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ORIGINAL ARTICLE

The Protective Effect of Melatonin in Vigabatrin Treatment on Newborn Rat Cerebellum

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ABSTRACT
According to the existing studies teratogenic effects of vigabatrin on fetuses and infants has been demonstrated. Also it has been proved that Melatonin can reduce toxicity and side effects of medications; this effect has been studied using the administration of melatonin, which is known today as antioxidants to eliminate free radicals in the mechanisms of infants' developing brain born from mothers treated with vigabatrin. The adult female rats were divided randomly into 5 groups, including one control and four experimental groups, after vaginal plagues. Group I received normal saline, group II received low-dose the vigabatrin, group III received low-dose vigabatrin + melatonin, Group IV received high dose vigabatrin, and group V received high doses of vigabatrin + melatonin on days 9, 10 and 11 of gestation. They received the drugs within gavages. infants born after morphometric procedures were placed within the 10% fixative solution for histotheqnic procedures. The Samples were stained with H&E general staining and special staining of Tunnel. The data were analyzed statistically using ANOVA followed by the Tukey's multiple comparison tests was performed. In the results of macroscopic studies low and high doses of vigabatrin had a significant decreasing effect compared with control group. Also, melatonin + vigabatrin had a high protective effect compared with vigabatrin group. The results of microscopic studies demonstrated tissue changes including incomplete villus in the cerebellum, lack of completion of cerebellar cortex's 3 layers, the presence of apoptosis or necrosis and congestion in the vigabatrin intervention groups. Meanwhile, the combined effect of melatonin+ vigabatrin was significant compared with only vigabatrin group.Based on the obtained results it was observed in the fetus growth parameters morphometry that the effect of melatonin is a protective effect as an antioxidant against free radicals created from administrating of vigabatrin. Also, it was observed that melatonin had no protective effect on decreasing of gray layer thickness undertreated with vigabatrin. The density of Purkinje cells was decreased because of reduced mitotic activity in the intervention groups; so, the incidence of apoptosis was observed. Whereas the combined effect of melatonin in the intervention groups had no protective effect on the incidence of apoptosis
Keywords: *Melatonin, Infant rat, Vigabatrin*

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INTRODUCTION

One of the main causes of congenital anomalies is useing certain drugs during pregnancy. Among the drugs that have been discussed in terms of teratology are anti-epileptic drugs(AEDs). This medicine is today one of the most common drugs in the treatment of epilepsy and some mental illness and its use is increasing [3,4,20]. The study was conducted in which the drug passes through the placental blood barrier and affect the fetus and thus cause abnormalities [10]. vigabatrin treatment during pregnancy reduces the total protein content of the embryo, which subsequently leads to decreased height and weight [12]. As the most important gland secretes melatonin epiphyseal highly effective antioxidant and neutralizing free radicals [18]. Melatonin is an antioxidant Because the activity or expression of melatonin antioxidant enzymes such as: Superoxide dismutase, glutathione reductase and glutathione peroxidase inducing(18). Melatonin can reduce toxicity and side effects [6]. Considering that studies on teratogenic effects on fetuses and infants born to drug vigabatrin has been demonstrated [9,10,17] And the use of

these drugs is on the rise. Therefore, in this study the use of animal models with administration of melatonin as an antioxidant that is relevant today Mechanism to eliminate free radicals in cerebellum development in infants born to mothers treated with lamotrigine, has been studied.

MATERIAL AND METHODS

A case study was Intervention. In this study of 96 adult male rat and 24 female rat weighing 30 ± 5 g were purchased from the Pasteur Institute. Cages in a controlled environment with a temperature of 22 ±2^{0}C, humidity 38% ± 2, 300 lux light intensity at the center of the room and consecutive 12-hour periods were kept in the dark.

Adequate food and water (concentrate) animals were put enough available. On the afternoon 3 females with a male mouse was placed in a cage for mating. Next morning the vaginal plug was evaluated in mice and in case of vaginal plug was separated from the rest. Date of vaginal plug was considered day zero of pregnancy. Pure powder of vigabatrin was prepared from Bakhtar biochemistry pharmaceutical company.

Net vigabatrin, milky powder and is insoluble in water. Ethanol is one of the best solvents for this drug [20]. So do the research and intraperitoneal injection of ethanol (Merle Germany) 20% of the volume is reached by 1ml of distilled water was used. In this study, melatonin (sigma, usa) was used at a dose of 10mg/kg intraperitoneally [1].

The solubility of melatonin in accordance Atessahim 1% ethanol was used. It is explained medicines during pregnancy period of organogenesis (days 11,10,9) was injected [10]. In this study group was a control group and four experimental groups.

First group (control group): It is only normal saline solution at 11,10,9 days of gestation received (12 head). The second group (experimental group A): That were pregnant rats on gestation 11,10,9 include 50 mg/kg vigabatrin (2) the gavage. The third group (experimental group II): Rat that were included in 11,10,9 days of pregnancy 50 mg / kg Vigabatrin and 10 mg / kg melatonin (1 and 2) via gavage(12head). The fourth group (intervention group three): Rats were 11,10,9 days of pregnancy, including 100 mg / kg by gavage to vigabatrin (12head).The fifth group (intervention group of four): Rats were 11,10,9 days of gestation 100mg/kg vigabatrin , and 10 mg / kg melatonin they were gavaged (12head). Number of babies born at term gestation and premature birth rates and mortality were recorded in different groups. After weight infants by digital balance with a sensitivity of 0.01 mg, based on the length from head to end the infant seat (CRL) from the caliper using 0/1mm carefully measured and recorded. Followed by 10% formalin fixative solution for babies in preparing histological slides were stored. In this study, samples were stained using public (H&E) To examine changes in cerebellar tissue staining (Tunnel) to investigate the induction of apoptosis and apoptosis of non-intervention groups were examined.

Statistical Analysis

The mean height and weight, length, head width, head, brain weight, brain size, ependymal, lateral ventricles, gray layer thickness and The various groups with the statistical methods of data expressed as Mean ± SEM and statistical methods to analyze data from ANOVA and Followed by Tukey's multiple comparison test to compare differences between groups were performed by the statistical software SPSS.

RESULTS

The results of macroscopic

Macroscopic study of vigabatrin in infants whose mothers affected by the drug at a low dose 50 mg / kg and a high dose of 100 mg / kg were compared with two control groups, Changes were observed. Between the two groups in melatonin vigabatrin in combination with low-dose, high dose and low-dose and high-dose vigabatrin both tests were evaluated. In each group of four quantitative traits, weight, length, different series - the seat and the largest transverse diameter of the fetal head between the left and right posterior parietal ridge head were studied.

Effects of vigabatrin on the weight:

Weight infants in the experimental groups with vigabatrin 50 mg / kg, 100 mg / kg was significantly decreased compared to the control group no significant difference was seen between the two groups(Table 1).

Effect of vigabatrin on the CRL:

CRL length experimental groups in infants with vigabatrin 50 mg / kg, 100 mg / kg was significantly decreased compared to the control group no significant difference was seen between the two groups.

Effect of vigabatrin on the anterior - posterior of the infant:

Along the anterior - posterior of the experimental groups in infants with vigabatrin 50 mg / kg, 100 mg / kg was significantly decreased compared to the control groups, no significant difference was seen between the two groups(Table 1).

Effects of vigabatrin on the transverse diameter of the head:
Length of the transverse diameter of the two groups of infants with vigabatrin 50 mg / kg, 100 mg / kg was significantly lower than the control group and There was also significant difference between the two groups(Table 1).
Vigabatrin and Melatonin combined effects on weight:
Weight infants in both groups II and IV combined melatonin vigabatrin than two experimental groups I and III were significantly increased. It appeared that melatonin has a significant additive effect(Table 1).
Vigabatrin and Melatonin on the combined effects of anterior - posterior of the infant:
Along the anterior - posterior babies in experimental groups II and IV combined melatonin vigabatrin compared to the first and third experimental group showed a significant increase. It appeared that melatonin has a significant additive effect (Table 1).
Vigabatrin and Melatonin combined effect of the transverse diameter of the head:
The transverse diameter of the infants in the experimental groups II and IV combined melatonin vigabatrin compared to the first and third experimental group showed a significant increase. It appeared that melatonin has a significant additive effect (Table 1).

Table 1: Comparison of mean (g) during the neonatal unit (mm) head length (mm) and the width (mm) in neonatal mice. Dissimilar letters in each vertical column indicate a significant difference Mean+SD, (P< 0/05).

Fourth experimental group	Third experimental group	Second experimental group	The first experimental group	Control group	Variables
Vigabatrin mg / kg 100 10mg/kg melatonin	Vigabatrin mg / kg 100	Vigabatrin mg / kg 50 10mg/kg melatonin	Vigabatrin mg/kg50	Saline normal	
^{b}**1/15 ±0/3**	^{ab}**0/9 ±0/8**	^{b}**1/3 ±0/2**	^{ab}**1/16 ±0/5**	^{a}**1/74 ±0/3**	Weight
^{b}**21/1 ±0/3**	^{ab}**19 ±0/3**	^{b}**23/2 ±0/2**	^{ab}**21/35 ±1/75**	^{a}**28/2 ±0/3**	infant length
^{b}**0/8 ±0/3**	^{ab}**0/65 ±0/3**	^{b}**0/8 ±0/2**	^{ab}**0/7 ±0/6**	^{a}**10/3 ±0/9**	Head length
^{b}**3/4±0/2**	^{ab}**2/8 ±0/9**	^{b}**3/8 ±0/9**	^{ab}**3/5±0/2**	^{a}**5/3 ±0/1**	Head Width

Table 2: Comparison of mean brain weight, brain volume, lateral ventricle ependymal and neonatal mice. Dissimilar letters in each vertical column indicate a significant difference Mean+SD, (P< 0/05).

fourth experimental group	Third experimental group	second experimental group	The first experimental group	Control group	Variables
Vigabatrin mg / kg 100 10mg/kg melatonin	Vigabatrin mg / kg 100	Vigabatrin mg / kg 50 10mg/kg melatonin	Vigabatrin mg/kg50	Saline normal	
^{b}**0/12 ±0/5**	^{ab}**0/11 ±0/7**	^{b}**0/13 ±0/8**	^{ab}**0/12 ±0/9**	^{a}**0/14 ±0/3**	brain volume
^{b}**78 ±0/82**	^{ab}**75 ±0/91**	^{b}**82 ±0/32**	^{ab}**80 ±0/44**	^{a}**88 ±0/53**	Lateral ventricles
^{b}**280 ±0/357**	^{ab}**270 ±0/231**	^{b}**295 ±0/921**	^{ab}**285 ±0/822**	^{a}**305/5 ±0/745**	ependymal

Macroscopic study of Vigabatrin in infants whose mothers affected by the drug at a low dose 50 mg / kg and a high dose of 100 mg / kg were compared with two control groups, Changes were observed. Between the two groups in combination with low-dose and high-dose melatonin vigabatrin and vigabatrin were evaluate .In each group varies slightly maximum thickness of gray, were compared in two experimental.
Evaluation of the maximum thickness of the cerebellar gray:
Measurement of the maximum gray layer of the cerebellum in the control and experimental groups showed infant of experimental groups of low and high doses of vigabatrin and vigabatrin experimental groups received melatonin, Cerebellar gray layer thickness is less than the control group, This reduction in the groups that received vigabatrin alone were highly significant and Melatonin had no significant additive effect of the combined use of lamotrigine (Table 3).

Table 3: Comparison of cerebellar gray maximum thickness (m µ) in neonatal mice. Dissimilar letters in each vertical column indicate a significant difference Mean+SD, (P< 0/05).

fourth experimental group Melatonin Vigabatrin	Third experimental Vigabatrin mg group / kg 100	second experimental Vigabatrin mg group / kg 50 10mg/kg melatonin	The first experimental group Vigabatrin mg/kg50	Control group (Saline normal)	Variables
^{b}**112/7**	^{ab}**105/5**	^{b}**115/7**	^{ab}**113/2**	^{a}**117/7**	Gray layer thickness

Based on microscopic observations of early development of the cerebellum in the control infants, three cell layers of cerebellar cortex were visible and distinguishable from each other. The primary form of cerebellar pia mater surrounding the cerebellum was observed in leaf shape and spread. Low-dose and high-dose vigabatrin group, Molecular cell layers, Purkinje and granular compared with the control group did not receive the order and disrupted tissue seemed. Leaf shape and form creases so that the cerebellum does not have a primary and elementary form completely observed. The groups that melatonin was used in both groups compared to vigabatrin, significant changes were seen.

The observations made about the cerebellar control of the fourth ventricle, choroid plexus tissue was fully developed and normal epithelial cells of the choroid. Low-dose and high-dose vigabatrin group of fourth ventricle choroid plexus was found congested and Choroidal epithelial cells and necrotic due to protests, their integrity is lost and choroidal tissue was observed disruptive. Following discharge, necrotic lesions with fibrin leakage of capillaries in the choroid plexus of the fourth ventricle area was clearly visible. The groups that melatonin was used in combination with vigabatrin, significant changes were observed in comparison with the situation in normal vigabatrin groups differed.

Apoptosis in epithelial cells of the choroid plexus and other observations intervention group compared with the control group. Eosinophilic cytoplasm with increased apoptosis, chromatin condensation and fragmentation, and the formation of apoptotic bodies were visible. The neuroglia cell apoptosis in sporadic cerebellar white matter was observed. Changes indicative of apoptosis in neurons in all layers of the cerebellar cortex was barely visible. Melatonin and vigabatrin in apoptosis was observed in both the intervention.

DISCUSSION

Studies show that most neurons are produced in mid-pregnancy and then start to migrate and reach the final places. Because even in human neuronal growth occurs during the prenatal period. But in the cerebellum continues even after birth so axis of the cerebellum and cerebral - spinal, are more sensitive. Cell migration and differentiation of neurons are simultaneously. Even though the work cell differentiation in humans as well as postnatal phase will continue [20]. Most of cerebellar injury in experimental models of pregnancy, fetal inflammation, brain Scheme prenatal are examined. In this study we conducted observations, descriptions intervention revealed to us that Cerebellar structures during organogenesis and late pregnancy are significantly more susceptible to irreversible damage of the cerebellar. As these injuries during birth, intrauterine or mental impairments may be evident during postnatal growth. Antiepileptic drugs including chemical teratogen is a lot of research has been done about it. Epilepsy is a chronic neurological disease that affects millions of people worldwide and these individuals are forced to use at least one of antiepileptic drugs. Drug treatment of epilepsy during pregnancy has always been associated with many complications such mothers and fetuses are more susceptible to various disorders. Root cause of congenital malformations after the use of antiepileptic drugs free radicals have been expressed by these drugs. In addition, a genetic disorder in the hydrolysis of these metabolites, teratogenic substances increases. Despite numerous studies, the issue of special days and fetal teratogenicity of antiepileptic drugs days still remains a scourge of society, is suggestive of Obstetrics and Gynecology. Melatonin is one of the epiphyseal gland secretions might be effective in controlling some physiological phenomena. Melatonin free radicals including hydroxyl radicals, and anions proxy-nitrate is removed and neutralized. Due to its small size and high lipophilic properties of melatonin readily passes through the cell membrane and the cell is spread. It is very high concentrations in the cell nucleus and DNA against damaging agents maintained. Melatonin is a potent antioxidant, melatonin, since the activity or expression of antioxidant enzymes such as superoxide dismutase, glutathione reductase and glutathione peroxidase inducing. Research conducted on behalf of other hypotheses about anomalies after taking antiepileptic drugs suggests. He believes that free radicals generated by antiepileptic drugs toxic to cells and embryos are individual consumers. In addition, a genetic disorder in the hydrolysis of the metabolites of these substances increases Organogenesis. Based on the results of the morphometric parameters of fetal growth, it seemed that the drug significantly reducing neonatal effects of vigabatrin is affected during pregnancy [17,19].

Based on the results of the morphometric parameters of fetal growth, it seemed The effect of melatonin as an antioxidant against free radicals caused by drug vigabatrin has a protective effect. So we found a significant difference compared to the control group. The results are consistent with the results presented [18]. Melatonin and the activity or expression of antioxidant enzymes such as superoxide dismutase, glutathione reductase and glutathione peroxidase inducing. Based on the results of the morphometric parameters of fetal growth, effect of vigabatrin resulted in a significant decrease in body weight compared to the control group had melatonin vigabatrin. Between the two groups there was no

significant difference between vigabatrin and melatonin controls. It appears that the protective effect of melatonin administration on weight parameter is treated with vigabatrin. vigabatrin significantly reduces the effect of length, head length and width of the neonatal vigabatrin group than the control group is melatonin. whereas no difference was detected between the groups in melatonin vigabatrin and Control group. It appears that the protective effect of melatonin administration on the length of the infants treated with vigabatrin. Effect of vigabatrin resulted in a significant reduction in brain weight and volume of infants born melatonin vigabatrin groups than control groups. whereas no difference was detected between the groups in melatonin vigabatrin and Control group. It seems that melatonin treatment increases the weight and size of the baby's brain is treated with vigabatrin. Effect of vigabatrin resulted in a significant decrease in the thickness of gray babies born melatonin vigabatrin group than the control group. whereas there was difference detected between the groups in melatonin vigabatrin and Control group. It appears that The protective effect of melatonin treatment on reducing the thickness of the gray layer is treated with vigabatrin. It is stated that such intervention by anti-mitotic drugs or X-rays in newborn mice, the damage in the pia mater layers of the meninges membrane provides. This study was based on observations of injuries to infants vigabatrin drug intervention, congestion of the pia mater layer was observed. Studies by Kampr and Bowman [9] and Kern [10] were done in the neuropathological point is the point that High percentage of injuries with loss of cerebellar Purkinje Purkinje cells of the cerebellum in number and in size is sometimes. The study of Purkinje cell density of state intervention group compared with the control group was expressed Indicating an effect on mitotic Purkinje cells and consequently saw little congestion in these groups were. However, the combined effects of melatonin in the intervention group had a protective effect on the incidence of apoptosis.

CONCLUSIONS

In summary, this study showed that the administration of vigabatrin reduced infant growth parameters, and quantitative parameters, and reduces brain tissue. Melatonin administration significantly from the beginning of gestation, infant growth parameters affect However, pathological changes in brain tissue and cerebellum tissue protective role attribute is not significant. This study suggests that melatonin may play an important role in clinical applications of cerebellum dysfunction after taking antiepileptic drugs should be And recommended melatonin treatment before conception and during the period should be long.

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