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The effects of melatonin and colchicine on ischemia–reperfusion injury in experimental rat testicular torsion model

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ABSTRACT

Purpose: The aim of the present study is to investigate the efficiency of colchicine and melatonın in an experimental rat testicular torsion model in the light of histological and biochemical data.
Methods: A total of 34 Wistar albino male rats were randomly divided into 5 groups as: Group C (control, n = 6), Group S (sham; underwent only left scrotal exploration, n = 7), Group TD (torsion and detorsion; 6 h of ischemia and 7 days of reperfusion, n = 7), Group TD/M (TD + Melatonin; 6 h of ischemia and 7 days of reperfusion and 7 days of 17 mg/kg intraperitoneal melatonin per day, n = 7), group TD/Col (TD + Colchicine; 6 h of ischemia and 7 days of reperfusion and 7 days of 1 mg/kg oral colchicine per day, n = 7). Histopathologic evaluation of seminiferous tubule deterioration was performed by Johnsen's scoring system. Total antioxidant status (TAS), total oxidant status (TOS), IL-6, TNF alpha levels were analyzed in each group.
Results: The histopathologic scores, total antioxidant status (TAS), total oxidant status (TOS), IL-6, TNF alpha levels in groups C and TD/Col were significantly lower than groups TD and TD/M ($P < .001$).
Conclusion: Our study results revealed that colchicine reduced testicular ischemia–reperfusion injury in experimental rat testis torsion model. Although detorsion of testis is crucial for the preserving the testicular viability, antioxidant and anti-inflammatory treatment modalities like colchicine might help to reduce ischemia–reperfusion injury in detorsed testis.

Testicular torsion is one of the most frequent urgent surgical urologic diseases particularly in young adults and children. Following spermatic cord twisting, edema and bleeding into tissue decrease venous blood flow, in addition to arterial flow occlusion. Prompt surgical exploration is crucial for the patients with testicular torsion. In order to salvage the torsed testicle, surgery should be done within 6 h of symptom onset [1,2]. Delayed surgery may result in orchiectomy, or diminished fertility, even if the testis is timely detorsed infertility risk is the most worrisome complication [3].

Various experimental studies have been conducted regarding the efficiency of melatonin on reducing tissue damage or ischemia–reperfusion injury [4–7,12]. However, providing and administration of melatonin are difficult.

Since the Ancient Greek Era, colchicine has been a well known plant-derived medicine, which has anti-fibrotic, anti-inflammatory, antihistaminic, and membrane stabilization effects, and inhibits lipid peroxidation. From those aforementioned characteristics of colchicine came the idea of utilizing colchicine for the diseases in which inflammatory processes and cell proliferation remain at forefront. Colchicine has been used in gouty arthritis for many years, and utilized as a referenced medicine for Familial Mediterranean Fever, Behcet's disease, scleroderma, and hepatic cirrhosis for the last 5 decades [8]. Although the anti-inflammatory mechanism of colchicine is not clear, it is well known that it down regulates tumor necrosis factor receptors, inhibits phospholipase activation, and decreases neutrophil chemotaxis, leukocyte function, and cytokine production [9]. The colchicine is routinely used in daily practice for the treatment of various diseases in children with the advantage of being a widely available and cost-effective medicine. In a rat ovary torsion model, beneficial role of colchicines on ischemia–reperfusion tissue injury has been demonstrated [10].

However, to date, there has not been any conducted study concerning the effect of colchicine on rat testicular torsion model, or

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comparative studies with melatonin. In this study, we aimed to investigate and compare the effects of colchicine and melatonin on ischemia–reperfusion injury in rat testicular torsion model.

1. Materials and methods

1.1. Animals

In this study, experimental procedures were performed per the guidelines suggested by the Turkish Institute of Health, and ethical committee approval was obtained (Approval No:2014/15, Selçuk University, Konya, Turkey). We carried out this study at Experimental Medical Research Center of Selçuk University. Thirty-four healthy 3-month-old male Wistar albino rats weighing between 350 and 400 g were used. Of the total 34 rats, 6 were included in control group, and remaining 28 rats were split into 4 groups. The rats were housed in individual solid-bottom plastic cages on sawdust bedding at a constant temperature of 21 °C and humidity of 55% with 12-h periods of light–dark exposure. The animals were allowed access to standard rat chow and water ad libitum. A 7-day period of acclimatization was used.

1.2. Experimental design

In this study, all surgical procedures were performed under intraperitoneal ketamine 60 mg/kg and xylazine HCl 10 mg/kg(Ketalar and Citanest, 2%, Eczacıbaşı, Turkey) for general anesthesia. All operations were performed under sterile conditions. In order to expose the testicle, scrotum was entered through a left inguinoscrotal incision. The tunica vaginalis was opened and the left testicle was delivered to the surgical field. The left testicle was rotated 720° in a clockwise direction and maintained in this torsion position by fixing the testicle to the scrotum with a 5/0 silk suture. After each surgical intervention, the incisions were closed again with 5/0 silk sutures. Torsion duration was 6 h. After 6 h, the spermatic cord was detorsed [12]. On day 7 re-explorations and orchiectomies were performed on each rat.

Rats were divided into 5 groups as follows; control group (Group C, n = 6), sham group (Group S, n = 7), torsion/detorsion group (Group TD, n = 7), torsion/detorsion and Melatonin group (Group TD/M, n = 7), torsion/detorsion and Colchicine group (Group TD/Col, n = 7).

In groups C and S, following left orchiectomy 3 mL blood samples were obtained via intracardiac aspiration, in order to determine the plasma Total Antioxidant Status (TAS) Total Oxidant Status (TOS), Tumor Necrosis Factor-Alpha (TNF-a), and IL-6 levels. TAS and TOS levels were analyzed with manual sandwich ELISA method with an automated measurement device. In Group TD, testicular torsion was performed, the testicle was fixed to the scrotum, and the scrotum was immediately closed. After 6 h of torsion, detorsion was performed, and scrotal incision was reclosed after fixing the testicle to scrotum.

In Group TD/M, surgical procedure was performed same as the Group TD. Differently, intraperitoneal injection of 17 mg/kg Melatonin (N-acetyl-5-methoxytrptamine, M-250 Lot 25HO904, Sigma Chemical Co, St. Louis, MO) was started 15 min before detorsion and continued for 7 days once per day. Before administration, Melatonin was dissolved in ethanol and then diluted in isotonic saline to a final concentration of 1% ethanol [4,5].

In Group TD/C, surgical procedure was performed same as the Group TD. Differently, oral administration of 1 mg/kg Colchicine (Sigma Chemical Co., St. Louis, MO) was started 15 min before detorsion and continued for 7 days once per day [10,11,13,14].

In groups TD, TD/M, and TD/C left orchiectomies were performed after one week. The Johnsen's score in testicular tissues was evaluated, and 3 mL blood samples were obtained in order to determine serum TNF-alpha (TNFa), IL-6, and plasma total antioxidant status (TAS) and total oxidative status (TOS) levels.

1.3. Biochemical analysis

The venous blood samples underwent centrifugation (at 4 °C) at 3500 rpm for 10 min and were stored at 80 °C until analysis. The tissues were first cleaned by saline solution and stored at −80 °C until the analysis time. In the analysis process, first they were homogenized in saline solution (20%, *w*/*v*), and homogenates were centrifuged at 5000 rpm for 30 min to remove debris and to obtain clear supernatant fraction. Then, the analyses were performed in this fraction. Serum and tissues TNF-alpha (eBioscience Vienna Austria, lot no: 110003042) and IL-6 (eBioscience Vienna Austria, lot no: 110003025) levels were studied by biochemists with manual sandwich ELISA methods. Plasma TAS and TOS levels were determined using an automated measurement method, and then oxidative stress index (OSI), which is the percent ratio of the TOS to the TAS, was calculated. For OSI value, first the result unit of TAS (mmol Trolox equivalent/l) was converted to mol equivalent/L and then calculation was made as follows; OSI = [(TOS, μmol H_2O_2 equivalent/L)/(TAS, μmol Trolox equivalent/L) × 100] [15,16].

1.4. Processing of histologic samples

Tissue samples from left testis were fixed in 10% formalin, and embedded in paraffin. Deparaffinized 4 μm-sized sections were stained with both hematoxylin–eosin (H&E) and periodic acid Schiff (PAS).

1.5. Histopathologic evaluation

Histopathologic examinations of the testes were done blindly. Johnsen's criteria were used to categorize the spermatogenesis using a score of 1–10 [17]. Each histopathologic characteristic was assessed on 20 seminiferous tubules using a microscope via × 10 and × 20 objective. Ischemic necrosis was graded according to the percentage of involved tubules. Presence of giant cells was also noted.

1.6. TUNEL

For Terminal Deoxynucleotidyl Transferase UTP Nick-End Labeling (TUNEL) Assay Paraffin embedded testis tissues were sectioned at 4 μm thick sections and collected on poly-l-lysine-coated slides then deparaffinized overnight at 60 °C incubator. TUNEL test application protocol was made according to Howard et al. [18].

1.7. TUNEL evaluation

The sections were examined in a microscope (Olympus BX51). To evaluate percentage of TUNEL-positive cells, 3–4 visual fields per section were randomly chosen and the numbers of TUNEL-positive cell nuclei were counted in seminiferous tubules with the same magnification (×40).

The percentage of apoptotic cells was detected on the monitor and the 'apoptotic index' was calculated according to the following formula. Apoptotic Index = Total number of apoptotic cells /100 [18].

1.8. Statistical analysis

Data were analyzed by using statistical software and variables were presented as median (min–max) for non-parametric values. The distribution of the variables was analyzed with the Kolmogorov–Smirnov test. Owing to non-parametric values, Bonferroni corrected Mann–Whitney U test, and Kruskal–Wallis test were used for comparisons between groups. Correlation analysis was carried out using Pearson's correlation test. A p value of <0.05 was considered as statistically significant for all tests.