Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Protective Effect of Fasudil on Testicular Ischemia-Reperfusion Injury in Rats

Cem Kaya[1], Alparslan Kapisiz[1], Sibel Eryilmaz[1], Ramazan Karabulut[1], Zafer Turkyilmaz[1], Mehmet Arda Inan[2], Gizem Yaz Aydin[3], Kaan Sonmez[1]

[1]Departments of Pediatric Surgery, Gazi University Faculty of Medicine, Yenimahalle, Ankara, Turkey; [2]Pathology, Gazi University Faculty of Medicine, Yenimahalle, Ankara, Turkey; [3]Biochemistry, Gazi University Faculty of Medicine, Yenimahalle, Ankara, Turkey

Correspondence: Ramazan Karabulut, Department of Pediatric Surgery, Gazi University, Faculty of Medicine, Emniyet Mahallesi, Mevlana Bulvarı, No. 29, Yenimahalle, Ankara, 06500, Turkey, Tel +90 312 2026210, Fax +90 312 2230528, Email karabulutr@yahoo.com; ramazank@gazi.edu.tr

Background: Ischemia-reperfusion (I/R) injury to the testis can lead to organ damage, infertility, and subfertility. The goal of this study was to investigate the effects of fasudil on this devastating condition.

Methods: Thirty male Long-Evans rats were divided into five groups: a control group (no torsion), rats administered fasudil (30 mg/kg, no torsion), rats subject to ischemia with no treatment (I) (I/R injury), injured rats that received treatment 1 (T1) (I/R with 30 mg/kg fasudil before detorsion), and injured rats that received treatment 2 (T2) (I/R with 30 mg/kg fasudil after detorsion). Serum levels of TNF-α and IL-6, along with tissue levels of glutathione (GSH), malondialdehyde (MDA), caspase-3, and Johnsen Tubular Biopsy Score (JTBS), were measured.

Results: Group I exhibited significantly higher levels of MDA and caspase-3 than all other groups except T2 ($p < 0.05$). Although the difference was not statistically significant, Group T2 exhibited lower MDA and caspase-3 levels than Group I ($p > 0.05$). Additionally, Group I displayed significantly higher TNF-α and IL-6 levels, and lower GSH and JTBS values, than the other groups ($p < 0.05$).

Conclusion: Our findings indicate that fasudil protects the testis from I/R injury, particularly when administered early.

Keywords: fasudil, testis, ischemia/reperfusion

Introduction

Testicular torsion is a critical urological emergency that affects approximately 1 in 4000 men under the age of 25 years. It occurs when the testis rotates on its own axis, leading to torsion of the spermatic cord and the subsequent impairment of blood flow. This condition can manifest at any age but is particularly prevalent during the perinatal and pubertal periods.[1,2] Rapid diagnosis and treatment are crucial in testicular torsion.[3] If treatment is not initiated within 4–8 hours of the onset of symptoms, spermatogenic cell loss may occur, leading to infertility and subfertility. In fact, an orchiectomy may become necessary.[1,4]

The pathophysiology of testicular torsion involves ischemia-reperfusion (I/R) injury. During the ischemic phase, the reduction in blood flow triggers anaerobic metabolism, leading to a decreased production of adenosine triphosphate (ATP) and antioxidant substances. This metabolic shift increases reactive oxygen species (ROS) due to the low antioxidant capacity of the tissue. Following reperfusion, the restoration of blood flow exacerbates the production of ROS generated by the mitochondrial electron transport chain, the NADPH oxidase system, and uncoupled nitric oxide synthase. The resulting ROS can damage cellular structures, causing a loss of cellular viability or even cell death.[5,6]

Rho-associated coiled-coil-containing kinase (ROCK) is a protein kinase that regulates several critical processes required for inflammatory response, including actin cytoskeleton organization, cell adhesion, migration, and apoptosis.[7] Previous studies have determined that ROCK activation is involved in the pathogenesis of I/R and that ROCK regulates ROS production. Fasudil, a selective ROCK inhibitor, has been shown to inhibit ROCK activation and reduce infarct size and myocyte apoptosis during myocardial I/R in rats. It has also been found to reduce lung damage caused by intestinal I/R.[8,9] Accordingly, this study was designed to investigate the potential protective effects of fasudil on testicular I/R injury.

Received: 1 August 2024
Accepted: 23 September 2024
Published: 25 September 2024

Materials and Methods

This experiment was approved by the Gazi University Animal Experiments Ethics Committee with the reference number G.U.ET-21.082 and the study was carried out in the Gazi University Faculty of Medicine Animal Research Laboratory. All experiments were conducted in strict accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. This experimental study was carried out using Long-Evans rats fed standard rat chow and kept in cages at proper ambient temperature and humidity in a laboratory environment with a 12:12 hour photoperiod. No food or water restrictions were applied before the study, and no medication was administered. Thirty rats weighing 250–300 g were randomly allocated into five groups of six rats each.

Experimental Testicular Torsion Model

Before every surgical procedure, ketamine hydrochloride (50 mg/kg; Ketalar, Eczacıbaşı, Turkey) and xylazine hydrochloride (5 mg/kg; Alfazyne 2%, Ege Vet, Turkey) were administered to each rat as anesthesia. Mid-scrotal incisions were used for torsion, detorsion, and sham procedures. After we created torsion by rotating the left testis clockwise 2 turns (720 degrees), it was fixed to the scrotum with 6/0 polypropylene. At the end of the torsion period, the testis was returned to its initial anatomical position. The following process were applied to the five groups (n=6/group):

Control group (Group C): Rats in this group only had their tissue and blood sampled without being subjected to any processing.

Fasudil group (Group F): 30 mg/kg fasudil was administered intraperitoneally (IP) without torsion. Tissue and blood samples were then taken.

Ischemia group (Group I): Two hours after 720° extravaginal left testis torsion, detorsion was carried out. At the fourth hour, an orchiectomy was carried out, and a blood sample was taken.

Treatment group 1 (Group T1): Thirty mg/kg of fasudil was administered IP 1.5 hours after torsion, and detorsion was performed 30 min after this procedure. At the fourth hour of detorsion, an orchiectomy was performed, and a blood sample was taken.

Treatment group 2 (Group T2): Two hours after torsion, detorsion was performed. Immediately afterward, 30 mg/kg of fasudil was administered IP. At the fourth hour of detorsion, an orchiectomy was performed, and a blood sample was taken.

Following the experiment, blood and tissue samples were obtained from each rat to measure malondialdehyde (MDA), glutathione (GSH), caspase-3, TNF-α, IL-6, and Johnsen Testicular Biopsy Score (JTBS). After the blood samples were centrifuged for 10 minutes at 3000 rpm to examine TNF-α and IL-6, the sera were placed in Eppendorf tubes and stored at −80°C. To facilitate histopathological analysis, half of the removed testis was preserved in 10% formalin; to measure MDA, GSH, and caspase-3, the other half of the tissue was frozen in liquid nitrogen and stored at −80°C.

Measurements of TNF-α and IL-6 levels in the blood samples were carried out in accordance with the instructions provided with the Enzyme-Linked ImmunoSorbent Assay (ELISA) kits we used (cat. no. E0764Ra and E0135Ra, respectively; Jiaxing Korain Biotech, Zhejiang, China). To measure tissue levels of MDA, GSH, and caspase-3, 100 mg of frozen testicular tissue from each rat was homogenized using phosphate-buffered saline (pH: 7.2) with a Teflon pestle shredder at 3500 rpm for 15 min. The supernatant was collected upon centrifugation. Levels of MDA, GSH, and caspase-3 were analyzed separately in each tissue sample obtained from each rat using commercial ELISA kits (cat. no. E0156Ra, E1101Ra, and E1648Ra, respectively; Jiaxing Korain Biotech, Zhejiang, China). Blood IL-6 and TNF-α levels and tissue MDA, GSH, and caspase-3 levels were evaluated by a single biochemist.

A 10% formaldehyde solution was used to fix the testicular tissues. These tissues were embedded in paraffin wax blocks, cut into 4-μm thick sections, and stained with hematoxylin-eosin. Using a light microscope, 60 transversely cut seminiferous tubules were assessed in each of the samples obtained from each rat to evaluate JTBS (Olympus BX53, Japan). All samples were evaluated blindly by two pathologists who had no knowledge of the study groups. The histopathological scoring was determined by taking the average of the values assigned by the two pathologists. When the pathologists reached a consensus on the grade, this was noted.

Statistical Analysis

The data are presented in the format mean ± standard deviation (SD). SPSS version 22.0 was used for the statistical analysis. The Shapiro–Wilk test was used to assess the normal distribution of each dataset. Tukey's post hoc test was utilized after a one-way analysis of variance for a statistical analysis of the normally distributed data. The level of statistical significance was set at $p < 0.05$. Kruskal–Wallis and Bonferroni post hoc tests were performed for MDA, caspase-3, and JTBS levels when the data were not normally distributed ($p < 0.005$).

Results

Group F exhibited the lowest amounts of tissue MDA and caspase-3, while Group I displayed the highest levels. While caspase-3 and MDA levels increased distinctly in the torsion group, a considerable decrease was observed in the T1 group treated with fasudil, although the difference was not statistically significant ($p = 0.036$ and 0.040, respectively). In terms of MDA ($p = 0.020$ and < 0.001, respectively) and caspase-3 ($p < 0.001$ and < 0.001, respectively), Group I had significantly higher levels than Groups C and F. Although MDA and caspase-3 levels were found to be lower in Group T2 than in Group I, no statistically significant differences were found (p Compared to Group I, which underwent torsion, there was a statistically significant increase in tissue GSH levels in Groups T1 and T2, which received fasudil treatment ($p = 0.005$ and 0.013, respectively). Additionally, there was a significant difference in GSH, as in MDA and caspase-3, in Group I relative to Groups C and F ($p < 0.001$ and < 0.001, respectively). Although the increase in Group T1 was greater than in Group T2, there was no statistically significant difference. Furthermore, we found that non-enzymatic antioxidant GSH levels increased in Group F, but no difference was found between Groups C and F. Decreases in the levels of lipid peroxidation product and apoptosis indicators MDA and caspase-3 were also detected in Group F (Table 1).

Group I exhibited the highest levels of IL-6 and TNF-α, marking a statistically significant difference compared to all other groups, while Group F displayed the lowest ($p < 0.001$; Table 1). Microscopy evaluation revealed that overall spermatic activity was preserved, with a JTBS score above 4 observed in all specimens. However, focal areas of reduced activity were observed in some samples. For example, Group T1 also shown a tubule with nearly perfect activity (Figure 1). In the ischemia group, tubular hyalinization and loss of function were seen (Figure 2). JTBS levels were highest in Group F and lowest in Group I (Figure 1). Fasudil administration without torsion (Group F) did not significantly change JTBS scores compared to Group C ($p > 0.05$). Group I showed statistically different spermatogenic activity in terms of JTBS compared to Groups C and F ($p < 0.001$ and < 0.001, respectively). Comparing Groups C and F with Groups T1 and T2 indicated no statistically significant differences in spermatogenic activity in terms of JTBS ($p = 0.049/0.006$ and $0.045/ 0.005$, respectively) (Figure 2). At the end of the experiment, an improvement in JTBS was detected in the treatment groups relative to the ischemia group (Table 1 and Figure 2).

Discussion

Testicular torsion is a medical emergency in which prompt identification and treatment are required to save the testis and its fertility, as testicular damage begins within 4–8 hours. Researchers have evaluated fasudil's antioxidant, anti-inflammatory, and histopathological healing effects in brain, heart, and intestine ischemia models, but we applied it to

Table 1 Distribution and Standard Deviations of Biochemical Parameters and JTBS According to Groups

	Group C	Group F	Group I	Group T^1	Group T^2	P Value
Malondialdehyde (MDA) (nmol/mL)	0.95±0.18	0.75±0.08	2.03±0.46	1.15±0.13	1.26±0.36	0.001
Glutathione (GSH)(mg/L)	481.41±43.47	492.48±28.35	373.06±53.70	468.91±47.23	459.60±35.25	0.001
Caspase-3 (ng/mL)	2.63±1.11	1.68±0.43	8.56±1.58	4.48±1.80	4.98±1.96	0.001
IL-6 (ng/L)	15.48±2.49	12.18±1.84	23.56±1.55	15.33±2.61	16.48±2.02	0.001
TNF-α (ng/L)	105.80±46.80	84.36±22.22	425.11±67.63	132.18±61.50	202.31±82.61	0.001
JTBS	9.76±0.10	9.77±0.12	7.49±0.39	8.72±0.29	8.32±0.31	0.001

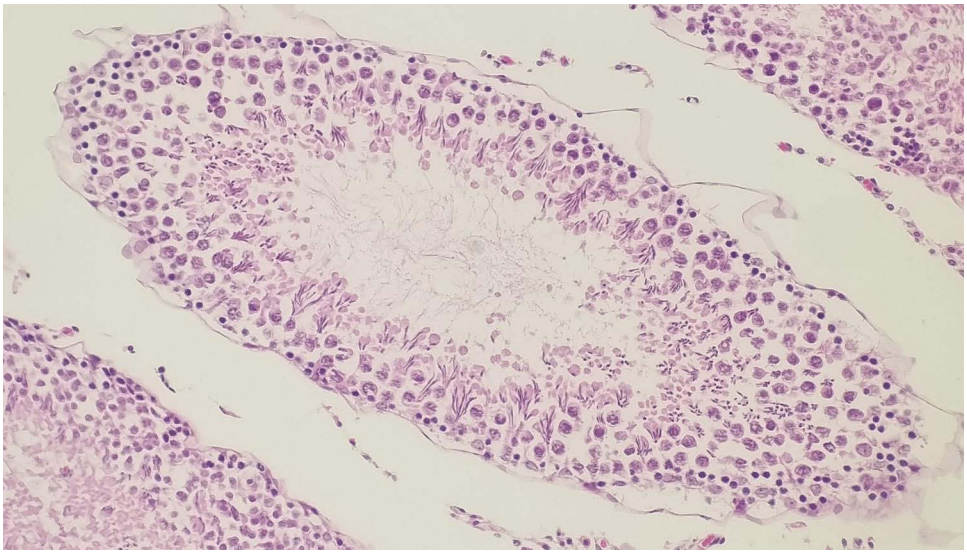

Figure 1 In the example of group T1; JTBS score 9 with mature sperms and spermatids.

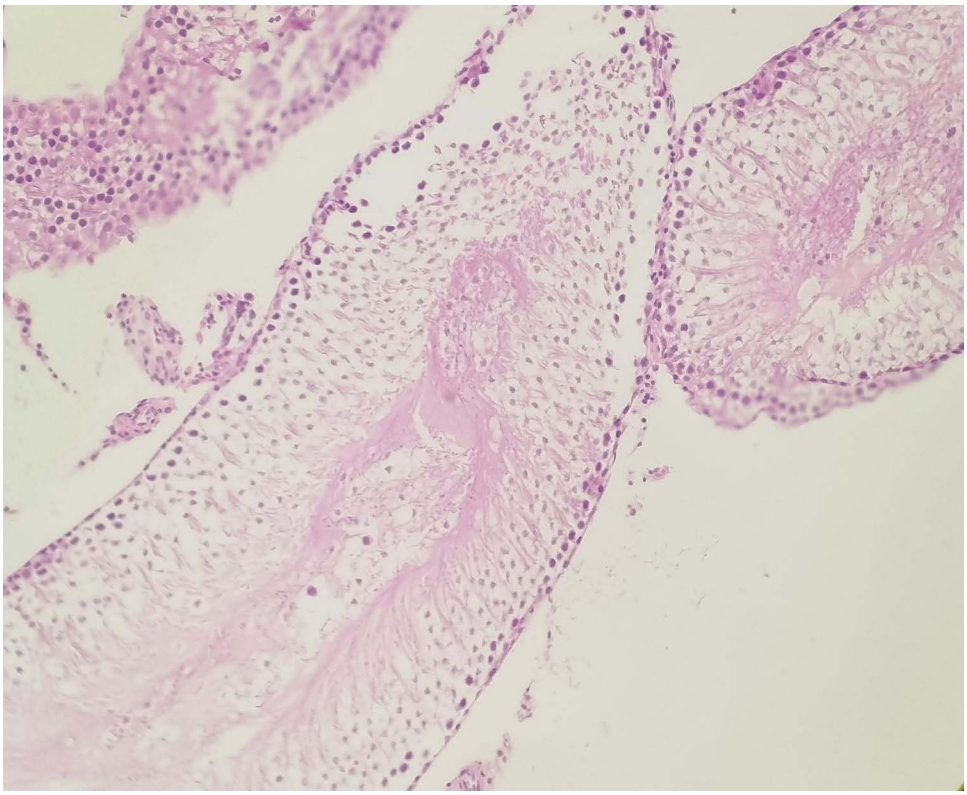

Figure 2 In the example of ischemia group; JTBS score 5 with alive spermatocytes but dead spermatids.

testicular ischemia for the first time.[8–10] Fasudil demonstrated antioxidant, anti-inflammatory, and tissue-healing properties by lowering MDA, IL-6, TNF-α, and caspase-3 levels while boosting GSH and JTBS. Even when administered alone, fasudil exerted anti-inflammatory and antioxidant effects on rats.

It was previously proven that experimental testicular I/R injury in rats begins at 540° torsion, and blood flow does not resume when the torsion lasts longer than 4 hours.[11–13] Accordingly, we applied 2-hour 720° extravaginal torsion to rats' left testes to comply with clinical models and generate adequate ischemia.

Anaerobic metabolism produces less ATP during ischemia, and chronic use generates purine metabolites, such as xanthine and hypoxanthine. During reperfusion, these metabolites are processed by the enzyme that transforms xanthine dehydrogenase into xanthine oxidase during ischemia, initiating excessive ROS production.[5,14] The resulting ROS produce oxidative stress in the testicular parenchyma, injure the cell genome, and induce apoptosis via caspase activation. These alterations are associated with increased testicular tissue necrosis.[15] The increased ROS can damage lipids, proteins, and DNA, causing cellular malfunction.[16] This can only be prevented by reducing ROS formation or increasing antioxidant defenses.

Several compounds have demonstrated antioxidant effects in testicular torsion models, as have hydrogen sulfide and memantine. MDA, GSH, SOD, and catalase were investigated as oxidant and antioxidant markers. These medicines used in these investigations exhibited antioxidant properties, benefitting the animals' pathophysiology.[1,17,18] Fasudil is a selective inhibitor of ROCK. RhoA belongs to the Ras superfamily, and the first effector recognized was ROCK. The Rho/ROCK signaling pathway influences cell contraction, motility, and morphology.[19–21] Researchers have shown that ROS stimulates the Rho/ROCK pathway and that fasudil has antioxidant, antiapoptotic, and anti-inflammatory properties while also regulating nitric oxide production.[19,22] Fasudil demonstrated antioxidant effects in acetaminophen-induced liver injury cases by lowering MDA, the end product of ROS-induced lipid peroxidation, and raising GSH, a nonenzymatic antioxidant thought to facilitate ROS scavenging.[23] Additionally, fasudil can decrease lipid peroxidation by lowering ROS levels in I/R injury cases.[24] This impact can be attributed to a reduction in ROS production via downregulation of NADPH oxidase.[20] Fasudil demonstrated antioxidant activity in our experiment by lowering MDA and raising GSH more notably in Group T1, who received it half an hour before detorsion, than in Group T2, who received it immediately afterwards.

Fasudil inhibits inflammatory response as well as neutrophil and oxygen free radical production in the circulation. Fasudil also transactivates peroxisome proliferator-activated receptor alpha (PPAR-α), which increases ROS detoxification defenses that protect cells from cytokine toxicity. Yang induced 1-hour cerebral ischemia in mice, demonstrating that cerebral necrosis, NADPH oxidase activity, and ROS content were reduced by fasudil treatment after 18 hours. It also displayed a neuroprotective effect, albeit not in PPAR-α knockout mice.[25]

During testis I/R, germ cells and interstitial macrophages produce more proinflammatory cytokines, and inflammation generally contributes significantly to the injury.[6,26–28] These cytokines cause chemotaxis of neutrophils to the testis, and neutrophils help overproduce reactive oxygen species during I/R.[6] ROCK inhibition was demonstrated to block NF-κB p65 signaling and inflammation in a study on lipopolysaccharide-induced renal failure. Consistent with these observations, in contrast-induced acute kidney injury, fasudil reduced p-NF-kB levels and the production of inflammatory cytokines, such as TNF-α and IL-6.[24] Similarly, these inflammatory cytokines, whose production also increased in acetaminophen-induced liver damage, decreased with fasudil treatment.[23] Consistent with previous research, we found a considerable increase in TNF-α and IL-6 levels after testicular ischemia. Fasudil produced a statistically significant decrease in the treatment groups compared to the ischemia group, particularly in Group T1. All these data reinforce the anti-inflammatory and antioxidant potential of fasudil.

Apoptosis of spermatogenic cells is a physiological process that occurs during normal spermatogenesis.[29] However, the abnormal process of testicular I/R frequently results in widespread spermatogenic cell death, which can lead to greater testicular dysfunction and infertility.[30] Several biomarkers, including Bax, Bcl-2, caspase-3, and p-53 protein, have been found useful in cases of testicular torsion to assess the apoptotic process. Lipid peroxidation-induced membrane instability allows mitochondrial cytochrome c to enter the cytoplasm. Cytochrome C release promotes apoptosome formation, which triggers caspase-9 and caspase-3, resulting in apoptosis.[31] In a flap survival model, fasudil inhibited apoptosis via its effect on the Bax/Bcl-2 ratio, an indicator of apoptosis; it also increased autophagy.[22] Additionally, fasudil exerted antiapoptotic effects by reducing apoptosis and caspase-3 in contrast-induced acute kidney injury, diabetic cardiomyopathy, and heart I/R injury models.[8,24,32] Fasudil can inhibit ROCK activity and upregulate p-Akt. This could influence caspase-3 by downregulating downstream apoptotic factor Bax and upregulating antiapoptotic protein Bcl-2.[24] In our testicular torsion model, consistent with these findings, fasudil reduced caspase-3 in the treatment groups, although not statistically significantly.

ROS overproduction during testicular reperfusion causes oxidative stress in the testicular parenchyma, destroys the cell genome, and initiates caspase cascades, resulting in apoptosis.[33] All these changes are directly related to increasing necrosis in the testicular tissues. Torsion of the testis causes injuries, which are evaluated using biochemical and histopathological approaches and based on the determination of fertility. The RhoA/ROCK pathway damages the testis's cytoskeleton, adherens junctions, and tight junctions, disrupting the blood–testis barrier and thus impairing spermatogenesis.[34]

Because ischemia strongly impacts spermatogenesis in the testes, we assessed the damage produced by torsion using JTBS, which is considered highly relevant in the quantitative histological evaluation of spermatogenesis.[35,36] Our JTBS results in the torsion group showed a substantial decrease, indicating histopathologically that testicular injury resulted. The development of values close to normal with fasudil treatment, while not quite matching the values in the control group, reflects the histological healing effects of fasudil, especially in Group T1. These results reinforce the protective effects of fasudil on tissue already demonstrated in previous studies using brain, acute kidney injury, and cardiac ischemia models. In sum, we demonstrated that fasudil exerts a histopathological healing effect, both by ameliorating JTBS and by preventing apoptosis through the inhibition of caspase-3.

The small number of animals in each group, the termination of the experiment after four hours, and the lack of measurements at the 24th hour or later are among the main limitations of the study. Moreover, parameters such as TNF-α, IL-6, GSH, MDA, and caspase 3 were evaluated only using ELISA, while quantitative real-time PCR and Western blot analyses were not performed. These deficiencies stem from the limitations on the use of laboratory animals in our country and our limited budget. We hope that firmer and more supportable results will be obtained in future studies that take these deficiencies into consideration.

Conclusions

Based on an experimental testicular torsion model, fasudil reduces I/R injury as an antioxidant, anti-inflammatory, antiapoptotic, and histopathological curative, especially when given early. Fasudil is easy to access and store, can be given intravenously, can spread rapidly to cells, is efficacious for up to eight hours with a single dose, and can be used in humans. Therefore, as shown in this study, fasudil offers beneficial protective effects before the detorsion stage in cases of testicular ischemia or similar ischemias and when starting the relevant surgery.

Data Sharing Statement

On reasonable request, the corresponding author will make available the data analyzed during the current investigation.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Vaos G, Zavras N. Antioxidants in experimental ischemia-reperfusion injury of the testis: where are we heading towards? *World J Methodol*. 2017;7(2):37–45. doi:10.5662/wjm.v7.i2.37
2. Bašković M, Krsnik D, Himelreich Perić M, et al. Astaxanthin Relieves Testicular Ischemia-Reperfusion Injury-Immunohistochemical and Biochemical Analyses. *J Clin Med*. 2022;11(5):1284. doi:10.3390/jcm11051284
3. Chen YT, Chuang FC, Yang CC, et al. Combined melatonin-adipose derived mesenchymal stem cells therapy effectively protected the testis from testicular torsion-induced ischemia-reperfusion injury. *Stem Cell Res Ther*. 2021;12(1):370. doi:10.1186/s13287-021-02439-x
4. Shunmugam M, Goldman RD. Testicular torsion in children. *Can Fam Physician*. 2021;67(9):669–671. doi:10.46747/cfp.6709669
5. Wu MY, Yiang GT, Liao WT, et al. Current Mechanistic Concepts in Ischemia and Reperfusion Injury. *Cell Physiol Biochem*. 2018;46(4):1650–1667. doi:10.1159/000489241

6. Wei SM, Huang YM. Baicalein Alleviates Testicular Ischemia-Reperfusion Injury in a Rat Model of Testicular Torsion-Detorsion. *Oxid Med Cell Longev*. 2022;2022:1603469. doi:10.1155/2022/1603469
7. Laschke MW, Dold S, Menger MD, Jeppsson B, Thorlacius H. The Rho-kinase inhibitor Y-27632 inhibits cholestasis-induced platelet interactions in the hepatic microcirculation. *Microvasc Res*. 2009;78(1):95–99. doi:10.1016/j.mvr.2009.04.003
8. Cheng C, Liu XB, Bi SJ, Lu QH, Zhang J. Inhibition of Rho-kinase is involved in the therapeutic effects of atorvastatin in heart ischemia/reperfusion. *Exp Ther Med*. 2020;20(4):3147–3153. doi:10.3892/etm.2020.9070
9. Li Y, Yao JH, Hu XW, et al. Inhibition of Rho kinase by fasudil hydrochloride attenuates lung injury induced by intestinal ischemia and reperfusion. *Life Sci*. 2011;88(1–2):104–109. doi:10.1016/j.lfs.2010.10.028
10. Guo MF, Zhang HY, Zhang PJ, et al. Fasudil alleviates cerebral ischemia-reperfusion injury by inhibiting inflammation and improving neurotrophic factor expression in rats. *Acta Neurobiol Exp*. 2023;83(1):97–110.
11. Janetschek G, Schreckenberg F, Grimm W, Marberger M. Hemodynamic effects of experimental testicular torsion. *Urol Res*. 1987;15(5):303–306. doi:10.1007/BF00258201
12. Turner TT. Acute experimental testicular torsion. No effect on the contralateral testis. *J Androl*. 1985;6(1):65–72. doi:10.1002/j.1939-4640.1985.tb00817.x
13. Tunçkiran A, Cayan S, Bozlu M, Yilmaz N, Acar D, Akbay E. Protective effect of vascular endothelial growth factor on histologic changes in testicular ischemia-reperfusion injury. *Fertil Steril*. 2005;84(2):468–473. doi:10.1016/j.fertnstert.2005.01.144
14. Flessas II, Papalois AE, Toutouzas K, Zagouri F, Zografos GC. Effects of lazaroids on intestinal ischemia and reperfusion injury in experimental models. *J Surg Res*. 2011;166(2):265–274. doi:10.1016/j.jss.2010.08.031
15. Kölükçü E, Firat F, Deresoy FA, Katar M, Atılgan D. The effects of pirfenidone on ischaemia-reperfusion injury in testicular torsion-induced rat model. *Andrologia*. 2021;53(2):e13922. doi:10.1111/and.13922
16. Wei SM, Wang RY, Chen YS. Sesamol Protects Testis from Ischemia-Reperfusion Injury through Scavenging Reactive Oxygen Species and Upregulating CREMτ Expression. *Oxid Med Cell Longev*. 2020;2020:9043806. doi:10.1155/2020/9043806
17. Yuksel S, Erginel B, Bingul I, et al. The effect of hydrogen sulfide on ischemia /reperfusion injury in an experimental testicular torsion model. *J Pediatr Urol*. 2022;18(1):16.e1–16.e7. doi:10.1016/j.jpurol.2021.11.019
18. Jafari A, Ghasemnejad-Berenji H, Nemati M, Pashapour S, Sadeghpour S, Ghasemnejad-Berenji M. Beneficial effects of memantine on ischemia/reperfusion injury following torsion/detorsion induced testicular damage in rats: improvement in histological and biochemical parameters. *J Pediatr Urol*. 2021;17(4):441.e1–441.e7. doi:10.1016/j.jpurol.2021.04.014
19. Xie Y, Song T, Huo M, et al. Fasudil alleviates hepatic fibrosis in type 1 diabetic rats: involvement of the inflammation and RhoA/ROCK pathway. *Eur. Rev Med Pharmacol Sci*. 2018;22(17):5665–5677.
20. Ma Z, Zhang J, Ji E, Cao G, Li G, Chu L. Rho kinase inhibition by fasudil exerts antioxidant effects in hypercholesterolemic rats. *Clin. Exp Pharmacol Physiol*. 2011;38(10):688–694. doi:10.1111/j.1440-1681.2011.05561.x
21. Seccia TM, Rigato M, Ravarotto V, Calò LA. ROCK (RhoA/Rho Kinase) in Cardiovascular-Renal Pathophysiology: a Review of New Advancements. *J Clin Med*. 2020;9(5):1328. doi:10.3390/jcm9051328
22. Ji E, Wang J, Wang L, Pan Z, Gao W. Pharmacological Effects of Fasudil on Flap Survival in a Rodent Model. *J Surg Res*. 2020;255:575–582. doi:10.1016/j.jss.2020.03.044
23. Ren X, Meng T, Ren X, Li X, Lu L. Fasudil alleviates Acetaminophen-induced liver injury via targeting Rhoa/ROCK signal pathway. *J Toxicol Sci*. 2021;46(6):255–262. doi:10.2131/jts.46.255
24. Wang Y, Zhang H, Yang Z, Miao D, Zhang D. Rho Kinase Inhibitor, Fasudil, Attenuates Contrast-induced Acute Kidney Injury. *Basic Clin Pharmacol Toxicol*. 2018;122(2):278–287. doi:10.1111/bcpt.12895
25. Yang X, Wang G. Fasudil mediates neuroprotection in ischemia/reperfusion by modulating the ROCK-PPARα-NOX axis. *Acta Cir Bras*. 2023;38:e387023.
26. Sarikaya K, Kölükçü E, Unsal V, Özdemir S. Protective Effects of Syringic Acid on Ischemia-Reperfusion Injury in Testicular Torsion: an Experimental Study in a Rat Model. *Cureus*. 2023;15(7):e42390. doi:10.7759/cureus.42390
27. Arabaci Tamer S, Yildirim A, Köroğlu MK, Çevik Ö, Ercan F, Yeğen BÇ. Nesfatin-1 ameliorates testicular injury and supports gonadal function in rats induced with testis torsion. *Peptides*. 2018;107:1–9. doi:10.1016/j.peptides.2018.07.005
28. Ahmed AI, Lasheen NN, El-Zawahry KM. Ginkgo Biloba Ameliorates Subfertility Induced by Testicular Ischemia/Reperfusion Injury in Adult Wistar Rats: a Possible New Mitochondrial Mechanism. *Oxid Med Cell Longev*. 2016;2016:6959274. doi:10.1155/2016/6959274
29. Dunkel L, Hirvonen V, Erkkilä K. Clinical aspects of male germ cell apoptosis during testis development and spermatogenesis. *Cell Death Differ*. 1997;4(3):171–179. doi:10.1038/sj.cdd.4400234
30. Xu LZ, He KX, Ning JZ, Cheng F. Oleuropein attenuates testicular ischemia-reperfusion by inhibiting apoptosis and inflammation. *Tissue Cell*. 2022;78:101876. doi:10.1016/j.tice.2022.101876
31. Yesil S, Ozdemir C, Arslan M, Gundogdu AC, Kavutcu M, Atan A. Protective effect of cerium oxide on testicular function and oxidative stress after torsion/detorsion in adult male rats. *Exp Ther Med*. 2022;25(1):1. doi:10.3892/etm.2022.11700
32. Fan X, Li X, Liu H, et al. A ROCK1 Inhibitior Fasudil Alleviates Cardiomyocyte Apoptosis in Diabetic Cardiomyopathy by Inhibiting Mitochondrial Fission in a Type 2 Diabetes Mouse Model. *Front Pharmacol*. 2022;13:892643. doi:10.3389/fphar.2022.892643
33. Cvetkovic T, Stankovic J, Najman S, et al. Oxidant and antioxidant status in experimental rat testis after testicular torsion/detorsion. *Int J Fertil Steril*. 2015;9(1):121–128. doi:10.22074/ijfs.2015.4216
34. Liu H, Zeng X, Ma Y, et al. Long-term exposure to low concentrations of MC-LR induces blood-testis barrier damage through the RhoA/ROCK pathway. *Ecotoxicol Environ Saf*. 2022;236:113454. doi:10.1016/j.ecoenv.2022.113454
35. Visser AJ, Heyns CF. Testicular function after torsion of the spermatic cord. *BJU Int*. 2003;92(3):200–203. doi:10.1046/j.1464-410X.2003.04307.x
36. Johnsen SG. Testicular biopsy score count-a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones*. 1970;1(1):2–25. doi:10.1159/000178170