EXPERIMENTAL STUDY

Comparison of hyperbaric oxygen and ozone treatment for ischemia/re-perfusion injury in an experimental testicular torsion model

Gökçe Karlı, M.D.,[1] Basak Erginel, M.D.,[1] Fatih Yanar, M.D.,[2] Esra Aycan Ustyol, M.D.,[3] Yasemin Ozluk, M.D.,[4] Meltem Savran Karadeniz, M.D.,[5] Burak İlhan, M.D.,[2] Feryal Gun Soysal, M.D.,[1] Erbug Keskin, M.D.[1]

[1]Department of Pediatric Surgery, İstanbul University İstanbul Faculty of Medicine, İstanbul-*Türkiye*
[2]Department of General Surgery, İstanbul University İstanbul Faculty of Medicine, İstanbul-*Türkiye*
[3]Department of Biochemistry, İstanbul University İstanbul Faculty of Medicine, İstanbul-*Türkiye*
[4]Department of Pathology, İstanbul University İstanbul Faculty of Medicine, İstanbul-*Türkiye*
[5]Department of Anesthesiology, İstanbul University İstanbul Faculty of Medicine, İstanbul-*Türkiye*

ABSTRACT

BACKGROUND: This study aims to compare the effects of medical ozone (MO) therapy and hyperbaric oxygen (HBO) therapy in an experimental testicular torsion model by measuring the oxidant and antioxidant markers and examining the histopathological tissue damage findings.

METHODS: Thirty-two Wistar rats are used and are divided into four groups; (1) sham group (SG), (2) only ischemia/reperfusion (I/R) by testicular torsion, (3) HBO administered group, and (4) MO administered group. No torsion was conducted in the SG. In all other groups, rats underwent testicular torsion followed by detorsion to create an I/R model. After I/R, HBO was injected in the HBO group, and in the MO group, intraperitoneal ozone was applied. At the end of 1 week, testicular tissues were obtained for biochemical analyzes and histopathological examinations. Biochemically, malondialdehyde (MDA) levels were measured for oxidant activity, and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels were measured for antioxidant activity. Furthermore, the testicles were evaluated histopathologically.

RESULTS: Both HBO and MO have significantly decreased MDA levels, compared with sham and I/R groups, resulting in decreased oxidation effects. The antioxidant GSH-Px levels in the HBO and MO groups were significantly higher than in the sham and I/R groups. In addition, the antioxidant SOD levels in the HBO group were significantly higher than sham, I/R, and MO groups. Therefore, the antioxidant effect of HBO was observed to be superior to MO, specifically considering SOD levels. Histopathologically, there was no significant difference between the groups ($p>0.05$).

CONCLUSION: The study may extrapolate that both HBO and MO are antioxidant agents that can be used in testicular torsion. HBO treatment might improve the cellular antioxidant capacity due to increased antioxidant marker levels more than MO therapy. However, further studies are needed with a larger sample size.

Keywords: Hyperbaric oxygen; mice; ozone; stress oxidative; testis.

INTRODUCTION

Testicular torsion is a surgical emergency that occurs in 1 in 4000 newborns or adolescents.[1,2] If not treated within 4–6 h, the tissue ischemia that occurs during torsion may cause germ cell death, resulting in infertility or reduced fertility.

Cite this article as: Karlı G, Erginel B, Yanar F, Ustyol EA, Ozluk Y, Savran Karadeniz M, et al. Comparison of hyperbaric oxygen and ozone treatment for ischemia/re-perfusion injury in an experimental testicular torsion model. Ulus Travma Acil Cerrahi Derg 2023;29:259-265.

Address for correspondence: Burak İlhan, M.D.
İstanbul Üniversitesi İstanbul Tıp Fakültesi, Genel Cerrahi Anabilim Dalı, İstanbul, Türkiye
Tel: +90 212 - 414 20 00 E-mail: burakmd@yahoo.com

Ulus Travma Acil Cerrahi Derg 2023;29(3):259-265 DOI: 10.14744/tjtes.2023.98861 Submitted: 16.11.2022 Revised: 23.01.2023 Accepted: 20.02.2023

[3,4] Reduced fertility in long-term follow-up was reported in 66.7% of affected patients.[5]

Numerous oxygen radicals are formed following testicular torsion. Evidence has shown that reactive oxygen radicals play an essential role in ischemia/reperfusion (I/R) injury.[6] Reactive oxygen species stimulate chain reactions that cause cellular dysfunction between lipids, proteins, and nucleic acids.[7,8] These reactive oxygen species include superoxide anions, hydroxyl radicals, hydrogen peroxide, and peroxynitrite. Studies have reported that biopsy results following reperfusion are more severe than those following pure ischemia.[9,10] In fact, marked histopathological changes have been encountered in groups where the reperfusion period, but not the ischemia period.[11] Therefore, the effect of different antioxidant agents has been evaluated and compared with each other to prevent I/R injury in testicular tissue.[12–14]

Medical ozone (MO) treatment is applying a certain amount of oxygen and ozone gaseous mixture into the body cavities or circulation system. MO is an adjuvant treatment modality, especially in pathological conditions with severe inflammation. While the oxygen and ozone mixture provides supplemental oxygen, it also increases some enzymes and cytokines.[15] Ozone applications can heal damaged tissues by increasing the secretion of different autocoids, cytokines, and growth factors. Some studies have investigated the protective effect of ozone therapy in testis injury by reducing levels of oxidative stress,[16–18] which has recently been tried in testicular torsion for these cytoprotective and antioxidant properties.[19] However, MO treatments are only sometimes used for treatment worldwide.

Hyperbaric oxygen (HBO) treatment is a non-invasive and systemic-based medical treatment method that increases partial oxygen pressure in blood. HBO treatment causes vasoconstriction in response to post ischemic vasodilatation. HBO has also been studied in testicular torsion.[20,21]

Biochemically, malondialdehyde (MDA) levels were measured for oxidant activity, and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels were measured for antioxidant activity. MDA is a marker of the oxidant system and shows lipid peroxidation. An increase in free radicals causes the overproduction of MDA. It indicates oxidative damage in the membrane of lipids. Antioxidant mechanisms develop a defense system against free radicals, which harm body tissues. SOD and GSH-Px is the cell's primary defense against free radicals. The accumulation of free radicals in the cell causes oxidative stress and cellular damage.

This is the first study comparing the biochemical and histopathological effects of ozone and HBO, both antioxidant oxygen provider agents, for I/R injury in an experimental testicular torsion model.

MATERIALS AND METHODS

This experimental study was performed under the guidelines for using laboratory animal subjects in research, as established by the Ethical Committee (Decision No: 2014/102). In total, 32 Wistar albino rats were included in this study. They were kept freely in metal cages under stable environmental conditions and fed water and a standard laboratory diet.

The surgical procedures were performed on the animals under sterile conditions. General anesthesia was achieved by intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi, Turkey) and 10 mg/kg xylazine hydrochloride (Rompun, Bayer, Germany).

The 32 rats were categorized into four groups of 8 rats as follows:

1. Sham group (SG) (n=8): The right testicle was removed from the scrotum. It was fixed to the scrotum with non-traumatic sutures, and an orchiectomy was performed 7 days later.
2. I/R group (n=8): The right testicle was twisted 720°, and detorsion was applied one h later. Orchiectomy was performed 7 days after detorsion.
3. I/R+HBO group (n=8): The right testicle was twisted 720°, and detorsion was applied one h later. HBO treatment was administered for 7 days, starting from postoperative day one. Orchiectomy was performed at the end of the 7th day.
4. I/R+MO treatment group (n=8): The right testicle was twisted 720°, and detorsion was applied one h later. Following detorsion, MO treatment was administered intraperitoneally for 7 days. Orchiectomy was performed at the end of the 7th day.

In the I/R groups, the rats were placed in the supine position following the achievement of adequate anesthetic depth. The inguinal and scrotal regions were shaved, and the skin was prepared with 10% Povidone-iodine (Isosol, Merkez Lab, Turkey). A scrotal pouch was created to place the testicle back after torsion easily. The testicle was then twisted along its longitudinal axis by 720° and fixated inside the scrotal pouch with 4/0 non-traumatic absorbable sutures, piercing through the dartos and tunica albuginea. A gauze dressing soaked with warm physiological saline solution was used to cover the incision. The fixation suture was released after one h of torsion. Following detorsion, the testicle was fixated with three non-traumatic absorbable sutures, and the layers were closed appropriately.

In the I/R+HBO group, the rats were administered over 98% oxygen at 2.4 ATM pressure for 7 days postoperatively in a total of 21 sessions of 60 min each in locally produced chambers, with a volume of 0.2 m^3 and a working pressure of 10 bars.

In the I/R+MO treatment group, the rats were administered 1 mg/kg ozone intraperitoneally for 7 days postoperatively. The ozone generator supplied ozone as 96% oxygen and 4% ozone. The mixture was applied intraperitoneally by polypropylene injectors without contamination.

The rats received a right orchiectomy, and the acquired tissues were cut into two pieces. The first piece was frozen immediately with liquid nitrogen and preserved at −80° until a biochemical investigation was conducted. The second piece underwent histological assessment and was fixed in a Bouin solution.

To measure MDA, the lipid peroxidation product, tissues were homogenized using an automatic tissue homogenizer (Heidolph DIAX900, Germany) after defrosting. Dahle's spectrophotometry principle was used to measure MDA. This principle is based on producing a pink-colored complex by incubating thiobarbituric acid under aerobic conditions at 90–95°C and a pH of 3.4. The resultant color is directly proportional to the MDA concentration, and its absorbance is read spectrophotometrically at 532 nm.[22]

Superoxide is a radical formed by the impact of fluorescent light from riboflavin, and it is reduced to hydrogen peroxide with SOD in the medium. Hydrogen peroxide is formed proportional to SOD activity. It reacts with o-Dianisidine, resulting in a colored product read spectrophotometrically at 460 nm.[23]

GSH-Px reduces hydrogen peroxide or lipid peroxide with glutathione. The measurement method was based on the oxidation of NADPH by the GSH reductase enzyme, which depends on the amount of GSSG after GSH (reduced glutathione) and H_2O_2 are converted to water and GSSG (oxidized glutathione) by the GSH-Px enzyme. NADPH is oxidized to NADP for as long as GSH is produced in the reaction. The activity of GSH-Px is determined by the reduction of NADPH absorbance at a wavelength of 340 nm.[24]

The tissue specimens reserved for histopathological assessment were fixed in the Bouin solution. After fixation, the specimens were embedded in paraffin. The standard 4-micrometer-thick cuts of specimens were stained with hematoxylin-eosin and prepared for microscopic evaluation. The same pathologist examined all specimens according to the scoring system of Cosentino et al.[25] (1998) under a light microscope (Nikon E600, Japan).

The following grades were given:

- Grade 1 (1 point): Normal testicular architecture with an orderly arrangement of germ cells.
- Grade 2 (2 points): Injury showed less orderly, non-cohesive germinal cells and closely packed seminiferous tubules.
- Grade 3 (3 points): The injury exhibited disordered sloughed germ cells with shrunken pyknotic nuclei and less distinct seminiferous tubule borders.
- Grade 4 (4 points): The injury-defined seminiferous tubules are closely packed with coagulative necrosis of the germ cells.

Statistical Analysis

The sample size was calculated with the G*Power Version 3.1.6 program. For the antioxidant levels measured in animal experiments between the groups, the difference in the medium effect size (effect size=1.0) was predicted to be statistically significant, and the sample size was determined as a total of 24 animals for 95% power at 0.05 alpha significance level. Although sample size is calculated 24 before the experiment, we added 2 rats to each group to compensate in case of loss of animals.

Statistical analysis was performed using the Statistical Package for the Social Sciences 21.0 (SPSS, Inc., Chicago, IL, USA). A Kolmogorov–Smirnov test was used to determine the variable distribution. Descriptive analyses were recorded as continuous variables' frequency, percentage, mean, and standard deviations. A one-way repeated measure analysis of variance (ANOVA) was used to compare the parameters of the groups, and the Bonferroni test was used to determine differences across groups. Pearson correlation analysis was performed to evaluate the relationship between grades and parameters. The results had a confidence interval of 95%, and $p<0.05$ was considered statistically significant.

RESULTS

Macroscopic changes due to ischemia were observed in all testicles removed for detorsion following 1 h of torsion. The changes included edema and color changes due to venous stasis.

MDA Measurement

MDA is an important marker of tissue peroxidation and occurs in I/R injury. The one-way ANOVA test showed a statistically significant difference between the mean MDA levels (nmol/mg protein) of the groups ($F=15.795$; $p=0$, <0.05). A post hoc Bonferroni correction was performed to determine which specific means differed.

The mean MDA level was measured as 100.25 nmol/mg prot in the SG, 232.12 nmol/mg prot in the I/R group, 129.12 nmol/mg prot in the HBO group, and 153.25 nmol/mg prot in the MO group (Fig. 1). Both HBO and ozone have decreased MDA, compared to sham and I/R groups, proving decreased oxidation effects.

GSH-Px Measurement

GSH levels were measured for the determination of antioxidant power. The one-way ANOVA test showed a statistically

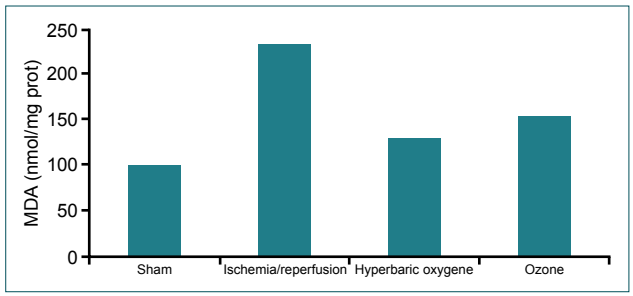

Figure 1. MDA levels of the groups (F=15.795; p=0<0.05).

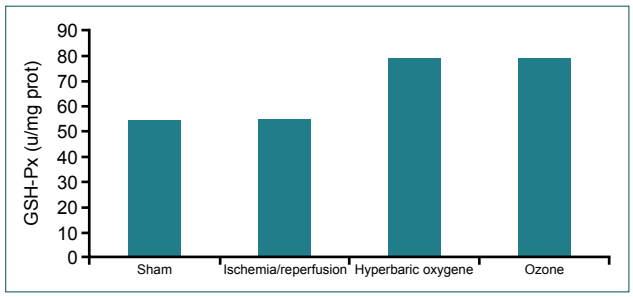

Figure 2. GSH-Px levels of the groups (F=7.647; p=0.001 <0.05).

significant difference between the means of the GSH-Px (u/mg prot) levels of the groups (F=7.647; p=0.001, <0.05). In addition, a post hoc Bonferroni correction was performed to determine which specific means differed.

The mean GSH-Px level was measured as 54.50 U/mg prot in the SG, 55.00 U/mg prot in the I/R group, 79.25 U/mg prot in the HBO group, and 79.12 U/mg prot in the MO group. The GSH-Px levels of the HBO and ozone groups were significantly higher than in the sham and I/R groups. No statistically significant difference was found between the other groups (p>0.05) (Fig. 2).

SOD Measurement

SOD levels were measured for the determination of antioxidant power. The one-way ANOVA test showed a statistically significant difference between the means of the SOD (u/mg prot) levels of the groups (F=6.332; p=0.002, <0.05). A post hoc Bonferroni correction was performed to determine which specific means differed.

The mean SOD level was measured as 15.86 U/mg prot in the SG, 12.40 U/mg prot in the I/R group, 21.15 U/mg prot in the HBO group, and 13.20 U/mg prot in the MO group. The SOD levels of the testicles in the HBO group were significantly higher than I/R and MO groups. No statistically significant difference was found between the other groups (p>0.05) (Fig. 3).

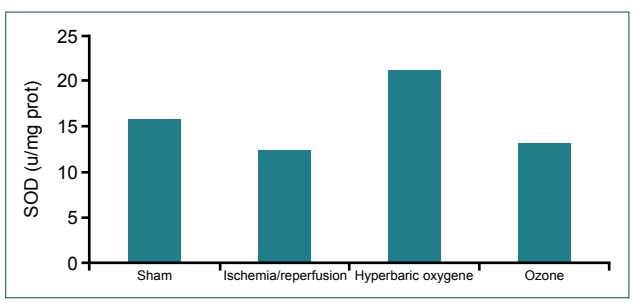

Figure 3. SOD Levels of the Groups (F=6.332; p=0.002 <0.05).

Histopathological Findings

The testicles were evaluated histopathologically according to the Cosentino scoring system.[25] The one-way ANOVA test showed a statistically significant difference between the groups' grades (F=7.391; p=0.001, <0.05). In addition, a post hoc Bonferroni correction was performed to determine which specific means differed.

The scores of the I/R group were significantly higher than those of the SG. No statistically significant difference was found between the other groups (p>0.05). In the SG, 5 (62.5%) testicles of rats revealed Grade 1, and 3 (37.5%) revealed Grade 2; in the I/R group, two testicles (25.0%) revealed Grade 2, 3 (37.5%) revealed Grade 3, and the other 3 (37.5%) revealed grade 4; in the HBO group, two testicles (25.0%) revealed Grade 1, 4 (50.0%) revealed Grade 2, 1 (12.5%) revealed Grade 3, and 1 (12.5%) revealed Grade 4; and in the MO group, 4 testicles (57.1%) revealed Grade 2, and 3 (42.9%) revealed Grade 3. Due to abscess findings in one of the rat testicles, one was not considered.

DISCUSSION

Several studies have been conducted to prevent I/R injury in testicular torsion, which can cause critical complications, such as infertility. An emergency surgical procedure is still the gold standard and the most commonly performed treatment modality.[26] To limit reperfusion injury following detorsion, several agents, such as allopurinol, melatonin, sildenafil, vitamin E, surfactant, poly-polymerase inhibitors, zinc aspartate, methylene blue, pentoxifylline, dexamethasone, catalase, heparin, verapamil, and acetylcysteine, or cold compress to the testicles were investigated.[24–26] These methods have yet to be included in routine clinical practice, and new agents still need to be investigated. Antioxidants are one of the most efficient agents protecting cell membrane integrity during I/R injury. In this study, we compared two antioxidant agents, MO and HBO, for I/R injury in an experimental testicular torsion model by evaluating the grade of I/R injury in terms of biochemical and histopathological effects.

MO treatment has shown promising results in wound healing, age-related macular degeneration, ischemia, and infectious diseases. The increased plasma concentration of hydrogen peroxide generated by MO treatment diffuses into the cell and triggers the synthesis of various interferons, interleukins, and transforming growth factors in leukocytes and endothelial cells.[27] Aslan et al.[28] evaluated the effect of MO treatment on an experimental ovary torsion–detorsion model and found that the ozone-administered group showed less

interstitial edema and congestion in ovarian and paraovarian tissues, and the MDA level decreased significantly compared to the torsion-detorsion group proving the antioxidants effects of ozone. Güven et al.[29] assessed the effect of MO treatment on esophageal burns by measuring the stenosis index, histopathologic damage score, oxidative stress, and antioxidant enzyme levels. They reported that the stenosis index and the histopathologic damage score were significantly lower, antioxidant enzymes, such as SOD and GSH-Px, were higher, and the MDA level was significantly lower in the group treated with MO. Tusat et al.[19] recently evaluated ozone use in testicular torsion.

HBO therapy increases oxygen pressure and the concentration of reactive oxygen species in the blood and tissues. HBO has also shown benefits in previous testicular torsion models.[20,21]

Koca et al.[30] compared the effects of HBO and MO treatment in rats with induced ischemic-reperfusion injury in skeletal bones. MDA and protein carbonyl (PCO) levels were found to be increased in the bones following 2 h of ischemia and 22 h of reperfusion. In the HBO and MO treatment groups, MDA and PCO levels decreased, and SOD and GSH-Px levels increased. In another study, Altinel et al.[31] compared the effect of HBO and MO on an experimental distal colitis model. They stated that MO treatment decreases inflammation and edema more than HBO and reduces oxidative stress. HBO and medical MO treatments have a wide range of indications in the surgical field. However, to the best of our knowledge, no study comparing the effect of HBO and MO treatment in I/R injury of the testicle has been published in the literature.

We measured the tissue levels of MDA for measuring oxidant, GSH-Px, and SOD for measuring the antioxidant system to evaluate I/R injury. Moreover, we performed a histopathological assessment.

MDA values showing the oxidation were significantly higher in the group with no treatment after torsion-detorsion compared to the other groups. For this reason, a successful I/R model was created in our study. Furthermore, the effects of both HBO and medical MO treatment on lipid peroxidation were similar; they both played a role in decreasing the oxidant situation, which is proved by the decrease in MDA levels in the experimental group (HBO and MO group).

GSH-Px and SOD are both markers for measuring antioxidant levels. In the HBO and MO treatment groups, GSH-Px was significantly higher than in Groups 1 and 2. As a result, HBO and MO treatment have an increasing effect on GSH-Px; therefore, they contribute to antioxidant capacity. Although the amount of GSH-Px was higher in the HBO group than in the MO group, the difference was not statistically significant. Another parameter of antioxidant capacity was SOD. The SOD level in the HBO treatment group was significantly higher than in every other group.

The levels of MDA, SOD, and GSH-Px might suggest that HBO treatment improves antioxidant activity better than MO treatment.

The final criterion evaluated was histopathologic assessment, in which the tubule structure and composition of stromal cells and germinal cells of the testicular tissue were considered in every group. According to the scoring system of Cosentino et al.,[25] one score was assigned for every grade. Due to abscess findings, one testicle was excluded. The histopathological results showed Grade 2 damage in 3 testicles only in Group 1, where the testicles were manipulated. We believe this might be attributed to the insult caused during the removal and refixation of the testicles. In testicles with torsion-detorsion, the damage score was significantly higher than in the other groups. We might state that detorsion after 1 h of torsion leads to histopathological damage in the testicles. There was no statistically significant difference regarding histopathology between the groups with HBO and MO treatment following torsion-detorsion and the group with testicular manipulation without torsion. Consequently, although HBO and MO treatment reduced the damaging effects of I/R injury, no significant difference was seen between them. Histopathological findings did not prove the positive effect of HBO and MO treatment on oxidative stress markers; this may be due to a limited number of animals to prove the cellular-based effect as histological manifestations.

This study's limitation arises from an experimental study with a small number of animals. On the other hand, the effect of HBO and MO on the contralateral testis in the testicular torsion model is not evaluated in this study. These beneficial effects may be the subject of another study. Furthermore, MO has yet to be approved everywhere, and HBO treatments are only sometimes readily available and may be costly.

Conclusion

We determined that HBO treatment improved antioxidant capacity more than MO treatment in cellular antioxidant levels. However, more studies with more animals with ethical approval are required to evaluate which antioxidant is superior to one another in I/R injury following testicle torsion.

Acknowledgment

We thank Ass. Prof. Dr. Bengisu Mirasoglu from Istanbul University, Istanbul Medical Faculty, Department of Underwater Medicine and Hyperbaric Medicine for their contributions.

Ethics Committee Approval: This study was approved by the İstanbul University Animal Experiment Ethics Committee (Date: 06.11.2014, Decision No: 2014/102).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: G.K.; Design: G.K., B.E.; Supervision: F.G.S., E.K.; Materials: G.K., B.E.; Data: G.K.,

B.E.; Analysis: G.K., B.E., F.Y., E.A.U., Y.O., M.S.K., B.İ.; Literature search: G.K., B.E.; Writing: G.K.; Critical revision: F.G.S., E.K.

Conflict of Interest: None declared.

Financial Disclosure: This study has been supported by İstanbul University Scientific Research Project Unit (Project number: 52821).

REFERENCES

1. Çay A, Alver A, Küçük M, Işık O, Eminağaoğlu MS, Karahan SC, et al. The effects of N-acetylcysteine on antioxidant enzyme activities in experimental testicular torsion. J Surg Res 2006;131:199–203. [CrossRef]
2. Nezami BG, Rahimpour S, Gholipour T, Payabvash S, Rahimian R, Tavangar SM, et al. Protective effects of immunophilin ligands on testicular torsion/detorsion damage in rats. Int Urol Nephrol 2009;41:93–9.
3. Wei SM, Yan ZZ, Zhou J. Protective effect of rutin on testicular ischemia reperfusion injury. J Pediatr Surg 2011;46:1419–24. [CrossRef]
4. Gezici A, Öztürk H, Büyükbayram H, Öztürk H, Okur H. Effects of gabexate mesilate on ischemia-reperfusion-induced testicular injury in rats. Pediatr Surg Int 2006;22:435–41. [CrossRef]
5. Wei SM, Yan ZZ, Zhou J. Psoralea corylifolia protects aganist testicular torsion/detorsion-induced ischemia/reperfusion injury. J Ethnopharmacol 2011;137:568–74. [CrossRef]
6. Aktaş BK, Bulut Ş, Bulut S, Baykam MM, Özden C, Senes M, et al. The effects of N-acetylcysteine on testicular damage in experimental testicular ischemia/reperfusion injury. Pediatr Surg Int 2010;26:293–8. [CrossRef]
7. Wei SM, Yan ZZ, Zhou J. Beneficial effect of taurine on testicular ischemia-reperfusion injury in rats. Urology 2007;70:1237–42. [CrossRef]
8. Taşkara E, Gör A, Kutlu Ö, Karagüzel E, Çobanoğlu Ü, Topbaş M, et al. Does propofol prevent ischemia-reperfusion injury due to torsion in the long term? Pediatr Surg Int 2011;27:1003–7. [CrossRef]
9. Blank ML, O'Neill PJ, Steigman CK, Cobb LM, Wilde RA, Havenstein PJ, et al. Reperfusion injury following testicular torsion and detorsion in prepubertal rats. Urol Res 1993;21:389–93. [CrossRef]
10. Kaya M, Harrison RG. An analysis of the effect of ischaemia on testicular ultrastructure. J Pathol 1975;117:105. [CrossRef]
11. Özen İO, Ekingen G, Taşlıpınar MY, Bukan N, Demiroğulları B, Karabulut R, et al. effect of melatonin on healing of colonic anastomosis in a rat model of peritonitis. Eur Surg Res 2007;39:122–7. [CrossRef]
12. Ekici S, Ekici AI, Öztürk G, Aksungar FB, Sinanoğlu O, Turan G, et al. Comparison of melatonin and ozone in the prevention of reperfusion injury following unilateral testicular torsion in rats. Urology 2012;80:899–906. [CrossRef]
13. Karaguzel E, Kadihasanoglu M, Kutlu O. Mechanisms of testicular torsion and potential protective agents. Nat Rev Urol 2014;11:391–9.
14. Zhang Y, Lv Y, Liu YJ, Yang C, Hu HJ, Meng XE, et al. Hyperbaric oxygen therapy in rats attenuates ischemia-reperfusion testicular injury through blockade of oxidative stress, suppression of inflammation, and reduction of nitric oxide formation. Urology 2013;82:489, e9–489, e15.
15. Bocci V. The case for oxygen-ozone therapy. Br J Biomed Sci 2007;64:44–9. [CrossRef]
16. Aydos TR, Başar MM, Kul O, Atmaca HT, Uzunalıoğlu T, Kisa Ü, et al. Effects of ozone therapy and taurine on ischemia/reperfusion-induced testicular injury in a rat testicular torsion model. Turk J Med Sci 2014;44:749–55. [CrossRef]
17. Mete F, Tarhan H, Çelik O, Akarken I, Vural K, Ekin RG, et al. Comparison of intraperitoneal and intratesticular ozone therapy for the treatment of testicular ischemia-reperfusion injury in rats. Asian J Androl 2017;19:43–6. [CrossRef]
18. Larson JL, Stephenson LL, Linda LB, Zamboni WA. Effect of hyperbaric oxygen on neutrophil CD18 expression. Plast Reconstr Surg 2000;105:1375–81. [CrossRef]
19. Tusat M, Menteşe A, Demir S, Alver A, İmamoğlu M. Medical ozone therapy reduces oxidative stress and testicular damage in an experimental model of testicular torsion in rats. Int Braz J Urol 2017;43:1160–6.
20. Alp BF, Cebi G, Özdemir A, Irkilata HC, Uzun G. Hyperbaric oxygen treatment for unilateral testicular torsion in a child. Diving Hyperb Med 2014;44:161–2.
21. Kolski JM, Mazolewski PJ, Stephenson LL, Texter J, Grigoriev VE, Zamboni WA. Effect of hyperbaric oxygen therapy on testicular ischemia-reperfusion injury. J Urol 1998;160:601–4. [CrossRef]
22. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8. [CrossRef]
23. Mylorie AA, Collins H, Umbles C, Kyle J. Erythrocyte superoxide dismutase activity and other parameters of copper status in rats ingesting lead acetate. Toxicol Appl Pharmacol 1986;82:512–20. [CrossRef]
24. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–69.
25. Cosentino MJ, Nishida M, Rabinowitz R, Cockett AT. Histopathology of prepubertal rat testes subjected to various durations of spermatic cord torsion. J Androl 1986;7:23–31. [CrossRef]
26. Turner TT, Brown KJ. Spermatic cord torsion: Loss of spermatogenesis despite return of blood flow. Biol Reprod 1993;49:401–7. [CrossRef]
27. Turner TT, Bang HJ, Lysiak JL. The molecular pathology of experimental testicular torsion suggests adjunct therapy to surgical repair. J Urol 2004;172:2574–8. [CrossRef]
28. Aslan MK, Boybeyi Ö, Şenyücel MF, Ayva Ş, Kısa Ü, Aksoy N, et al. Protective effect of intraperitoneal ozone application in experimental ovarian ischemia/reperfusion injury J Pediatr Surg 2012;47:1730–4. [CrossRef]
29. Güven A, Gündoğdu G, Sadir S, Topal T, Erdoğan E, Korkmaz A, et al. The efficacy of ozone therapy in experimental caustic esophageal burn. J Pediatr Surg 2008;43:1679–84. [CrossRef]
30. Koca K, Yurttas Y, Bilgic S, Cayci T, Topal T, Durusu M, et al. Effect of preconditioned hyperbaric oxygen and ozone on ischemia-reperfusion induced tourniquet in the skeletal bone of rats. J Surg Res 2010;164:e83–9.
31. Altinel O, Demirbas S, Cakir E, Yaman H, Ozerhan IH, Duran E, et al. Comparison of hyperbaric oxygen and medical ozone therapies in a rat model of experimental distal colitis. Scand J Clin Lab Invest 2011;71:185–92. [CrossRef]

DENEYSEL ÇALIŞMA - *ÖZ*

Deneysel testiküler torsiyon modelinde iskemi/yeniden perfüzyon yaralanması için hiperbarik oksijen ve ozon tedavisinin karşılaştırılması

Dr. Gökçe Karlı,[1] Dr. Basak Erginel,[1] Dr. Fatih Yanar,[2] Dr. Esra Aycan Ustyol,[3] Dr. Yasemin Ozluk,[4] Dr. Meltem Savran Karadeniz,[5] Dr. Burak İlhan,[2] Dr. Feryal Gun Soysal,[1] Dr. Erbug Keskin[1]

[1]İstanbul Üniversitesi İstanbul Tıp Fakültesi, Çocuk Cerrahisi Anabilim Dalı, İstanbul
[2]İstanbul Üniversitesi İstanbul Tıp Fakültesi, Genel Cerrahi Anabilim Dalı, İstanbul
[3]İstanbul Üniversitesi İstanbul Tıp Fakültesi, Biyokimya Anabilim Dalı, İstanbul
[4]İstanbul Üniversitesi İstanbul Tıp Fakültesi, Patoloji Anabilim Dalı, İstanbul
[5]İstanbul Üniversitesi İstanbul Tıp Fakültesi, Anestezi Anabilim Dalı, İstanbul

AMAÇ: Bu çalışmanın amacı, deneysel bir testis torsiyon modelinde medikal ozon (MO) tedavisi ile hiperbarik oksijen (HBO) tedavisinin etkilerini, oksidan ve antioksidan belirteçleri ölçerek ve histopatolojik doku hasarı bulgularını inceleyerek karşılaştırmaktır.
GEREÇ VE YÖNTEM: Otuz iki Wistar sıçan kullanıldı ve dört gruba ayrıldı; (1) sahte grup (SG), (2) sadece testis torsiyonu ile iskemi/reperfüzyon (I/R), (3) HBO uygulanan grup ve (4) MO uygulanan grup. SG'de burulma yapılmadı. Diğer tüm gruplarda, sıçanlara bir I/R modeli oluşturmak için testis torsiyonu ve ardından detorsiyon uygulandı. İ/R sonrası HBO grubuna hiperbarik oksijen, MO grubuna intraperitoneal ozon uygulandı. Bir hafta sonunda biyokimyasal analizler ve histopatolojik incelemeler için testis dokuları alındı. Biyokimyasal olarak oksidan aktivite için malondialdehit (MDA), antioksidan aktivite için süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GSH-Px) seviyeleri ölçüldü. Ayrıca testisler histopatolojik olarak da değerlendirildi.
BULGULAR: Hem HBO hem de MO, sahte ve I/R grupları ile karşılaştırıldığında, oksidasyon etkilerinin azalmasıyla sonuçlanan, MDA seviyelerini önemli ölçüde azaltmıştır. HBO ve MO gruplarındaki antioksidan GSH-Px seviyeleri, sham ve I/R gruplarına göre anlamlı derecede yüksekti. HBO grubundaki antioksidan SOD seviyeleri sham, I/R ve MO gruplarına göre anlamlı olarak yüksekti. Bu nedenle, özellikle SDO seviyeleri dikkate alındığında, HBO'nun antioksidan etkisinin MO'dan daha üstün olduğu gözlenmiştir. Histopatolojik olarak gruplar arasında anlamlı fark yoktu ($p>0.05$).
TARTIŞMA: Çalışma, hem HBO hem de MO'nun testis torsiyonunda kullanılabilecek antioksidan ajanlar olduğunu tahmin edebilir. HBO tedavisinin, MO tedavisine göre antioksidan belirteç düzeylerinin artması nedeniyle hücresel antioksidan kapasiteyi iyileştirebileceğini belirledik. Bununla birlikte, daha büyük bir örneklem büyüklüğü ile daha fazla çalışmaya ihtiyaç vardır.
Anahtar sözcükler: Fareler; hiperbarik oksijen; ozon; stres oksidatif; testis.

Ulus Travma Acil Cerrahi Derg 2023;29(3):259-265 doi: 10.14744/tjtes.2023.98861