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Higenaminin İskemi Reperfüzyon Tarafından İndüklenen Testis Hasarına Etkileri: Biyokimyasal Bir Çalışma

The Effects of Higenamine on Testicular Damage Injured by Ischemia Reperfusion: A Biochemical Study

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Öz: Amaç: Bu araştırmanın amacı, higenaminin iskemi reperfüzyonunun neden olduğu testis hasarındaki koruyucu etkilerini incelemektir.
Gereç ve Yöntem: Deneyimizde, sıçanlar sham kontrol, iskemi reperfüzyon ve iskemi reperfüzyon + higenamin grupları olmak üzere 3 gruba ayrıldı. Bazı oksidan, antioksidan ve inflamatuar parametreler deney sonunda elde edilen testis dokularında değerlendirildi.
Bulgular: Mevcut çalışmada, iskemi reperfüzyon grubunda oksidan ve inflamatuar parametrelerin arttığını ve antioksidan parametrelerin azaldığını, ancak tek doz higenaminin uygulanmasının, iskemi reperfüzyon tarafından indüklenen testis oksidatif hasarına karşı koruyucu olduğu öne sürülen tedavi grubunda antioksidan parametrelerin arttığını ve oksidan parametrelerinin azaldığını gözlemledik.

Anahtar Kelimeler — İskemi reperfüzyon, higenamin, testis, oksidatif stres, inflamasyon, sıçan.

Abstract: Purpose: The purpose of this research is to examine protective effects of higenamine on testis injured by the ischemia reperfusion.
Material and Method: In our experiment, the rats were separated as 3 groups including sham control, ischemia reperfusion, and ischemia reperfusion+higenamine. Some oxidant, antioxidant and inflammatory parameters were evaluated in testis tissues obtained at the end of the experiment.
Findings: In current study, we observed that the oxidant and inflammatory parameters increased and antioxidant parameters decreased in the ischemia reperfusion group but the antioxidant parameters increased and oxidant parameters decreased in treatment group suggesting that single dose administration of higenamine is protective against testicular oxidative damage resulted from ischemia reperfusion.

Keywords — Ischemia reperfusion, higenamine, testis, oxidative stress, inflammation, rat.

1.INTRODUCTION

Testicular torsion is an emergency that can occur in young fertile males and causes testicular damage. In testicular torsion, the duration and degree of torsion are important in determining the severity of the damage (1; 2). Damage after torsion occurs during both ischemia and reperfusion processes due to disruption of tissue oxygenation in ischemia and release of reactive oxygen species (ROS) by re-supply of blood flow in reperfusion (3-5). Detorsion can

therefore increase the damage to the testicles. Inflammatory cells and immature sperm cause the formation of unstable ROS molecules with unpaired electrons (6). Increased ischemia and inflammation can cause increment in ROS synthesis (7). Antioxidant endogenous enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which increase in tissue by reperfusion, help to protect the tissue from oxidant stress (8; 9).

Higenamine (1-[(4-hydroxyphenyl) methyl]-1,2,3,4-tetrahydroisoquinoline-6,7-diol) is a phenolic alkaloid obtained in 1976 from the aconite radical (10). It is a powerful antioxidant because it prevents the formation of inflammation, apoptosis and thrombosis (11-14). Higenamine is regarded as a traditional cardiostimulant and anti-inflammatory in China. According to published reports, higenamine possesses a variety of pharmacological properties, including dilation of blood vessels and bronchi, immunomodulation and antioxidation. This study was planned to investigate the protective effect of higenamine against testicular oxidative damage caused by ischemia reperfusion (I/R).

2. MATERIALS AND METHODS

Ethical Approval and Animals

This experimental study was approved (28.03.2019/63) by Atatürk University Experimental Animal Ethics Committee before the experiment. Our experimental study was carried out at Atatürk University Experimental Animals Research and Application Center using healthy male Wistar-albino rats weighing 260-290 g, obtained from Atatürk University Experimental Animal Research and Application Center. 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.

Experimental Design and Groups

The 24 rats used in our study were weighed and divided into 3 groups, including 8 rat in each group;

Sham Control Group; in this group, each rat was fixed to the surgical setup in a supine position. Later, the abdominal midline incision was opened using sterile tecniques and the incision area closed again without performing testis I/R model.

I/R Group; in this group, the abdominal field was shaved and washed via 10 % povidone-iodine, and a 2-cm midline abdominal incision was performed using sterile techniques. It was

performed laparotomy under anesthesia. Spermatic cord was found and clamped to create ischemia for 2 hours. The clamp was removed and testis reperfusion allowed for 2 hours. At the end of the reperfusion period, testis tissue samples were rapidly taken.

I/R + higenamine 10 mg/kg; as a defined in the I/R group, ischemia was induced for 2 hours by clamping. Higenamine was administered intraperitoneally at dose of 10 mg/kg 30 minutes before reperfusion. Then the clamp was removed and the reperfusion period started. At the end of the experiment, testis tissues were taken. Rats were sacrificed by high-dose anesthesia. All the experimental steps were complated under controlled and continuous general anesthesia (ketamin/ksilazin 60/10 mg/kg bw, intraperitoneally). At the end of the reperfusion, tissue samples were taken from the section of the testis tissue exposed to I/R and purified by washing with normal cold saline.

Biochemical Analysis

Tissue samples were weighed for 100 mg and homogenized with 2 mL of phosphate buffer. Homogenized tissues were centrifuged at 5000 rpm for 20 minutes at +4 °C and the supernatants were carefully transferred to ependorfs and maintained at -80 °C. The principle of measurement of MDA, as a result of lipid peroxidation, is based on measuring the absorbance at 532 nm of the pink color compound formed as a result of the reaction of malondialdehyde (MDA) and thiobarbituric acid (TBA) (15). Total antioxidant status (TAS) value was determined with the commercially available kit (Rel Assay Diagnostics). Total oxidant status (TOS) measurement was performed with commercially available kit (Rel Assay Diagnostics). The ratio of TOS to TAS was accepted as the oxidative stress index (OSI). OSI value was calculated as follows: OSI = [(TOS, μmol/L)/(TAS, mmol/L) × 10] (16). The activity of myeloperoxidase (MPO) measurement is based on the kinetic measurement of the absorbance at 460 nm wavelength of the yellowish-orange colored complex in result the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide (17). The xanthine oxidase enzyme catalyzes the formation of uric acid from xanthine. The resulting superoxide radical forms the molecular oxygen and hydrogen peroxide with the superoxide dismutase enzyme. The resulting superoxide reacts with the tetrazolium salt to form a formazan dye in situations where the effect of the SOD enzyme is insufficient, and the SOD activity is measured with the inhibition degree of this reaction (18).

Statistical Analysis

TAS, TOS, OSI, MPO, SOD and MDA results obtained from our study were analyzed using a statistical analysis (SPSS 21, USA) program. Descriptive statistics of the values in the groups

were expressed as mean ± SD. P value was taken as 0.05. Shapiro Wilk test was used for the assumption of normality test. One-Way ANOVA test was used for normal distribution and Tukey test was used for post hoc paired comparisons. Kruskal Wallis test, which is a nonparametric test, was used for the parameters that do not conform to normal distribution. When the significance was determined, the Mann Whitney U test was performed.

3.RESULTS

Table 1 shows the concentrations of TAS, TOS, MPO, SOD, MDA and level of OSI in testis tissue in the sham control group, I/R and I/R + higenamine groups. Compared with the sham control group, the concentrations of TOS, MPO, MDA and level of OSI were significantly increased, while TAS and SOD values were decreased in the I/R group. When the sham control and treatment groups were compared, no statistical significance was found in the biochemical parameters. Compared with the I/R group, TOS, OSI, MPO, and MDA, oxidant parameters, were significantly decreased and TAS and SOD, antioxidant parameters, were significantly increased in I/R + higenamine group (Table 1, p=0.00).

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 control group (I)	1.337±0.094	7.943±0.677	0.597±0.079	395.743±86.700	36907.947±6754.149	213.379±28.742
I/R (II)	0.762±0.094	10.812±0.880	1.436±0.191	193.524±22.670	83630.530±8338.628	403.037±74.827
I/R + higenamine (III)	1.253±0.084	8.304±0.665	0.667±0.090	390.099±57.830	36865.317±8157.824	218.914±30.215
p value (Meaningful intergroup comparisons)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)

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. $p<0.05$.

4. DISCUSSION

One of the typical I/R injury of acute scrotal cases in urological department, namely testicular torsion, is excess rotation of the testis along with the spermatic cord causing infertility in males (19). So preserving the fertility capacity early diagnosis and treatment of testicular torsion especially surgical detortion which is believed to be gold standard treatment should be performed as soon as possible in order to provide reperfusion of testicular tissues (20; 21). Because the insufficiently oxygenated ischemic tissues may suffer conditions ranging from functional disorders to necrosis (22) re-establishment of blood flow should be the first intervention in testicular I/R injury of the torsed testis. However detorsion also leads to reperfusion injury (23) making too much ROS that the antioxidant capacity can not scavenge. Those events cause protein modification and lipid peroxidation making subsequent cellular dysfunction. The organism contains variable antioxidant enzymes that repair oxidative damage (24). Oxidative stress, apoptosis and necrosis are the mechanisms of damage in I/R (25). There is an increase in oxidative stress between ROS production and antioxidant defense mechanisms in favor of ROS. MDA increase, which is one of the best indicators of lipid peroxidation caused by ROS formation, occurs and increased in the I/R group in our study (26). After the increase in ROS with reperfusion following ischemia, MDA increases with lipid peroxidation and antioxidant preservatives such as SOD try to restore the balance (27-29). SOD is protective against the harmful effects of free radicals, such as O_2^- and H_2O_2, which are released in oxidative stress (30). TAS shows antioxidant activity and TOS reflects the intensity of oxidative stress. Any condition that exceeds the antioxidant capacity causes oxidative stress and OSI indicates this stress state (31-33). I/R study was showed that the TOS and OSI values increased, by contrast the TAS decreased in I/R group (34). In a study, the reduction of SOD caused by doxorubusin is increased by application of higenamine (35). In addition, in a collagen-induced arthritis study, the decreased GSH level was increased by administration of higenamine in the arthritis-induced group. And antioxidant properties of higenamine have been shown (36).

ROS and MDA, which we have evaluated in this study, are indicative of oxidative damage and increased with I/R in our study and decreased with higenamine administration. (26). Cell membrane damage is indicated by increased MDA elevation by peroxidation of fatty acids containing three or more double bonds (37) (38; 39). MDA causes cross-linking of molecules in the cell membrane and disrupts cell function as a result of changes in ion permeability and enzyme activity in the cell (40; 41). MPO is the main indicator of neutrophil accumulation in

I/R (42) and in our study, the increase in I/R decreased to control levels with higenamine administration. In a study, the increase in doxorubusin-induced MDA decreased by higenamine treatment (35). In the intestinal I/R study in mice, it was reported that inflammatory parameters such as MPO, TNF-α and IL-6 were increased in I/R group and higenamine administration decreased these levels and showed antiinflammatory effect (43).

The most important biochemical data of higenamine treatment can be expressed as follows: I/R injury in testes was related to dramatic increases of MDA level, TOS and OSI and MPO, and a decreases in SOD activity and TAS value in the testicular tissue. The novel result of the present study is that higenamine significantly derogated testicular tissue damage induced by I/R. Most importantly, higenamine treatment effected in the positive direction changes of the findings of MDA, TOS, OSI and MPO and stimulated an overproduction of enzymatic antioxidant SOD activity and increased TAS value.

4.1.Conclusion

These results recommend that higenamine may protect the testis by diminishing oxidative injury caused by ischemia reperfusion. We have indicate that treatment with higenamine at single dose (10 mg/kg) reduces testicular damage induced by ischemia reperfusion in testis in experimental animals exposed to a torsion for 2 hours and detorsion for 2 hours model. Part of the mechanisms of these protective effects of higenamine may be caused from supporting the antioxidant capacity by higenamine. Moreover, further researches are necessarry for explain the other protective mechanism on testicular tissue damage induced by ischemia reperfusion.

Conflict of Interest.

None declared.

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