

The effect of prophylactic melatonin administration on reperfusion damage in experimental testis ischemia-reperfusion

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Abstract

AIM: Torsion of testis, which is a urologic emergency case, is generally treated by surgical detorsion procedure. However, the resulting reperfusion and both ipsilateral and contralateral testis damage caused thereby are important problems. This study aims at investigating the administration of prophylactic melatonin in order to reduce free radical damage that is caused due to reperfusion after experimental testis torsion-detorsion procedure.

MATERIAL AND METHOD: The rats used in the study were allocated to four groups, each containing 10 rats. Rats in Group I had six hours of torsion (ischemia) followed by orchietomy. Rats in Group II had six hours of torsion, then received melatonin (10 mg/kg/IM) and after that had detorsion (reperfusion). Rats in Group III had detorsion after six hours of torsion and serum physiologic administration (the same volume as melatonin). Torsion and/or detorsion procedures were not applied in Group IV (control). Ipsilateral, contralateral testis and plasma Malondialdehyde (MDA) levels were determined in all groups.

RESULTS: Tissue and plasma MDA levels in the group which had detorsion were found to be significantly higher than those in the group that had orchietomy only ($p < 0.02$). Ipsilateral and contralateral testis MDA levels were identified to be significantly lower in the group receiving prophylactic melatonin in comparison to the group receiving serum physiologic ($p < 0.02$). Plasma and contralateral MDA levels correlated positively with MDA levels identified in ipsilateral testis in all groups ($r_s = +0.89$, $n = 40$, $p = 0.000$).

CONCLUSION: In cases where testis torsion is identified, administration of melatonin just before torsion may reduce local and systemic free radical damage.

Introduction

Testis torsion, a condition that is held responsible in the etiology of male infertility, requires emergent detorsion of the testis. Detorsion aims at saving the affected testis, but the concerned procedure leads to cellular damage due to ischemia-reperfusion [1, 2]. Free oxygen radicals are held responsible for tissue damage that is caused in reperfusion after ischemia. A number of medications have been studied in the literature in order to prevent reperfusion damage [3–5].

This study investigates the effect of prophylactic systemic melatonin administration before reperfusion in tissues left to ischemia on the reperfusion damage caused in testis.

Material and method

This study was performed at the Selçuk University Experimental Medicine Research and Application Center (SUDAM). All experiments procedures were approved by the SUDAM Ethics Committee. Male, adult Sprague-Dawley rats (weighing between 200 to 250 g) were used in the study. Rats were kept in the same places, given food and water ad libitum. Animals were randomly chosen and allocated to four groups, each containing 10 rats.

Rats in Group I had six hours of torsion (ischemia) followed by orchietomy. Rats in Group II had six hours of torsion, then received melatonin (10 mg/kg/IM) and had detorsion (reperfusion). Rats in Group III had detorsion after six hours of torsion and serum physiologic administration (the same volume as melatonin). Torsion and/or detorsion procedures were not applied in Group IV (control).

Within the experimental context, all rats were anesthetized by 50 mg/kg intramuscular ketamin hydrochlorur (Ketalar, Eczacıbaşı, Istanbul, Turkey) and 5 mg/kg Xsilazin hydrochloride (Rompun, Bayer, Istanbul, Turkey). The method used by Ozokutan BH et al. was modified for torsion and detorsion procedures. Torsion and detorsion procedures were performed on the left testis after testes were taken out by mid-scrotal incision. After 720 degrees torsion of the left testis and fixation of it to scrotum by prolen, continuation of torsion was ensured. After that, incision sites were closed by suturing with 2/0 silk [5].

In Group I immediately after orchietomy following six-hour torsion, in groups that had detorsion after twelve-hour reperfusion following detorsion procedure, all rats were decapitated, bilateral orchietomy was performed and blood samples were collected. The collected blood samples were examined for plasma Malondialdehyde (MDA) values and orchietomy material for tissue MDA levels.

In the statistical analysis, all values were shown as mean \pm standard deviation. Kruskal Variance analysis was employed for comparisons among groups. Upon finding $p < 0.05$, double comparisons were made with Mann Whitney U test. $P < 0.02$ was considered significant. Spearman correlation analysis was made between ipsilateral and contralateral testis tissue MDA level and plasma levels.

Results

Throughout the study no death or slowing down of activity was observed in subjects. MDA levels of the plasma and the operated and contralateral testes are shown in Table I.

The comparison between tissue MDA levels revealed that detorsion significantly increased MDA levels in relation to the group that had only torsion and orchietomy ($p < 0.02$). However when the latter group was compared with the control group where no procedure was performed, it was seen that only ischemia significantly increased tissue MDA level ($p < 0.02$) (Table I).

Comparison of the MDA levels of orchietomy tissue taken 12 hours after detorsion procedure demonstrated that melatonin administration significantly reduced MDA level in relation to the group which was administered serum physiologic ($p < 0.02$) (Table I).

The comparison between the group that was administered melatonin and the one that had orchietomy only did not give a significant difference in terms of both tissue and plasma MDA levels ($p < 0.02$) (Table I).

The differences among groups in terms of MDA levels were found to be similar with significant differences obtained in comparisons with regard to tissue MDA. Similarly, MDA levels found in contralateral testes had the same significance with orchietomy material taken from torsioned testes (Table I). MDA levels of operated tested were significantly higher than MDA levels of contralateral testes in all groups (Table I).

Spearman correlation analysis showed a positive correlation between ipsilateral and contralateral testis tissue MDA levels and also between plasma MDA levels of the two ($r_s = 0.89$, $n = 40$, $p = 0.000$).

Discussion

Oxygen radicals that appear in ischemia-reperfusion cases (superoxide, hydroxyl, peroxy, alkoxy and singlet oxygen radicals) have a destructive effect on lipids of all membranes. Consequently cellular destruction, particularly in membranes, is seen. The end product of this phenomenon called lipid peroxidation is MDA. MDA is a reliable indicator of lipid peroxidation [7]. In our study, the level of this product is used to show the extent of biochemical influence. It has been put forward in previous studied that there is a positive correlation between MDA level and the level of histopathological

Table I: Plasma and tissue malondialdehyde levels (Mean \pm SD).

	Testis (nmol/g)		Plasma (nmol/ml)
	Ipsilateral	Contralateral	
Group I (n=10)	45 \pm 4 ^a	36 \pm 3 ^d	2.2 \pm 0.4 ^h
Group II (n=10)	51 \pm 4 ^a	38 \pm 5 ^d	2.8 \pm 0.8 ^h
Group III (n=10)	81 \pm 6 ^b	61 \pm 8 ^e	5.8 \pm 0.5 ^g
Group IV (n=10)	9 \pm 3 ^c	10 \pm 2 ^f	0.9 \pm 0.2 ^k

Note: The letters in superscript on averages in the columns show that the difference obtained from double comparisons between groups is significant ($p < 0.03$, Mann Whitney U test).

influence. Therefore, we did not perform histopathological scoring on the torsioned testes and contralateral testes. However, both testes had histopathological findings, primarily interstitial hemorrhage, reported in previous studies.

Melatonin is effective on such oxygen radicals as hydroxyl radical, singlet oxygen, peroxy radical and superoxide anion. It protects nucleus DNA, membrane lipids and cytosolic proteins against oxidative damage [8]. It also supports SOD, GSH-Px, glutathione reductase and glucose-6-phosphate dehydrogenase of the antioxidant system [9] and has an inhibitor influence on nitric oxide synthetase [10]. It has a protective effect on organelles, particularly mitochondria [11].

In our study it was demonstrated that MDA levels were lower in both torsioned and contralateral testes in the cases that were given melatonin when compared to those given serum physiologic.

Literature information about whether contralateral testis is or is not affected in unilateral torsion is contradictory. Besides those literature studies reporting that contralateral testis is not affected [5, 12], there are also those reporting that it is [13, 14]. It was claimed that immunological mechanism were responsible for the affected contralateral testis and that the time that lapses during reperfusion was important for the formation of the required immune response. In our study affection at the biochemical level was observed both in tissues that had ischemia and in those that had reperfusion together with ischemia. In the study, the time that lapsed during reperfusion ranged between 6 to 18 hours and this time can be thought to be sufficient for the formation of an immune response. However in consideration of the studies that report affection of the contralateral testis and that have 4 hours for ischemia and 8 hours for ischemia with reperfusion [5], it appears that there may be other factors involved. One factor may be related to the technique used for torsion. Since in cases where torsion is less than 720 degrees testicular blood flow continues and therefore there is no definite ischemia [15]. Another possible factor is that conditions like edema or inflammation in the adnexa exposed to ischemia may have directly affected neighboring tissues.

In addition to these, affection of the contralateral testis may be attributed to the free radical products', formed during reperfusion, entering the systemic circulation first and then reaching to the testis where blood circulation continues. There are studies supporting this last opinion of ours. One of these is the study by Çelik et. al. It was shown in the concerned study that non-cardiogenic pulmonary edema was formed in lungs due to the effect of free radical products that entered systemic circulation following adnexal torsion and detorsion [16]. Studies addressing the same mechanisms demonstrated that remote organs were affected due to local reperfusion [17, 18].

Testicular torsion is among the important reasons of male infertility. Reperfusion that develops due to the detorsion procedure performed to save testis tissue leads to the emergence of free radical products,

which affect both torsioned and contralateral testes. The main damage to the tissues happens during reperfusion. Systemic melatonin administration just before reperfusion reduces biochemical tissue damage due to reperfusion.

In this study melatonin was used prophylactically. Level of lipid peroxidation at the 12th hour after reperfusion was measured. Further studies are needed in order to clarify the long-term effects of prophylactic melatonin administration and to reveal its influence on infertility rates attributed to torsioned testes, as well as to demonstrate the effects of various doses and routes of melatonin administration.

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