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Effects of melatonin on behavioral changes of neonatal rats in a model of cortical dysplasia

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Abstract. – BACKGROUND: Cortical dysplasia (CD) is associated with several behavioral disorders in both the pediatric and the adult population. The effect of melatonin on behavioral disorders in rats generated CD has not been investigated so far.

AIM: To investigate the effects of melatonin administration on activity and anxietic behavior of neonatal rats in a model of CD.

MATERIALS AND METHODS: Newborn Sprague-Dawley rats (n=21) were randomized into three groups. On postnatal day 1, one freeze lesion was carried out in 14 rats between bregma and lambda to create a CD model. Another group of neonatal rats served as control group (n=7). Those 14 rats were either administered melatonin (n=7) or vehicle solution (n=7). Melatonin treatment (4 mg/kg/day, i.p.) was initiated ten days after induction of cold injury and continued for three weeks. Animal activity and anxiety were analyzed by using open field and elevated plus maze tests 24h after the last melatonin administration (day 32) in a blind manner.

RESULTS: It was observed that CD induced animals spent significantly less time in the open field area when compared to the other groups ($p < 0.01$). Additionally, the time spent in the open field area was significantly elevated in the melatonin-treated animals compared to both the control and the CD groups ($p < 0.01$). Accordingly, anxiety scores in the CD group was significantly increased ($p < 0.01$), and this effect could be reversed by administration of melatonin.

CONCLUSIONS: Melatonin exerts protective behavioral effects against cortical dysplasia in newborn rats. Further clinical investigations may prove melatonin as a useful therapeutic adjunct to prevent from possible behavioural damages of cortical dysplasia.

Key Words:
Melatonin, Cortical dysplasia, Behavior

Introduction

Cortical Dysplasia (CD) is defined as cortical malformation caused by the varying degrees of developmental errors occuring during the intrauterine period. Cerebral dysplasias are also accepted as epileptic foci. In that cases, in addition to involuntary movements, the neurological tests related to intelligence and learning reveal that they can also cause growth retardation[1].

One of the most common reasons of drug resistant epilepsy and developmental anomalies are the malformations of the cerebral cortex. Malformations of cortical development are shown to cause 25% of the childhood epilepsies[1,2]. Various behavioral disorders are demonstrated in epileptic patients. Consuming that the patients with abnormalities of cortical development are epileptic, the patients' behavioral disorders may depend on abnormalities of cortical development.

Melatonin, is an indoleamine that secreted from the pineal gland and a potent free radical scavenger. Its' antioxidant properties are indirectly by activating the major antioxidant enzymes and by increasing the effectiveness of mitochondrial electron transport system[3,4]. In experimental studies, it has been reported that melatonin decreases cAMP production in rats after hypoxia-ischemia (HI), inhibits the activity of free radicals and reduces microglial activation[5,6]. Furthermore, melatonin can disperse to all tissues and cell compartments including the brain with its lipophilic property through all the morpho-physiological barriers. Some other studies also reported that melatonin is beneficial as a neuroprotective agent with its anticonvulsive, sedative and hypnotic properties in the neonatal HI model[7,8]. Additionally, melatonin has been proposed to reduce the proconvulsant effects of Pentineltetrazol[9]. However, the effect of melatonin on behavioral disorders in rats generated cortical dysplasia has not been investigated so far.

Up-to-date, there are only a few studies demonstrating the disorder of learning and memory in animals related to the abnormalities of the cortical development[10,11]. Therefore, we aimed to

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investigate the effects of melatonin administration on activity and anxietic behavior in neonatal rats in a model of cortical dysplasia.

Materials and Methods

After the approval of the Ethical Committee of Yeditepe University Faculty of Medicine, newborn Sprague-Dawley rats (n= 21) were used for this study. The rats were accommodated in dry cages, laboratory environment in 1 atmospheric pressure in 22°C degrees (± 2), 12 hours light/12 hours dark. Standard rat chow and city water were used for nutrition. 75 mg/kg ketamine and 10 mg/kg xylazine intraperitoneally (i.p.) were implemented for anesthesia. During the operations, sterile conditions were provided for each rat.

A total of 21 rats were randomized into 3 groups;

Group-1 (Control): Vehicle solution (10% ethanol in saline i.p.) was administered to 7 newborn Sprague-Dawley rats. Open field (activity) and the "elevated plus maze" (anxiety) tests were blindly perfomed on 32nd day.

Group-2 (Cortical dysplasia): A freeze lesion was carried out on 7 rats between bregma and lambda on the skull in the right hemisphere for 5 seconds using a cooled 0.5 mm steel probe. Vehicle solution (10% ethanol in saline i.p.) was administered. Open field (activity) and the "elevated plus maze" (anxiety) tests were blindly perfomed on 32nd day.

Group-3 (Cortical Dysplasia + Melatonin): A freeze lesion was carried out on 7 rats between bregma and lambda on the skull in the right hemisphere for 5 seconds using a cooled 0.5 mm steel probe. Melatonin (4 mg/kg/day i.p.) was administered, 10 days after the lesion created and continued for 3 weeks. 24 hours after the implementation of the last melatonin (32nd day), the open field (activity) and the elevated plus maze (anxiety) tests were blindly performed.

Open-field (Activity) Test

Apparatus is an area which is used for activity test and consists of 36x36 cm white melamine. Each rat was placed in a corner of the apparatus and movements were recorded during 5 minutes. Points considered in this record were; the distance traveled in this area, average speed, stretching movements and evaluation of the time spent during the mobile and immobile states.

Elevated-plus Maze (Anxiety) Test

The test was consisting of 2 open and 2 closed arms, Apparatus which is prepared seems like a plus shape and 40-70 cm height from floor. Anxiety reduction was indicated by increasing the time spent in open arms (time spent in open arms/total time) and increasing the number of input to open arms (open arm entry number/total number). The total number of the introduction to open and closed arms were recorded.

After all transactions were completed including the operation, subjects were sacrificed by decapitation method under general anesthesia. SPSS (Statistical Package for Social Sciences) 13.0 program was used for statistical analyses (SPSS Inc., Chicago, IL, USA). All the obtained numerical and nominal data were compared using one way ANOVA test. Numerical values were expressed as mean ± standard deviation (SD). $p < 0.05$ was considered statistically significant.

Results

The comparison of all groups regarding the level of their activites is demonstrated in the Figure 1. It was observed that cortical dysplasia induced animals spent significantly less time in the open field area when compared to the control group ($p < 0.01$). Additionally, the time spent in the open field area was significantly elevated in the melatonin-treated animals compared to both the control and the CD groups (control group: 327.3±44.5 seconds, CD group: 198.2±35.7 seconds, melatonin+CD: 435.1±40.2 seconds, $p < 0.01$).

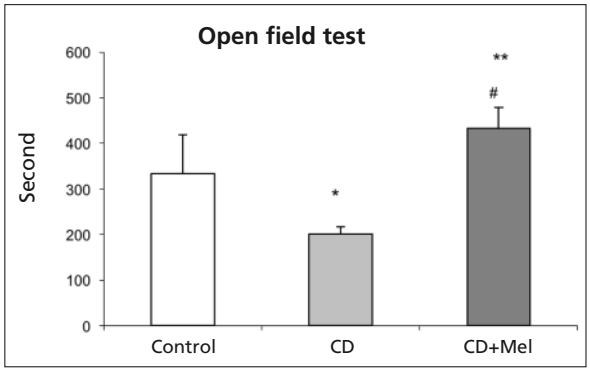

Figure 1. Comparison of groups according to their level of activity. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with control groups; #$p < 0.05$, comparison of CD + Melatonin vs CD Groups.

Furthermore, the levels of the anxiety scores are shown in Figure 2. It was found that the anxiety scores in the cortical dysplasia group were significantly increased when compared to the control group ($p < 0.01$). This effect was significantly reversed by administration of melatonin ($p < 0.01$). Moreover the anxiety levels of melatonin+CD group and the control group were found to be comparable (Control group: 382.4±48.2 seconds, CD group: 237.7±34.2 seconds, melatonin+CD: 285.7±37.6 seconds, $p < 0.05$).

Discussion

Data of this experimental study reveals that CD causes anxiety and reduced activity in newborn Sprague-Dawley rats. We have demonstrated that those behavioral deteriorations could be reversed with the administration of systemic melatonin. Therefore, melatonin exerts protective behavioral effects against cortical dysplasia in newborn rats. Further clinical investigations may prove melatonin as a useful therapeutic adjunct to prevent from possible behavioural damages of cortical dysplasia.

Most of the neurons in the developing nervous system migrate from where they proliferate to their own locations[12]. Studies on neuronal migration are important in understanding the etiology of diseases caused by abnormal migration errors and possible novel therapeutic approaches against neurological diseases[13]. Cell-based applications which are used in the therapy of neurodegenerative diseases demonstrate the importance of the migration of neurons or cells to the target area[9]. As previously reported, for normal cortical cycle, neurons and glias should spend the processes of proliferation, migration and maturation[14,15]. This synchronization of developmental events is achieved by the transmission of the signals to different regions of the brain via the neurons.

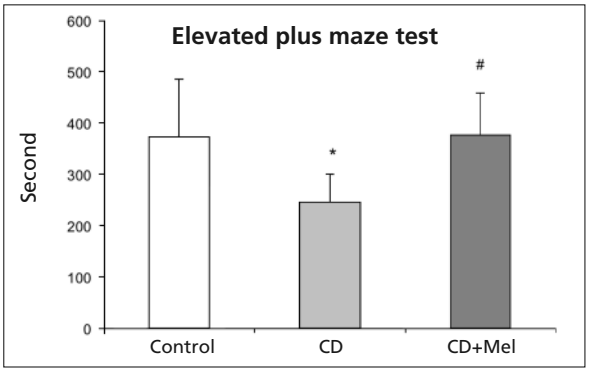

Figure 2. Comparison of groups according to the level of anxiety. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with control groups; #$p < 0.05$, comparison of CD + Melatonin vs CD Groups.

Cortical dysplasia is a cortical malformation caused by developmental defects occuring during the intrauterine proliferation process (neuronal differentiation, neuro-glial interaction), neuronal migration and maturation periods[12]. It has been demonstrated that CD is strongly associated with epilepsy in both the pediatric and the adult population[13]. Children with CD are also being faced with seizures and mental retardation.

There are various methods to create an experimental model of cortical dysplasia. Freezing the lesion, implementation metilazomethanol acetate which is an antimycotic agent, the radiation and the implementation of carmustine an antineoplastic agent during pregnancy[14,15]. In our study, the cortical dysplasia was created by making contact with frozen probe for 5 seconds which was created between bregma and lambda of the right side of skulls in new born rats.

Melatonin is an indirect antioxidant which has stimulating effects on the antioxidative enzymes and is used in both experimental and clinical applications[20]. Beyond its antioxidant properties, it has also been tested and successfully used in other clinical situations. Melatonin was initially used by passengers in trans-meridian flights in order to reduce the severity of the effect of jet-lag. Afterwards, it has gained popularity as a sleeping pill[21,22]. In addition to the cleaning ability of free radicals, one other effect of melatonin is the reduction of neutrophil accumulation[23]. The high success of melatonin in reducing the oxidative damage includes both receptor-independent and receptor-mediated processes. It also depends on a synergistic effect with conventional antioxidants and antioxidative functions, the unique intracellular distribution and the ability to induce gene expression[24,25]. In another study, melatonin has been shown to be effective in protecting the epileptic seizures in the new born rats which were experimentally pinealectomized[26]. By creating a model of cortical dysplasia,the negative behavioral impacts of CD and the positive effects of melatonin treatment on behavior disorders are clearly demonstrated. Data of that experimental study revealed that; the availability of melatonin treatment should be further investigated for the treatment of behavioral disorders that occur in patients with CD.

Therefore, we have adopted melatonin in our work for several above explained reasons plus the very low toxicity, the availability of a pure state and having a reasonable price[19]. Our primary goal was to elucidate the possible protective effects of melatonin administration on activity and anxietic behavior of neonatal rats in a model of CD.

Open field (activity) test is used for measuring behavioral responses such as locomotor activity, hyperactivity and anxiety. Such an environment lead to anxiogenic stimuli for rats because they normally avoid from staying in open areas where the bright light is. This environment allows assessment of the locomotor activity induced by anxiety and behaviors related to the research. Activity test was used due to its very limited impact on subsequent behavior of experimental animal. We have observed that cortical dysplasia induced animals spent significantly less time in the open field area when compared to the control group. Additionally, the time spent in the open field area was significantly elevated in the melatonin-treated animals compared to both the control and the CD groups.

Elevated-plus maze (anxiety) is a screening test that used on rats and mice. Test anxiety is a general research for neurobiological investigations and the default anxiolytic and anxiogenic components are evaluated. We have found that the anxiety scores in the cortical dysplasia group were significantly increased when compared to the control group. This effect was significantly reversed by administration of melatonin.

In the group of CD without treatment, a significant impairment in anxiety scores and a significant decrease in activity test compared with the control group were determined. Furthermore, it has been found that melatonin treatment improves the deterioration of behavioral parameters due to CD. This treatment decreased the anxiety scores by inverting these varying parameters depending on the formation of CD.

Conclusions

In this experimental study, we have found that CD causes anxiety and reduced activity in newborn Sprague-Dawley rats. We have demonstrated that those behavioral deteriorations could be reversed with the administration of systemic melatonin. Melatonin exerts protective behavioral effects against cortical dysplasia in newborn rats. Further clinical investigations may prove melatonin as a useful therapeutic adjunct to prevent from possible behavioural damages of cortical dysplasia.

Conflict of Interest

None.

References

1) Young-Kuk L, Seong-Ryong L, Choong-Young K. Melatonin attenutes the changes in polyamine levels induced systemic kainate administration in rat brains. J Neurol Sci 2000; 178: 124-131.
2) Taylor DC, Falconar MA, Bruton CJ, Corsellis JA. Focal dysplasia of the cerebral cortex in epilepsy. J Neurol Neurosurg Psychiatry 1971; 34: 369-387.
3) Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007; 42: 28-42.
4) Tomas-Zapico C, Coto-Montes A. A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. J Pineal Res 2005; 39: 99-104.
5) Husson I, Mesples B, Bac P, Vamecoj, Evrand P, Grennss P. Melatoninergic neuroprotection of the murine periventricular white matter against neonatal excitotoxic challenge. Ann Neurol 2002; 51: 82-92.
6) Wellin AK, Svedin P, Lapatto R, Sultan B, Hagberg H, Gressens P, Kjellmerr I, Mallard C. Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. Pediatric Res 2007; 61: 153-158.
7) Tutunculer F, Eskiocak S, Basaran UN, Ekuklu G, Ayvaz S, Vatansever U. The protective role of melatonin in experimental hypoxic brain damage. Pediatrics Int 2005; 47: 434-439.
8) Gitto E, Karbownik M, Reiter RJ, TAN DX, Cuzzocrea S, Chiurazzi P, Cordara S, Corona G, Trimarchi G, Barberi I. Effects of melatonin treatment in septic newborns. Pediatric Res 2001; 50: 756-760.
9) Vanecek J. Cellular mechanism of melatonin action. Physiol Rev 1998; 78: 687-721.
10) Xikota JC, Rial D, Ruthes D, Pereira R, Figueiredo CP, Prediger RD, Walz R. Mild cognitive deficits associated to neocortical microgyria in mice with genetic deletion of cellular prion protein. Brain Res 2008; 1241: 148-156.
11) Rial D, Xikota JC, Miozzo A, Cruz VE, Prediger RD, Walz R. Differential gender-related susceptibility to learning and memory deficits in mice submitted to neonatal freezing microgyria model. Brain Res Bull 2009; 79: 177-181.

12) Leon J, Acuna-Castrvijeo D, Escames G, Tan DX, Reiter RJ. Melatonin mitigates mitochondrial malfunction. J Pineal Res 2005; 38: 1-9.

13) Berger-Sweeney J, Hohmann C. Behavioral consequences of abnormal cortical development: insights into developmental disabilities. Behav Brain Res 1997; 86: 121-142.

14) Rakic P. Neuron-glia relationship during granule cell migration in developing cerebellar cortex. J Comp Neurol 1971; 141: 283-312.

15) Rakic P. Mode of cell migration to the superficial layers of fetal monkey neocortex. J Comp Neurol 1972; 145: 61-84.

16) Barkovich AJ. Pediatric neuroimaging, Lippincott William & Wilkins III, 2000. Edition Chapter 5, pp. 253-381.

17) Kuzniecky R, Murro A, King D, Morawetz R, Smith J, Powers R, Yaghmai F, Faught E, Gallagher B, Snead OC. Magnetic resonance imaging in childhood intractable partial epilepsies: pathologic correlations. Neurology 1993; 43: 681-687.

18) Smyth MD, Barbaro NM, Baraban SC. Effects of antiepileptic drugs on induced epileptiform activity in a rat model of dysplasia. Epilepsy Res 2002; 50: 251-264.

19) Benardete EA, Kriegstein AR. Increased excitability and decreased sensitivity to GABA in an animal model of dysplastic cortex. Epilepsia 2002; 43: 970-982.

20) Rodriquez C, Mayo JC, Sainz RJ. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 2004; 36: 1-9.

21) Cardinali DP, Borfman GP, Liotta G, Pérez Lloret S, Albornoz LE, Cutrera RA, Batista J, Ortega Gallo P. A multifactorial approach employing melatonin to accelerate resynchronization of sleepwake cycle after a 12 timezone westerly transmeridian flight in elite soccer athletes. J Pineal Res 2002; 32: 41-46.

22) Cardinali DP. Clinical perspectives for the use of melatonin as a neuroprotective chronobiotic in Alzheimer's disease. Aktual Neurol 2003; 3: 188-204.

23) Reiter RJ, Melchiorri D, Sewerynek E. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 1995; 18: 1-11.

24) Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress: a review. J Biomed Sci 2000; 7: 444-458.

25) Gilad E, Cuzzocrea S, Zingarelli B, Salzman AL, Szabó C. Melatonin is a scavenger of peroxynitrite. Life Sci 1997; 60: 169-174.

26) Turgut M, Uyanikgil Y, Ates U, Baka M, Yurtseven ME. Pinealectomy stimulates and exogenous melatonin inhibits harmful effects of epileptiform activity during pregnancy in the hippocampus of newborn rats: an immunohistochemical study. Childs Nerv Syst 2006; 22: 481-488.