Effect of melatonin on spinal cord injury induced lipid peroxidation in rats: prognostic value of malondialdehyde

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Ratlarda lipid peroksidasyonunun indüklediği spinal kord hasarında melatoninin etkisi: malondialdehitin tanısal önemi

Özet

Amaç: Melatonin, pineal bez tarafından üretilen güçlü bir antioksidan ajandır. Bu çalışmada biz, ratlarda spinal kord iskemisinde melatoninin nöroprotektif etkisini araştırmayı amaçladık. **Yöntem:** Abdominal aortun oklüzyonu için 30dk süre ile aortic klemp kullanıldı. Çalışmada 24 Winstar erkek rat kullanıldı ve ratlar rastlantısal olarak 4 gruba ayrıldı: kontrol grubu (sham grup, n:5), sham + melatonin verilen grup (10mg/kg melatonin intraperitoneal olarak verildi, n:5), iskemi/reperfüzyon grubu (oklüzyon yapılan ancak herhangi bir farmakolojik ajan verilmeyen grup, n:5), melatonin + iskemi/reperfüzyon grubu (oklüzyon yapılan ve iskemiden 10 dk sonra intraperitoneal olarak 10mg/kg melatonin verilen grup). Spinal kord örnekleri malondialdehit (MDA) analizi için alındı. **Sonuçlar:** Aort oklüzyonu yapılan grupta, lipid peroksidasyonunun olduğunu gösteren MDA düzeyleri diğer gruplardan belirgin olarak daha yüksekti ($p<0.05$). Aort oklüzyonu+melatonin verilen grupta da aort oklüzyonu yapılan grup ile karşılaştırıldığında MDA düzeylerinin belirgin olarak azaldığı görüldü ($p<0.05$). **Tartışma:** Bu çalışmada biz, melatonin enjeksiyonunun sekonder hasarın etkilerini azalttığını ve hasarlı spinal kordun iyileşmesini kolaylaştırdığını gösterdik.

Anahtar Kelimeler: melatonin, iskemi/reperfüzyon, spinal kord, oksidatif stress, malondialdehit

Abstract

Aim: Melatonin is a potent antioxidant agent produced by the pineal gland. In this study we aimed to investigate the neuro-protective effect of melatonin on ischemic spinal cord in the rats. **Method:** Aortic clamp was used for the occlusion of the abdominal aorta for 30 minutes. Twenty four Winstar male rats were used for this study and rats were divided randomly into 4 groups: control group (sham group, n: 5), sham+melatonin treated group (receiving 10mg/kg melatonin intraperitoneally, n: 5), ischemia/reperfusion group (undergoing occlusion but receiving no pharmacologic intervention, n: 7), melatonin + ischemia/reperfusion group (undergoing occlusion and receiving 10mg/kg melatonin intraperitoneally 10 minutes after ischemia, n: 7). Spinal cord samples were taken for the malondialdehyde (MDA) analyses. **Results**: In the aort occlusion group, the MDA levels, indicating the extend of lipid peroxidation, were significantly higher than the other groups ($p<0.05$). Also in the aort occlusion + melatonin group the levels of MDA were significantly reduced compared to the aort occlusion group ($p<0.05$). **Discussion:** In this study, we showed that injection of melatonin reduces the effects of secondary injury and facilitate the recovery of the damaged spinal cord.

Key Words: melatonin, ischemia-reperfusion, spinal cord, oxidative stres, malondialdehyde

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Müracaat tarihi: 13.09.2011
Kabul tarihi: 30.09.2011

Introduction

Spinal cord injury has resulted in a classification scheme of primary and secondary injury. Primary injury occurs as a result of the destructive nature of the initial

impact and the secondary injury occurs after a complex array of pathophysiological processes including ischemia, inflammation, excitotoxicity, oxidative cell damage in the minutes to years following the initial injury (1) and contributes to the worsening of motor functions (2). In the underlying mechanisms of the secondary injury, increasing and activation of neutrophilic infiltration in the traumatized spinal cord takes an important role (3). Oxygen radicals, released from neutrophils, damage the cell membrane and free radical-induced lipid peroxidation is important in the destruction of the injured spinal cord (4,5).

Melatonin, a secretory product of pineal gland, takes part in many important physiologic functions like regulation of circadian rhythms (6). High lipophilicity of melatonin is important for providing oxidative protection (7). Also melatonin plays an important role in protection from free-radical damage and neutrophil-induced toxicity by limiting the increased myeloperoxidase activity (8).

In this study we aimed to see the protective effect of melatonin on lipid peroxidation due to secondary injury of the spinal cord.

Materials and methods

Animal Model

In the study twenty four Winstar-Albino male rats weighed between 200-250 g were used. All animals received care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research" and the " Guide for Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). The animals were provided with free acces to food and water. They were kept under controlled temperature (25 ± 2 ºC) and illumination (12 hours each of light and darkness).

Melatonin preparation

Melatonin (Sigma, St Louis, MO, USA) was dissolved in ethanol (0.5 ml) and diluted with physiological saline.

Technique of aortic ischemia-reperfusion

The rats were anesthesized with an intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) and were placed under a heating lamp. The skin was aseptically prepared and a midline laparatomy was performed. 10 ml of warm normal saline was instilled into the peritoneal cavity to help maintain fluid balance. The abdominal aorta was exposed by gently deflecting the loops of intestine to the left with moist gauze swabs. An atraumatic microvascular clamp (vascu-statts II, midi straight 1001-532 ; Scanlan Int., St. Paul, MN, USA) was then placed across the infrarenal abdominal aorta for 30 minutes. The abdomen was then closed and the wound was covered with plastic wrap to minimize the heat and fluid losses. Aortic occlusion and reperfusion were confirmed by the loss and reappearance of satisfactory pulsation in the distal aorta. All animals were sacrified with intramuscular 100 ml/kg ketamine hydrocloride (Ketalar®, Parke-Davis, Eczacibaşı, Istanbul, Turkiye) and 25 mg/kg xylazine hydrochloride (Rompun®, Bayer, Germany) then tissue samples were obtained. The spinal cord was removed, and washed in ice-cold phosphate buffered saline, and frozen immediately in a deep freezer until further use.

Experimental design

Twenty four rats were randomly allocated to four groups as follows: SHAM (n= 5), SHAM + Melatonin (n= 5), Aort Occlusion (n= 7), and Aort occlusion + Melatonin (n= 7). In the sham group, the animals operated and underwent the same procedures as the control group except aortic occlusion. In the sham + melatonin group, the animals operated and underwent the same procedures and melatonin 10 mg/kg was given intraperitoneally. In the aort occlusion group, the animals operated and ischemia/reperfusion was developed by

clamping the thoraco-abdominal aorta for 30 minutes. In the aort occlusion+melatonin group, the animals operated and ischemia/reperfusion was developed by clamping the thoraco-abdominal aorta for 30 minutes and melatonin 10 mg/kg was given intraperitoneally.

Biochemical procedure

The frozen tissue samples of spinal cord were homogenized (Ultra Turrax T25, Janke & Kunkel GmbH & Co., KG, Staufen, Germany) (1:10,w7v) in 100 mmol/liter phosphate buffer (pH 7.4) keeping in an ice bath. MDA measurement was performed from homogenate.

Malondialdehyde assay

MDA levels, indicating the extent of lipid peroxidation and increased at the end of the reperfusion, were estimated by the double heating method of Draper and Hadley (9). The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. Because of this, 2.5 ml of 100 g/l trichloroacetic acid solution was added to 0.5 ml homogenate in each centrifuge tube, and the tubes were placed in a boiling water bath for 15 min. After cooling in tap water, the tubes were centrifuged at 1000×g for 10 min and 2 ml of the homogenate was added to 1 ml of 6.7 g/l TBA solution in a test tube. The tube was then placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Japan) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of the MDA–TBA complex (absorbance coefficient $\varepsilon = 1.56 \times 105$ cm-1 M-1) and is expressed as nanomoles per gram protein. The protein content of was determined using the Lowry method (10).

Statistical analysis

Data are expressed as means – standart deviation (SD) values. In the comparison of the groups the Kruskall-Wallis nonparametric analysis is used and post hoc comparisons were made using the Mann-Whitney U test. P values < 0.05 were considered as statistically significant.

Results

In this study, 24 male Winstar rats were used. Results were shown in the figure. In the aort occlusion group, the MDA levels were significantly higher than the other groups ($p<0.05$). In the aort occlusion and aort occlusion + melatonin group the levels of MDA, as an index of lipid peroxidation, were significantly increased in the spinal cord tissue samples compared to the sham and sham + melatonin groups ($p<0.05$). In addition, in the aort occlusion + melatonin group the levels of MDA was significantly reduced compared to the aort occlusion group ($p<0.05$).

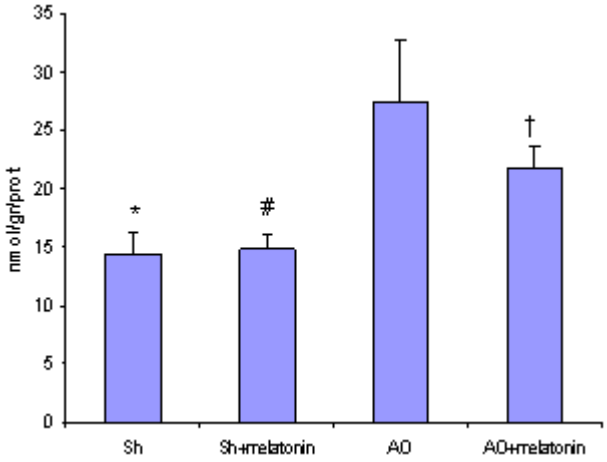

Figure 1: Effect of Melatonin on aort occlusion (AO) in the spinal tissue samples MDA levels * $p<0.01$ when compared with AO and AO+melatonin groups
# $p<0.01$ when compared with AO and AO +melatonin groups, † $p<0.01$ when compared with AO

Discussion

Acute spinal cord injury occurred by experimental models support the primary and secondary injury in which the initial mechanical insult is followed by a series of events that promote progressive tissue damage and ischemia. Therapeutic modulation is important for the secondary

injury. Our experiment tested a hypothesis that whether melatonin has a preventive role on acute spinal cord injury-induced lipid peroxidation.

The change in MDA levels in the current study was regarded as an indicator of increased reactive oxygen species production occurring during the ischemic period and may reflect the pathological process of the spinal cord injury.

Bracken et al. (11) reported that as the treatment delays the secondary injury increases and it becomes to be more difficult to slow the injury cascade of neurodestructive events. The human central nervous system, mostly composed of lipids, can be easily damaged by hydroxyl radicals or lipid peroxidation (4).

Lipid peroxidation is an important parameter for evaluating the cellular disturbances caused by spinal cord injury (12-15). Protection against lipid peroxidation and free radicals that occurs after the onset of neuronal ischemia is the main purpose for the prevention of the secondary injury. Many studies have showed a positive correlation between increased antioxidant enzymes and decreased antioxidant defenses (16).

Melatonin, an antioxidant agent for free oxygen radicals, takes place in the treatment of a variety of cerebral pathologies including ischemia, vasospasm and brain edema in experimental settings (14, 17) as a highly soluble and easily diffusible molecule (18). Fujimoto et al. (2) showed that MDA levels increased after the injury and melatonin has a protective effect on experimental trauma induced on rat spinal cord. They determined the reduction of TBA reactive substance content, which shows the changes in malonaldehyde precursors in tissue.

Our results indicate the increase in MDA levels as an indicator of the ischemia/reperfusion injury in correlation with the literature.

The role of oxygen free radicals in ischemic neuronal damage has raised the interest in antioxidants (14).

Erten et al. (14) showed that antioxidant enzyme levels due to the extensive presence of free radicals in damaged tissue were significantly changed in the presence of melatonin. They had found that there was a significant decrease in the free radical scavengers such as superoxide dismutase and catalase in the melatonin given pre-occlusion. Also Korkmaz et al reported that melatonin administration may reduce the incidence of spinal cord injury, but they used melatonin 10 minutes before ischemia (19). In our study we showed that melatonin given post-occlusion is also decreases the effects of the secondary injury.

In conclusion, the present study shows that melatonin has an effect in preventing secondary damage due to peroxidation of lipid membranes in the ischemic spinal cord. Treatment with melatonin may have a potential role in an alternative treatment for spinal cord ischemia. In addition, it can be suggested that MDA levels may have a prognostic value and may be an important marker showing the degree of spinal changes and the success in the treatment of neuronal impairment with melatonin.

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