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Original Research

Effect of melatonin on cytokine levels in a hyperthermia-induced febrile seizure model

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Abstract: Higher serum cytokine levels have been reported in children admitted with febrile seizures and in some experimental models. However, other studies have shown that cytokine levels are influenced by melatonin. In this study, we investigated serum cytokine levels in a hyperthermia-induced febrile rat seizure model and the effect of melatonin. A total of 28 male Sprague–Dawley rats were divided into four groups: the control (C) group, healthy melatonin (MT) group, and hyperthermia-induced febrile seizure groups with (HIFS-MT) and without (HIFS) administration of melatonin. Melatonin (80 mg/kg) was given intraperitoneally 15 min before the seizure. HIFS was induced by placing the rats in 45°C water. The rats were sacrificed under anesthesia after the seizure. Blood samples were drawn by transcardiac puncture to measure serum cytokine and melatonin levels. Serum interleukin (IL)-1β, IL-6, IL-10, and tumor necrosis factor (TNF)-α levels were lower in the HIFS group than those in the C group ($p = 0.005$, $p = 0.200$, $p = 0.011$, and $p = 0.016$, respectively). All serum cytokine levels of rats in the MT and HIFS-MT groups were similar to those in the C group. This experimental rat model demonstrated that serum cytokine levels decrease with HIFS and that administering melatonin maintains serum cytokine levels. These results suggest that cytokines may play role in the anticonvulsive activity of melatonin in rats with febrile seizures.

Key words: Hyperthermia-induced febrile seizures; Anticonvulsive effect; Melatonin; Cytokine; Serum.

Introduction

Febrile seizure (FS), the most commonly encountered neurologic disorder in children (1-3), is generally known to have a benign course. However, some experimental animal studies have reported 30%–40% prolonged complicated FSs resulting in temporal lobe epilepsy (4). In this regard, understanding FS pathogenesis is crucial for preventing seizures and diminishing seizure severity and duration.

A number of clinical and experimental studies have investigated the role of various cytokines including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-10 in FS pathogenesis (5-12). However, serum cytokine levels have not been studied in a hyperthermia-induced febrile seizure (HIFS) rat model.

Some clinical studies have reported lower serum cytokine levels in children with FS compared to those in healthy controls (13-15). Administering melatonin in addition to anticonvulsive treatment in children with FS has been investigated (16-17). In addition, the anticonvulsive activity of melatonin was shown experimentally in the maximal electroshock mouse model, pilocarpine-induced epilepsy rat model, pentylenetetrazol-induced seizure model, and penicillin-induced seizure rat model (18-22). We previously demonstrated the anticonvulsive efficacy of melatonin in a HIFS model (23). In addition, some studies have shown that melatonin affects serum levels of pro-inflammatory (IL-1β, IL-6, and TNF-α) and anti-inflammatory cytokines (24-26).

In this study, we investigated serum cytokine levels of rats in the HIFS model and the effect of melatonin.

Materials and Methods

Experimental Animals and Study Groups

In the present study, 22–30-day-old male Sprague–Dawley rats were divided randomly into four groups ($n = 28$); the control (C) group, melatonin-administered (MT) group, and groups experiencing hyperthermia-induced febrile seizure with (HIFS-MT) and without melatonin (HIFS). Rats were placed in standardized laboratory conditions under a 12-hour day/night photoperiod, and received food and water *ad libitum*. We allowed the rats to adapt to their new environment during the first 3 days.

General experimental procedure

All procedures were conducted between 09.00 and 12.00 am. Melatonin (80 mg/kg) was administered intraperitoneally 15 min before the seizure. Body temperature was measured rectally before and after the seizure. HIFS was achieved by placing the rats in 45°C water. Seizure severity was evaluated according to Racine's scale. Rats were sacrificed under anesthesia just after the febrile seizure, and blood was drawn by transcardiac puncture. Serum melatonin and cytokine levels were measured by enzyme-linked immunosorbent as-

say (ELISA).

Melatonin Preparation and Administration

Melatonin (M5250; melatonin powder, ≥98% TLC; Sigma, St. Louis, MO, USA) was dissolved in 2.5% (v/v) ethanol/saline solution just before use and administered intraperitoneally (i.p) at a dose of 80 mg/kg 15 min before the seizure (23,27).

Hyperthermia-induced Febrile Seizure Model

HIFS was achieved by placing the rats in 45°C water that did not reach the rat's neck. The rats were removed from the water and placed on a dry towel when the seizure began (myoclonic spasm at the edge of the lips or the rat stayed still and sank to the bottom) (28). Seizure latency, total duration of clinical seizure, and seizure stage were evaluated. Seizure latency was defined as the time interval between contact of the rat with the water and the beginning of the seizure. Clinical seizure duration was the time period from the beginning of the seizure until the rat was conscious and regained movement. Seizure severity was evaluated according to Racine's scale, which assigns scores as follows: 0, no convulsive behavior; 1, facial clonus; 2, nodding; 3, anterior limb clonus; 4, rearing up movement; and 5, rearing up and falling on one side due to imbalance (29).

Rectal Temperature Measurement

The body temperature of the rats was measured rectally before and as soon as possible after the febrile seizure (pre- and post-experimental) using the Medical Grade YSI 400 temperature probe for medical purposes (ABD; BIOPAC Systems, Inc. Santa Barbara, CA).

Blood Samples

The rats were sacrificed just after the febrile seizure by administering xylazine/ketamine (10 mg/kg and 100 mg/kg, i.p, respectively) (30). Blood samples were drawn by transcardiac puncture as soon as possible after sacrifice and centrifuged at 1,500 rpm for 6 min. All serum samples were labelled and stored at −80°C until biochemical analyses were performed (31).

ELISA

Protein levels of the serum samples were checked by the Bradford method. Then, serum melatonin (Bio-Assay) and cytokine (RAYBIOTECH, Norcross, GA, USA) (IL-1β, IL-6, TNF-α, and IL-10) levels were measured by ELISA according to the manufacturer's instructions (32).

Statistical Analysis

The Shapiro-Wilk test of normality was used to determine the normality of the distributions of continuous numeric variables. If parametric test assumptions were met, numerical variables are presented as mean and standard deviation; if these assumptions were not met, the numerical variables are presented as median and range. The study groups were compared in terms of numerical dependent variables including weight, serum melatonin level, pre-experimental rectal temperature, and serum IL-1, IL-6, TNF-α, and IL-10 levels using one-way analysis of variance for variables that met the parametric test assumptions and the Kruskal–Wallis analysis of variance for variables that did not. Between-group differences in numerical independent variables, such as post-experimental rectal temperature, latency, seizure duration, and seizure severity, were compared using Student's *t*-test if the parametric test assumptions were met and the Mann–Whitney *U*-test if not. Type-I error probability was $\alpha = 0.05$ for all tests. SPSS v17.0 software was used for the analysis (SPSS, Inc., Chicago, IL, USA, September 2012 license number: 1093910, Baskent University).

Table 1. Body weights and pre-experimental rectal temperatures in groups.

	Body Weight (gr) Mean±SD	Pre-experimental Rectal Temperature (°C) Median (Min-Max)
C	44.0 ± 4.62	35.9(34.7-35.9)
MT	43.7 ± 3.72	35.9(33.9-37.1)
HIFS	43.6 ± 5.53	35.7(35.5-36.0)
HIFS -MT	45.6 ± 4.57	35.7(34.7-35.9)
p	0,843[1]	0,780[2]

[1] One way analysis of variance test, [2] Kruskal Wallis variance analysis test

Results

The experimental animals in all groups were similar in terms of weight and pre-experimental rectal temperature (Table 1). No animal died during the study.

Rats in the MT and HIFS-MT groups had higher serum melatonin levels than did those in HIFS and C groups ($p < 0.05$) (Figure 1).

Post-experimental rectal temperature was lower in the HIFS-MT group than that in the other groups ($p = 0.02$). No significant difference was observed in seizure latency between the HIFS and HIFS-MT groups (79.0 ± 10.0 sec vs. 80.9 ± 27.46 sec, respectively, $p = 0.435$). The melatonin-administered seizure group (HIFS-MT) had a shorter mean seizure duration (174.0 ± 109.85 sec vs. 285.0 ± 121.50 sec, respectively, $p = 0.049$) and a lower mean seizure scale score ($p = 0.0005$) than those of the HIFS group (Table 2).

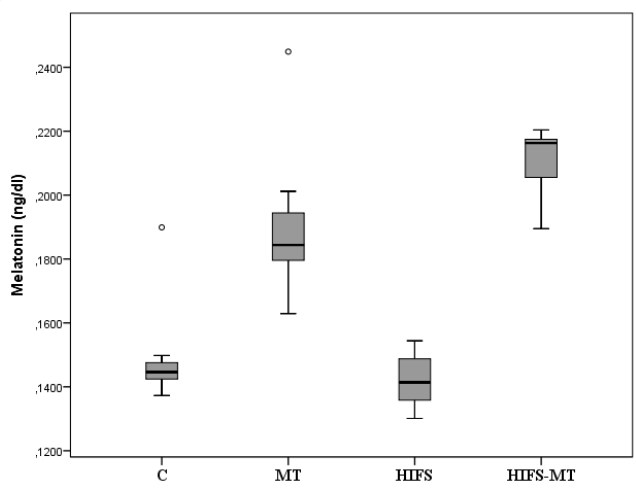

Figure 1. Serum melatonin levels of the study groups. Serum melatonin (MT) levels in the hyperthermia-induced febrile seizure with (HIFS-MT) and without (HIFS) MT and the control (C) groups ($p < 0.05$).

Table 2. Post-experimental rectal temperatures, latencies, seizure durations and scales in groups.

	Post-experimental Rectal Temperature (°C)	Latency (sec)	Seizure Duration (sec)	Seizure Scale
	Median (min-max)	Mean±SD	Mean±SD	Median (min-max)
HIFS	44.4(42.2-45.1)	79.0 ± 10.00	285.0 ± 121.50	5(4-5)
HIFS-MT	42.4(39.4-44.1)	80.9 ± 27.46	174.0 ± 109.85	3(1-3)
p	**0.02**	0.435	**0.049**	**0.0005**

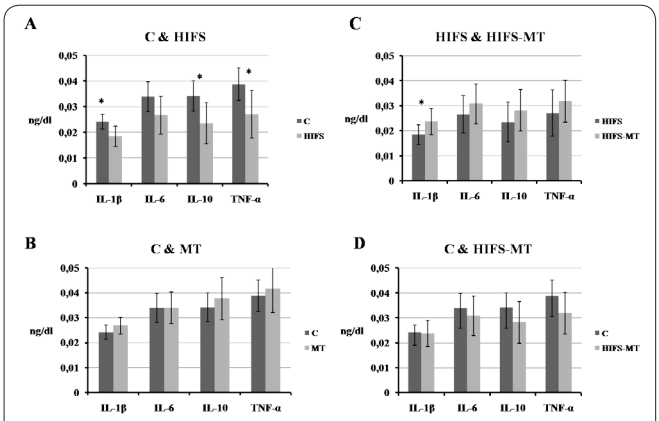

Figure 2. Serum cytokine levels of the study groups. Cytokine levels of A) control (C) and hyperthermia-induced febrile seizure (HIFS) groups, B) C and melatonin (MT) groups, C) HIFS and HIFS-MT groups, D) C and HIFS-MT groups. (* $p < 0.05$)

The HIFS group had lower serum IL-1β (0.019 ± 0.0040 ng/ml), IL-6 (0.027 ± 0.0074 ng/ml), IL-10 (0.024 ± 0.0080 ng/ml), and TNF-α (0.027 ± 0.0093 ng/ml) levels compared to those in the C group ($p = 0.005$, $p = 0.200$, $p = 0.011$, and $p = 0.016$, respectively) (Figure 2A).

No significant difference was observed in the serum cytokine levels between the C and MT groups ($p > 0.05$) (Figure 2B).

The HIFS-MT group had higher serum IL-1β (0.024 ± 0.0053 ng/ml), IL-6 (0.031 ± 0.0080 ng/ml), IL-10 (0.028 ± 0.0084 ng/ml), and TNF-α levels (0.032 ± 0.0084 ng/ml) than those in the HIFS group ($p < 0.05$ for IL-1β and $p > 0.05$ for others) (Figure 2C). No significant difference in serum cytokine levels was observed between the HIFS-MT and C groups ($p > 0.05$) (Figure 2D).

Discussion

The present study is the first to investigate the effects of serum cytokine levels and melatonin in a HIFS model. Rats in the HIFS group had low serum cytokine levels (IL-1β, IL-6, IL-10, and TNF-α). However, both the MT and HIFS-MT groups had serum cytokine levels similar to those for the controls.

Previous studies showed that patients with febrile seizure had higher TNF-α gene expression and serum IL-1β and IL-6 levels compared to healthy children (6,10,11). In addition, plasma IL-1β level, IL-10 (an anti-inflammatory/regulatory cytokine) level, and the IL-1β/IL-10 ratio were higher in patients with febrile seizure; this ratio has been suggested to be useful in predicting febrile seizure risk (12). Previous experimental studies demonstrated that both hyperthermia of the brain and increased IL-1β levels led to seizures by aggravating neuronal excitability (5.9). IL-1β receptor-deficient rats were more resistant to febrile seizures, and lowering the seizure threshold by administering IL-1β also supported previous clinical studies (8). Heida et al. (33) explored the role of IL-1β in the development of febrile seizure and found that 14-day-old rats that experienced febrile seizure had higher hippocampal and hypothalamic IL-1β levels compared to controls after an i.p lipopolysaccharide (LPS) injection. In our study, we found lower serum cytokine levels in the HIFS group. This result was probably due to rapid seizure induction by external hyperthermia and early blood collection to measure serum cytokine levels, which did not allow adequate time for cytokine-mediated inflammatory processes to occur. Cytokine-mediated inflammatory processes are expected to play a role in more slowly developing febrile seizures due to infectious and endogenous processes. In addition, no study has investigated cytokine levels in a febrile seizure model induced by external hyperthermia. On the other hand, a number of previous studies have explored the effects of applying external hyperthermia to the body that did not lead to febrile seizures, such as the 'whole body hyperthermia (WBH)' model (34-38). Yamashita et al. (36) investigated the role of cytokines in the cardioprotective effect of hyperthermia. They conducted a WBH model by leaving adult rats in 42°C water for 15 min and measuring serum cytokine levels 5 days after WBH. The results indicated that IL-1β and TNF-α levels increased significantly. Nelson et al. (37) studied the effects of hyperthermia on neonatal mortality and serum cytokine (IL-1β and IL-6) levels. They placed 10–14-day-old rats in water at various temperatures (38, 39, and 40°C) for 1 hour, and collected blood 1 hour after WBH; they reported similar serum IL-1β levels, but increased IL-6 levels with higher external hyperthermia. Haveman et al. (34) applied WBH at 41.5°C for 1 hour. They showed that serum TNF-α level remained unchanged, serum IL-1 level reached a peak 15 min after WBH, and serum IL-6 level remained unchanged during WBH, but increased significantly 1 hour later. On the other hand, Ostberg et al. (35) conducted an experimental study on BALB/c and C57BL/6 mice and reported that long-duration WBH did not affect serum IL-1β, IL-6, or TNF-α levels. In a cell-culture study, Fairchild et al. (38) evaluated the effects of hyperthermia on LPS-induced cytokine expression in neonatal and adult monocytes and macrophages maintained at 40°C. They reported that no increase in TNF-α or IL-1β expression was observed initially, but TNF-α and IL-1β concentrations increased by 18–50% in all cell cultures, and IL-6 concentrations decreased by 26–29% in all cell cultures except adult macrophages after 24 hours in a hyperthermal condition. All of these studies clarify that a certain time period is necessary for inflammatory processes to take place. The differences in serum cytokine levels are mostly due to a difference in the environmental temperature that experimental animals are exposed to, the exposure duration, and the

time interval between hyperthermia exposure and blood collection. In the present study, we exposed the rats to 45°C, and the exposure duration to hyperthermia (equal to seizure latency, sec-min) was relatively shorter compared to other studies. We collected blood samples just after the application. This was also a rather short delay when compared to previous studies (hours/days).

Previous studies have shown that melatonin administration affects serum cytokine levels (24-26). Additionally, there is evidence to support anticonvulsive activity of melatonin in various seizure models and in the HIFS model (16.17,18-23). However, the relationship between cytokines and the anticonvulsive efficacy of melatonin is unknown. In this study, we demonstrated that serum cytokine levels decrease with febrile seizures, whereas melatonin administration maintains serum cytokine levels stable at a level similar to that for controls in the HIFS rat model. Maestroni et al. (24) showed that melatonin diminished T cell apoptosis and increased T cell-mediated cytokine expression. Yuan et al. (26) studied human umbilical vein endothelial cells and found that melatonin administration decreased IL-1β levels. Long-term melatonin treatment decreased pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), which increase with aging and increase anti-inflammatory cytokine levels (25). Melatonin (30 mg/kg) was injected i.p into C57BL/6J mice to investigate the protective effects of melatonin on brain injury, and blood and brain samples were collected 48 hours from a surgical model. Melatonin administration decreased IL-1β and TNF-α levels (39). Ersoy et al. (40) conducted a septic shock model and injected 10 mg/kg i.p melatonin into Wistar albino rats twice at a 6-hour interval to investigate the effects of melatonin during the early inflammatory phase of sepsis. They collected blood samples at 16 hours and at 3 and 7 days after injection. In the groups receiving melatonin, serum IL-6 levels were significantly lower than those in the controls in the 16-hour sample, whereas it was higher than that of the control group on days 3 and 7. Serum IL-10 level was significantly higher than that of controls after 16 hours, whereas it was significantly lower than the controls on days 3 and 7. Carrillo-Vico et al. (41) injected 10 mg/kg i.p melatonin twice into adult female Swiss LPS-induced septic shock model mice. They collected blood samples 2, 4, and 6 hours after the model and decapitated the mice. Serum IL-1, IL-6, and TNF-α levels remained unchanged in all samples in the groups receiving melatonin, whereas IL-10 was significantly higher only in the 6-hour blood sample. Kahya et al. (42) conducted a study of diabetic rats to investigate the effects of melatonin and selenium on oxidative stress in the brain and plasma cytokine levels. They injected 10 mg/kg i.p melatonin into rats for 14 days and collected blood samples 12 hours after the last melatonin injection. They found similar IL-1β levels in the melatonin group and the controls. Consistently, we found similar serum IL-1β levels in the MT and C groups in our study model. Our results support studies by Carrillo-Vico et al. (41) and Kahya et al. (42). We also found significantly higher IL-1β levels in the HIFS-MT group compared to the HIFS group. This result parallels that in a study by Maestroni et al. (24), but contradicts the results of Yuan et al. (26), Rodríguez et al. (25), and Zhao et al. (39). On the other hand, no significant difference in serum IL-6 levels was observed between our study groups, a finding consistent with the results of Carrillo-Vico et al. (41). In the present study, serum IL-10 level in the MT group was significantly higher than that in the HIFS and HIFS-MT groups, which is consistent with Maestroni et al. (24), Rodríguez et al. (25), the 16 hour results of Ersoy et al. (40), and the 6 hour results of Carrillo-Vico et al. (41).

Some previous studies have shown that the serum TNF-α level decreases after melatonin is administered (25,39), whereas it remained unchanged after 2, 4, and 6 hours in Carrillo-Vico et al. (41).

Consequently, some previous studies are consistent with our results regarding changes in cytokine levels after melatonin administration, whereas other studies contradict our results. These controversial results are most probably due to differences in the melatonin dose, the number of repetitions in different experimental models, or different blood sampling times.

In the present study, serum IL-6 levels did not change, whereas IL-1β, IL-10, and TNF-α levels decreased significantly just after seizure in the HIFS rat model. Furthermore, administering melatonin to the rats resulted in no change in serum IL-6 levels, whereas it increased serum levels of the other cytokines (IL-1β, IL-10, and TNF-α) to the levels of the control. These results suggest that the anticonvulsive efficacy of melatonin may be related to cytokines. Further studies with blood sampling at different times after seizure and investigating cytokine levels, especially in the hippocampus, are needed to clarify the role of cytokines in the anticonvulsive efficacy of melatonin in the HIFS model.

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Interest Conflict
The authors declare that there is no conflict of interest regarding the publication of this paper.

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Ethical Approval
This study was approved by Baskent University Ethics Committee for Animal Experiments (Project # DA16/42).

References

1. Stenklyft PH, Carmona M. Febrile seizures. Emerg Med Clin North Am 1994;12:989-99.

2. Baram TZ, Shinnar S. Febrile seizures. In: The incidence and prevalence of febrile seizures. Stafstrom CE. San Diego: Academic Press; 2002. pp. 1–25.

3. Ostergaard JR. Febrile seizures. Acta Paediatr 2009;98:771-3.

4. Dubé CM, Ravizza T, Hamamura M, Zha Q, Keebaugh A, Fok K, et al. Epileptogenesis provoked by prolonged experimental febrile seizures: mechanisms and biomarkers. J Neurosci 2010:30(22):7484–94.

5. Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A. Prud'homme JF, et al. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. Nat Genet 2001;28:46–48.
6. Virta M, Hurme M, Helminen M. Increased plasma levels of pro- and anti-inflammatory cytokines in patients with febrile seizures. Epilepsia 2002;43(8):920-3.
7. Turrin NP, Rivest S. Innate immune reaction in response to seizures: implications for the neuropathology associated with epilepsy. Neurobiol Dis 2004;16(2):321-34.
8. Dube CM, Vezzani A, Behrens M, Bartfai T, Baram TZ. Interleukin-1b Contributes to the Generation of Experimental Febrile Seizures. Ann Neurol 2005:57:152–155.
9. Ichiyama T, Suenaga N, Kajimoto M, Tohyama J, Isumi H, Kubota M, et al. Serum and CSF levels of cytokines in acute encephalopathy following prolonged febrile seizures. Brain and Development 2008;30:47-52.
10. Sasaki K, Matsuo M, Maeda T, Zaitsu M, Hamasaki Y. Febrile seizures: characterization of double-stranded RNA-induced gene expression. Pediatr Neurol 2009;41(2):114-8.
11. Choi J, Min HJ, Jeon-Soo Shin JS. Increased levels of HMGB1 and pro-inflammatory cytokines in children with febrile seizures. Journal of Neuroinflammation 2011;8:135-44.
12. Gunawan A, Muid M, Suyudi H, Wisnu B, Subandiyah K. The Correlation Between IL-1β and IL-10 Levels in Estimating The Risk of Febrile Seizures. Journal of Tropical Life Science 2014;4(2):131-136.
13. Guo JF, Yao BZ. Serum melatonin levels in children with epilepsy or febrile seizures. Zhongguo Dang Dai Er Ke Za Zhi 2009;11(4):288-90.
14. Ardura J, Andres J, Garmendia JR, Ardura F. Melatonin in epilepsy and febrile seizures. J Child Neurol. 2010;25(7):888-91.
15. Dabak O, Altun D, Arslan M, Yaman H, Vurucu S, Yesilkaya E, et al. Evaluation of Plasma Melatonin Levels in Children With Afebrile and Febrile Seizures. Pediatr Neurol. 2016;57:51-5.
16. Peled N, Shorer Z, Peled E, Pillar G. Melatonin effect on seizures in children with severe neurologic deficit disorders. Epilepsia 2001;2(9):1208-10.
17. Elkhayat HA, Hassanein SM, Tomoum HY, Abd-Elhamid IA, Asaad T, Elwakkad AS. Melatonin and sleep-related problems in children with intractable epilepsy. Pediatr Neurol. 2010;42(4):249-54.
18. Borowicz KK, Kamiński R, Gsior M, Kleinrok Z, Czuczwar SJ. Influence of melatonin upon the protective action of conventional anti-epileptic drugs against maximal electroshock in mice. Eur Neuropsychopharmacol 1999;9:185–190.
19. Costa-Lotufo LV, de Fonteles MM, Lim ISP, de Oliveira AA, Nascimento VS, de Bruin VM, et al. Attenuating effects of melatonin on pilocarpine-induced seizures in rats. Comp Biochem Physiol C 2002,131:521–529.
20. Yildirim M, Marangoz C. Anticonvulsant effects of melatonin on penicillin-induced epileptiform activity in rats. Brain Res 2006;1099:183-188.
21. Yahyavi-Firouz-Abadi N, Tahsili-Fahadan P, Riazi K, Ghahremani MH, Dehpour AR. Melatonin enhances the anticonvulsant and proconvulsant effects of morphine in mice: Role for nitric oxide signaling pathway. Epilepsy Res 2007;75:138–144.
22. Solmaz I, Gurkanlar D, Gokcil Z, Cuneyt G, Ozkan M, Erdogan E. Antiepileptic activity of melatonin in guinea pigs with pentylenetetrazol-induced seizures. Neurol Res 2009;31:989–995.
23. Aydin L, Gundogan NU, Yazici C. Anticonvulsant efficacy of melatonin in an experimental model of hyperthermic febrile seizures. Epilepsy Res. 2015;118:49-54.
24. Maestroni GJ. The photoperiod transducer melatonin and the immune hematopoietic system. J Photochem Photobiol B 1998;43:186-192.
25. Rodríguez MI, Escames G, López LC, López A, García JA, Ortiz F, et al. Chronic melatonin treatment reduces the age-dependent inflammatory process in senescence-accelerated mice. J Pineal Res 2007;42(3):272-9.
26. Yuan X, Li B, Li H, Xiu R. Melatonin inhibits IL-1β-induced monolayer permeability of human umbilical vein endothelial cells via Rac activation. J Pineal Res 2011;51(2): 220-5.
27. Moezi L, Shafaroodi H, Hojati A, Dehpour AR. The interaction of melatonin and agmatine on pentylenetetrazole-induced seizure threshold in mice. Epilepsy Behav 2011;22(2):200-6.
28. Łotowska MES, Joanna M, Łotowska JM. The neuroprotective effect of topiramate on the ultrastructure of pyramidal neurons of the hippocampal CA1 and CA3 sectors in an experimental model of febrile seizures in rats. Folia Neuropathol 2011;49 (3):230-6.
29. Jiang W, Duong TM, de Lanerolle NC. The neuropathology of hyperthermic seizures in the rat. Epilepsia 1999;40:5-19.
30. Rossato LG, Costa VM, Dallegrave E, Arbo M, Dinis-Oliveira RJ, Santos-Silva A, et al. Cumulative mitoxantrone-induced haematological and hepatic adverse effects in a subchronic in vivo study. Basic Clin Pharmacol Toxicol 2014;114(3):254-62.
31. Pervaiz N, Hoffman-Goetz L. Immune cell inflammatory cytokine responses differ between central and systemic compartments in response to acute exercise in mice. Exerc Immunol Rev 2012;18:142-57.
32. Benot S, Goberna R, Reiter RJ, Garcia-Mauriño S, Osuna C, Guerrero JM. Physiological levels of melatonin contribute to the antioxidant capacity of human serum. J Pineal Res 1999;27(1):59-64.
33. Heida JG, Pittman QJ. Causal links between brain cytokines and experimental febrile convulsions in the rat. Epilepsia 2005;46:1906-1913.
34. Haveman J, Geerdink AG, Rodermond HM. Cytokine production after whole body and localized hyperthermia. Int J Hyperthermia 1996;12:791–800.
35. Ostberg J R, Taylor SL, Baumann H, Repasky EA. Regulatory effects of fever-range whole-body hyperthermia on the LPS-induced acute inflammatory response. Journal of leukocyte biology 2000,68(6):815-820.
36. Yamashita N, Hoshida S, Otsu K, Taniguchi N, Kuzuya T, Hori M. The involvement of cytokines in the second window of ischaemic preconditioning. Br J Pharmacol 2000;131(3):415-22.
37. Nelson EA, Wong Y, Yu LM, Fok TF, Li K. Effects of hyperthermia and muramyl dipeptide on IL-1beta, IL-6, and mortality in a neonatal rat model. Pediatr Res 2002;52(6):886-91.
38. Fairchild KD, Viscardi RM, Hester L, Singh IS, Hasday JD. Effects of hypothermia and hyperthermia on cytokine production by cultured human mononuclear phagocytes from adults and newborns. Journal of Interferon & Cytokine Research 2000;20(12):1049-1055.
39. Zhao L, An R, Yang Y, Yang X, Liu H, Yue L, et al. Melatonin alleviates brain injury in mice subjected to cecal ligation and puncture via attenuating inflammation, apoptosis, and oxidative stress: the role of SIRT1 signaling. Journal of Pineal Research 2015;59(2):230-239.
40. Ersoy OF, Özkan N, Özsoy Z, Kayaoğlu HA, Yenidoğan E, Çelik A, et al. Effects of melatonin on cytokine release and healing of colonic anastomoses in an experimental sepsis model. Ulus Travma Acil Cerrahi Derg 2016;22(4):315-321.
41. Carrillo-Vico A, Lardone PJ, Naji L, Fernández-Santos JM, Martín-Lacave I, Guerrero JM, et. al. Beneficial pleiotropic actions of melatonin in an experimental model of septic shock in mice: regulation of pro-/anti-inflammatory cytokine network, protection against oxidative damage and anti-apoptotic effects. J Pineal Res. 2005;39(4):400-8.

42. Kahya MC, Naziroğlu M, Çiğ B. Melatonin and selenium reduce plasma cytokine and brain oxidative stress levels in diabetic rats. Brain Inj. 2015;29(12):1490-6.