Research Article

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Possible protective effect of remifentanil against testicular ischemia-reperfusion injury

https://doi.org/10.1515/tjb-2024-0111
Received May 7, 2024; accepted August 20, 2024;
published online September 19, 2024

Abstract

Objectives: This study aims to evaluate the protective efficacy of remifentanil against testicular ischemia-reperfusion injury.

Methods: The study included 24 male rats. The rats were randomized into three groups: Group 1 was the control group. Group 2 was subjected to a testicular torsion/detorsion model. Group 3 underwent similar procedures and additionally received remifentanil (0.6 µg/kg/min) intravenously for the first 20 min of reperfusion. Blood samples were taken for biochemical analyses, and orchiectomy was performed for histopathologic examination.

Results: Biochemical analysis of blood samples showed a significant increase in antioxidant enzyme activity, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in Group 3 compared to Group 2 (p:0.004 and p:0.002, respectively). There was a dramatic decrease in the levels of proinflammatory cytokines, including interleukin-1 beta (IL-1 Beta), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-alpha) in Group 3 compared to Group 2 (p:0.001, p:0.046, and p:0.004, respectively). Similarly, malondialdehyde (MDA) levels decreased in Group 3 compared to Group 2 (p:0.004). Histopathologic examination of Group 3 rats showed positive changes in inflammation, hemorrhage, edema, and congestion levels compared to Group 2 (p<0.001). Similarly, there was a positive effect on the Johnsen and Cosentino score in Group 3 compared to Group 2 (p:0.001 and p<0.001, respectively).

Conclusions: In our study, it has been documented that remifentanil protects against testicular ischemia-reperfusion injury.

Keywords: remifentanil; testis; ischemia-reperfusion injury

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Introduction

Testicular torsion is the failure of testicular blood supply secondary to rotation of the spermatic cord. It is one of the most critical emergencies in urology and pediatric surgery departments [1–3]. The primary treatment approach for testicular torsion is immediate scrotal exploration and testicular detorsion [4]. Epidemiologic studies estimate that approximately 1 in 1,500 men will face testicular torsion surgery by the age of 18 [3]. Current results of these surgical interventions revealed that the testicular salvage rate is approximately 75 %, which is directly related to ischemia

time [5]. However, clinical follow-up of these cases reports that testicular atrophy may develop at a rate of 9–43 % [6]. The leading cause of this condition is ischemia-reperfusion injury that occurs after testicular detorsion [1]. During the ischemia-reperfusion process, many reactive oxygen radicals (ROS) are produced in the testicular tissue, such as hydrogen peroxide, superoxide, and hydroxyl radicals. The antioxidant defense system cannot eliminate the ROS that occur in the environment in a very short time [1, 7]. They are highly reactive and disrupt cellular membrane structures, lipid peroxidation, protein denaturation, deoxyribonucleic acid, and carbohydrate destruction, negatively impacting male reproductive health [1, 2].

Remifentanil is an ultra-short-acting, synthetic μ-opioid analgesic [8, 9]. Anesthesiologists widely use it in surgical foci because it does not cause adverse postoperative effects such as respiratory depression and intestinal motility disturbance, even at high doses intraoperatively [8]. In addition to its anesthetic properties, this pharmacological agent has protective and immune modulatory effects against oxidative stress [10]. Due to these functions, experimental studies on many different tissues, such as the intestinal system, uterus, liver, ovary, and heart have determined that it has protective effects against ischemia-reperfusion injury [8–13].

With the understanding of the role of ischemia-reperfusion injury in the surviving testicular tissues of patients with testicular torsion, many pharmacological agents with ROS elimination, antioxidant, and anti-inflammatory properties have been investigated to add to surgical procedures [14]. This study aimed to determine the possible protective effects of remifentanil against testicular ischemia-reperfusion injury.

Materials and methods

Study design

Our study involved twenty-four 12-week-old male Wistar Albino rats with a mean weight of 300 g. We processed the rats in the laboratory following institutional guidelines and the National Research Council's Guide for the Care and Use of Laboratory Animals. All procedures followed the provisions of the Strasbourg Universal Declaration on Animal Welfare of 1986 and were approved by the Animal Studies Ethics Committee of Tokat Gaziosmanpaşa University (2023 HADYEK-01/51879863-15).

Sample handling, preparation and storage

Blood samples taken from the inferior vena cava during the formation of experimental groups and surgical procedures were placed in tubes for biochemical measurements. The bloods placed in the tubes was centrifuged at 4,000 rpm at 4 °C for 10 min, then frozen as serum samples and stored at −20 °C until the study day. The testicular tissues from the rats were preserved in a buffered formalin solution.

Surgical procedure

Rats were injected with ketamine hydrochloride 50 mg/kg and xylazine 10 mg/kg intraperitoneally for anesthesia. A single dose of anesthesia was sufficient. Skin or finger pinch responses were evaluated to monitor the depth of anesthesia. Then, surgical procedures were performed after ensuring appropriate anesthetic depth and under sterile conditions at constant temperature (22 ± 2 °C) and 50 ± 5 % humidity with a 12-h light/dark cycle.

Group 1 was assigned as the control group. Blood samples were collected from rats in this group for baseline biochemical and histopathologic analyses, and left orchiectomy was performed.

Group 2 was the testicular ischemia-reperfusion group. Rats in this group underwent a testicular torsion/detorsion model as previously described in the literature. In this context, a left inguinoscrotal incision was made; the left testicle was rotated 720° counterclockwise and fixed to the scrotum with a 5.0 Prolene suture for 3 h. After the torsion was released, reperfusion was allowed for another 3 h. Afterward, blood samples were taken for biochemical and histopathologic analysis, and left orchiectomy was performed [15].

Group 3 was designated as the treatment group. The testicular torsion/detorsion model was similar to that in Group 2. In addition, remifentanil (0.6 μg/kg/min) was administered intravenously for the first 20 min of reperfusion [13].

Tissue processing and hematoxylin-eosin staining

After orchiectomy, testicular tissues were removed and fixed in a 4 % buffered neutral formalin solution for 48 h for histological analysis. After fixation, the testes were washed in running water throughout the day, dehydrated in ascending alcohol series (70 %, 80 %, 90 %, 96 %, 100 %), cleared in xylene series, and paraffin impregnated. Then, each testis was cut in midtransfers and blocked by paraffin embedding in the same orientation. Consecutive, thin serial sections of 5 μm thickness taken from the blocked testes with a rotary microtome (Leica RM2135, Germany) were placed on frozen slides for hematoxylin and eosin staining. Hematoxylin and eosin-stained preparations of testicular tissue of the study groups were analyzed

under a 40x objective with a light microscope (Nikon, Eclipse 200; Japan). Microscopic examinations were performed on an average of 5–6 consecutive sections of each individual, and an average of 25 seminiferous tubules were evaluated in five different random areas in each section. Analyses were performed in a blinded study with a coding system. Testicular tissues were evaluated histopathologically and spermatogenically with the Johnsen scoring system, and the mean Johnsen score was calculated [16, 17]. As the second step of microscopic examination, hemorrhage, inflammation, edema, and congestion were evaluated in testicular parenchymal damage. These parameters were scored as follows: 0 for normal, 1 for mild, 2 for moderate, and 3 for severe [18]. On the other hand, ischemic changes were evaluated according to the Cosentino scoring system (Grade 1=normal, Grade 4=coagulative necrosis) [19].

Biochemical analyzes

Determination of superoxide dismutase (SOD) activity

SOD uses O_2–radical as a substrate. SOD activity is based on the reading of the optical density (OD) at 560 nm wavelength of the blue-colored formazan dye formed by superoxide radicals generated using xanthine and xanthine oxidase with nitro blue tetrazolium (NBT). SOD in the sample inhibits the formazan reaction by removing superoxide radicals from the medium [20]. We used the U/mL unit for SOD activity in our study.

Malondialdehyde (MDA) level determination

The determination of MDA, one of the lipid peroxidation products, is based on the principle that thiobarbutyric acid and MDA react to give a colored compound that can be measured at a wavelength of 532 nm [21]. In our study, μmol/mL unit was used for the MDA level.

Glutathione peroxidase (GSH-Px)

It is a method based on measuring the activity of glutathione peroxidase at 340 nm, which catalyzes the oxidation of reduced glutathione to oxidized glutathione by reacting with hydrogen peroxide [22]. We used the U/L unit for GSH-Px activity in our study.

Measurement of tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), and interleukin-6 (IL-6) in serum

TNF-alpha, IL-1beta, and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were purchased commercially. TNF-alpha, IL-1beta, and IL-6 levels in sera obtained from rats were determined using ELISA kits (BT LAB, China) and according to the brochures included in the kits. The units used in our study for TNF- alpha, IL-1beta, and IL-6 levels were ng/dL, μg/dL, and ng/L, respectively. The intra-assay coefficient of variation (CV) values are 30.52 % for TNF-alpha, 42 % for IL-1beta and 26.7 % for IL-6.

Statistical analyzes

Descriptive statistics were used to provide information about the general characteristics of the study groups. The data of the variables were defined using mean ± standard deviation (SD) and min-max. The compliance of the series related to the variables to normal distribution was examined with skewness and kurtosis values. The fact that the skewness and kurtosis values are between (–1.5) and (+1.5) shows that the normal distribution condition is met. One-way analysis of variance (ANOVA) was used to evaluate intergroup differences in variables. Post-hoc Tukey HSD or Tamhane's T2 was used for further comparisons. P-Values less than 0.05 were considered statistically significant. For calculations, off-the-shelf statistical software (IBM SPSS Statistics 22, SPSS Inc., an IBM Co., Somers, NY) was used.

Results

Histopathological results

Our histopathologic analyses are detailed in Table 1 and Figure 1. We evaluated seminiferous tubule structures and spermatic activity using the Johnson score in all sections. In Group 1 and 3, the Johnson score was observed as 9.514 and 8.299, respectively. In Group 2, the Johnson score was 6.47, significantly lower than the control group ($p<0.001$). On the other hand, there was a significant improvement in this Johnson score in Group 3 (p:0.001). Similarly, the Cosentino score in Group 1 was calculated as 1.125. In Group 2, this value was significantly increased and measured as 3.5 ($p<0.001$). A significant improvement was detected in Group 3, with a Cosentino score of 2.25 observed. ($p<0.001$). Again, our histopathologic examinations revealed very intense inflammation in Group 2. In Group 1, no inflammation of mild or higher was observed. In Group 3, the inflammation score was reported to be 0.96 and suppressed compared to Group 2 ($p<0.001$). Similarly, the vasocongestion, edema, and hemorrhage scores in Group 3 were 1.11, 1.14, and 1.15, respectively, which were significantly lower than in Group 2 ($p<0.001$) (Figures 2 and 3). In Group 1, vasocongestion, edema, and hemorrhage scores of 1 and above were not observed.

Table 1: Comparison of histopathological scores of testicular damage between groups.

	Groups	n	Mean ± SD	Min-max	p-Values	Post Hoc p-Values
Edema	Control	8	0.620 ± 0.17	0.45–0.85	**<0.001**[a]	1–2: <0.001[a]
	I/R	8	2.24 ± 0.27	2–2.64		1–3: <0.001[a]
	I/R+treatment	8	1.14 ± 0.18	0.79–1.45		2–3: <0.001[a]
Inflammation	Control	8	0.28 ± 0.13	0.18–0.55	**<0.001**[a]	1–2: <0.001[a]
	I/R	8	2 ± 0.09	1.85–2.17		1–3: <0.001[a]
	I/R+treatment	8	0.96 ± 0.19	0.8–1.4		2–3: <0.001[a]
Vasocongestion	Control	8	0.39 ± 0.11	0.3–0.64	**<0.001**[a]	1–2: <0.001[a]
	I/R	8	2.32 ± 0.21	2.11–2.69		1–3: <0.001[a]
	I/R+treatment	8	1.11 ± 0.18	0.93–1.46		2–3: <0.001[a]
Hemorrhage	Control	8	0.15 ± 0.05	0.08–0.25	**<0.001**[a]	1–2: <0.001[a]
	I/R	8	2.23 ± 0.23	2.02–2.71		1–3: <0.001[a]
	I/R+treatment	8	1.15 ± 0.16	0.89–1.29		2–3: <0.001[a]
Johnsen scores	Control	8	9.514 ± 0.225	9,01–9,77	**<0.001**[a]	1–2: <0.001[a]
	I/R	8	6.471 ± 0.434	5.85–7.04		1–3: 0.012[a]
	I/R+treatment	8	8.299 ± 0.833	7–9.36		2–3: 0.001[a]
Cosentino scores	Control	8	1.125 ± 0.35	1.0–2.0	**<0.001**[a]	1–2: <0.001[a]
	I/R	8	3.500 ± 0.535	3.0–4.0		1–3: <0.001[a]
	I/R+treatment	8	2.250 ± 0.463	2.0–3.0		2–3: <0.001[a]

I/R, ischemia-reperfusion; SD, standard deviation. Test: One-Way Analysis of Variance (ANOVA). Differences between groups were examined with Post Hoc Tukey HSD or Tamhane's T2. [a]indicates statistically significant (p<0.05).

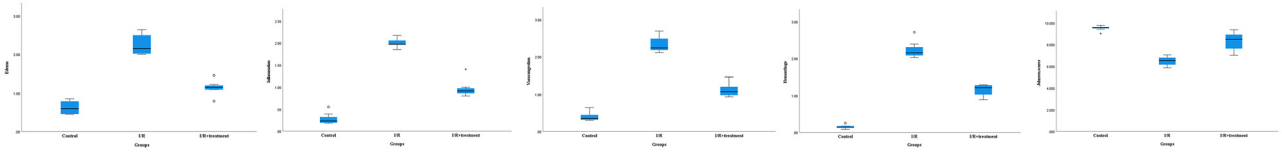

Figure 1: Box plot for comparison of edema, inflammation, vasocongestion, hemorrhage and Johnsen scores in study groups.

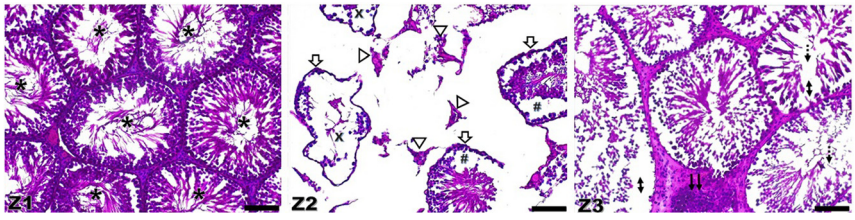

Figure 2: Representative microscopic testis images from our study groups. In the control group (Z1), a normal histological appearance is observed with seminiferous tubule epithelium, interstitial tissue, and numerous spermatozoa (thin arrows) in the tubular lumen. In group 2, (Z2), deformed tubules (arrow), fragments of eroded interstitial tissue (arrowhead), hollow tubules (x) with basal lamina detachment, complete or mostly shed seminiferous epithelium, germinal epithelial separation, accumulation of germinal epithelium in the lumen (square), and tubules without spermatozoa in the lumen are visible. In images of group 3, (Z3), although some seminiferous tubules show epithelial irregularities (double-headed arrow), absence of mature spermatozoa in the lumen (dashed arrow), and inflammatory areas in some interstitial regions (double arrow), overall, the testicular tissue more closely resembles a normal testis tissue with reduced damage, displaying seminiferous tubules and interstitial tissues (Hematoxylin eosin, Scale bar: 50 µm).

Biochemical results

In the control group, IL-1beta, IL-6, and TNF-alpha levels were calculated as 1.12, 9.61, and 46.89, respectively. Biochemical analysis showed a significant increase in proinflammatory cytokine levels, including IL-1beta, IL-6, and TNF-alpha, in Group 2 compared to the control group (p:0.001, p:0.004, and p:0.014, respectively). There was a significant suppression of inflammation in Group 3 compared to Group 2 (p:0.001 for IL-1beta, p:0.046 for IL-6, p:0.004 for TNF-alpha). On the other hand, the highest MDA level was in Group 2 (p<0.001). Group 1 MDA value was

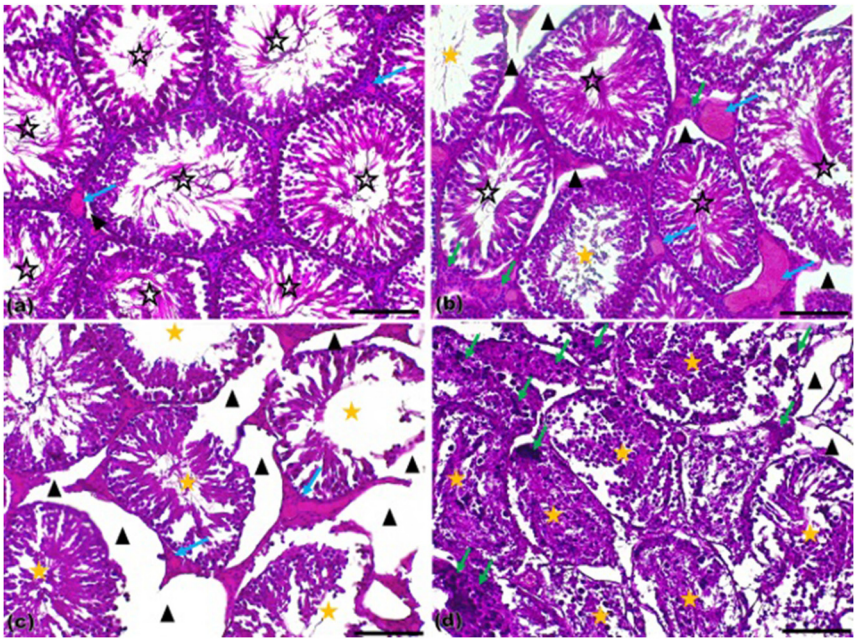

Figure 3: In the control group, intact seminiferous tubule epithelium, interstitial tissue, and a normal histological appearance with numerous spermatozoa in the tubular lumen are observed in seminiferous tubules (black star) (a). In the treatment group, there is a significant reduction in tubular damage, along with mild edema, inflammation, congestion, and hemorrhage (b). In group 2, severe tubular damage and deformations, along with intense inflammatory cell infiltrations, edema, congestion, and hemorrhage, are observed (c, d). Black star: Seminiferous tubules with a normal histological structure containing spermatozoa in the lumen. Yellow star: Disrupted normal histological arrangement in damaged seminiferous tubules. Arrowhead: Edema in the tubular interstitial spaces. Blue arrow: Areas of congestion and hemorrhage. Green arrow: Foci of inflammatory cell infiltration (Hematoxylin eosin, Scale bar: 100 μm).

measured as 5.76, which was quite low compared to the other groups (p<0.001). The MDA level in Group 3 was 6.59, which was dramatically decreased compared to Group 2 (p:0.004). In terms of antioxidant levels, SOD and GSH-Px enzyme activities in Group 3 were 6.55 and 518.06, respectively. These values were significantly higher than in Group 2 (p:0.004 and p:0.002, respectively). On the other hand, SOD and GSH-Px enzyme activities in Group 1 were measured as 7.27 and 580.84, respectively, which were higher than the antioxidant enzyme activities in the other groups (p:0.006 and p<0.001, respectively) (Table 2 and Figure 4).

Table 2: Comparison of SOD and GSH-Px activity, TNF-alfa, IL-1beta, IL-6 and MDA levels obtained from blood between rat groups.

	Groups	n	Mean ± SD	Min-max	p-Values	Post Hoc p-Values
SOD, U/mL	Control	8	7.27 ± 1.04	5.63–8.6	**0.006**[a]	1–2:0.005[a]
	I/R	8	4.91 ± 1.84	2.21–7.21		1–3:0.529
	I/R+treatment	8	6.55 ± 0.95	5.43–8.2		2–3:0.004[a]
GSH-PX, U/L	Control	8	580.84 ± 70.81	445.4–665.3	**<0.001**[a]	1–2:<0.001[a]
	I/R	8	387.46 ± 73.35	307.5–508.2		1–3:0.521
	I/R+treatment	8	518.06 ± 55.67	391.1–565.3		2–3:0.002[a]
MDA, μmol/mL	Control	8	5.76 ± 2.11	3.71–10.2	**<0.001**[a]	1–2:<0.001[a]
	I/R	8	9.61 ± 1.41	7.56–11.32		1–3: 0.566
	I/R+treatment	8	6.59 ± 1.35	4.87–8.6		2–3:0.004[a]
TNF-alpha, ng/dl	Control	8	46.89 ± 10,55	31.2–63.43	**0.002**[a]	1–2:0.014[a]
	I/R	8	76.04 ± 20.51	41.2–95.4		1–3:0.599
	I/R+treatment	8	58.98 ± 10.16	46.2–72.1		2–3:0.004[a]
IL-1beta, μg/dL	Control	8	1.12 ± 0.25	0.89–1.65	**<0.001**[a]	1–2:0.001[a]
	I/R	8	2.24 ± 0.52	1.54–3.1		1–3:0.440
	I/R+treatment	8	1.16 ± 0.24	0.78–1.53		2–3:0.001[a]
IL-6, ng/L	Control	8	9.61 ± 2.2	6.32–13.2	**0.001**[a]	1–2:0.004[a]
	I/R	8	14.7 ± 2.86	10.1–18.34		1–3:0.491
	I/R+treatment	8	11.19 ± 2.12	9.21–15.2		2–3:0.046[a]

SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; TNF- alpha, tumor necrosis factor alpha; IL-1beta, interleukin 1 beta; IL-6, interleukin 6; SD, standard deviation; I/R, ischemia-reperfusion. Test: One-Way Analysis of Variance (ANOVA). Differences between groups were examined with Post Hoc Tukey HSD or Tamhane's T2. [a]statistically significant (p<0.05).

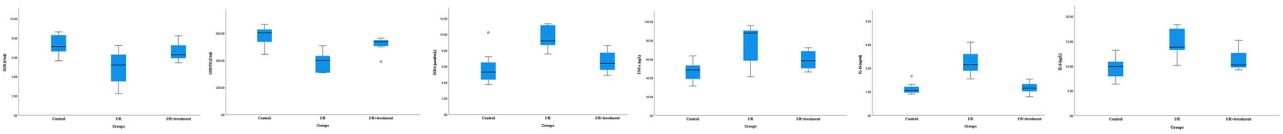

Figure 4: Box plot for comparison of GSH-px, SOD, MDA, TNF-alpha, IL-1 and IL-6 in study groups. SOD (U/L), superoxide dismutase; MDA (U/L), malondialdehyde; GSH-px (nmol/mL), glutathione peroxidase; TNF-alpha (ng/dL), tumor necrosis factor alpha; IL-1beta (µg/dL), interleukin 1 beta; IL-6 (ng/L), interleukin 6.

Discussion

In our study, it was determined that remifentanil, a synthetic µ-opioid analgesic agent, has a protective effect on ischaemia – reperfusion injury occurring after testicular torsion/detorsion in rats. In our study, against testicular ischemia reperfusion damage, remifentanil decreased the level of MDA, the end product of lipid peroxidation, showed antioxidant activity by increasing the activity of antioxidant enzymes such as SOD and GSH-Px, and on the other hand, had anti-inflammatory activity by suppressing the level of pro-inflammatory cytokines such as IL-1 beta, IL-6 and TNF-alpha. However, in histopathological evaluation, a significant improvement was observed in edema, inflammation, vasocongestion, hemorrhage and Johnsen scores. To our knowledge, this is the first experimental study in the English literature where remifentanil was administered in rats with a testicular ischemia-reperfusion model. In testicular torsion, tissue oxygenation cannot be maintained. It is critical to restore the blood supply by bringing the spermatic cord to its normal position for testicular tissue viability. However, reoxygenation of tissues paradoxically causes ischemia-reperfusion injury with highly destructive effects. Although testicular ischemia-reperfusion injury is highly complex and multifactorial, its etiopathogenesis remains unclear [23]. The prevailing ischemic condition breaks down the high-energy adenosine triphosphate hypoxanthine in the environment, and xanthine dehydrogenase turns into xanthine oxidase. Restoration of the blood supply leads to intense oxygen xanthine oxidase enzyme, which converts hypoxanthine into uric acid and ROS [23, 24]. In testicular ischemia-reperfusion injury, germ cells and macrophages produce high amounts of proinflammatory cytokines, which induce a chemotactic effect for neutrophils. These neutrophils migrating into the testicular tissue cause an increase in the concentration of ROS through a chain of reactions in which the enzyme nicotinamide adenine dinucleotide phosphate oxidase plays a key role [1]. As a result, antioxidant defense systems such as superoxide dismutase, glutathione peroxidase, and catalase, which are responsible for scavenging reactive oxygen species, become ineffective against the rapidly increasing amount of ROS in the environment [2, 24]. However, changes in cellular and mitochondrial calcium stores are critical in testicular ischemia-reperfusion injury. Increased calcium affects the formation of pores in membrane structures, leading to a significant increase in permeability and triggering apoptosis [24].

The effect of ROS is directly related to their concentration. Within physiologic limits in the testicular tissue, ROS have essential functions such as bacterial elimination, protein phosphorylation, cell proliferation, cell differentiation, and apoptosis [2]. Low levels of ROS are essential for spermatogenesis and the maintenance of reproductive health [25]. However, ROS in high amounts in the environment have a highly destructive effect. Testes are highly sensitive to toxins due to the high content of polyunsaturated fatty acids [23]. In this context, ROS can cause structural defects in seminiferous tubules, decreased testosterone production, testicular atrophy, DNA fragmentation, mitochondrial damage, abnormal head morphology, and cytoplasmic irregularities in sperm, leading to disruptions in critical steps of spermatogenesis and a significant decline in fertility capacity [4, 25]. Considering that testicular torsion is most common in children and young men, a detailed understanding of the etiopathogenesis of ischemia-reperfusion injury after testicular torsion treatment and the development of treatment strategies are critical for the continuation of generations. In the literature, studies show that spermatogenesis can be impaired in a time interval ranging from 2 h to three months after testicular detorsion [4]. In Ekşi et al.'s experimental study in which testicular torsion for 2 h followed by testicular detorsion for 2 h, rats exposed to ischemia-reperfusion injury showed diffuse desquamation and focal necrosis areas in the seminiferous tubule epithelium. However, they observed a significant decrease in the Johnsen testicular biopsy scores [26]. Wei et al. analyzed the testicular tissues of rats exposed to testicular ischemia for 2 h in a rat model of testicular torsion detorsion at the end of the third month. They observed significant decreases in testicular weight, seminiferous tubular diameter, number of germ cell layers, and the Johnsen testicular biopsy score secondary to testicular ischemia-reperfusion injury. The same study reported that spermatogenesis was adversely affected critical-level due to ischemia-reperfusion injury in

connection with spermatogenic arrest and sperm absence [1]. Similarly, our histopathologic examinations showed that the Johnsen score significantly decreased in rats exposed to ischemia-reperfusion injury compared to the control group. Similarly, we found that inflammation, hemorrhage, edema, and congestion scores were all impaired in the ischemia-reperfusion group directly related to tissue damage.

The efficacy of remifentanil, a 4-anilinopiperidine derivative of fentanyl, starts quite rapidly, and its half-life is between 3 and 5 min [8, 27]. Remifentanil has a synergistic effect with inhaled anesthetics, hypnotics, and benzodiazepines [10]. Remifentanil is rapidly converted to inactive metabolites by plasma and tissue esterases independent of the liver and kidneys [28]. Remifentanil is widely preferred because it has a very mild effect on hemodynamics [29]. A large series of clinical analyses have shown that remifentanil use in cardiac surgery is associated with lower cardiac enzyme levels, reduced hospitalization, and duration of mechanical ventilation [30]. A recent retrospective large series study in Japan involving 926 hospitals similarly reported less postoperative mortality with remifentanil in neuro-anesthesia [31]. Recent studies show that remifentanil has important protective properties against ischemia-reperfusion injury, in addition to its analgesic use in operating rooms and intensive care units. Although the molecular mechanisms of this effect have been intensively investigated, though not yet fully elucidated.

Remifentanil inhibits the interleukin-18 (IL-18) signaling pathway and decreases the production of IL-1beta, TNF-alpha, and interferon-gamma [32]. Suppression of this cytokine also inhibits neutrophil migration and adhesion molecule expression [33]. Zongze et al. reported that remifentanil decreased inflammatory factor production, inhibited myeloperoxidase activity, and suppressed nitric oxide synthase expression, thus playing a protective role against sepsis [34]. Cho et al. observed that remifentanil significantly improved mucosal damage by reducing oxidative stress and systemic inflammation in experimental studies in which intestinal ischemia-reperfusion injury was induced [8]. Liu et al. similarly observed that remifentanil provided a hepatoprotective effect by modulating IL-18/IL-18BP balance in their rat liver ischemia-reperfusion model [35]. The clinical study by Jiang et al. reported that remifentanil showed hepatoprotective activity against liver ischemia-reperfusion by decreasing intercellular adhesion molecule-1 expression and inflammatory factors [28]. Our study similarly observed that remifentanil suppressed inflammation secondary to testicular ischemia perfusion injury. We found a significant decrease in IL-1, IL-6 and TNF-alpha levels and a significant regression of inflammation scores in direct microscopic evaluation of tissues. Remifentanil also plays a critical role in antioxidant defense mechanisms that regress to inadequate levels due to ischemia-reperfusion injury. An experimental study analyzing a rat uterine ischemia-reperfusion model revealed that remifentanil decreased tissue leukocyte density and cell degeneration. The same study also reported that remifentanil showed antioxidant activity by increasing catalase and superoxide dismutase enzyme activities [9]. Another study by Atalay et al. similarly reported that remifentanil significantly attenuated ovarian ischemia-reperfusion injury by increasing catalase enzyme activities [11]. Also, tissue biochemical examinations in our study revealed that SOD and GSH-px values, which regressed secondary to testicular ischemia-reperfusion injury, increased after remifentanil administration.

Remifentanil also activates intracellular anti-apoptotic signaling pathways through the activation of opioid receptors and attenuates cell apoptosis secondary to oxidative damage [36]. Kim et al. reported that remifentanil showed cardioprotective activity by increasing the anti-apoptotic protein B-cell lymphoma 2 (BCL-2), extracellular signal regulated kinase ½ (ERK ½), and sarcoplasmic reticulum gene expression levels related to calcium homeostasis in their rat cardiac ischemia-reperfusion model [13]. An experimental study by Zhao et al. demonstrated that remifentanil blocked apoptosis associated with preserved mitochondrial function while reducing oxidative stress and inflammatory response. As a result, they documented a significant reduction in liver ischemia-reperfusion injury [12]. Erkılıç et al. revealed that remifentanil decreased the number of necrotic cells and regressed the severity of tubular cell damage in rats exposed to renal ischemia-reperfusion injury [37]. In our study, we observed a dramatic improvement in inflammation, hemorrhage, edema, congestion, and the Johnsen scores in rats treated with remifentanil compared to the ischemia-reperfusion group.

Conclusions

Our study was the first in the English literature to evaluate the efficacy of remifentanil in rats with a testicular torsion/detorsion model. With our study data, we found that remifentanil is a highly effective anesthetic agent in minimizing oxidative stress secondary to testicular ischemia/reperfusion injury and protecting the testicular tissue. Large series of randomized studies are needed for our study to guide reproductive health and sexual life disciplines.

Limitations of this study

The main limitation of our study is the lack of an immunohistochemically detailed histopathologic examination. Other

deficiencies of our study are that the late effects of remifentanil on testicular tissue could not be demonstrated, and the efficacy of different dose curves could not be analyzed.

Acknowledgments: The authors would like to thank to staff of Experimental Animals Research Unit of Tokat Gaziosmanpasa University for providing valuable supports.
Research ethics: All procedures followed the provisions of the Strasbourg Universal Declaration on Animal Welfare of 1986 and were approved by the Animal Studies Ethics Committee of Tokat Gaziosmanpaşa University (2023 HADYEK-01).
Informed consent: Not applicable.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: Authors state no conflict of interest.
Research funding: None declared.
Data availability: The data used to support the findings of this study can be obtained from the corresponding author on request.

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