ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

Protective Effect of Melatonin on the Oxidative Stress Caused by Diabetes and Forced Swimming Exercise on Rat Brain Tissue

Diyabetin ve Zorlu Yüzme Egzersizinin Sıçan Beyin Dokusunda Yol Açtığı Oksidan Hasar Üzerine Melatoninin Koruyucu Etkisi

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ABSTRACT Objective: The objective of the present study is to examine how melatonin supplementation affects lipid peroxidation in the brain tissue of diabetic rats subjected to acute swimming exercise. **Material and Methods:** The study was carried out on 80 Spraque-Dawley type adult male rats, which were equally allocated to 8 groups: Group 1, general control; Group 2, melatonin-supplemented control; Group 3, melatonin-supplemented diabetic control; Group 4, swimming control; Group 5, melatonin-supplemented swimming; Group 6, melatonin-supplemented diabetic swimming; Group 7, diabetic swimming; and Group 8, diabetic control. The animals were injected with 40 mg/kg subcutaneous streptozotocin (STZ). The same dose was repeated after 24 hours. The rats were supplemented with 3 mg/kg/day intraperitoneal (ip) melatonin for 4 weeks. Brain tissue samples were collected from the animals, which were decapitated at the end of the study, to determine malondialdehyde (MDA) (nmol/gram/protein) and glutathione (GSH) (mg/dL/gram protein) levels. **Results:** The highest MDA values in brain tissue were in Group 7 and 8. Groups 3 and 6 had the highest brain GSH values. Brain GSH values in Group 2 were lower than the values in Groups 3 and 6, but higher than those in all other groups. The lowest GSH values in brain tissue were established in Groups 7 and 8. **Conclusion:** Results of the present study indicate that the oxidative stress caused by diabetes and acute swimming exercise in rat brain tissue can be offset by melatonin supplementation.

Key Words: Oxidative stress; exercise; melatonin; brain; lipid peroxidation; rat

ÖZET Amaç: Bu çalışmanın amacı, akut yüzme egzersizi yaptırılan diyabetik sıçanlarda melatonin uygulamasının, beyin dokusundaki lipit peroksidasyonunu nasıl etkilediğinin araştırılmasıdır. **Gereç ve Yöntemler:** Spraque-Dawley cinsi 80 adet erişkin erkek sıçan kullanılan çalışmada, deney hayvanları eşit sayıda 8 gruba ayrıldı: Grup 1, genel kontrol; Grup 2, melatonin uygulanan kontrol; Grup 3, melatonin uygulanan diyabetli kontrol; Grup 4, yüzme kontrol; Grup 5, melatonin uygulanan yüzme; Grup 6, melatonin uygulanan diyabetli yüzme; Grup 7, diyabetli yüzme ve Grup 8, diyabetli kontrol. Diyabet oluşturmak için hayvanlara 40 mg/kg dozunda intraperitoneal (ip) streptozotosin (STZ) enjekte edildi. Enjeksiyonlar 24 saat sonra aynı dozda tekrarlandı. Son enjeksiyonlardan 6 gün sonra kan glukoz düzeyi 300 mg/dL ve üzerinde olan hayvanlar diyabetik olarak kabul edildi. Hayvanlara 4 hafta boyunca 3 mg/kg/gün deri altı melatonin verildi. Çalışmanın bitiminde dekapite edilen hayvanlardan alınan beyin dokusu örneklerinde malondialdehit (MDA) (nmol/gram/ protein) ve glutatyon (GSH) (mg/dL/gram protein) düzeyleri tayin edildi. **Bulgular:** Beyin dokusundaki en yüksek MDA değerleri Grup 7 ve 8'de elde edildi. Grup 3 ve 6 en yüksek beyin GSH değerlerine sahipti. Grup 2'nın aynı değerleri Grup 3 ve 6'dan düşük, diğer grupların tamamından yüksekti. Beyin dokusundaki en düşük GSH değerleri Grup 7 ve 8'de elde edildi. **Sonuç:** Mevcut çalışmanın sonuçları, diyabetin ve akut yüzme egzersizinin sıçan beyin dokusunda yol açtığı oksidan hasarın melatonin uygulamasıyla önlenebileceğini göstermektedir.

Anahtar Kelimeler: Oksidatif stres; egzersiz; melatonin; beyin; lipit peroksidasyon; sıçan

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Diabetes mellitus (DM) is a major health problem associated with increased cardiovascular mortality, neuropathy, nephropathy and retinopathy resulting from abnormal insulin secretion and impairments in the carbohydrate and lipid metabolisms.[1] Life style changes, as well as exercise, nutrition and behavioural changes are highly important in the prevention and/or treatment of diabetes.[2] Type I diabetes develops as a result of severe damage in pancreatic cells and commonly results in insulin dependency. Oxidative stress is among the leading mechanisms that are believed to be involved in beta cell damage. Therefore, studies on the treatment and prevention of diabetes have focused on both conditions in which oxidative stress is involved and prevention by antioxidants.[3] Regular exercising strengthens antioxidant defences and may reduce oxidative stress during rest after acute exercise.[4] However, information is lacking on the benefits and risks of acute and chronic exercise in groups of people who have increased sensitivity to oxidative stress, like diabetes patients.[5] Melatonin is a strong antioxidant which eradicates hydroxyl (-OH), the most harmful radical.[6,7] A principal characteristic of melatonin as an antioxidant is its ability to reach almost all organelles and nucleus of the cell and also to easily pass through barriers like the blood-brain barrier. Thus, melatonin has a wide spectrum of antioxidant activity. A crucial advantage of melatonin is that it is not toxic even when used at high doses and for long periods of time.[6-8] Consequently, besides being effective as a direct free radical scavenger, melatonin also indirectly activates the antioxidant defence systems.[6,9] Melatonin, which is known to contribute to the carbohydrate metabolism, is also claimed to play a protective role against diabetes.[10,11] In experimental studies, diabetes is used as a model of oxidative stress.[12] It was demonstrated in diabetic rats that lipid peroxidation increased in various tissues, including the brain tissue, and antioxidant defences were impaired in diabetes.[12] It was also reported that lipid peroxidation caused by diabetes in the brain tissue of rats was inhibited by melatonin supplementation.[13] Of the various methods available to monitor oxidative stress, the most frequently used in clinical and experimental studies is the thiobarbituric acid assay for the determination of malondialdehyde (MDA), one of the end products of lipid peroxidation. In the present study, the MDA and glutathione (GSH) levels were chosen as indicators of oxidative damage and of antioxidant system status, respectively. The objective of the present study was to examine how melatonin supplementation influenced lipid peroxidation in the brain tissue of diabetic rats subjected to acute swimming exercise.

MATERIAL AND METHODS

ANIMAL MATERIAL AND GROUPS

The study included 80 Spraque-Dawley type adult male rats obtained from the Akdeniz University Experimental Medicine Practice and Research Centre and was run in the Experimental Animals Unit of the Selçuk University School of Veterinary Medicine. The study protocol was approved by the local ethics committee. The animals used in the study were equally divided into 8 groups:

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

Group 2 (n=10) Melatonin-Supplemented Control Group: The group fed on a normal diet and was supplemented with 3 mg/kg/day intraperitoneal (ip) melatonin for 4 weeks.

Group 3 (n=10) Melatonin-Supplemented Diabetic Control Group: The group in which diabetes was induced by subcutaneous injection of 40 mg/kg streptozotocin (STZ) and which was supplemented with 3 mg/kg/day ip melatonin for 4 weeks.

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

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

Group 6 (n=10) Melatonin-Supplemented Diabetic Swimming Group: The group in which diabetes was induced by subcutaneous injection of 40

mg/kg STZ, and that was then supplemented with 3 mg/kg/day ip melatonin for 4 weeks and was subjected to 30-minute acute swimming exercise.

Group 7 (n= 10) Diabetic Swimming Group: The group in which diabetes was induced by subcutaneous injection of 40 mg/kg STZ and which was subjected to 30-minute acute swimming exercise.

Group 8 (n= 10) Diabetes Group: The group in which diabetes was induced by subcutaneous injection of 40 mg/kg STZ.

EXPERIMENTAL ANIMALS

The experimental animals were fed in steel cages, which were washed clean daily. Their feed was provided in special steel bowls and their water (normal tap water) in glass feeding bottles. The animals were given about 10 g feed per 100 g of body weight daily. They were kept in an environment where it was 12 hours dark and 12 hours light and standard room temperature (21±1 °C) was maintained. All injections were given at 9.00 and 10.00 AM. At the end of the four-week procedures, the animals were decapitated at 9.00-10.00 AM and brain tissue samples were obtained to be used in the analyses. The brain tissue samples collected were kept at -80 °C until analysis.

EXPERIMENTAL PROCEDURES

Inducement of Diabetes in Experimental Animals

In order to induce diabetes, 40 rats were selected as the diabetes groups. The rats were injected with 40 mg/kg subcutaneous STZ (Sigma, S-0130). Same dose was repeated after 24 hours. Six days after the last injection, blood glucose levels of the animals were determined in blood samples drawn from the tail vein using a diagnostic glucose kit. The animals whose blood glucose was 300 mg/dL or higher were considered diabetic.[14]

SWIMMING EXERCISE

Swimming exercise was performed in a heat-resistant glass swimming pool, 50 cm deep and wide with a thermostat that kept the temperature fixed at 37°C. The exercise was conducted once for 30 minutes, 24 hours after the procedures ended. The experimental animals were made to swim in pairs, and then were decapitated to collect brain tissue samples.

MELATONIN SUPPLEMENTATION

After dissolving 40 mg melatonin (Sigma M-5250) in 3ml pure ethanol, the suspension was sealed and was stored in dark in the deep freeze until the time of use. From this stock solution 0.1ml was added in 0.9 ml NaCl (3 mg/kg/day) and was then injected to the rats through the intraperitoneal route at 9.00 AM. Melatonin supplementation was carried out at the same hours for 4 weeks.

BIOCHEMICAL ANALYSES

MDA Analyses in Brain Tissue

Brain tissue samples were homogenized at 4°C with 150 mMol KCl to obtain a 10% homogenate (Microsan Ultrasonic Cell Disruptor Misonic). To 2 ml of the homogenate, 2 ml $HClO_4$ was added and was centrifuged at 3000 rpm for 15 minutes. MDA level was measured in the supernatant. Of the hemogenate, 0.5 ml was mixed with 3 ml H_3PO_4 and 1ml 0.675% thiobarbituric acid and then was kept in a boiling water bath for 45 minutes. MDA levels in brain tissue were measured at 532 nm and were expressed as nmol/gram/protein.[15]

Determination of GSH in Brain Tissue

Brain tissue samples were homogenized at 4°C with 150 mMol KCl to obtain a 10% homogenate (Microsan Ultrasonic Cell Disruptor Misonic) and were centrifuged at 3000 rpm for 15 minutes. GSH level in the supernatant was measured with the Ellman method. Protein concentration in the brain tissue was identified in accordance with the biuret method. GSH level was expressed as mg/g/protein.[16]

STATISTICAL ANALYSES

Computer package software (SPSS 16.0) was used in the statistical analyses of results. Data were expressed as median (lowest, highest). The significant differences between groups were determined by the Kruskal-Wallis test; individual comparisons between groups were made with the Mann-Whitney-

U test. Bonferroni correction was done for pairwise comparisons (α^*=0.05/8=0.006). P<0.006 was considered statistically significant.

RESULTS

The highest MDA levels in the brain tissue were in Group 7 (p<0.006). MDA values in Group 8 were lower than the levels in Group 7 but was higher than those in all other groups (p<0.006). Group 6 had lower brain MDA values than Groups 7 and 8 (Table 1). Groups 2, 3 and 6 had the highest brain GSH values (p<0.006). The same values in Groups 1, 4 and 5 were lower than in Groups 2, 3 and 6 (p<0.006). The lowest GSH values in the brain tissue were obtained in Groups 7 and 8 (p<0.006) (Table 2).

DISCUSSION

The highest MDA levels in the brain tissue were obtained in the diabetes group subjected to swimming exercise (Group 7). MDA levels in Group 8 (diabetes group) were lower than those in Group 7 but were higher than the MDA levels in all other groups. Experimental studies reported that exhaustive exercise increased oxidant damage in the blood and various tissues, including the brain tissue.[17, 18] However, the effect of diabetes and exercise on lipid peroxidation in the brain tissue of diabetic rats is controversial. Neither exercise nor melatonin-supplementation was reported to have a significant effect on oxidative mechanisms of the brain tissue in rats subjected to 30-minute swimming exercise.[19]

TABLE 1: Levels of malondialdehyde (nmol/gram/protein) in the brain tissue of groups.

Groups	MDA (Median)	Min	Max
1 General Control	77.12^{D}	72.41	90.68
2 Melatonin-Supplemented Control	75.49^{D}	71.46	85.69
3 Melatonin-Supplemented Diabetic Control	81.86^{D}	72.06	85.75
4 Swimming Control	80.60^{D}	75.60	81.58
5 Melatonin-Supplemented Swimming	88.04^{D}	75.12	88.44
6 Melatonin-Supplemented Diabetic Swimming	106.76^{C}	105.25	108.07
7 Diabetic Swimming	156.20^{A}	141.70	181.80
8 Diabetes	132.89^{B}	131.70	140.54

*Means with different superscripted letters in the same column are statistically significant α^*=0.05/8=0.006 (Bonferroni Correction), P<0.006.
Mann Whitney–U P Values
MDA: Malondialdehyde: A>BCD, B>CD, C>D.
Grup 1-2: 0.268, 1-3: 0.876, 1-4: 0.456, 1-5: 0.639, 1-6: 0.003, 1-7: 0.003, 1-8: 0.003, 2-3: 0.548, 2-4: 0.149, 2-5: 0.095, 2-6: 0.005, 2-7: 0.005, 2-8: 0.005, 3-4: 0.639, 3-5: 0.222, 3-6: 0.005, 3-7: 0.005, 3-8: 0.005, 4-5: 0.530, 4-6: 0.003, 4-7: 0.003, 4-8: 0.003, 5-6: 0.008, 5-7: 0.008, 5-8: 0.008, 6-7: 0.005, 6-8: 0.005, 7-8: 0.005.

TABLE 2: Levels of glutathione (mg/dL/gram protein) in the brain tissue of groups.

Groups	GSH (Median)	Min	Max
1 General Control	24.27^{B}	21	25
2 Melatonin-Supplemented Control	31.60^{A}	29	32
3 Melatonin-Supplemented Diabetic Control	36.73^{A}	35	47
4 Swimming Control	22.08^{B}	18	27
5 Melatonin-Supplemented Swimming	22.26^{B}	20	27
6 Melatonin-Supplemented Diabetic Swimming	35.89^{A}	34	36
7 Diabetic Swimming	14.61^{C}	12	16
8 Diabetes	15.08^{C}	14	16

*Means with different superscripted letters in the same column are statistically significant α^*=0.05/8=0.006 (Bonferroni Correction), P<0.006.
Mann Whitney–U P Values.
GSH: Glutathione: A>BC, B>C.
Grup 1-2: 0.003, 1-3: 0.003, 1-4: 0.805, 1-5: 0.432, 1-6: 0.003, 1-7: 0.003, 1-8: 0.003, 2-3: 0.008, 2-4:0.003, 2-5: 0.008, 2-6: 0.008, 2-7: 0.008, 2-8: 0.008, 3-4: 0.003, 3-5: 0.008, 3-6: 0.310, 3-7: 0.008, 3-8: 0.008, 4-5: 0.755, 4-6: 0.003, 4-7: 0.003, 4-8: 0.003, 5-6: 0.008, 5-7: 0.008, 5-8: 0.008, 6-7: 0.008, 6-8: 0.008, 7-8: 0.151.

Rauscher et al. showed that antioxidant activity was markedly inhibited in the brain tissue of rats which had diabetes induced by STZ.[12] Consequently, many researchers reported that diabetes increased lipid peroxidation in experimental animals.[20-22] In any case, diabetes is used as a model of oxidative damage in experimental animals.[12,22] Elevated MDA levels we obtained in the brain tissue of the diabetes group (Group 8), which was not subjected to any additional procedure, are consistent with the results of the above-cited researchers. However, in the present study, we established the highest brain MDA values in the diabetes group subjected to swimming exercise (Group 7). This finding of ours indicates that MDA levels that increase in diabetes are further elevated by acute swimming exercise. The report by Atalay et al.[23] suggesting that lipid peroxidation which increased in diabetic rats showed a further increase with exercise is a remarkable result lending support to the elevated brain MDA levels we found in Group 7. Furthermore, we found the lowest brain GSH values in the diabetic groups, which were not supplemented with melatonin (Groups 7 and 8). Increased oxidative stress has an important place in the pathogenesis of diabetes; therefore, there is a clear correlation between diabetes and oxidative stress.[24,25] However, it was noted that the major cause of increased lipid peroxidation in diabetes was the impaired antioxidant defence system.[26] Elevated brain MDA levels we obtained in non-melatonin-supplemented groups in our study may be attributed to the disruption of antioxidant activity. Similarly, reduced GSH levels we found in non-supplemented diabetic groups (Groups 7 and 8) are supportive of this conviction of ours.

Melatonin-supplemented groups-Group 3 (melatonin-supplemented diabetes) and group 6 (melatonin-supplemented diabetic swimming)-had the highest brain GSH values. Melatonin has been shown in numerous studies to activate the antioxidant system.[27,28] The fact that melatonin-supplementation brought about a strong antioxidant effect in diabetic rats has made researchers focus on the protective effect of melatonin in diabetes.[29,22] It has even been noted that low circulatory levels of melatonin may be associated with the development of diabetes.[30] Baydas et al. have impressively shown that the oxidant damage in the brain tissue of diabetic rats is inhibited in parallel to elevated GSH levels with melatonin supplementation.[13] Their result is also consistent with the elevated GSH levels we found in the brain tissue in Groups 3 and 6. Elevated GSH levels in the brain tissue of diabetic rats supplemented with melatonin (Groups 3 and 6) is an indicator of the fact that melatonin supplementation may be important in diabetics and exercise.

CONCLUSION

The results of the present study indicate that the oxidant damage that occurs in diabetic rats and/or diabetic rats subjected to acute swimming exercise can be prevented by melatonin supplementation.

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