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The Effects of Fentanyl on Testicular Ischemia-Reperfusion Injury

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Abstract

Objective: Testicular torsion is a condition that often occurs as a result of the rotation of the spermatic cord in childhood and adolescence in men, manifests with acute pain and causes infertility in the future even if emergency intervention is performed. The aim of this study is to investigate the protective and preventive effects of fentanyl, a potent analgesic agent frequently used in anaesthesia practice, on testicular ischemia–reperfusion injury, which manifests through acute pain.

Methods: A total of 16 adult male Wistar rats, weighing 200-250 g, were used in this study. They were divided into two groups, consisting of eight animals in each group. Torsion was created in all rats by rotating left testicles 720° clockwise on the day of the experiment. 3 μM of fentanyl was applied intraperitoneally 30 minutes before detorsion to the fentanyl group. Following an hour of ischemia, the left testicle was reinstated, and tissues were repaired according to their physiology. Following 24 hours of reperfusion, the animals were euthanised after taking left testes and blood samples.

Results: Fentanyl, administered prior to testicular detorsion, significantly suppressed germ cell damage in torsioned tissue, catalase activity and malondialdehyde levels in blood samples taken from the heart. No significant differences were observed in plasma total thiol concentration, histological score, Leydig cell counts, percentage of necrosis and tubule rupture.

Conclusion: These findings show that fentanyl administered before detortion creates a protective effect by preventing testicular ischemia–reperfusion injury leading to infertility in the future.

Keywords: Opioid, catalase activity, malondialdehyde, germ cell, testicular torsion, testicular detorsion

Introduction

Testicular torsion is an emergency surgical state that is frequently seen in men during childhood and adolescence, resulting in acute pain caused by the rotation of the spermatic cord.[1] The actual therapy is urgent surgical detorsion; if this cannot be done, manual detorsion can be also applied.[2] Even though an urgent detorsion is performed, 40-60% of the cases develop testicular injury resulting in infertility.[3] The damage that occurs during the torsion is not limited to ischemia but continues even after the detorsion due to the reperfusion. Ischemia-related damage occurs due to the impairment of tissue oxygenation, reduction of cellular energy sources and activation of the xanthine oxidase system, and deepens with the formation of reactive oxygen species (ROS), such as superoxide, hydroxyl radicals, nitric oxyde, peroxynitrite, and the increase in proinflammatory cytokines and cell adhesion molecules during reperfusion.[4] The main source of ROS is polymorphonuclear leukocytes (PMNs), which adhere to the vascular endothelium and migrate to damaged tissue during reperfusion, but the contribution of the xanthine oxidase system activation in parenchymal cells cannot be excluded.[5] ROS overwhelms the scavenging capacity of the enzymatic antioxidant defence system (ie, superoxide dismutase, catalase (CAT) and glutathione peroxidase) or

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the nonenzymatic antioxidant defences, impairs DNA and protein functions, causes lipid oxidisation of both the cell and mitochondrial membranes and triggers apoptosis in germ cells, further leading to a decrease or loss of sperm stem cells, resulting in infertility.[5]

Fentanyl is a synthetic opioid agonist used in anaesthetic practice and pain management.[6] Having stronger analgesic effect than morphine, fentanyl mimics the effects of endogenous opioids by stimulating μ, δ and κ opioid receptors bound to G proteins, which are distributed in both central and peripheral tissues.[6,7] When the opioid receptors are stimulated, voltage-gated calcium channels close and intracellular calcium level decreases, while potassium channels are open and intracellular potassium conduction increases; neuronal inhibition and hyperpolarisation result in an analgesic effect at the level of brain and spinal cord.[7] Previous studies have shown that opioid agonists increase survival of mice under severe hypoxia and protect the organs to be transplanted.[8] Fentanyl and other opioids are known to suppress ischemia–reperfusion injury (IRI) of tissues and organs such as the heart, kidney, brain, liver, endothelium and skeletal muscle.[6] There are no studies investigating the effect of fentanyl on testicular torsion. In a recent study, it has been reported that morphine inhibits lipid peroxydation and increase antioxidant markers in testicular tissue.[9]

In this study, we aimed to investigate the protective and preventive effects on histopathological changes in the testicular tissue, in which IRI was created, and biochemical changes in the blood by applying fentanyl prior to testicular detorsion in a 1 hour 720° testicular torsion rat model.

Methods

Animals

A total of 16 adult male Wistar rats, weighing 200-250 g, were obtained from Tekirdag Namık Kemal University Experimental Animals Practice and Research Center. They were divided into two groups, consisting of eight animals in each group. The research protocol was approved by the Tekirdag Namık Kemal University Animal Experiments Local Ethics Committee (approval number: 3/2019-11-11).

Main Points

- Testicular torsion is an emergency surgical condition that is frequently seen in childhood and adolescence in men and causes infertility even when treated in a timely manner.
- Fentanyl, a synthetic opioid agonist and having a stronger analgesic effect than morphine, significantly suppressed germ cell damage in torsioned tissue.
- Fentanyl significantly suppressed catalase activity and malondialdehyde level in blood.

The rats were housed with a reversed 12 hour light/dark cycle at 21 ± 3°C and 50 ± 5% humidity. They were allowed unlimited access to standard rat chow and water.

Experimental Procedure

Surgical torsion–detorsion procedure was performed under ketamine-xylazine (100 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine, intraperitoneal (i.p.)) anaesthesia following 1 week of adaptation period for all the animals randomly divided into two groups (fentanyl and control). Torsion was created by rotating the left testes 720° in a clockwise direction when viewed cranially following transcrotal incision.[10] 30 minutes before detorsion, 3 µM (1 mg kg^{-1}) fentanyl (Sigma-F013) was applied i.p. to the fentanyl group and the same volume of vehicle was applied i.p. to the control group. Following an hour of ischemia, the left testicle was restored to its initial state, tissues were repaired according to their physiology, and the animals were left to rest. The next day, at the end of 24 hours of reperfusion, the left testes were taken for histopathological examination as well as blood samples from the heart for biochemical analysis under ketamine-xylazine (100 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine, i.p.) anaesthesia, and the animals were then euthanised.[11]

Histopathologic Examination

All paraffin-embedded sections of the tissue processing using paraffin block were taken from the testicular tissues already preserved with 10% formaldehyde solution. They were stained with haematoxylin and eosin (H&E) after being deparaffinised. As well, the sections were stained using Masson trichrome staining (Bench Mark Special Stains device), CD68 (Bench Mark XT model device) and applying androgen receptor antibodies. The sections were examined under an Olympus CX41 model light microscope.

A four-level grading scale similar to that of Cosentino et al.[12] was used to quantify histologic injury. Grade 1 showed normal testicular architecture with an orderly arrangement of germinal cells. Grade 2 injury showed less orderly, noncohesive germinal cells and closely packed seminiferous tubules. Grade 3 injury exhibited disordered, sloughed germinal cells with shrunken, pyknotic nuclei and less distinct seminiferous tubule borders. Grade 4 injury defined seminiferous tubules that were closely packed with coagulative necrosis of the germinal cells. Johnson tubular biopsy score (JTBS) was developed to examine spermatogenesis histopathologically after the testicular damaging.[13] For this purpose, loss of sperm cells and spermatids, absence of germ cell layers, degeneration of germ cell layers, structural damage to germ cells, tubule rupture and reactions in Leydig cells in ruptured tubules were examined in seminiferous tubules, and proliferation of Leydig cells, oedema, haemorrhage, granuloma and fibrosis were evaluated in interstitium according to the Cosentino et al.[14] classification. The degree

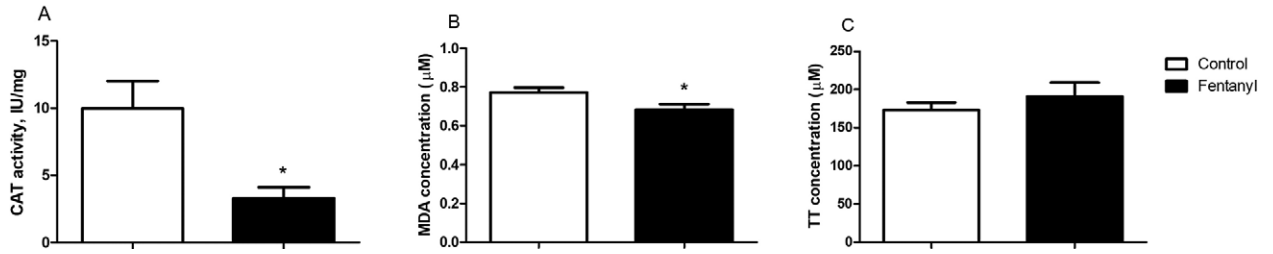

Figure 1. a-c. The effects of 3 μM fentanyl administered i.p. 30 minutes before left testicular detorsion to the blood catalase activity (A), malondialdehyde concentration (B) and total thiol concentration (C). Six animals were used in each group, and results were expressed as mean ± SEM, *$P < .05$.

of damage was evaluated according to the classifications of Johnson.[13] In addition to these evaluations, the percentage of necrosis, tubule rupture and damage of the germ cell layer was calculated.

Biochemical Analysis

Blood samples taken immediately after the left testicle was removed were placed in heparinised tubes and centrifuged immediately. In the separated plasma, CAT activity being part of the antioxidant defence system, malondialdehyde (MDA) levels indicating the increase in lipid peroxidation and total thiol (TT) level, as the early indicator of protein oxidation through ROS, were analysed. The CAT activity, the plasma levels of MDA and TT were measured according to the Aebi method,[15] modified Hammode method based on double boiling[16] and Sedlak and Lindsay's method,[17] respectively.

Statistical Analysis

The GraphPad Prism 5.01 software was used for the analysis of the data. Parametric CAT activity (n = 6), MDA concentration (n = 6), TT concentration (n = 6), germ cell damage (n = 8), Leydig cell count (n = 8), tubule rupture (n = 8) and percentage of necrosis (n = 8) results were calculated by two-tailed unpaired t-test (demonstrated mean ± standard error of the mean (SEM)). The Mann–Whitney U test was used for the nonparametric histological score (n = 8) analysis (demonstrated median and interquartile range). This study has 80.0% power to detect an effect size of E= S × E/S = 0.287 (n = 6). For all statistical calculations, significance was considered as $P < .05$.

Results

Biochemical Analysis Following 24 hours of Reperfusion Time

Fentanyl-administered group (3.32 ± 0.79) statistically significantly decreased CAT activity (t = 3.044; df = 10; $P = .0124$; Figure 1A) compared with the control group (9.99 ± 2.04). Fentanyl group (0.68 ± 0.03) statistically significantly decreased MDA concentration (t = 2.324; df = 10, $P = .0425$; Figure 1B) compared with the control group (0.77 ± 0.02). There was no statistical significance in the TT concentration (t = 0.854; df = 10; $P = .4131$; Figure 1C) between fentanyl (190.9 ± 18.35) and control groups (173.0 ± 9.92).

Histopathological Examination Following 24 hours of Reperfusion Time

Fentanyl-administered group (6.25 ± 0.81) statistically significantly decreased germ cell damage in torsioned tissue (t = 2.837; df = 14; $P = .0132$; Figure 2A) compared with control group (14.38 ± 2.74). There was no statistical significance in the histological score ($P = .95$; Mann–Whitney U = 32; Figure 2B), Leydig cell counts (t = 0.7373; df = 14; $P = .4731$; Figure 2C), tubule rupture (t = 1.440; df = 14; $P = .1718$; Figure 2D) and percentage of necrosis (t = 0.5092; df = 14; $P = .6186$; Figure 2E) between fentanyl (respectively, 3.62 ± 3.19-4.06; 13.0 ± 0.68; 1.87 ± 0.91; 10.0 ± 2.11) and control (respectively, 3.62 ± 3.19-4.06; 12.13 ± 0.97; 4.37 ± 1.47; 8.75 ± 1.25) groups. The effects of fentanyl on the signs of damage in seminiferous tubules compared to the control group are presented in Figure 3.

Discussion

The primary finding of our study is that fentanyl suppresses germ cell damage. Damage to germ cells, known to be the most susceptible to hypoxia in the testicular tissue, develops due to excessively increased ROS during reperfusion.[18] The sources of ROS are PMNs that adhere to the vascular endothelium and migrate to damaged tissue, and the xanthine oxidase system activation in parenchymal cells.[5] According to this information, fentanyl, applied before testicular detorsion, may suppress ROS caused by the PMN and/or xanthine oxidase system, thus preventing germ cell damage. Indeed, in IRI studies, it has been previously reported that opioids suppress endothelial and PMN activities, reduce adhesion molecules essential for PMN migration and show anti-inflammatory effects in this way.[18,19] In addition, in IRI studies with anti-inflammatory drugs and free radical scavengers, suppression of germ cell damage was similarly

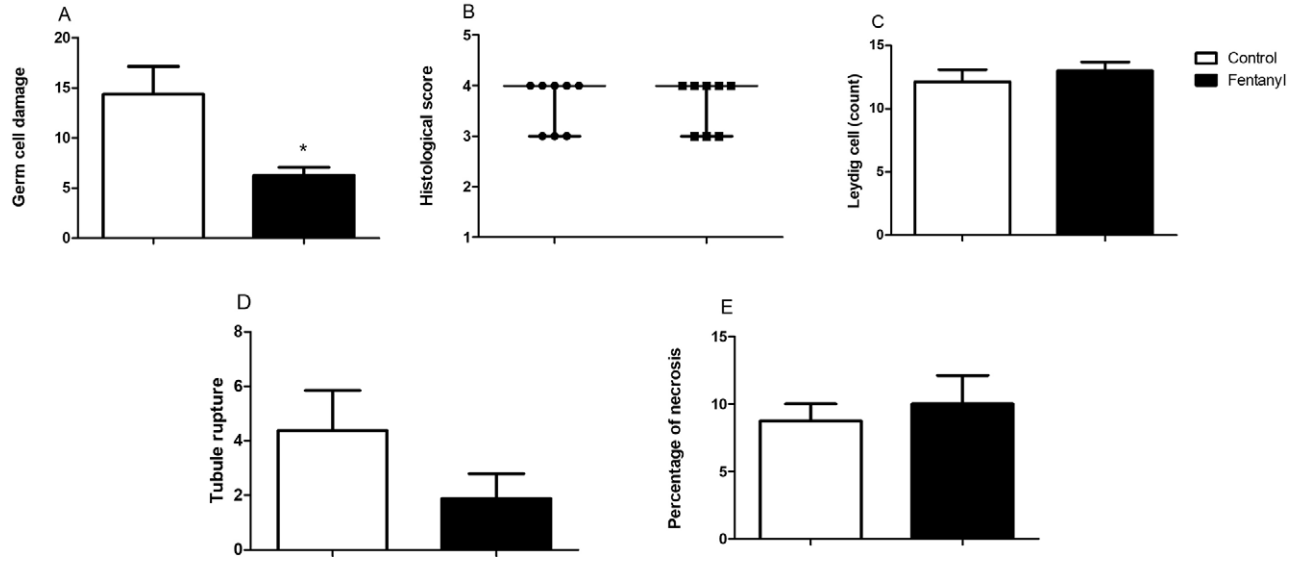

Figure 2. a,e. The effects of 3 μM fentanyl administered i.p. 30 minutes before left testicular detorsion to the germ cell damage (a), histological score (b), Leydig cell count (c), tubule rupture (d) and percentage of necrosis (e). Eight animals were used in each group, and results were expressed as mean ± SEM (a, c, d and e) and median and interquartile range (B), *$P < .05$.

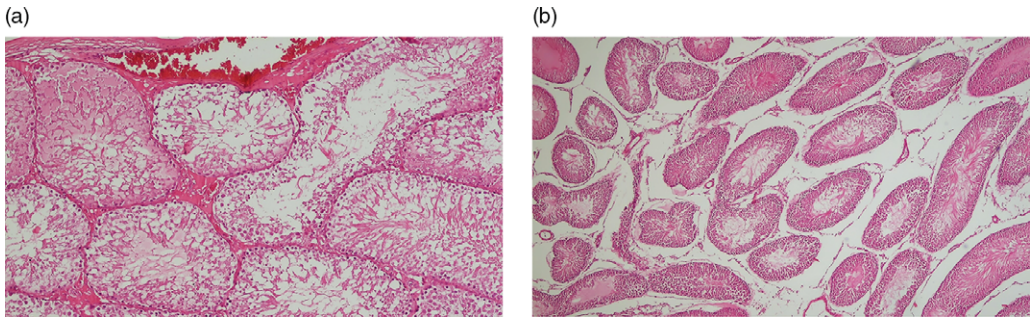

Figure 3. a,b. The effects of 3 μM fentanyl administered i.p. 30 minutes before left testicular detorsion on the signs of damage in seminiferous tubules. (a) Control group (H&E stain, original magnification × 400) and (b) fentanyl group (H&E stain, original magnification × 100).

observed.[5] Opioids have also been shown to prevent cell death due to peroxynitrite, a more toxic ROS, that results from the interaction of superoxide radicals and nitric oxide.[20]

Fentanyl, application in our study, may have prevented germ cell damage by stimulating opioid receptors. Fentanyl, like other opioids, is known to stimulate μ, δ and κ opioid receptors that distribute in the central and peripheral tissues.[8] According to previous studies, opioids suppress IRI through localised δ opioid receptors in peripheral tissue.[21] While the μ opioid receptors are thought to play no role in IRI, the contribution of κ opioid receptors is controversial.[21] Based on this information, fentanyl stimulates μ, δ and κ opioid receptors; the presence of which is shown in testicular tissue. It may suppress germ cell damage directly, by binding to δ receptors and also indirectly by preventing the activation and migration of PMN, the main source of ROS, and preventing the conversion of superoxide radicals to more toxic ROS.[22,23]

The secondary and tertiary findings of our study are that fentanyl suppresses CAT activity and MDA concentration. In the testicular IRI study with morphine, there was an increase in CAT activity and a decrease in MDA level; it was thought that CAT activity increased in order to scavenge the excessive ROS, and thus decreased the level of MDA.[9] Considering this, following fentanyl application, an increase in membrane lipid peroxidation and MDA level due to ROS increased excessively as a result of suppression in CAT activity and would be expected instead of suppression. The decrease in MDA level as well as the suppression of CAT

activity, noted in our study, showed that fentanyl prevents the formation of ROS at a level that will increase CAT activity as much as the control group by showing protective activity, and the level of MDA decreases since too much ROS cannot be formed. Fentanyl only lowered the level of MDA as it prevented the formation of PMN-induced ROS, but was unable to reset it and completely suppress CAT activity.

Fentanyl, which we used in our study, could decrease MDA level by stimulating opioid receptors. Following the stimulation of opioid receptors, protein kinase C is activated, K(ATP) dependent channels, which impaired function during ischemia, are stabilised and increased calcium level in mitochondria and cytosol is decreased and protects against lipid peroxidation at the level of membrane.[24] This process fosters tissue protection by increasing the production of extracellular adenosine, cellular accumulation of energy reserves and decreased PMN adhesion.[25] Based on this information, fentanyl may suppress CAT activity and MDA concentration directly by binding to opioid receptors and indirectly by preventing the PMN-induced ROS.

The last biochemical finding we obtained in our study is that fentanyl does not change TT concentration, which is an early symptom of ROS-mediated protein oxidation. This may be due to fentanyl preventing the formation of ROS from PMN and does not affect ROS from the xanthine oxidase system.

Other histopathological findings that we obtained in our study are that fentanyl did not change histological score, Leydig cell counts, necrosis percentage and tubule rupture. According to the studies carried out, by providing reperfusion after detorsion, cells other than germ cells in the testicular tissue are protected.[10,18] In our study, we achieved local tissue reperfusion by detortion after 1 hour of torsion in both fentanyl and control groups, and we did not observe changes in germ extracellular tissues as stated above.

Conclusions

The first thing to be done in testicular torsion, which manifests itself through severe pain and requires immediate intervention, is to relieve the pain and as soon as possible to perform a surgical operation. Otherwise, manual detorsion is required. Fentanyl, an opioid agonist, which is more effective in severe pain than morphine is frequently used in anaesthetic procedures. According to the findings we obtained in our study, fentanyl directly suppresses IRI by probably stimulating the δ receptor and indirectly prevents PMN migration, reduces the formation of PMN-induced ROS and blocks the production of more toxic ROS. These findings indicate that fentanyl administration will decrease the rate of infertility in patients with testicular torsion before detortion. The limitation of our study may exist due to the fentanyl has been given in a single dose prior to testicular detorsion.

Ethics Committee Approval: Ethical committee approval was received from the Tekirdag Namık Kemal University Animal Experiments Local Ethics Committee (2019-11-11, 09:00).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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