Annals of Medical Research
DOI: 10.5455/annalsmedres.2019.09.535 2019;26(11):2549-52

Original Article

Cryptotanshinone mitigates ischemia reperfusion-induced testicular damage: A experimental study

Ayhan Tanyeli[1], Ersen Eraslan[2], Fazile Nur Ekinci Akdemir[3], Mustafa Can Guler[1], Derya Guzel Erdogan[4], Elif Polat[5], Omer Topdagi[6]

[1]Ataturk University, Faculty of Medicine, Department of Physiology, Erzurum, Turkey
[2]Yozgat Bozok University, Faculty of Medicine, Department of Physiology, Yozgat, Turkey
[3]Agri Ibrahim Cecen University, High School of Health, Department of Nutrition and Dietetics, Agri, Turkey
[4]Sakarya University, Faculty of Medicine, Department of Physiology, Sakarya, Turkey
[5]Ataturk University, Faculty of Medicine, Department of Biochemistry, Erzurum, Turkey
[6]Ataturk University, Faculty of Medicine, Department of Iternal Medicine, Erzurum, Turkey

Abstract

Aim: The aim of this study is to investigate the protective effects of cryptotanshinone on ischemia reperfusion-induced testis damage.

Materials and Methods: In this study, the rats were divided into 3 groups. Groups of the study are planned as follows; sham, ischemia reperfusion, and ischemia reperfusion+cryptotanshinone. Oxidant molecules such as total oxidant status (TOS), malondialdehyde (MDA), myeloperoxidase (MPO) and antioxidant molecules like total antioxidant status (TAS), superoxide dismutase (SOD) with oxidative stress index (OSI) were evaluated in the testicular tissues obtained at the end of the experiment.

Results: In ischemia reperfusion group, TAS and SOD levels decreased while other oxidant parameters were increased. But in ischemia reperfusion+cryptotanshinone group, the levels of anti¬oxidant parameters increased while the levels of oxidant parameters decreased.

Conclusion: These results have shown us that cryptotanshinone is highly effective against oxidative testis damage caused by ischemia reperfusion.

Keywords: Testicular ischemia reperfusion; cryptotanshinone; oxidative stress; inflammation; rat.

INTRODUCTION

Testicular torsion is an emergency that causes extensive damage on the testis and the blood flow must be restored (1-3). When ischemic tissue is reperfused again, increased blood flow causes the formation of free radicals (4,5). Damage occurs after reperfusion following blockage of both arterial and venous systems in the testis, called ischemia reperfusion (I/R) injury (6,7). Following I/R injury, as a result of the release of reactive oxygen species (ROS), structural components such as nucleic acid, lipids and proteins are damaged and cause cellular inflammation and dysfunction. (8). Endogenous antioxidant enzymes like superoxide dismutase (SOD) protect the testicular tissue from I/R injury, but they are insufficient when excessive ROS production occurs (9,10). Oxidative damage caused by I/R has been shown in many tissues and there are studies showing that antioxidant treatment is effective in this damage (11). Therefore, we used cryptotanshinone for antioxidant defense and antiinflammatory properties that we thought it would be effective against I/R.

Cryptotansinone (CT) is a compound obtained from the root of Salvia miltiorrhiza Bunge (Danshen) (12,13). CT has many pharmacological properties including anticancer, antiinflammatory, antioxidative, antidiabetic functions (14). Positive results have been obtained in the treatment of cardiovascular diseases, ulcerative colitis and various inflammatory diseases (15). CT has been proved to be antioxidative (16), and antiinflammatory (17,18) bioactivities. The present study evaluated effects of CT on I/R-induced testicular oxidative damage.

MATERIAL and METHODS

Ethical Approval and Animals

Current experimental study was approved (2019-67) by Atatürk University Experimental Animal Ethics Committee

Received: 13.09.2019 **Accepted:** 19.10.2019 **Available online:** 05.12.2019
Corresponding Author: Ayhan Tanyeli , Atatürk University, Faculty of Medicine, Department of Physiology, Erzurum, Turkey
E-mail: ayhan.tanyeli@atauni.edu.tr

before the experiment. Wistar albino male rats obtained from Atatürk University Experimental Animals Research and Application Center (ATADEM). The experimental study was carried out at ATADEM using healthy male. Rats were housed in cages in laboratory conditions such as 12 hours of light, 12 hours of darkness, humidity of 55 % and a mean temperature of 25 °C. Rats were fed with standard rat feed, and provided drinking water. All animals were deprived of food 12 hours before the experiment, but were allowed to drink water.

Groups and Testicular I/R Model

In the present study, 24 Wistar albino male rats were weighed (250±10 g) and randomly divided into 3 groups.
In sham group, the abdominal area was shaved and cleaned. Also, scrotal area was bilaterally opened with an incision under the anesthesia and closed again without I/R model and any medication. Testicular I/R model was applied as described in Halici et al. method. In I/R group, after anesthesia administration, the rats were fixed in the dorsal horizontal position. The incision area was cleaned with povidone iodine. Scrotal incisions were made in 1-2 cm size. The spermatic cord was detected and the testes were rotated 720 degree in the scrotal area and clamped with microvascular clamp to initiate a 2 h ischemia process. The clamp was removed and testis reperfusion allowed for 2 h. When the reperfusion period is over, testicular tissues were taken rapidly (19).

I/R + CT (50 mg/kg); as a defined in the I/R group, ischemia was induced for 2 hours. Cryptotanshinone was administered intraperitoneally at the dose of 50 mg/kg 30 minutes before reperfusion. After 2 h of ischemia, the clamp was removed and 2-hour reperfusion period started. Finally, testis tissues were kept frozen until the biochemical analysis.

Biochemical Assessments

Total antioxidant status (TAS) was detected with the commercial kit (Rel Assay Diagnostics) Kit. Total oxidant status (TOS) measurement was applied with commercially available kit (Rel Assay Diagnostics). The ratio of total oxidant status to total antioxidant capacity was accepted as the oxidative stress index (OSI). OSI value was calculated as: OSI = [(TOS, μmol H2O2 equivalent L)/(TAS, mmol Trolox equivalent/L) × 10]. We used OSI as another indicator of oxidative stress. SOD evaluation was based on the production of superoxide radicals produced by xanthine and the xanthine oxidase system, which reacts with nitroblue tetrazolium to form formazan dye. The measurements were made as described by Sun et al. (20). The lipid peroxidation levels in testicular samples were measured using the thiobarbituric acid reactive substance method described by Ohkawa and colleagues (21). The activity of myeloperoxidase (MPO) in the testes tissue was estimated according to methods described by Bradley et al. (22).

Statistical Analysis

Normality was verified by Shapiro–Wilk test. The homogeneity of variances was tested using Levene method. Analysis was performed using one-way analysis of variance (ANOVA) for comparison among groups followed by post hoc multiple comparisons with Bonferroni adjustment. All the results were presented as Mean±SD. The differences were accepted significant when P<0.05.

RESULTS

There was no morbidity or mortality in rats during experimental applications. In the I/R group, compared to the sham operation group, TAS (from 1.438±0.256 to 0.700±0.113, p=0.000) level decreased, whereas the TOS (from 7.294±0.720 to 11.304±1.188, p=0.000), OSI (from 0.520±0.102 to 1.634±0.202, p=0.000) levels increased. I/R+CT group, compared to the I/R group, TAS (from 0.700±0.113 to 1.300±0.196, p=0.000) level increased, while TOS (from 11.304±1.188 to 7.876±0.600, p=0.000), OSI (from 1.634±0.202 to 0.622±0.137, p=0.000) levels decreased (see table 1).

Table 1. Mean values of biochemical parameters and comparison among groups

Experimental Groups n=8)	TAS (mmol/L)	TOS (μmol/L)	OSI (arbitrary unit)	SOD (U/mg protein)	MPO (U/g protein)	MDA (μmol/g protein)
Sham operation (I)	1.43±0.25[a]	7.29±0.72[a]	0.52±0.10[a]	429.77±83.24[a]	33808.22±4235.63[a]	223.94±35.754[a]
I/R (II)	0.70±0.11[b]	11.30±1.18[b]	1.63±0.20[b]	186.00±20.66[b]	82998.80±6678.11[b]	410.60±80.83[b]
I/R +CT (III)	1.30±0.19[ac]	7.87±0.60[ab]	0.62±0.13[ac]	413.24±44.59[ac]	35950.77±4297.95[ac]	186.00±20.66[ac]

TAS = Total Antioxidant Status; TOS = Total Oxidant Status; OSI = Oxidative Stress Index; SOD=Superoxide Dismutase; MPO=Myeloperoxidase; MDA=Malondialdehyde. Data are presented as mean ± S.D. a,b and c, p<0.001.

In the I/R group compared to the sham operation group, SOD (from 429.773±83.245 to 186.007±20.669, p=0.000) level decreased, while MPO (from 33808.223±4235.635 to 82998.805±6678.113, p=0.000), MDA (from 223.942±35.754 to 410.608±80.833, p=0.000) levels increased. I/R + CT group, compared to the I/R group, while the level of SOD (from 186.007±20.669 to 413.240±44.596, p=0.000) increased, MPO (from 82998.805±6678.113 to 35950.770±4297.958,p=0.000),MDA(from410.608±80.833 to 186.007±20.669, p=0.000) levels decreased (Table 1).

DISCUSSION

Testicular torsion is one of the urological emergencies associated with impaired arterial and venous circulation

(23). If early intervention is not performed, orchiectomy may be necessary preventing edema and necrosis on the side of the torsion, resulting in infertility (24,25). Studies have shown that SOD and TAS levels decrease and MDA level increases in relation to increased oxidative stress in testicular torsion/detorsion. Some compounds have been shown to protect testicular tissue from damage by normalizing these molecules to normal levels (9,26,27). It has been shown in the literature that spermatogenesis and sexual hormones necessary for spermatogenesis are affected even if torsion is corrected without infarction (28). Although surgical detorsion is performed for these reasons, testicular tissue and spermatogenesis benefit from therapeutic additional treatments (29). Inflammation is an important factor in testicular torsion and causes molecules such as MPO released from leukocytes to play a role in inflammatory cascades such as nitrate and peroxidation phases (30).

Many CT-related studies are available in the literature supporting the results of current study. There are many studies showing that CT reduces damage due to oxidative stress. It prevents the damage in renal epithelium and tubular cells, reactive oxygen species production and apoptosis (31) and also contributes to the recovery of kidney functions (32,33). Cryptotanshinone plays role in antioxidant system by inhibiting tumor necrosis factor-α (TNF-α) induced ROS formation in endothelial cells (34). In a previous study, Zhang et al. reported that Cryptotanshinone increases antioxidant defense in cardiomyocytes and prevents mitochondrial dysfunction (35). In parallel with these studies, in current study, antioxidant and antiinflammatory properties of CT have been shown in testis I/R model in rats. In the I/R group, TAS and SOD decreased while MDA, MPO, TOS, OSI levels were increased and CT treatment reversed these levels.

Due to our results, reduction of MDA, MPO, TOS, OSI levels in testicular I/R model in rats by CT, suggesting that CT alleviated I/R-induced testis injury. We assessed oxidative stress in the testis tissue to investigate the improving effects of the CT against I/R-induced testis injury and observed that oxidative stress decreased with CT. The fact that there is no study related with the protective effects of CT in the literature review of I/R-induced testis injury model makes this study original.

Understanding of cellular damage mechanisms of I/R is important for planning new and effective treatment methods. I/R studies demonstrated that inflammation and oxidative stress suppression can provide significant contributions to the I/R treatment. In this study, inflammation, oxidative stress pathways are suppressed by CT and this encourages hope in the treatment of I/R.

CONCLUSION

These results recommend that CT may protect the testis by diminishing oxidative injury caused by I/R. We have indicate that treatment with CT at single dose reduces I/R-induced testicular damage in experimental animals exposed to I/R. Part of the mechanisms of these protective effects of CT may be caused from supporting the antioxidant capacity via CT. Moreover, further researches are necessarry to explain the other mechanisms on testis injury induced by I/R.

Acknowledgement: There is no financial support organization in the implementation of this study. We would like to thank all participants for contributing in the present survey and also thanks to Kardelen Erdoğan and Yaylagülü Yaman, undergraduates of Atatürk University Nursing Faculty, for their effort, help and support during the experiment
Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: There are no financial supports.
Ethical approval: Current experimental study was approved (2019-67) by Atatürk University Experimental Animal Ethics Committee before the experiment.

Ayhan Tanyeli ORCID: 0000-0002-0095-0917
Ersen Eraslan ORCID: 0000-0002-4000-2815
Fazile Nur Ekinci Akdemir ORCID: 0000-0001-9585-3169
Mustafa Can Guler ORCID: 0000-0001-8588-1035
Derya Guzel Erdoğan ORCID: 0000-0002-7618-5043
Elif Polat ORCID: 0000-0003-0042-4084
Omer Topdagi ORCID:0000-0002-9690-4447

REFERENCES

1. Williamson RC. The continuing conundrum of testicular torsion. The British journal of surgery 1985;72:509-10
2. Kurt O, Yazici CM, Erboga M, et al. Mannitol has a protective effect on testicular torsion: An experimental rat model. J of pediatric urology 2016;12:1-8.
3. Shimizu S, Tsounapi P, Dimitriadis F, et al. Testicular torsion-detorsion and potential therapeutic treatments: A possible role for ischemic postconditioning. International journal of urology : official journal of the Japanese Urological Association 2016;23:454-63.
4. Erol B, Tokgoz H, Hanci V, et al. Vardenafil reduces testicular damage following ischemia/reperfusion injury in rats. The Kaohsiung journal of medical sciences 2009;25:374-80.
5. Turner TT, Bang HJ, Lysiak JL. The molecular pathology of experimental testicular torsion suggests adjunct therapy to surgical repair. The J of urology 2004;172:2574-8.
6. Bodur A, Alver A, Kahraman C, et al. Investigation of N-acetylcysteine on contralateral testis tissue injury by experimental testicular torsion: long-term effect. The American journal of emergency medicine 2016;34:1069-74.
7. Cvetkovic T, Stankovic J, Najman S, et al. Oxidant and antioxidant status in experimental rat testis after testicular torsion/detorsion. International J of fertility & sterility 2015;9:121-8.
8. Tang D, Kang R, Zeh HJ, et al. High-mobility group box 1, oxidative stress, and disease. Antioxidants & redox signaling 2011;14:1315-35.

9. Mestrovic J, Drmic-Hofman I, Pogorelic Z, et al. Beneficial effect of nifedipine on testicular torsion-detorsion injury in rats. Urology 2014;84:1194-8.
10. Rashid S, Ali N, Nafees S, et al. Alleviation of doxorubicin-induced nephrotoxicity and hepatotoxicity by chrysin in Wistar rats. Toxicology mechanisms and methods 2013;23:337-45.
11. Chandra AK, Chatterjee A, Ghosh R, et al. Vitamin E-supplementation protect chromium (VI)-induced spermatogenic and steroidogenic disorders in testicular tissues of rats. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 2010;48:972-9.
12. Zhou L, Zuo Z, Chow MS. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J of clinical pharmacology 2005;45:1345-59.
13. Cheng TO. Cardiovascular effects of Danshen. International J of cardiology 2007;121:9-22.
14. Zhu W, Qiu W, Lu A. Cryptotanshinone exhibits therapeutical effects on cerebral stroke through the PI3K/AKTeNOS signaling pathway. Molecular medicine reports 2017;16:9361-6.
15. Jiang Y, You F, Zhu J, et al. Cryptotanshinone Ameliorates Radiation-Induced Lung Injury in Rats. Evidence-based complementary and alternative medicine : eCAM 2019;1908416.
16. Jin HJ, Li CG. Tanshinone IIA and Cryptotanshinone Prevent Mitochondrial Dysfunction in Hypoxia-Induced H9c2 Cells: Association to Mitochondrial ROS, Intracellular Nitric Oxide, and Calcium Levels. Evidence-based complementary and alternative medicine : eCAM 2013;610694.
17. Maione F, Piccolo M, De Vita S, et al. Down regulation of pro-inflammatory pathways by tanshinone IIA and cryptotanshinone in a non-genetic mouse model of Alzheimer's disease. Pharmacological research 2018;129:482-90.
18. Ma S, Zhang D, Lou H, et al. Evaluation of the anti-inflammatory activities of tanshinones isolated from Salvia miltiorrhiza var. alba roots in THP-1 macrophages. J of ethnopharmacology 2016;188:193-9.
19. Dogan C, Halici Z, Topcu A, et al. Effects of amlodipine on ischaemia/reperfusion injury in the rat testis. Andrologia 2016;48:441-52.
20. Sun Y, Oberley LW, Li Y. A Simple Method for Clinical Assay of Superoxide-Dismutase. Clin Chem 1988;34:497-500.
21. Ohkawa H, Ohishi N, Yagi K. Assay for Lipid Peroxides in Animal-Tissues by Thiobarbituric Acid Reaction. Anal Biochem 1979;95:351-8.
22. Bradley PP, Priebat DA, Christensen RD, et al. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982;78:206-9.
23. Cattolica EV, Karol JB, Rankin KN, et al. High testicular salvage rate in torsion of the spermatic cord. The Journal of urology 1982;128:66-8.
24. Karaguzel E, Kadihasanoglu M, Kutlu O. Mechanisms of testicular torsion and potential protective agents. Nature reviews Urology 2014;11:391-9.
25. Mogilner JG, Lurie M, Coran AG, et al. Effect of diclofenac on germ cell apoptosis following testicular ischemia-reperfusion injury in a rat. Pediatric surgery international 2006; 22:99-105.
26. Ozbek O, Altintas R, Polat A, et al. The protective effect of apocynin on testicular ischemia-reperfusion injury. The Journal of urology 2015;193:1417-22.
27. Sener TE, Yuksel M, Ozyilmaz-Yay N, et al. Apocynin attenuates testicular ischemia-reperfusion injury in rats. J of pediatric surgery 2015;50:1382-7.
28. Turner T, Tung KS, Tomomasa H, et al. Acute testicular ischemia results in germ cell-specific apoptosis in the rat. Biology of reproduction 1997;57:1267-74.
29. Payabvash S, Salmasi AH, Kiumehr S, et al. Salutary effects of N-acetylcysteine on apoptotic damage in a rat model of testicular torsion. Urologia internationalis 2007;79:248-54.
30. Ozturk H, Ozturk H, Gideroglu K, et al. Montelukast protects against testes ischemia/reperfusion injury in rats. Canadian Urological Association journal = Journal de l'Association des urologues du Canada 2010;4:174-9.
31. Zhu R, Wang W, Yang S. Cryptotanshinone inhibits hypoxia/reoxygenation-induced oxidative stress and apoptosis in renal tubular epithelial cells. J of cellular biochemistry 2019.
32. Wang W, Wang X, Zhang XS, et al. Cryptotanshinone Attenuates Oxidative Stress and Inflammation through the Regulation of Nrf-2 and NF-kappaB in Mice with Unilateral Ureteral Obstruction. Basic & clinical pharmacology & toxicology 2018;123:714-20.
33. Wang W, Zhou PH, Hu W, et al. Cryptotanshinone hinders renal fibrosis and epithelial transdifferentiation in obstructive nephropathy by inhibiting TGF-beta1/Smad3/integrin beta1 signal. Oncotarget 2018;9:26625-37.
34. Ran X, Zhao W, Li W, et al. Cryptotanshinone inhibits TNF-alpha-induced LOX-1 expression by suppressing reactive oxygen species (ROS) formation in endothelial cells. The Korean journal of physiology & pharmacology : official journal of the Korean Physiological Society and the Korean Society of Pharmacology 2016;20:347-55.
35. Zhang Y, Chen L, Li F, et al. Cryptotanshinone protects against adriamycin-induced mitochondrial dysfunction in cardiomyocytes. Pharmaceutical biology 2016;54:237-42.