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EXPERIMENTAL STUDY

Protective effect of melatonin on lipid peroxidation in various tissues of diabetic rats subjected to an acute swimming exercise

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Abstract: *Background:* The present study aimed to explore the effect of melatonin administration on lipid peroxidation in various tissues of rats with streptozocin induced diabetes and subjected to an acute swimming exercise.
Methods: The study used 80 adult male rats, which were equally allocated to 8 groups: Group 1, general control; Group 2, melatonin-administered control; Group 3, melatonin-administered diabetic control; Group 4, swimming control; Group 5, melatonin-administered swimming; Group 6, melatonin-administered diabetic swimming; Group 7, diabetic swimming; Group 8, diabetic control. Diabetic rats were administered 3 mg/kg/day ip melatonin for 4 weeks. At the end of the study, the animals were decapitated to collect samples from liver, lung and spleen tissues, which were then analyzed to determine levels of liver MDA (nmol/gram/protein) and GSH (mg/g/protein).
Results: The highest MDA values in liver, lung and spleen tissues were obtained in the Group 7. The values in the Group 8 were lower than those in the Group 7, but higher than in all other groups. The Group 5 and 6 had the highest liver, lung and spleen GSH values.
Conclusion: Results obtained from the study indicate that the increase in free radical production and the inhibition of antioxidant activity in diabetes and acute exercise are both prevented by melatonin administration *(Tab. 2, Ref. 30)*. Full Text in PDF *www.elis.sk.*
Key words: diabetes, exercise, melatonin, lipid peroxidation, rat.

Melatonin, which is secreted by the pineal gland, is associated with the regulation of many different neuroendocrine functions (1). It was shown to contribute to carbohydrate mechanism (2), as well as having critical effects on free fatty acids and lipogenesis (3, 4). Melatonin is also claimed to play a preventive role in diabetes (5). Besides, it is known to have a potent antioxidant effect (6). The beneficial effects of exercise on cardiovascular risk factors, insulin sensitivity, glucose disposal and body fat distribution in diabetic patients have been well-defined (7). Therefore, exercise is strongly recommended to diabetic patients (8). However, although the data are conflicting, physical exercise is also thought to increase free radical formation (9). It has been reported that all tissues of diabetic rats underwent significant impairments in antioxidant defense (10, 11) and thus, experimental diabetes could be used as a model of oxidative damage in animals (12, 13). The present study aims to explore the effect of melatonin administration on lipid peroxidation in various tissues of rats with streptozocin induced diabetes and subjected to an acute swimming exercise.

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Methods

Animal material and groups

This study was carried out on 80 Spraque-Dawley type male rats supplied by the Experimental Medicine Application and Research Center of Mediterranean University in the Experimental Animals Unit of Selcuk University, School of Veterinary Medicine. The study protocol was approved by the Ethics Committee of the Selcuk University, School of Physical Education and Sports. The animals used in the study were equally allocated to 8 groups:

Group 1, (n: 10) The General Control Group: The group which was not subjected to any procedure and fed on a normal diet.

Group 2, (n: 10) The Melatonin-Administered Control Group: The group which was fed on a normal diet and was additionally administered 3 mg/kg/day intraperitoneal (ip) melatonin for 4 weeks.

Group 3, (n: 10) The Melatonin-Administered Diabetic Control Group: The group with diabetes induced by subcutaneous "40 mg/kg" streptozotocin (STZ) injection and then administered 3 mg/kg/day intraperitoneal (ip) melatonin for 4 weeks.

Group 4, (n: 10) The Swimming Control Group: The group, which was fed on a normal diet and subjected to a 30-minute acute swimming exercise.

Group 5, (n: 10) The Melatonin-Administered Swimming Group: The group, which was fed on a normal diet, was additionally administered 3 mg/kg/day intraperitoneal (ip) melatonin for 4 weeks, and was subjected to a 30-minute acute swimming exercise.

Group 6, (n: 10) The Melatonin-Administered Diabetic Swim-

ming Group: The group with diabetes induced by subcutaneous "40 mg/kg" streptozotocin (STZ) injection and then administered 3 mg/kg/day intraperitoneal (ip) melatonin for 4 weeks and subjected to a 30-minute acute swimming exercise.

Group 7, (n: 10) The Diabetic Swimming Group: The group with diabetes induced by subcutaneous "40 mg/kg" streptozotocin (STZ) injection and subjected to a 30-minute acute swimming exercise.

Group 8, (n: 10) The Diabetes Group: The group with diabetes induced by subcutaneous "40 mg/kg" streptozotocin (STZ) injection.

Experimental animals

Experimental animals were kept in special steel cages which were cleaned daily by washing. They were fed from special steel bowls and given (tap) water in glass feeding bottles. The animals were fed with 10 g rat pellet per 100 g body weight daily. They were kept under 12 hour dark/12 hour light cycles and standard room temperature (21±1°C). All injections were given between 9.00 and 10.00 a.m. At the end of the 4-week procedures, liver, lung and spleen tissue samples were collected from all animals by decapitation between 9.00 and 10.00 a.m. to be used in analyses. The samples were stored at -80°C until analyses.

Experimental procedures

Inducement of diabetes in experimental animals

In order to induce diabetes, 40 rats were allocated to the diabetic groups. The rats were injected 40 mg/kg subcutaneous streptozotocin (STZ) "Sigma, S-0130". The injections were repeated in the same dose 24 hours later. The glucose level of the blood taken from the tail vein of the animals 6 days after the last injection was measured using a diagnostic glucose kit. The animals whose blood glucose was at or above 300 mg/dlt were accepted as diabetic (14).

Swimming exercise

The exercise was performed in a heat-resistant glass swimming pool, 50 cm in length and width, with a thermostat keeping the temperature fixed at 37°C. The exercises were performed once for 30 minutes, 24 minutes after the end of procedures. The experimental animals were made to swim in pairs, and then decapitated to collect blood samples for analysis.

Melatonin administration

For melatonin administration, 40 mg of melatonin (Sigma M-5250) was dissolved in 3 ml pure ethanol. After that, this suspension was stored capped and in the dark in a refrigerator until use. Then, 0.1 ml was taken from the stock solution, added 0.9 ml NcCl (3 mg/kg/day) and injected by ip route to the rats at 09.00 a.m. Melatonin administration was repeated in the same hours for 4 weeks.

Biochemical analyses

Tissue MDA analyses: In order to obtain a 10 % homogenate, liver, lung and spleen tissue samples were homogenized at +4 °C with 150 mMol KCl (Microsan Ultrasonic Cell Disruptor Misonic). The homogenate was added 2 ml $HClO_4$ and centrifuged at 3000 rpm for 15 minutes. MDA level was evaluated in the supernatant. Then, 3 ml H_3PO_4, 1 ml 0.675 % thiobarbituric acid and 0.5 ml from the homogenate were mixed and kept in a boiling water bath for 45 minutes. MDA levels were read at 532 nm and determined as nmol/gram/protein (15).

Tissue GSH determination: In order to obtain a 10 % homogenate, liver, lung and spleen tissue samples were homogenized at +4 °C with 150 mMol KCl (Microsan Ultrasonic Cell Disruptor Misonic) and centrifuged at 3000 rpm for 15 minutes. The level of GSH in the supernatant was determined by the Ellmann method. Tissue protein concentration was determined using a biuret method. GSH level was specified as mg/g/protein (16).

Statistics

A computer software package was used in the statistical evaluation of results. The arithmetic means and standard errors of all parameters were calculated. Variance analysis was used to determine differences between the groups. The Least Significant Difference "LSD" Test was employed to compare group means in the statistically significant variance analysis results. Differences for which $p<0.05$ were accepted significant.

Results

In the study, the highest MDA values in liver, lung and spleen tissues were found in the Group 7 ($p<0.001$). The values in the Group 8 were lower than the values in the Group 7, but higher than in all other groups ($p<0.001$). MDA values in liver, lung and spleen tissues in the Groups 3, 4 and 6 were lower than the values in the Groups 7 and 8, but higher than those in the Groups 1, 2 and 5 ($p<0.001$ (Tab. 1).

The Group 5 had the highest liver, lung and spleen GSH values ($p<0.001$). GSH values of liver, lung and spleen tissues in the

Tab. 1. Levels of MDA in liver, lung and spleen tissues of groups (nmol/ml).

Groups (n=10)	liver	lung	spleen
1 General Control	59.08±10.99D	22.41±8.20D	22.11±4.74D
2 Melatonin Supplemented Control	62.47±6.73D	21.50±5.30D	23.06±5.45D
3 Melatonin Supplemented Diabetic Control	83.77±13.82C	60.30±8.75C	43.41±10.20C
4 Swimming Control	85.34±14.38C	61.70±9.90C	47.70±8.90C
5 Melatonin Supplemented Swimming	58.08±11.69D	25.30±6.45D	23.56±5.72D
6 Melatonin Supplemented Diabetic Swimming	87.87±10.65C	60.34±5.52C	45.34±9.22C
7 Diabetic Swimming	112.65±15.01A	82.41±8.08A	75.63±11.16A
8 Diabetes	90.12±11.33B	71.50±5.73B	63.06±10.60B

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

Tab. 2. Levels of GSH in liver, lung and spleen tissues of groups (mg/dl).

Groups (n=10)	liver	lung	spleen
1 General Control	25.94±6.45D	22.95±6.26D	17.25±2.85D
2 Melatonin Supplemented Control	43.93±5.72C	45.55±7.10C	43.22±5.80C
3 Melatonin Supplemented Diabetic Control	44.82±8.70C	43.80±6.39C	41.60±6.75C
4 Swimming Control	46.36±6.08C	45.75±5.54C	46.28±9.30C
5 Melatonin Supplemented Swimming	70.48±7.95A	69.80±4.69A	72.76±7.50A
6 Melatonin Supplemented Diabetic Swimming	56.93±5.04B	57.89±6.50B	60.25±9.09B
7 Diabetic Swimming	22.42±4.03D	23.83±4.92D	18.47±3.90D
8 Diabetes	23.61±5.25D	220.35±3.80D	15.56±2.98D

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

Group 6 were lower than the values in the Group 5, but higher than those in all other groups ($p<0.001$). Values of GSH in the concerned tissues in the Groups 2, 3 and 4 were lower than the values in the Groups 5 and 6, but higher than those in the Groups 1, 7 and 8 ($p<0.001$) (Tab. 2).

Discussion

In the present study, the highest MDA levels in liver, lung and spleen tissues were obtained in the Group 7 (diabetic swimming). It is known that lipid peroxidation significantly increases in diabetic conditions (17, 18). It has also been shown by many researchers that physical exercise causes oxidative stress (19–22). High MDA values we obtained in the Group 7 with induced diabetes and subjected to an acute swimming exercise, are consistent with the studies presented above. MDA values in the Group 8 (diabetic group) were lower than the values in the Group 7 (diabetic swimming group), but higher than all others. Besides being a chronic metabolic disorder, diabetes is a condition of increased oxidative stress (23). Elevated free radicals in diabetes interact with lipids, proteins and nucleic acids to cause loss of membrane integrity, structural and functional changes in proteins, and genetic mutations (23). High MDA levels obtained in the Group 8 can be considered as an expected result, and this result is parallel to the results of researchers who reported increased lipid peroxidation in diabetes.

MDA values in liver, lung and spleen tissues in the Groups 3, 4 and 6 were lower than the values in the Groups 7 and 8, but higher than those in the Groups 1, 2 and 5. Low levels of MDA found in the Groups 3 and 6, relative to the Groups 7 and 8, are particularly important, as the Groups 3 and 6 are melatonin-administered groups, and the results obtained in these groups indicate that melatonin administration inhibits MDA values in diabetic groups, irrespective of exercise. The fact that elevated MDA levels in various tissues of rats subjected to swimming exercise were reported to be inhibited by melatonin administration (24) also supports the results we obtained in the Groups 3 and 6. Additionally, the decrease we obtained in MDA with melatonin administration is consistent with the report showing that melatonin has a protective effect by preventing an increased lipid peroxidation in liver injury induced by alpha-naphthylisothiocyanate (ANIT) and another study (25), by Guven et al. (26) showing that melatonin administration prevents the tissue damage in the liver of diabetic rats. The Group 5 (melatonin-administered swimming group) had the highest liver, lung and spleen GSH values. This result indicates that melatonin administration significantly increased GSH levels in an acute swimming exercise. The fact that melatonin administration to rats subjected to a half-hour acute swimming exercise was shown to increase levels of GSH in various tissues, in comparison to the exercise group, which was not administered melatonin (27), is consistent with high GSH levels we found in our study.

GSH values in the group 6, which was the melatonin-administered diabetic swimming group, were lower than in the Group 5, but higher than in all other groups. Many researchers have reported that exogenous melatonin may increase antioxidant activity and prevent oxidant damage in diabetic rats (28–30). Elevated GSH levels obtained with melatonin administration in the present study prove that melatonin administration to diabetic rats subjected to swimming exercise increases antioxidant activity. This result also demonstrates that melatonin administration may be important for exercising diabetics.

Conclusion

The results obtained from the study indicate that melatonin administration prevents an increase in free radical production and the inhibition of antioxidant activity in diabetes and acute swimming exercise.

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