Experimental Study

Histological effects of zinc and melatonin on rat testes

Tuncer İ¹, Sunar F², Toy H³, Baltaci AK², Mogulkoc R²

Department of Anatomy, Meram Medical School, Selcuk University, Konya, Turkey. fusunar@yahoo.com

Abstract: Background: The objective of this study is to examine the histological effects of zinc and melatonin, alone or in combination, on rat testes.

Methods: For this purpose four study groups of ten Sprague-Dawley male rats were formed and treated for one month as follows: (1) Controls; (2) Rats injected with 3 mg/kg/day zinc, as zinc sulfate; (3) Rats injected with 3 mg/kg/day melatonin and (4) Rats injected with 3 mg/kg/day each zinc and melatonin.

Results: After four weeks the rats treated with melatonin showed inhibited spermatogenesis, testicular tubular degeneration and necrosis, obstruction of tubular lumen and lymphocytic infiltration. The two zinc-treated groups showed no histological differences to controls but the melatonin-only group showed inhibited spermatogenetic activity, tubular degeneration and necrosis, as well as obstruction of tubule lumens and lymphocytic infiltration.

Conclusion: The obtained results suggest that 4-week treatment with melatonin leads to histological and physiological impairments of testis and that zinc supplementation might offset these damaging effects (Tab. 2, Fig. 4).

Key words: zinc, melatonin, testes, rat.

Evaluation of the histopathological effects of zinc on the reproductive system together with those of melatonin on rat testes is expected to be a positive contribution to the current knowledge on the subject.

Methods

Study groups

The experiments were conducted at the Experimental Medicine Application and Research Center of Selcuk University (SUDAM), which provided the forty adult Sprague-Dawley male rats that were used in the study. The study protocol was approved by the local Ethics Committee.

The experimental animals were housed in stainless steel cages that were washed and cleaned daily and maintained at 21±1 °C in a 12-h light/dark cycle. The rats were fed a standard diet (Ayetkin’s Grain Co. (Konya, Turkey) and allowed to drink tap water ad libitum.

Four study groups of ten rats each were formed and treated as follows during one month:

Group 1. Normal controls receiving a standard diet and not subjected to any procedure.

Group 2. Rats that received I.P. doses of 3 mg/kg zinc (as zinc sulfate, in saline).

Group 3. Rats receiving I.P. injections of saline containing 3 mg/kg melatonin.

Group 4. The rats were injected I.P. with saline containing 3 mg/kg each zinc (as zinc sulfate) and melatonin.

The injections were administered daily between 9 AM and 10 AM.

References


Fax: +90.332.3205654

Indexed and abstracted in Science Citation Index Expanded and in Journal Citation Reports/Science Edition
Tab. 1. Histological changes in the testis tissues of study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tubular degeneration and necrosis (Median value)</th>
<th>Obstruction in tubule lumens and lymphocytic infiltration (Median value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 General control (n=10)</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 Zinc administered (n=10)</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 Melatonin administered (n=10)</td>
<td>5.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 Zinc+Melatonin administered (n=10)</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

P = 0.05 0.05

*Means with different superscripted letters in the same column have statistical significance (p<0.001)

Tab. 2. Spermatogenesis scoring of study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Spermatogenetic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 General control (n=10)</td>
<td>7.00±0.00</td>
</tr>
<tr>
<td>2 Zinc administered (n=10)</td>
<td>7.00±0.00</td>
</tr>
<tr>
<td>3 Melatonin administered (n=10)</td>
<td>6.50±0.50</td>
</tr>
<tr>
<td>4 Zinc+Melatonin administered (n=10)</td>
<td>3.50±0.25</td>
</tr>
</tbody>
</table>

P = 0.01

*Means with different superscripted letters in the same column are statistically significant (p<0.001)

Preparation of the solutions
Zinc sulfate was dissolved in doubly distilled water and aliquots were taken and diluted with saline so that the dose administered intraperitoneally would be 3 mg/kg.

40-mg melatonin (Sigma M-5250) were suspended in 3 ml ethanol. The suspension was stored in a freezer until required. For injection 0.1 ml of the stock solution were dissolved in 0.9 ml saline to a concentration of 3 mg/kg.

Histological methods
After the rats were sacrificed by decapitation under anesthesia, the testes were removed and 5 μm cross sections were taken from each sample. The cross sections were stained with hematoxylin-eosin and examined under a microscope for evaluation of the spermatogenic activity of 20 seminiferous tubules in five different fields in each cross section. The findings of the examination were assessed according to modified Johnson criteria: 1) Azoospermia; 2) Presence of Sertoli cells; 3) Spermatogonia; 4) Presence of spermatocytes; 5) Presence of spermatids; 6) Presence of spermatozoa and 7) Normal spermatogenesis (sufficient amount of cells).

Treatment of the data
Statistical evaluation of results was conducted through computer software. The data did not meet the prerequisites of variance analysis, so they were subjected to square root conversion. The values obtained after conversion were subjected to variance
analysis. The Duncan test was used to establish differences between groups.

Results

The microscopic examination of tissues from groups 1, 2 and 4 did not reveal inhibition of spermatogenetic activity, tubular degeneration and necrosis, obstruction in tubule lumens or lymphocytic infiltration.

The controls and zinc-supplemented rats (groups 1 and 2) were seen to contain a sufficient amount of cells (score 7), while the rats administered with zinc and melatonin reached a score 6. The melatonin-only group had scores 3 or 4, displaying inhibited spermatogenetic activity (Tab. 1), tubular degeneration and necrosis, obstruction in tubule lumens and lymphocytic infiltration (Tab. 2, Figs 1–4).

Discussion

Melatonin is a neurohormone secreted from the pineal gland that has a circadian rhythm. Consequently, animals used in experimental studies are subjected to controlled short or long photoperiods. Vaughan et al implanted 15 mm Silastic testosterone pellets in rats subjected to short and long photoperiods and found that the decrease in testicular weight in the rats subjected to a short photoperiod was less than that in rats subjected to a longer photoperiod (10). In a subsequent study the authors injected melatonin to rats that were implanted testosterone pellets and subjected them to longer photoperiods, finding that melatonin enhanced the effects of testosterone causing gonadal atrophy in reproductive organs (11). Jarrige et al reported that there was no change of testicular dihydrotestosterone content despite absolute atrophy, but a decrease in the plasma dihydrotestosterone levels in the rats born to pinealectomized mothers (12).

In agreement with these reports, in the present study we observed that testes of rats given melatonin experienced histological changes and inhibited spermatogenetic activity when compared to the other groups. When zinc is given in combination with melatonin the differences observed were not significant in comparison with normal controls or rats treated with zinc alone. These results suggest that zinc might have a protective effect against the testicular damage caused by melatonin.

References


Received April 5, 2011.
Accepted May 4, 2011.