 ± 0.03[a]	11.64 ± 1.08[a]	5.20 ± 0.97[a]	174.32 ± 7.05[a]	77222.10 ± 42575.30[a]	141.64 ± 11.83[a]
T/D+Laz	0.82 ± 0.11[b]	6.99 ± 0.37[b]	0.84 ± 0.09[b]	407.85 ± 20.51[b]	284813.46 ± 24588.28[b]	68.60 ± 5.33[b]

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

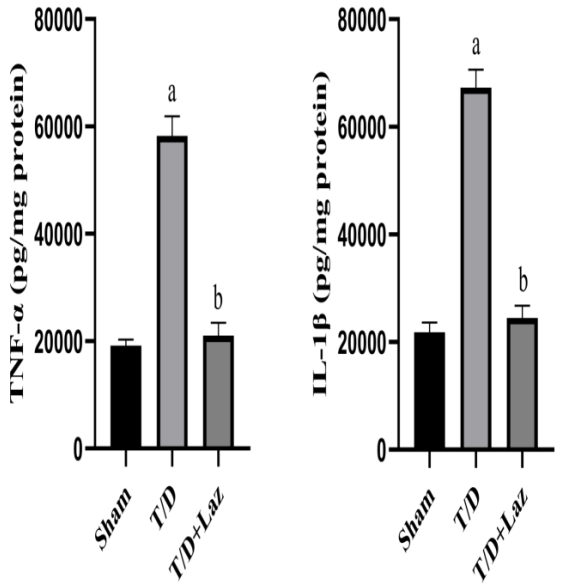

Figure 1: Results of IL-1β and TNF-α among all the experimental groups.

6. Discussion

Ovarian torsion (O/T) mostly occurs during reproductive period (28). Ischemic tissue injury is based on insufficient materials for energy supply. Ovarian detorsion means the recovery of blood flow. But replenishment of blood flow results in ovarian tissue injury (29). O/T is an emergency situation with a 3% prevalence (30; 31).

During ovarian T/D, blood reflow elevates in lactic acid, proinflammatory cytokine and lipid peroxide levels (7; 32; 33). Increased ROS damages cells via lipid peroxidation (34). MDA is a lipid peroxidation product and harmful for tissues. It reflects oxidative stress (6). It is created by ROS during I/R (35). It has been proven that oxidative stress causes tissue damage in various animal models (36-39). Neutrophil infiltration also leads to I/R injury besides oxidative stress. MPO activity represents neutrophil activation and infiltration (40). Neutrophil infiltration, TNF-α, IL-1β and several proinflammatory cytokine production accompany I/R (41). IL-1β plays role in apoptosis and inflammation (10; 42).

Besides ischemic injury, reperfusion also leads to injury in tissues (43). Antioxidant enzymes like SOD compose cellular defense system against oxidative injury (44). SOD is a crucial antioxidant enzyme and SOD activity declined during ovarian T/D in previous studies (45; 46). Oxidative stress is the surpass of oxidant activity against antioxidant system. OSI is the ratio of TOS to TAS. It is preferred for the determination of oxidative stress (47; 48). TOS and TAS play role in I/R injury assessment (49).

Laz has been studied in various I/R injury models including renal I/R injury (19), intestinal I/R injury in rats (50). Different agents with feature anti-inflammatory, antioxidant and radical scavengers have beneficial effects have been reported in alleviation or elimination of I/R injuries (51-56). In the current study, we thought that Laz could minimize T/D damage with its antioxidant and anti-inflammatory effects. Therefore, current study was planned to investigate possible protective effects of Laz on ovarian tissue by using an ovarian T/D model.

Here, several inflammatory mediators and oxidative stress biomarkers were diminished and antioxidant activity was enhanced by Laz treatment in ovarian T/D injury model.

7. Conclusions

Laz alleviated T/D-induced ovarian injury in experimental rat model through declining oxidative mediators and elevating antioxidant parameters. This is a hope-inspiring result in order to evaluate for T/D pathologies.

8. Acknowledgement

None.

9. Conflict of interest statement

None.

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