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Original Article

Histopathologic Evaluation of Intraperitoneal Administration of Cerium Oxide Nanoparticles on Ischemia Reperfusion Injury in Rat Testicular Torsion and Detorsion Model

Seyedeh Shima Ashraf-Talesh, Amir Amniattalab*

[1] *Department of Pathology, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran.*

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ABSTRACT

Testicular torsion and detorsion are important clinical problems for infertility in men. In fact, torsion of the spermatic cord is an emergency that results from the rotation of the testis and epididymis around the axis of the spermatic cord. Male factor infertility accounts for up to half of all cases of infertility and affects one man in 20 in the general population. Using a rat testis model, effects of cerium oxide nanoparticles (NCER) were studied on ischemia-reperfusion injury. Eighteen healthy male Wistar rats were used. The animals were subjected to three experimental groups (n = 6): Group Sham: Only laparotomy was performed. Group IS/REP: A 3-hour ischemia and 3-hour reperfusion were performed. Group IS/REP/NCER: The procedure included 3-hour ischemia, 3-hour reperfusion, and 20 µL (0.3 mmol/lit) of NCER 30 min before the cessation of ischemia. Significantly amended development of ischemia/reperfusion tissue injury was observed in animals treated with NCER compared to those of other groups (p = 0.001). Mean values of histomorphometric indices were significantly more improved than those observed for other groups (p = 0.001). Where testicular tissue is exposed to ischemia intraperitoneal administration of NCER could bear clinical benefits in diminishing ischemia-reperfusion injury.

Introduction

Testicular torsion and detorsion are important clinical problems for infertile man. In fact, torsion of the spermatic cord is an emergency that results from rotation of the testis and epididymis around the axis of the spermatic cord. Male factor infertility accounts for up to half of all cases of infertility and affects one man in 20 in the general population.[1] While the annual incidence of testicular torsion is between one in 4,000 males and one in 158 males younger than 25 years, with peaks of incidence occurring in neonates and adolescents entering puberty.[2,3] Urgent operative interventions are required to re-establish the blood flow and circumvent the perpetual damaging effects on the testis which may turn out to be decreased spermatogenesis in majority of cases thus permanently lowering fertility chances.[4]

A suggested pathogenesis of tissue injury in the course of reperfusion is buildup of the activated

* Correspondence to: Amir Amniattalab, Department of Pathobiology, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, 3737, Iran, E-mail: a.amniattalab@iaurmia.ac.ir

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neutrophils that produce reactive oxygen species.[5] Lipid peroxidation in the cell is the most injurious consequence of free radicals that result in decrease in the membrane potential and subsequently, cell injury. Malondialdehyde (MDA), one of the end products of lipid peroxidation, also results in serious cell damage through induction of polymerization and cross linking in membrane components.[6] Free oxygen radicals react with DNA and form 8- hydroxy guanine (8-OHGua) that is one of the damage products of DNA.[7] In spite of the fact that generation of free oxygen radicals occurs continuously in cells, the presence of endogenous antioxidant defense systems preserves tissues from the harmful effects of free oxygen radicals.[8]

Cerium oxide (CeO_2) is a technologically important material due to its properties and applications in several areas that range from engineering to biological sciences.[9] Cerium oxide nanoparticle have found great potential applications as nanofiller due to its low cost, high surface area and quick transformation between Ce^{+3} and Ce^{+4} which enhance its antioxidant properties.[10] CeO_2 nanoparticle is an effective biocide against various bacterial strains.[10-12] The Cerium oxide nanoparticle very low cytotoxic effects on nervous tissue cell lines, making them suitable candidates for various biological applications.[13-15]

The use of nanoparticles besides improving the solubility also provides controlled releasing. The physiologic characteristic of the peritoneal cavity which helps remove toxic metabolites from the body has been successfully exploited to provide peritoneal dialysis in end stage renal disease patients.[16] The same characteristics of the peritoneal membrane also provide a useful portal of entry in the body for several pharmacological agents. One advantage would be that the drug achieves therapeutic efficacy in the region of interest while minimizing the systemic toxicities. Intraperitoneal administration seems more effective and available where oral administration of an agent may cause difficulties. It is clear that transperitoneal absorption of the agent is far faster than oral administration.[17] To the best knowledge of authors, the literature is poor regarding using cerium oxide nanoparticles on testicular ischemia/reperfusion injury. Aimed to study peritoneal effects of cerium oxide nanoparticles on ischemia/reperfusion injury, a study was designed to determine if cerium oxide nanoparticles could in fact protect against ischemia/reperfusion induced testicular damage. The assessments were based on histological parameters.

Materials and Methods

Experimental Information

All chemicals used were of analytical grade, used as received without any further purification and obtained from Sigma-Aldrich.

Preparation of NCER Nanoparticles

The nanoparticles were kindly gifted from Faculty of Chemical Engineering, Sahand University of Technology. The cerium oxide nanoparticles were prepared using a modified method described by others.[18,19] The reagents required for the synthesis namely; Cerium nitrate hexahydrate solution (0.1M) and sodium hydroxide solution (0.3M) were dissolved in deionized water. NaOH solution was added to the precursor solution drop wise for 10 minutes at room temperature under constant stirring. A pinkish white solution was obtained that was centrifuged at 9000 rpm for 15 min. The pellet was collected by discarding the supernatant. The obtained pellet was washed thrice with deionized water. It was then dried at 80 in hot air oven for 30min and then annealed at 270° C for 24 h. The same procedure was followed for the synthesis of CeO_2 nanoparticles, using different surfactants such as 0.05 M SDS, 0.05 M CTAB and 0.05 M PVP. The surfactant was added along with cerium nitrate hexahydrate solution. The structural characterization and size of cerium oxide nanoparticles were studied using field emission scanning electron microscope (FESEM) (Figure 1).

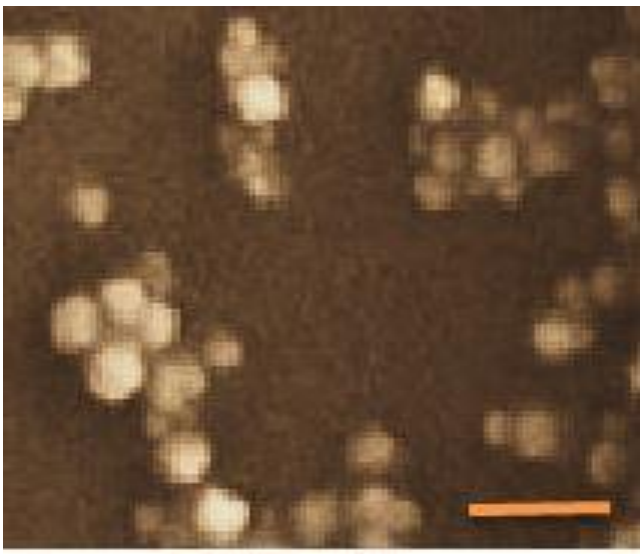

Figure 1. Transmission electron microscopic (TEM) images of cerium oxide nanoparticles. Scale bar: 300 nm.

Design of Study

Two weeks before and during the experiments, the animals were housed in individual plastic cages with an ambient temperature of (23 ± 3)° C, stable air humidity and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. All measurements were made by two blinded observers unaware of the analyzed groups. The present study was designed and modified based on a method described by others.[20] Following randomization of eighteen healthy male Wistar rats ~250 g, the animals were subjected to three experimental groups (n = 6): Group Sham: Only laparotomy was performed. Group IS/REP: A 3-hour ischemia and a 3-hour reperfusion were were performed. Group IS/REP/NCER: The procedure included a 3-hour ischemia, a 3-hour reperfusion and 20 µL (0. 3 mmol/lit) of NCER 30 min before cessation of ischemia. The rats were kept for one week and then the testes were taken for further histopathological assessments. The right testes were transferred to a 10% formaldehyde solution for histopathological assessments.

Surgery

Animals were anesthetized by intraperitoneal administration of ketamine-xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain.[21] The ethical Committee of the Islamic Azad University approved all the experiments under code#. IAU-103670-20/12/2019. Adult male rats were anaesthetized using 20 mg/ml of xylazine and 10 mg/ml of ketamine and the testis was rotated as described.[20]Briefly, the testis was exteriorized through a low midline laparotomy, the gubernaculums were divided and the testis was freed from the epididymo-testicular membrane. The testis was twisted (720°) and kept wet using sterile normal saline soaked gauze. At the appropriate time the testis was counter-rotated to the natural position for reperfusion. Testes were collected at appropriate specified time intervals under the experimental conditions. The animals were euthanized via overdose of anesthetic agents.

Histology

Testes were fixed in 10% buffered formalin for 24 hours. The tissue samples were then processed and embedded in paraffin. A 5-µm semi-thin section was paraffin-embedded. The samples were then dewaxed, rehydrated and stained routinely with hematoxylin and eosin. The sections were then observed under a light microscope. For semithin sections, ovaries were fixed in 2.5% buffered glutaraldehyde and post fixed in 2% OsO_4 for 2 h, dehydrated through an ethanol series and were next stained with hematoxylin eosin. To evaluate histopathological changes of the somniferous tubules for both experimental and control testis, the degree of damage for each section was evaluated.[22] Using 10× objective lens, ten tubules were scored for each sample. Seminiferous tubule profiles were evaluated and each was given a score from 1 to 10 on the basis of the criterion based on a scoring system described in previous studies (Table 1).[23] Scoring was based on 10 tubule profiles for each testis and the average for each score was recorded. Scores below 10 were considered evidence of impaired spermatogenesis.

Statistical Analysis

Data were analyzed by a commercially available Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) program for Windows software. *p*-values < 0.05 were regarded as statistically significant. One-way Analysis of Variance (ANOVA) test was performed and post hoc multiple comparisons were done with least-squares differences (LSD).

Results

Histology

Figure 2 shows the effects of ischemia/reperfusion on spermatogenesis in testicular tissue. The level of damage caused by testicular torsion/ detorsion compared to the SHAM and testicular biopsy scores for the testicular sections from experimental groups are also presented. Sham operated rats demonstrated normal seminiferous tubule morphology and no obvious damage was inflicted on the testis of the control sham operated group of experimental animals (Figure 2A). This was demonstrated by the high testicular biopsy score of 9.3 ± 0.5 (mean ± SD) recorded for this group (Table 1). Cross sections from testes of IS/REP group showed seminiferous tubules with sloughing and disorganization. Damage was at the spermatozoa, spermatids and some spermatocytes level. The interstitial was disorganized and contained some blood (Figure 2B.). The testicular biopsy score of

7.3 ± 0.5 (mean ± SD) was observed (Table 2). In IS/REP/NCER, seminiferous tubules showed normal spermatogenesis, exhibiting all stages of spermatogenic cells including abundant spermatozoa. Some sloughing and tissue disorganization were observed (Figure 2C). The testicular biopsy score of 8.3 ± 0.6 (mean ± SD) was observed that was significantly different from those of other experimental groups (p = 0.001) (Table 2).

Table 1. Scoring system for testicular biopsy for the testicular sections based on others [23].

Score	Explanations
1	There are no cells in the tubule.
2	Only Sertoli cells are observed
3	The only germ cells observed are spermatogonia
4	Few spermatocytes (less than 5-10 per tubular cross-section) are observed; however, no spermatids or spermatozoa are observed
5	Many spermatocytes are observed; however, no spermatids or spermatozoa are observed
6	Few spermatids (less than 5-10 per tubular cross-section) are observed; however, many spermatocytes are observed.
7	No spermatozoa are observed; however, many round and elongated spermatids are observed.
8	Few spermatozoa (less than 5-10 per tubular cross- section) are observed, however, many round and elongated spermatids are observed.
9	Many spermatozoa, however, the germinal epithelium is disorganized with marked sloughing.
10	Complete spermatogenesis with many mature and shed spermatozoa is observed. The germinal epithelium is originated normally and an open lumen is observed.

Table 2. Scores of testicular biopsies for the testicular sections from experimental groups. 10 tubule profiles for each testis were basis for scoring. Scores below 10 were considered as impaired spermatogenesis.

Scores	Experimental groups		
	Sham	**IS/REP/**	**IS/REP/NCER**
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	2	2
7	-	2	3
8	1		
9	4	3	4
10	5	3	4
Mean ± SD	9.3 ± 0.5	7.3 ±0.5	8.3 ± 0.6*

*p = 0.001 vs. other experimental groups.

Discussion

In the present study, it was investigated whether intraperitoneal administration of cerium oxide nanoparticles was useful or not in the prevention of ovarian damage in ischemia/reperfusion conditions in rat testes and it was found to have beneficial effects. Histopathological assessments were performed in Sham, IS/REP,IS/REP/NCER groups. Scoring system for testicular biopsy for the testicular sections were performed. The scores for IS/REP/NCER were significantly different from those of IS/REP group showing that the cerium nanoparticles could improve damages induced by ischemia.

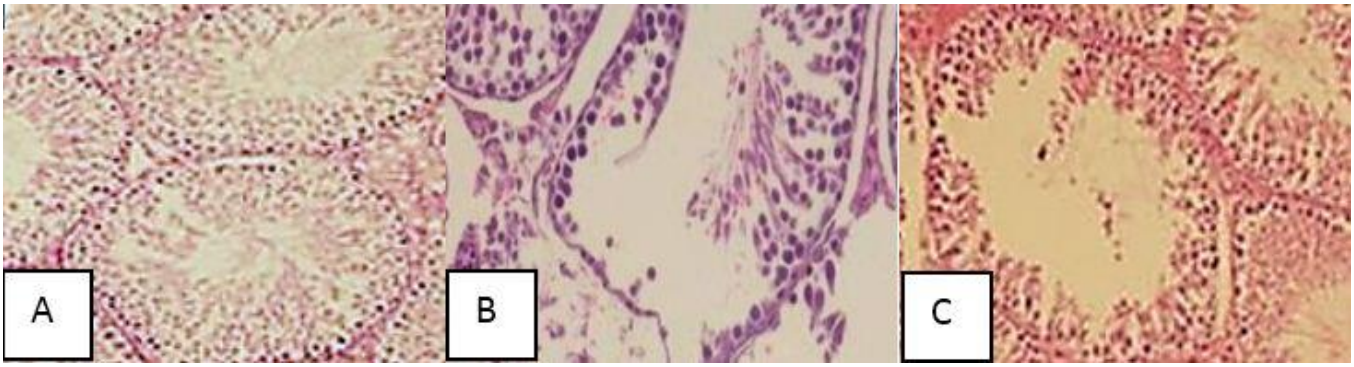

Figure 2. Histologic micrographs of the testicular tissue in Sham (A), IS/REP (B), IS/REP/NCER (C) groups. **A:** Photomicrograph of a cross section of testis from Sham group. Seminiferous tubules showing normal spermatogenesis, exhibiting all stages of spermatogenic cells including abundant spermatozoa. **B:** Photomicrograph of a cross section from IS/REP group. Seminiferous tubules showing sloughing and disorganization. Damage is at the level of spermatozoa, spermatids, spermatocytes and spermatogonia. The interstitial is disorganized and contains some blood. Sertoli cells formed multinuclear giant cells. Some tubules are affected at the level of Sertoli cells. **C:** Photomicrograph of a cross section of testis from IS/REP/NCER group. Seminiferous tubules showing normal spermatogenesis, exhibiting all stages of spermatogenic cells including abundant spermatozoa. Some sloughing and tissue disorganization are noted (H&E, 10×).

Testicular torsion is a urological emergency that induces biochemical and morphological changes.[24] Testicular torsion can affect males of any age but it occurs more often in neonates, boys and young men.[25] To our knowledge the impact on prognosis of age at testicular torsion is unknown. The prognosis of testicular torsion is related to the duration and degree of torsion, resulting in different levels of parenchymal injury by oxidative stress.[26] Therefore, beyond rapid diagnosis and treatment several methods have been investigated to minimize the injury caused by testicular torsion.[27,28] Although rat testes differ somewhat from human testes, rats have been widely used as experimental models in testicular torsion studies because lesions in rat testes are comparable to those in human testes after torsion.[29]

Several antioxidants have been investigated with promising results in rats submitted to testicular torsion.[30-33] Of these antioxidants resveratrol and arginine have shown good results when used in testicular ischemia and reperfusion situations.[34,35] Arginine, an amino acid with antioxidant properties, is important for nitric oxide synthesis.[36] Resveratrol is a potent antioxidant present in many food sources that has inhibitory activity against reactive oxygen species and also enhances nitric oxide bioavailability.[37]

It has been demonstrated that cerium oxide nanoparticles showed protective effect on hepatic ischemia reperfusion injury by injecting intravenously 0.5 mg/kg of 10–30 nm spherical nanoparticles into Sprague Dawley rats 1 h prior to inducing hepatic ischemia in left lateral and median lobes. Rats injected with cerium oxide nanoparticles were shown to decrease the hepatic ischemia reperfusion injury induced levels of alanine aminotransaminase and lactate dehydrogenase in serum accompanied by a significant decrease in hepatic ischemia reperfusion injury induced hepatic injury score, hepatocyte necrosis and several serum inflammatory markers including macrophage derived chemokine, macrophage inflammatory protein-2, KC/GRO, myoglobin and plasminogen activator inhibitor-1.[38]

Substances are administered by a wide variety of routes. A key factor determining the route selected is whether the agent is being administered for a local or systemic (either enteral or parenteral effect. Parenteral administration methods typically produce the highest bioavailability of substances because these methods avoid the first-pass effect of hepatic metabolism, which occurs commonly with orally administered chemicals and therapeutics. Intraperitoneal administration seems more effective and available where oral administration of an agent may cause difficulties. It is clear that trans-peritoneal absorption of the agent is far faster than oral administration.[39] It seems time saving is very important in emergency conditions like ovarian torsion.

In conclusion, histopathological results obtained from all the experimental groups indicated that intraperitoneal administration of cerium oxide nanoparticles could be helpful in minimizing ischemia-reperfusion injury in testicular tissue exposed to ischemia. Regarding the trans-peritoneal absorption of the cerium oxide nanoparticles that is far faster than its oral administration, it could be considered in clinical practice where that testicular torsion is the case and testicular functions must be resumed as early as possible to preserve and prevent future infertility. The present study demonstrated that intraperitoneal administration of cerium oxide nanoparticles could improve ischemia-reperfusion injury in testicular tissue exposed to ischemia.

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Conflict of Interest

The authors declare no conflict of interest.

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