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The effects of etomidate on testicular ischemia reperfusion injury in ipsilateral and contralateral testes of rats

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Abstract. – OBJECTIVE: Testicular torsion is a condition that manifests with acute pain and can lead to infertility despite urgent surgical intervention. The aim of this study is to investigate the protective and preventive effects of etomidate, an imidazole derivative, a non-barbiturate general anesthetic agent, without analgesic effect, on testicular ischemia-reperfusion injury.

MATERIALS AND METHODS: Twenty-four adult male Wistar rats weighing 200-250 g were used in the study. Torsion was created in all rats by rotating left testes 720° clockwise on the day of the experiment. 30 minutes before detorsion, 4 mg/kg etomidate and 10 mg/kg propofol were administered intraperitoneally to the etomidate and propofol groups, respectively. After an hour of ischemia, the left testis was reinstated, and the tissues were repaired according to their physiology. Following 24 hours of reperfusion, the animals were euthanized after ipsilateral and contralateral testes were removed.

RESULTS: Etomidate applied before testicular detorsion significantly suppressed germ cell damage and Leydig cell loss in ipsilateral tissue. It did not cause any significant changes in the percentage of necrosis, histological score, and tubule rupture in ipsilateral tissue. Propofol administered before testicular detorsion significantly suppressed the percentage of necrosis only in the ipsilateral tissue. In addition, no signs of damage were observed in the contralateral testis.

CONCLUSIONS: These findings show that etomidate administered before detortion creates a protective effect by preventing testicular ischemia-reperfusion injury.

Key Words:
General anesthetic, Germ cell, Leydig cell, Testicular torsion and detorsion, Ischemia reperfusion injury, Ipsilateral and contralateral testes.

Introduction

Testicular torsion is a condition that manifests with acute pain as a result of the spermatic cord rotation, requires urgent surgical detorsion, and causes 40-60% infertility despite emergency intervention[1,2]. Damage in the torsioned testicular tissue occurs due to both impaired perfusion and post-detorsion reperfusion[3,4]. In perfusion impairment, the oxygen level in the tissues decreases and the cellular energy sources are depleted. In addition, the xanthine oxidase system is activated and the reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals are formed. All these changes lead to ischemic damage[3]. As a result of reperfusion following detorsion, the release of ROS originating from polymorphonuclear leukocytes and proinflammatory cytokines is abrupt and excessive. This triggers oxidative stress and deepens the damage[4]. Over-produced ROS in a short time cannot be eliminated by the enzymatic and non-enzymatic antioxidant defense system. ROS, which cannot be removed from the damaged tissue, cause polyunsaturated fatty acid peroxidation in cell and mitochondrial membrane lipoproteins, inhibition of mitochondrial respiratory chain enzymes, DNA

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damage, and protein dysfunction. These changes trigger germ cell destruction, reduce sperm stem cells and cause infertility[4].

Etomidate, an imidazole derivative, is a non-barbiturate intravenous general anesthetic agent[5]. Etomidate used in general anesthesia induction lacks analgesic effect, with minimal effect on blood gases, ventilation or cardiovascular system[6,7]. Etomidate is also used off-label for procedural sedation[8]. Etomidate increases the effect of gamma-aminobutyric acid (GABA), which is the main inhibitory neurotransmitter, by stimulating the GABA-A receptor and prolongs its depressing effect on the central nervous system (CNS)[9]. Etomidate also affects the adrenergic alpha 2 receptor, but its clinical significance is not yet known[10]. Previous studies have shown that etomidate suppresses ischemia-reperfusion injury (IRI) of tissues and organs such as the heart, lung, bone, skeletal muscle, spinal cord, retina[11-16]. There are no studies investigating the effect of etomidate on testicular torsion.

Propofol, like etomidate, is an intravenous anesthetic agent. It is used in the induction and maintenance of general anesthesia, as well as refractory status epilepticus. Propofol, like etomidate, increases GABAergic transmission by stimulating the GABA-A receptor[9]. In previous studies, the effects of propofol on testicular torsion have been investigated and it has been shown to suppress ischemia-reperfusion injury[14,17,18].

In this study, it was aimed to investigate the protective and preventive effects of etomidate applied prior to testicular detorsion in 1 hour 720° testicular torsion rat model on ipsilateral (torsioned) and contralateral (not torsioned) testicular tissue. In addition, the effects of propofol, which has a protective effect on ischemia-reperfusion injury in previous studies and increases GABAergic transmission like etomidate, will be investigated on both ipsilateral and contralateral testis.

Materials and Methods

Animals

In the study, a total of 24 male Wistar rats (aged 2–4 months, weighing 200-250 g) were used in 3 groups, with 8 animals in each group. The research protocol was approved by the local Ethics Committee TNKU DHEK (approval number: T2021/662-6). The rats obtained from TNKU-DHUAM were housed with a reversed 12 h light/dark cycle at 21±3°C and 50±5% humidity. There was unlimited access to standard rat chow and water.

Experimental Procedure

The animals were first given a seven-day adaptation period. Animals randomly divided into control, propofol and etomidate groups were anesthetized with a ketamine-xylazine mixture. After making sure that deep anesthesia was provided, surgical torsion-detorsion was performed. The left testis, which was made visible by the transcrotal incision, was rotated 720° clockwise for one-hour torsion[19]. 30 minutes before the detorsion, 4 mg/kg etomidate was administered to the etomidate group, 10 mg/kg propofol to the propofol group, and the same volume of saline to the control group[14,16]. 30 minutes after the treatment, the left testis was brought to its initial state, the tissues were repaired according to their physiology, and the animals were left to rest for 24 hours. Animals with a 24-hour reperfusion period were sacrificed with high-dose anesthesia, and both ipsilateral and contralateral testes were removed for histopathological examination[17].

Histopathologic Examination

Histopathological evaluation was performed blindly by the same pathologist. All paraffin-embedded sections of tissue processing using paraffin block taken from the testicular tisssues already preserved with 10% formaldehyde solution. They were deparaffinized, stained with haematoxylin and eosin (H&E), Masson trichrome staining (Bench Mark Special Stains device), CD68 (Bench Mark XT model device) and androgen receptor antibodies (cell marque, Bench Mark XT model device) were applied. Light microscope (Olympus CX41 model) was used to examine the sections. The grading scale of Cosentino et al[22], and Johnson tubular biopsy score (JTBS) were used to examine histological damage and spermatogenesis in testicular tissue, respectively (Table I)[20,21]. Injury degree evaluations were made according to Johnson classification[19]. Evaluations such as loss of sperm cells, germ cell degeneration and damage, tubule rupture, proliferation of Leydig cells, hemorrhage, edema, and fibrosis were made according to Cosentino et al[22] classification. In addition to these evaluations, the percentage of necrosis, tubule rupture and damage of the germ cell layer was calculated. The number of Leydig cells in 1 large magnification field was calculated by androgen receptor immunohistochemistry staining.

Table I. Grades and explanations of testicular histological damage[19].

Grades	Explanations
1	Normal testicular architecture with an orderly arrangement of germinal cells
2	Less orderly, noncohesive germinal cells and closely packed seminiferous tubules
3	Disordered, sloughed germinal cells with shrunken, pyknotic nuclei and less distinct seminiferous tubule borders
4	Seminiferous tubules that were closely packed with coagulative necrosis of the germinal cells

Drugs and Solutions

All animals underwent surgical torsion-detorsion under anesthesia of 100 mg/kg ketamine (Alfamine, Alfasan, Kuipersweg, Woerden, The Netherlands) and 10 mg/kg xylazine (Basilazine, Bavet, Tuzla, Istanbul, Turkey). Etomidate (E6530, Sigma-Aldrich, St. Louis, MO, USA) administered at a dose of 4 mg/kg (16.4 μM) was dissolved in propylene glycol (81350, Sigma-Aldrich, St. Louis, MO, USA) and saline, respectively[16]. Propofol (P076, Sigma-Aldrich, St. Louis, MO, USA) was administered at a dose of 10 mg/kg (56.1 μM)[14]. All administrations were done intraperitoneally[14,16].

Statistical Analysis

Data were analyzed using GraphPad Prism 5.01 software (La Jolla, CA, USA). All data were shown as mean±standard error of mean (SEM). Germ cell damage, Leydig cell counts, percentage of necrosis and histological score analyzed with two-way ANOVA Bonferroni post-tests. One-way ANOVA Kruskal-Wallis Dunn's post-test was used for tubule rupture. $p<0.05$ were considered statistically significant.

Results

Etomidate administered group (6.25±0.82) showed statistically significant decreased germ cell damage in ipsilateral tissue ($F_{Interaction}=5.98$, $p=0.005$; $F_{Treatment\ affect}=2.91$, $p=0.06$; $F_{Enj\ side\ affect}=0.96$, $p=0.33$; Figure 1A) compared to control group (14.38±2.74). There was no statistical significance in ipsilateral germ cell damage between etomidate and propofol (16.43±2.10) groups, in the contralateral germ cell damage between control (7.86±2.14), propofol (11.88±1.62) and etomidate (12.50±2.11) groups (Figure 1A).

Leydig cell counts in ipsilateral tissue was statistically significant ($F_{Interaction}=5.07$, $p=0.001$;

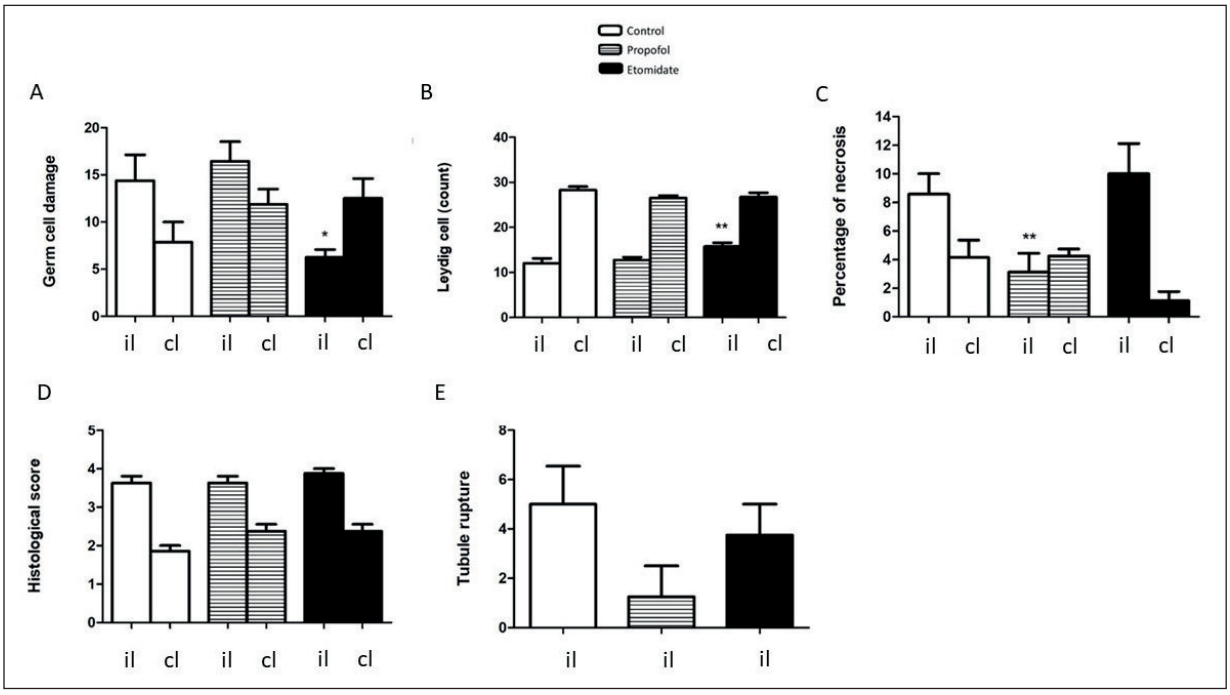

Figure 1. The effects of 4 mg/kg etomidate (n=8) and 10 mg/kg propofol (n=8) administered i.p. 30 min before left testicular detorsion to the ipsilateral and contralateral germ cell damage (**A**), Leydig cell counts (**B**), percentage of necrosis (**C**), histological score (**D**), and to the ipsilateral tubule rupture (**E**). All data are shown as mean±SEM. *p <0.05 and **p <0.01 compared compared with the control group. il: ipsilateral, cl: contralateral.

$F_{Treatment\ affect}$=2.13, p=0.0108; $F_{Enj\ side\ affect}$=428, p=0.001; Figure 1B) in etomidate administered animals (15.75±0.84) compared to control group (12.00±1.11). There was no statistical significance in ipsilateral Leydig cell counts between etomidate and propofol (12.75±0.62) groups, in the contralateral Leydig cell counts between control (28.29±0.78), propofol (26.50±0.60) and etomidate (26.75±0.94) groups (Figure 1B).

Percentage of necrosis in ipsilateral tissue was statistically significant ($F_{Interaction}$=7.55, p=0.001; $F_{Treatment\ affect}$=2.16, p=0.12; $F_{Enj\ side\ affect}$=14.20, p=0.001; Figure 1C) in propofol group (3.12±1. 31) compared to control group (8.57±1. 43). There was no statistical significance in ipsilateral percentage of necrosis between etomidate (10.00±2.11) and the control groups, in the contralateral percentage of necrosis between control (4.14±1.22), propofol (4.25±0.49) and etomidate (1.12±0.64) groups (Figure 1C).

There was no statistical significance ($F_{Interaction}$=1.15, p=0.32; $F_{Treatment\ affect}$=2.61, p=0.08; $F_{Enj\ side\ affect}$=118, p=0.001; Figure 1D) in ipsilateral and contralateral histological score between control (respectively, 3.62±0.18; 1.86±0.14), propofol (respectively, 3.62±0.18; 2.38±0.18) and etomidate (respectively, 3.87±0.12; 2.37±0.18) groups (Figure 1D).

There was no statistical significance in ipsilateral tubule rupture between control (5.00±1.54), propofol (1.25±1.25) and etomidate (3.75±1.25) groups (Figure 1E).

The effects of etomidate and propofol on the signs of damage in seminiferous tubules compared to the control group, are presented in Figure 2.

Discussion

The primary and secondary findings of our study are that etomidate suppresses germ cell damage and Leydig cell loss in the ipsilateral testis. Studies show that germ cells are most susceptible to IRI damage in testicular tissue, and if urgent detorsion is not performed on time and ischemia time is prolonged, Leydig cells may be also lost[23,24]. Rapidly and excessively produced ROS cannot be removed from the environment and ischemia-reperfusion damage deepens[4]. Etomidate decreases germ cell damage and prevents Leydig cell loss in the ipsilateral testis possibly by suppressing oxidative stress and inflammation associated with IRI, and possibly by decreasing the ROS level. As a matter of fact, similar results were obtained in previous studies with etomidate. Studies investigating its effects on myocardial IRI have shown that etomidate inhibits myocar-

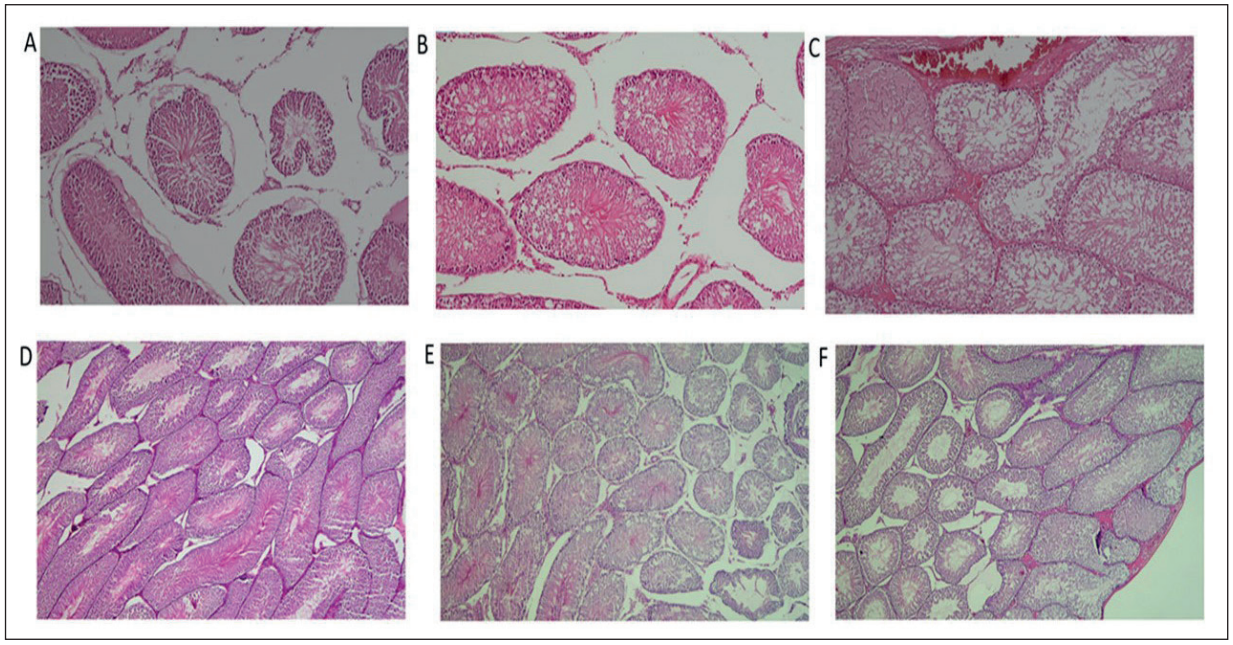

Figure 2. The effects of 4 mg/kg etomidate and and 10 mg/kg propofol administered i.p. 30 min before left testicular detorsion on the signs of damage in seminiferous tubules. (**A**) Ipsilateral control group (H&E stain, original magnification x 100), (**B**) Ipsilateral propofol group (H&E stain, original magnification x 100), (**C**) Ipsilateral etomidate group (H&E stain, original magnification x 200), (**D**) Contralateral control grup (H&E stain, original magnification x 100), (**E**) Contralateral propofol grup (H&E stain, original magnification x 100), (**F**) Contralateral etomidate grup (H&E stain, original magnification x 100).

dial apoptosis and fibrosis by suppressing oxidative stress and inflammation, and by decreasing percentages of infarct/risk ratio and recoveries of cardiac function[11]. Studies on CNS IRI have shown that etomidate exerts a neuroprotective effect, mitigates spinal cord and brain damage, by suppressing oxidative stress and inflammation[15]. It has been reported that etomidate suppresses skeletal muscles IRI as well as IRI occurring after tibial fracture surgery with the same mechanism[13,14].

Etomidate is known to increase GABAergic activity in the brain by stimulating the GABA-A receptor, to suppress the release of excitatory neurotransmitters such as glutamate and substance P, and to inhibit the excitability of cells by inhibiting synaptic transmission[9]. GABA not only creates a depressive effect in the CNS, but also mediates vital effects in the peripheral tissue. GABA has been reported to exert anti-inflammatory effects in rheumatoid arthritis mouse models[25]. The administration of GABA precursor glutamate into the interpositus nucleus region, which is responsible for the regulation of gastric activity in the brain is attenuates gastric IRI by preventing apoptosis and proliferation in the gastric mucosa, while the application of 3-mercaptopropionic acid, a glutamate decarboxylase inhibitor, which mediates the production of GABA from glutamate, into the same brain area, or administration of bicuculline, a GABA-A receptor antagonist, into the lateral hypothalamic area (LHA), another brain region responsible for gastric activity reverses this mitigating effect[26]. In another study, it has been shown that GABA-A receptor, which is overexpressed in the LHA using an adenovirus vector, suppresses gastric ischemia-reperfusion injury[27]. GABA therapy suppresses intestinal and renal IRI, while the GABA-A antagonist picrotoxin induces IRI and aggravates acute kidney injury[28]. An *in vivo* study showed that the GABA-A agonist muscimol reduces hepatic IRI and oxidative stress, but this result was not supported by an *in vitro* study[29]. It is claimed that GABA suppresses IRI indirectly rather than directly and increases the activity of GABA by binding to the GABA-A receptor[30]. In the light of this information, etomidate may have suppressed oxidative stress and inflammation, inhibited apoptosis by directly and/or indirectly affecting GABAergic pathways in the CNS and peripheral nervous system (PNS), thus preventing germ cell damage and Leydig cell loss.

Etomidate administration has been reported to be associated with adrenal suppression in some clinical studies[31,32]. It has been shown that etomidate inhibits the 11beta-hydroxylase, which is responsible for the conversion of 11-deoxycortisol to cortisol, thereby suppressing cotrizol production[31,32]. Male infertility is often seen in conditions such as Cushing's disease, exogenous hormone therapy, or stress, which are thought to be associated with high cortisol levels[33,34]. High cortisol levels suppress the expression of steroidogenic enzymes, induce oxidative stress in testicular tissue, reduce the testicular response to gonadotropins and thus accelerate apoptosis of Leydig and germ cells[34]. It can be concluded that etomidate suppresses ischemia-reperfusion injury by inhibiting excessive cortisol production through adrenal suppression, in addition to its effect on GABAergic pathways, thus preventing germ cell damage and Leydig cell loss.

Other histopathological findings obtained in our study are that etomidate did not change histological score, necrosis percentage and tubule rupture in ipsilateral testis. According to the studies providing reperfusion after detorsion, Sertoli and Leydig cells other than germ cells in the testicular tissue are protected[19,23,24].

Propofol, which was reported to attenuate IRI by showing antioxidant and anti-inflammatory effects in previous studies, is another intravenous general anesthetic agent[18]. In our study, while propofol did not affect germ cell damage and Leydig cell loss, etomidate was effective in the ipsilateral testis, by suppressing the percentage of necrosis in the ipsilateral testis, but they did not affect other histopathological findings. Propofol, which increases GABAergic transmission by binding to the GABA-A receptor like etomidate, was expected to act similarly to etomidate, suppressing germ cell damage and Leydig cell loss[9]. These findings obtained in our study indicate that etomidate, like propofol, not only increases GABAergic transmission, but also has a protective effect on IRI by directly or indirectly affecting different substances and/or pathways. More studies are needed to better understand the mechanism of action of etomidate.

Conclusions

Although there are publications that the contralateral testis may be affected after ipsilateral testicular torsion, this opinion is still controversial[35]. It is claimed that sudden and excessive production of ROS in IRI, immunological response, acrosomal enzyme release, congenital testicular dysplasia and/or reflexive vasospasm can lead to changes in the contralateral testes[4]. However, we did not observe any damage to the contralateral testicular tissue in our study.

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Conflict of Interests
The authors declare that they have no conflict of interest

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