Protective Effects of Zofenopril on Testicular Torsion and Detorsion Injury in Rats

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Purpose: To investigate the protective effect of zofenopril on torsion/detorsion-induced biochemical and histopathological changes in experimental testicular ischemia or reperfusion injury in rats.

Materials and Methods: A total of 35 prepubertal male Wistar-Albino rats were divided into five groups, including 7 rats in each group: Group I (sham, S), sham operation; group II (torsion/detorsion-early orchiectomy, T/D-E), 2 hours ischemia and 4 hours reperfusion; group III (torsion/detorsion-late orchiectomy), T/D-L), 2 hours ischemia and 5 days reperfusion; group IV (zofenopril-early orchiectomy, Z-E), 2 hours ischemia, 4 hours reperfusion, and a single dose of zofenopril; and group V (zofenopril-late orchiectomy, Z-L), 2 hours ischemia, 5 days reperfusion, and 5 doses of zofenopril. We determined the tissue levels of malondialdehyde, nitric oxide, glutathione peroxidase, and superoxide dismutase enzyme activities. Histopathologically, mean seminiferous tubule diameter measurements were used.

Results: Malondialdehyde (3.490 ± 0.89 versus 1.729 ± 0.25 in early period; 3.837 ± 1.694 versus 1.694 ± 0.47 in late period) and nitric oxide levels (3.507 ± 0.44 versus 2.853 ± 0.54 in early period; 4.010 ± 0.72 versus 2.446 ± 0.29 in late period) significantly reduced and glutathione peroxidase (0.012 ± 0.001 versus 0.017 ± 0.001 in early period; 0.013 ± 0.002 versus 0.018 ± 0.001 in late period) and superoxide dismutase enzyme activities (58.030 ± 5.97 versus 70.773 ± 3.85 in early period; 57.421 ± 7.81 versus 76.329 ± 4.09 in late period) significantly increased in the testis tissue in zofenopril pretreated groups compared to group T/D both in early and late period ($P < .05$). The mean seminiferous tubule diameter was significantly better in pretreated group (210.33 ± 17.32) than group T/D (185.02 ± 22.45) only in late period ($P < .05$), but not in early period (209.38 ± 30.40 versus 208.21 ± 13.57; $P > .05$).

Conclusion: Treatment with zofenopril decreased damage in ipsilateral testis caused by ischemia/reperfusion, and clinical application of zofenopril might be a new approach for the treatment of testicular torsion in addition to conventional detorsion.

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INTRODUCTION

Testicular torsion is a common urologic emergency condition usually affecting newborns, children, and adolescent boys. Early diagnosis and immediate treatment are crucial for the preservation of the sperm production and fertility. It seems that the main pathophysiology of testicular torsion/detorsion is ischemia/reperfusion (I/R) injury of the testis.[1,2]

These I/R injuries are associated with the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), with the return of blood flow following a period of ischemia, as shown in other organs, such as the brain, myocardium, kidneys, and testes.[3] It has been demonstrated that ROS increase in the areas of ischemia and reperfusion, and is thought to play a crucial role in the loss of ipsilateral testicular spermatogenesis.[4]

Zofenopril, a derivative of the proline amino acid and an inhibitor of angiotensin-converting enzyme and angiotensin II,[5] ameliorates experimental cardiac and renal I/R injury or doxorubicin-induced cardiac injury in animal models[6,7] and has beneficial cardiovascular effects in patients with myocardial infarction.[8] Mak and colleagues demonstrated that angiotensin-converting enzyme inhibitor agents, including zofenopril, can protect endothelial cells against free radical-induced lipid peroxidation and cell injury.[9]

The successful result of zofenopril in different organs led us to the use of this treatment in the model of testicular torsion. The aim of this study was to investigate the protective effect of zofenopril, on torsion/detorsion-induced biochemical and histopathological changes in experimental testicular I/R injury.

MATERIALS AND METHODS

Animals and Reagents

The experimental protocol was approved by the Ethics Committee. This study was carried out on 35 prepubertal male Wistar-Albino rats (170 to 220 g). The experimental animals were housed at room temperature under a 12 h light/12 h dark cycle and had free access to both tap water and standard pellet diet for rats. Zofenopril (Zoprotec, Menarini Group, Italy) was given orally (15 mg/kg/day).

Experimental Groups

A total of 35 rats were randomly divided into five groups (n = 7). Group I (sham, S), sham operation; group II (torsion/detorsion-early orchiectomy, T/D-E), 2 hours ischemia and 4 hours reperfusion; group III (torsion/detorsion-late orchiectomy), T/D-L), 2 hours ischemia and 5 days reperfusion; group IV (zofenopril-early orchiectomy, Z-E), 2 hours ischemia, 4 hours reperfusion, and a single dose of zofenopril; and group V (zofenopril-late orchiectomy, Z-L), 2 hours ischemia, 5 days reperfusion, and 5 doses of zofenopril.

Surgical Procedure

The rats were anesthetized with intraperitoneal ketamine injection (50 mg/kg). All operations were performed under sterile conditions. The scrotum was entered through a scrotal midline incision. The tunica vaginalis was opened, and the left testis was delivered to the surgical field. The left testis was rotated 720° in a clockwise direction and then left in the same position by fixing it to the scrotum with a 4–0 silk suture.[10-13] The ischemia period was 2 hours and orchiectomy was performed after 4 hours of detorsion in the early orchiectomy model. In the late orchiectomy model, the ischemia period was 2 hours and orchiectomy was performed after 5 days of detorsion. In the early orchiectomy groups, zofenopril (15 mg/kg/day, po) was administered only once, 30 minutes prior to detorsion. In the late orchiectomy groups, zofenopril (15 mg/kg/day, po) was administered 30 minutes before detorsion, once daily for 5 days. In order to be consistent, all the control animals (both the early and late orchiectomy models) were gavaged with pro-rated volumes of normal saline. At the end of the study, ipsilateral orchiectomy was performed to determine biochemical and histopathological changes in all groups.

Biochemical Analysis

All testes tissue specimens were washed with 0.9% NaCl to remove hematoma and then air dried. They were stored in plastic bottles individually at -20°C until biochemical analyses were carried out.

The testicular tissue was homogenized with 1.5% potassium chloride to make a 10% homogenate using a glass homogenizer. Testicular malondialdehyde (MDA) in tissue homogenate was measured using the thiobarbituric acid reactive substance assay, as described by Ohkawa and associates.[14] The principle of the method is based

on measurement of the concentration of the pink chromogen compound that forms when MDA reacts with thiobarbituric acid. The MDA level is expressed as nanomoles per milligram protein.

Nitric oxide (NO) measurement was performed using the Griess method for detection of nitrite levels.[15] Nitric oxide is unstable and has a short lifetime; in the presence of oxygen, it reacts rapidly to form nitrite or nitrates. Since the direct determination of NO radicals is difficult, and since nitric oxide synthase (NOS) activity can only be determined in tissue or cell homogenates, the determination of nitrite, the stable end product of NO radicals, is most often used as a measure of NO production.

Superoxide dismutase activity in the tissue was measured according to the method described by Fridovich.[16] This method employs xanthine and xanthine oxidase to produce superoxide radicals that react with p-iodonitrotetrazolium violet to form a red Formazon dye, which was measured at 505 nm. Superoxide dismutase activity was expressed in units per milligram of protein (units/mg).

Glutathione peroxidase (GPx) activity was measured by the method of Paglia and Valentine.[17] The enzymatic reaction in the tube, which contained nicotinamide adenine dinucleotide phosphate, reduced glutathione, sodium azide, and glutathione reductase, was initiated by addition of H_2O_2, and the change in absorbance at 340 nm was monitored by a spectrophotometer.

Histopathological Evaluation

The testicular tissue was fixed in Bouin's solution, post fixed in 70% alcohol, and embedded in a paraffin block. A 5-μm section was obtained, deparaffinized, and stained with Hematoxylin and Eosin. Histological evaluation using the light microscope was done by an observer in a blind, randomly numbered fashion without any knowledge of which testis had or had not undergone torsion.

The mean seminiferous tubular diameter (MSTD) was estimated by measuring ten separate roundest seminiferous tubules with a microscope-adaptable micrometer for each testis.

Statistical Analysis

Statistical analyses were accomplished using the SPSS software (the Statistical Package for the Social Sciences, Version 15.0, SPSS Inc, Chicago, Illinois, USA). All results were reported as mean ± SD. Differences between groups of continuous data were compared by Mann–Whitney *U* test. *P* values less than .05 were considered statistically significant.

RESULTS

Biochemical Results

Malondialdehyde and NO levels, and SOD and GPx activities from the testicular tissue samples are demonstrated in Table. The MDA and NO levels were significantly higher in the T/D-E and T/D-L groups compared to the sham group ($P < .01$).

Tissue MDA, SOD, GPx, and NO levels and MSTD values of all groups*

	MDA (nmol/mg protein)	SOD (units/mg protein)	GPx (units/mg protein), µm	NO (units/mg protein)	MSTD
S	1.327 ± 0.30	76.359 ± 7.05	0.018 ± 0.001	2.353 ± 0.29	205.17 ± 11.21
T/D-E	3.490 ± 0.89[a]	58.030 ± 5.97[a]	0.012 ± 0.001[a]	3.507 ± 0.44[a]	208.21 ± 13.57
T/D-L	3.837 ± 1.52[b]	57.421 ± 7.81[b]	0.013 ± 0.002[b]	4.010 ± 0.72[b]	185.02 ± 22.45[b]
Z/E	1.729 ± 0.25[c]	70.773 ± 3.85[c]	0.017 ± 0.001[c]	2.853 ± 0.54[c]	209.38 ± 30.40
Z/L	1.694 ± 0.47[d]	76.329 ± 4.09[d]	0.018 ± 0.001[d]	2.446 ± 0.29[d]	210.33 ± 17.32[d]

*MDA indicates malondialdehyde; SOD, superoxide dismutase; GPx, glutathion peroxidase; NO, nitric oxide; MSTD, mean seminiferous tubular diameter; group S, sham operation; group T/D-E, 2 hours ischemia and 4 hours reperfusion; group T/D-L, 2 hours ischemia and 5 days reperfusion; group Z-E, 2 hours ischemia, 4 hours reperfusion, and a single dose of zofenopril; and group Z-L, 2 hours ischemia, 5 days reperfusion, and 5 doses of zofenopril.
£ The values are expressed as mean ± SD, *n* = 7 for each group.
a *P* < .01 when T/D-E compared with S
b *P* < .01 when T/D-L compared with S
c *P* < .05 when Z/E compared with T/D-E
d *P* < .05 when Z/L compared with T/D-L

In groups Z-E and Z-L, there was significant reduction of the ipsilateral testicular MDA and NO levels after zofenopril treatment both in early and late period ($P < .05$). The GPx and SOD activities of ipsilateral testes in groups T/D-E and T/D-L reduced in comparison with group S ($P < .01$). In the treatment groups, groups Z-E and Z-L, the SOD and GPx activities increased after treatment with zofenopril ($P < .05$).

Histopathological Results

The values of MSTD of each group are listed in Table. After testicular I/R, the MSTD from the group T/D-L significantly decreased in the ipsilateral testes when compared with the group S ($P < .01$). But in the group T/D-E, the value of MSTD did not reduce.

The findings of the histopathological evaluation for each group are shown in Figures 1 to 5. The testes of rats in group S indicated the presence of normal testicular structure and uniform seminiferous tubular morphology with normal spermatogenesis and the presence of primary and secondary spermatocytes, spermatids, and spermatozoa (Figure 1). In the I/R group, there was a significant reduction in the seminiferous tubular diameter. Furthermore, there were severe distortion of tubules and presence of peritubular fibrosis. These findings were observed only in the late orchiectomy model (Figure 2), but not in the early orchiectomy model (Figure 3). Zofenopril-treated animals showed an improved histological appearance in the left testis in the Z-L group compared with T/D-L group ($P < .05$).

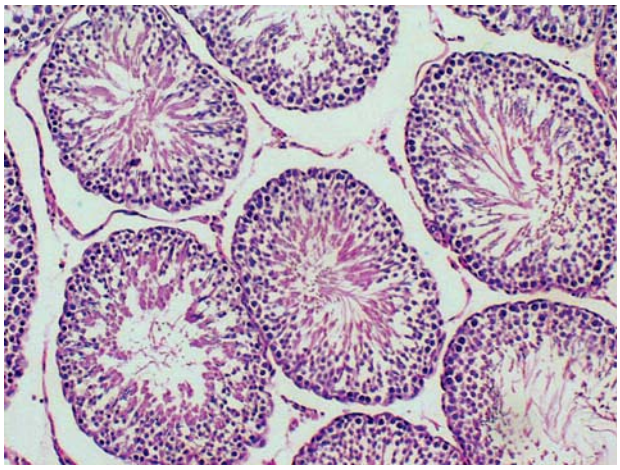

Figure 1. Histologic findings of ipsilateral testes in sham-operation

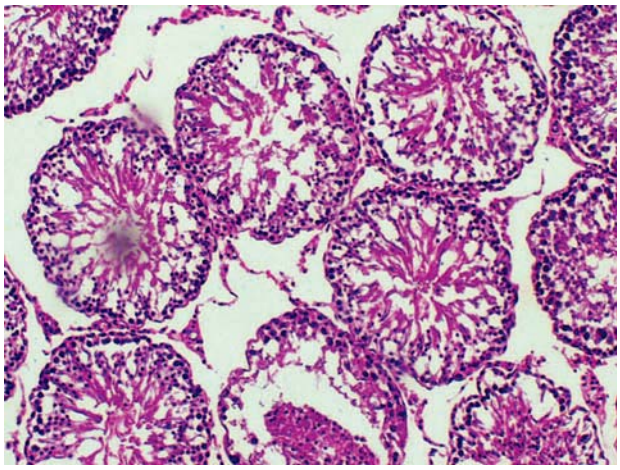

Figure 3. Histologic findings of ipsilateral testes in torsion/detorsion-early orchiectomy

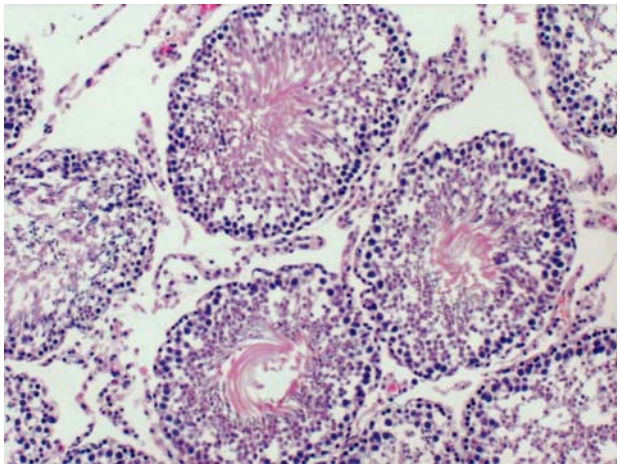

Figure 2. Histologic findings of ipsilateral testes in torsion/detorsion-late orchiectomy

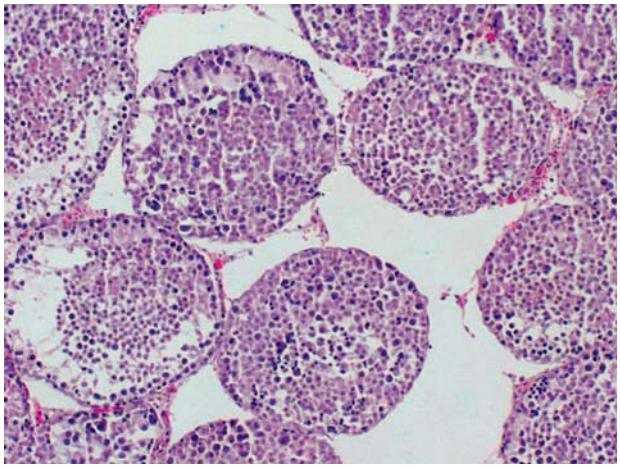

Figure 4. Histologic findings of ipsilateral testes in zofenopril pretreatment-late orchiectomy (Hematoxylin and Eosin, 100×)

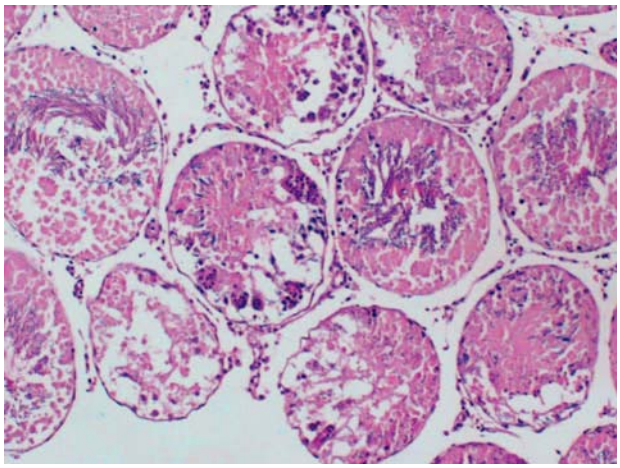

Figure 5. Histologic findings of ipsilateral testes in zofenopril pretreatment-early orchiectomy

Administration of zofenopril caused significant rescue of testicular function by preserving the intact seminiferous tubular morphology in the left testis (Figure 4). On the other hand, in Z-E group, treatment with zofenopril led to findings similar to those of the T/D-E group (Figure 5).

DISCUSSION

Testicular torsion is a surgical emergency that needs prompt intervention to torsioned gonad. Late presentation or failures in diagnose or inadequate management will lead to testicular injury.[18] Ischemia-reperfusion injury to the testes is associated with overgeneration of ROS, such as hypochlorous acid, nitric oxide, hydrogen peroxide, superoxide anion, hydroxyl radicals, and so forth.[19] Mammalian testes are highly susceptible to oxidative stress.[1,12] High concentrations of ROS play an important role in the pathophysiology of damage to human spermatozoa.[19] The ROS are difficult to quantify directly in tissue because of their high reactivity and short half-life. [12,20]

Malondialdehyde is used widely as an oxidative stress indicator in tissues induced by I/R.[20] Malondialdehyde is the stable final product of lipid peroxidation produced by ROS and is a well-known parameter for determining the increased free radical formation in reperfused tissue.[21] Many studies showed that MDA levels in testicular tissue increase after testicular injury.[10-13,22] The level of MDA significantly increased in the T/D group when compared to the sham group. Our findings agree with these testicular torsion studies. Furthermore, pretreatment with zofenopril (15 mg/kg/day) prevented lipid peroxidation, resulting in decreased MDA accumulation.

Nitric oxide is an important mediator of cell death either through apoptosis or necrosis, depending on the duration and severity of injury.[23] Our study showed that I/R injury due to testicular torsion/detorsion increased NO production in ipsilateral testicular tissue. Treatment with zofenopril before reperfusion prominently decreased the concentration of testicular NO level when compared with the sham group.

Enzymatic antioxidant defense systems, such as SOD and GPx, protect tissues from ROS and oxidative damage.[11] Superoxide dismutase and GPx are major enzymes that scavenge harmful ROS in male reproductive organs. Superoxide dismutase, one of the major intracellular antioxidant enzymes, is a potent protective enzyme that can selectively and rapidly reduces O_2 to H_2O_2. The GPx system constitute the first step of antioxidant defense system in I/R injury in the testis tissue, among the antioxidant defense enzymes.[22] Glutathione peroxidase catalyzes the conversion of H_2O_2 to H_2O. This balance is disrupted under high oxidative stress, such as reperfusion injury. In our study, we observed that the level of SOD and GPx activities significantly decreased by I/R injury due to testicular torsion/detorsion in testicular tissue. Previous studies have shown the same findings that I/R leads to inactivation of antioxidant enzymes in rat testes.[24,25] Pretreatment with zofenopril significantly increased SOD and GPx activity after testicular torsion.

Mogilner and colleagues concluded that ischemia leads to histological damage in the ipsilateral testis.[26] In this study, we evaluated testicular damage by observing changes in tubular architecture. Using a rat model, it has been demonstrated that testicular torsion/detorsion caused a prominent reduction in MSTD. In our experiment, we observed morphologic changes in ipsilateral testis following unilateral testicular torsion/detorsion.

According to our biochemical and histological results, treatment with zofenopril decreased damage in ipsilateral testis caused by I/R. It can be explained that zofenopril had protective effects against I/R injury by reducing the production of free radicals, scavenging free radicals, and preventing inflammation.

Various antioxidants and free radical scavengers have been proposed in recent years for treatment of testicular torsion-induced male infertility. Moreover, application of some antioxidants and ROS scavengers, such as SOD, catalase, allopurinol,[27] caffeic acid phenethyl ester,[28] melatonin,[29] selenium,[10] resveratrol,[30] and N-acetylcysteine[11] have been shown to prevent I/R injury in testes. However, none have been tested and confirmed the efficacy in clinical trials.

Zofenopril has been used as an antihypertensive drug in humans, and it may have the clinical applicability in patients with testicular torsion. The use of this drug in cardiac patients clinically without significant side effects and successfully in previous experimental I/R injury in the heart and kidney can make its use in testicular torsion more attractive. According to the results presented in this study, it is reasonable to propose that clinical application of zofenopril might be a new approach for the treatment of testicular torsion in addition to conventional detorsion.

CONCLUSION

In conclusion, exogenous administration of zofenopril reduced oxidative damage biochemically in the early and late stage and histopathologically only in the late stage of testicular torsion/detorsion in our rat model. Antioxidant treatment with compounds, such as zofenopril, may contribute to the salvage of surgically untwisted testis, and zofenopril might play a role in the treatment of testicular torsion in the future. Further studies should be performed in the models comparable to clinical testicular T/D cases, both from the point of timing of administration and the dosage needed.

CONFLICT OF INTEREST

None declared.

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