Brief Communication

The effect of melatonin on ductus epididymis. *Unilateral testicular torsion in rats*

Ayse Yildirim, MD, PhD, Murat Akkus, BSc, PhD, Ahmet R. Ersay, MD, Yusuf Nergiz, BSc, PhD, Ozlem P. Baran, MD, PhD.

The main pathophysiology of testicular torsion is ischemia/reperfusion (I/R) injury of the testis caused by the twisted spermatic cord and its release.[1] Although extensive research has been conducted on the pathophysiology of testicular torsion, the effect of ischemia on the epididymis has not been primarily investigated due to the testis and epididymis share a common blood supply (testicular, cremasteric, and vasal vessels).[2] The main cause of testicular damage after torsion is oxygen free radicals produced during reperfusion. Oxygen free radicals oxidize membrane lipids, proteins, and DNA, leading to cellular dysfunction and sometimes cell death. Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone synthesized and secreted predominantly by the pineal gland in the dark and has been shown recently to be a potent free radical scavenger.[3,4] This study was designed to investigate the protective effect of melatonin, which is more effective than other free radical scavengers, in I/R injury after experimental testicular torsion to evaluate the ultrastructural findings of ductus epididymis with or without melatonin in both torsion and detorsion of testis. All procedures complied with the standards for care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Dicle University, DÜSAM). Sprague-Dawley male rats (250-300 g) fed on a standard diet and with tap water ad libitum, in a 12 hour light-dark cycle was used. All animals were anesthetized with an intraperitoneal injection of 50 mg/kg of pentobarbital sodium. Testis I/R injury was induced by torsion of the left testis, with a 720 degree twisting of the spermatic cord so as to produce a total occlusion of testis for 2.5 hours. The same testis was then detorsioned. The Sprague-Dawley male rats were divided into 5 groups, each containing 7 rats. After 2.5 hours of torsion and detorsion, unilateral orchiectomies were performed for histopathological examination. The groups were labeled as first group (control), second group (torsion); third group (torsion plus detorsion), fourth group (torsion plus melatonin 20 mg/kg/IP plus detorsion), fifth group (torsion plus melatonin 50 mg/kg/IP plus detorsion). For the histological examination, epididymal tissues were fixed in 2.5% glutaraldehyde and postfixation 1% osmic acid solutions, the araldit blocks prepared by routine electron microscopic techniques were cut in a thickness of 300-400 A°. They were then examined under transmission electron microscopy after application of contrast stain. Tissue sections were evaluated using a JEOL 1010 transmission electron microscope. The epididymal tissues were intact in controls. In the torsion group, when the microphotographs were examined, the nuclei of basal cells was normal, however, mitochondrial crystolysis and mitochondrial swelling were present in the cytoplasm of columnar cells, dilatation, and sometimes fragmentation in the tubuli of rough endoplasmic reticulum was also detected. Myelin figures were seen in some areas due to cytoplasmic edema. As well as the presence of seconder lysosomes, autophagic vacuoles were noticeable and there was increase in the lipid droplets compared with controls. When the detorsion group was examined, there was an increase of lipid contents in the basal cells, electron dense lipid accumulation, more autophagic vacuoles, increase of seconder lysosomes, not only activated Golgi complex but also mitochondrial crystolysis were seen in the cytoplasm of columnar cells. In some tissue sections of the detorsion group, necrosis had started in the columnar cells, extensive degeneration and increased numbers of autophagic vacuoles in the rough endoplasmic reticulum were also detected.

In the melatonin 50 group, electron dense lipid droplets with mitochondrial crystolysis and mitochondrial swelling in the cytoplasm of basal cells were observed. In other sections of this group, there were myelin figures in some areas, and an increase in seconder lysosomes was observed. Therefore, active Golgi complex and lipid accumulation were also noticeable. In the melatonin 20 group, when the microphotographs were investigated, we detected only slight lipid accumulation in the basal cells, the lipid accumulation in the columnar cells was slightly more than the basal cells, and mitochondrial crystolysis with more myelin figures due to the edema of the cytoplasm was also detected. Therefore, the columnar cells had extense cytoplasm and few seconder lysosomes (**Figure 1**). This study investigated the effects of prolonged ischemia on the ultrastructure of the epididymis and the protective role of melatonin hormone. Kristo et al[2] reported that the macroscopic and microscopic observations of their study support the hypothesis that the epididymis is more resistant to ischemia than the testis during testicular torsion. In their study, the group that underwent 4 hours of left testicular and epididymal ischemia showed light microscopic findings, which demonstrated intact epithelia with microvilli, normal nuclear chronicity, and no cellular debris. However, in the same study, the group that underwent 8 hours of left testicular and epididymal ischemia, demonstrated minimal sloughing of the luminal cells in the epididymis. Thus, there was no reported ultrastructural study of epididymal ischemia,

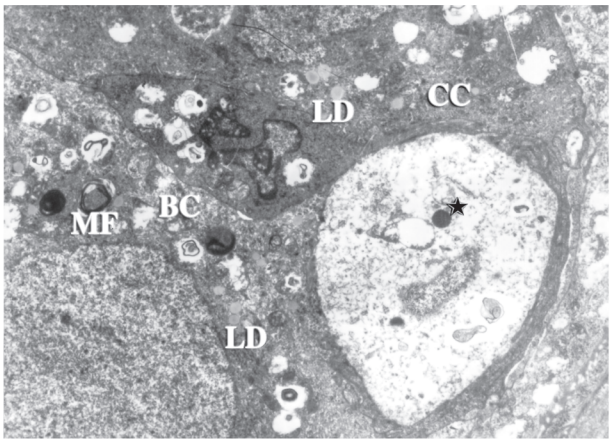

Figure 1 - Melatonin 20 group demonstrating rat ductus epididymis to which 2.5 hours of torsion performed with administration of 20 mg melatonin. Very few lipid accumulation (LD) compared with the melatonin 50 group in the basal cells (BC), mitochondrial crystolysis (MC) and more myelin figures (MF) seen. Few seconder lysosomes (*) in the columnar cells (CC) has been demonstrated. (Uranyl acetate-lead citrate staining, original magnification x4400).

and when we compared the light microscopic findings of Kristo's study[2] with ours in the torsion group, which underwent 2.5 hours of testicular and epididymal ischemia, we detected mitochondrial swelling and crystolysis in the columnar cells of ductus epididymis. In addition, the presence of seconder lysosomes and autophagic vacuoles was noticeable and there was increase in the lipid droplets. Kristo et al[2] considered both the structural and functional differences of the testicular and epididymal response to ischemia.[2] In our study, we saw the structural and functional degenerations as Kristo et al had reported, and our observations were similar with theirs.

In recent years, several antioxidant agents have been used to prevent I/R-induced tissue damage in experimental testicular torsion such as, superoxide dismutase (SOD), catalase, calcium channel blockers, oxypurinol, and allopurinol, except melatonin.[5] Melatonin is a potent antioxidant agent more effective than allopurinol in preventing testicular damage after acute experimental torsion. Even though this is an animal model, melatonin may clinically be used as an antioxidant agent in testicular torsion.[6] In our study, we also examined the prophylactic effect of melatonin in 20 and 50 mg/kg doses. Prillaman and Turner[5] also studied to determine whether testicular function after one hour of torsion can be rescued by the administration of antioxidant agents (SOD, catalase, verapamil, and allopurinol). These investigators showed that SOD and catalase treatments provide a significant rescue of functioning testicular parenchyma after one hour of torsion, and neither allopurinol nor verapamil adds benefit. However, the dose of allopurinol could have been inappropriate. No significant rescue was seen in the testes undergoing 2 hours of torsion.

In our study, the 2.5 hours of torsion showed similar results to that of Abasiyanik and Dagdonderen.[6] The results of melatonin 20 and melatonin 50 were nearest the controls. The prophylactic effect was present, but there was no complete rescue of the tissue. However, Akgur et al[7] reported that allopurinol treatment prevents reperfusion injury after testicular torsion lasting as long as 5 hours. In our study, we observed that melatonin 20 was more prophylactic than melatonin 50 according to the results. In Abasiyanik and Dagdonderen study,[6] they determined that melatonin treatment prevents I/R injury in testicular tissue both biochemically and histopathologically after 6 hours of torsion.[6] In our study, as the melatonin 20 group had little lipid accumulation in the basal cells, and the lipid accumulation in the columnar cells slightly more than the basal cells, we suggest that melatonin 20 mg administration has a protective effect in ischemia of 2.5 hours of the ductus epididymis.

In conclusion, after investigation of the ductus epididymis of the rats undergoing ischemia and reperfusion to their testes, the detorsion group had more degeneration than the torsion group. In addition, 20 mg/kg melatonin had a more protective role than 50 mg/kg melatonin on the tissue of the ductus epididymis in damaging testis by ischemia-reperfusion.

Received 14th February 2006. Accepted 14th August 2006.

From the Department of Histology and Embryology (Yildirim, Akkus, Nergiz, Baran), Faculty of Medicine, Dicle University, Diyarbakir, and the Department of Urology (Ersay), Faculty of Medicine, Onsekiz Mart University, Canakkale, Turkey. Address correspondence and reprint requests to: Dr. Ozlem P. Baran, Department of Histology and Embryology, Faculty of Medicine, Dicle University, 21280 Diyarbakir, Turkey. Tel. +90 (412) 2488001 Ext. 4650. Fax. +90 (412) 2488440. E-mail: obaran@dicle.edu.tr

References

1. Turner TT, Brown KJ. Spermatic cord torsion: loss of spermatogenesis despite return blood flow. ***Biol Reprod*** 1993; 49: 401-407.
2. Kristo BA, Palmer JS, Cromie WJ. Possible mechanisms for epididymal sparing during testicular ischemia. ***Urology*** 1999; 53: 435–439.
3. Ambrosio G, Flaherty JT, Duilio C. Oxygen radicals generated at reflow induce peroxidation of membrane lipids in reperfused hearts. ***J Clin Invest*** 1991; 87: 2056-2066.
4. Pieri C, Marra M, Moroni F. Melatonin: A peroxyl radical scavenger more potent than vitamin E. ***Life Sci*** 1994; 55: 272-276.
5. Prillaman HM, Turner TT. Rescue of testicular function after acute experimental torsion. ***J Urol*** 1997; 157: 340-345.
6. Abasiyanik A, Dagdönderen L. Beneficial effects of melatonin compared with allopurinol in experimental testicular torsion. ***J Pediatr Surg*** 2004; 39: 1238-1241.
7. Akgur FM, Kilinc T, Aktug T, Olguner M. The effect of allopurinol pretreatment before detorting testicular torsion. ***J Urol*** 1994; 151: 1715-1717.