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Influence of melatonin treatment on the survival and seizures frequency in pilocarpine-induced epilepsy in rats

Influência do tratamento com melatonina na sobrevivência e frequência das crises epiléticas em ratos induzidas por pilocarpina

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RESUMO

Objetivo: verificar se o tratamento com melatonina no período crônico pode alterar a frequência de crises e sobrevida de ratos Wistar submetidos ao modelo de epilepsia induzido por pilocarpina. **Métodos:** os animais foram divididos em dois grupos: Epi+MEL (n=8), animais tratados com melatonina (10 mg/kg) no período crônico; Epi+VEI (n=5), animais tratados com solução veículo no período crônico. Para analisar a duração e a frequência de crises os animais foram vídeo-monitorados antes do tratamento no 5 º e 7º mês de vida e após o início do tratamento no 9°, 11° e 16° mês de vida. **Resultados:** os animais tratados com melatonina não apresentaram alterações na duração e frequência de crises. Embora tenhamos observado uma taxa de sobrevida de 87,5% nos animais tratados com melatonina e 40% nos animais tratados com veículo, não observamos diferença estatística. **Conclusão:** o tratamento com melatonina não foi eficaz no controle da frequência e duração das crises, bem como não alterou a sobrevida dos animais. Contudo, acreditamos que a melatonina tenha forte potencial no aumento da expectativa de vida, porém mais estudos são necessários para uma melhor compreensão da sua ação neuroprotetora, bem como seu papel na expectativa de vida.

Palavras-chave: epilepsia, melatonina, status epilepticus, neuroproteção, modelo experimental

ABSTRACT

Objective: to verify if treatment with melatonin in the chronic period can modify the seizures frequency and survival in Wistar rats submitted to pilocarpine-induced model of epilepsy. **Methods:** animals were divided in two groups: Epi+MEL (n=8) animals treated with melatonin (10 mg / Kg) in the chronic period; EPI+VEH (n=5) animals treated with vehicle solution in the chronic period. To analyze duration and frequency of seizure, all animals were video-monitored during the 5th and 7th month of life and during the treatments in the 9th, 11th and 16th month of life. **Results:** the animals treated with melatonin in the chronic phase not presented changes in the duration and frequency of seizures. Although, the animals treated with melatonin have shown a survival rate of 87.5% and the animals treated with vehicle 40%, this finding was not statistically significant. **Conclusion:** Chronic treatment with melatonin was not effective in the control of frequency and duration of seizures, as well did not modify the survival of the animals. Nevertheless, we believe that melatonin has strong potential to increase life expectancy, however, more studies are needed for a better understanding of its neuroprotective action, as well as their role in life expectancy.

Keywords: epilepsy, melatonin, status epilepticus, neuroprotection, experimental model

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INTRODUCTION

Epilepsy is a chronic disorder characterized by recurrent seizures. This condition presents cognitive, neurobiological, psychosocial and social consequences that can affect the quality of life[1]. Approximately 1% of the population has epilepsy, the equivalent of 50 million people in world[2], and the temporal lobe epilepsy (TLE) is the most common epilepsy in adults, accounting for 40% of all cases[3,4].

Neuroprotective substances have been studied in epilepsy with the objective to prevent or decrease characteristics like inflammation, excitotoxicity and neuronal death in the brain. In this context, the melatonin receives attention due to present anti-inflammatory and antioxidant action[5,6] for its inhibitory effects on the central nervous system and prevent neuronal death[7,8,9].

In the last decades several studies showed a relationship between epilepsy and melatonin. In humans, the melatonin in addition to their antiepileptic drug (AED) can decrease the seizures frequency in children with severe intractable seizures[10]. In addition, patients with epilepsy present lower levels of melatonin salivary in the interictal period and higher levels after a seizure[11].

Studies in animal models of epilepsy can contribute to better understanding of behavioral, physiological and molecular aspects of this condition in human. In this context, our team's recent data showed that melatonin treatment after status epilepticus improves behavioral and morphological aspects of TLE and the pinealectomy promotes increased neuronal excitability facilitating the epileptogenic process. This facilitation can be reversed by subsequent melatonin administration[12].

Thus, the aim of this study was to verify if melatonin treatment can modify the seizure frequency and survival in chronic phase of pilocarpine-induced epilepsy.

METHODS

1. Animals

All experimental protocols were approved by the Ethical Committee of the Federal University of São Paulo (Unifesp) nº 1390/07 and all efforts were made to minimize animal suffering following the proposal of International Ethical Guideline for Biomedical Research[13].

Wistar adult male rats, (200–250g – two months of age), were used under a 12:12-h light–dark cycle (light on 7:00h), room temperature of 21±2°C, and granted free access to food and water for all the period of the experiment. These animals were divided in two groups: 1 - Group - (Epi+MEL) - Animals submitted to pilocarpine model and treated with melatonin (n=8). 2 - Group (Epi+VEH) - Animals submitted to pilocarpine model and treated with vehicle solution (n=5).

2. Pilocarpine administration

All animals received a systemic injection of pilocarpine HCl (350 mg/kg, ip, Merck S.A.) 30 min after scopolamine methylnitrate administration (1 mg/kg s.c., Sigma Co., MO, USA to prevent the peripheral cholinergic effects).

3. Treatment

The animals were treated with melatonin (10mg/Kg) or vehicle solution (ethanol 1%)[14] during the night. These solutions were prepared daily, the melatonin 10mg/Kg was diluted in 100% ethanol followed by dilution in drinking water (final ethanol 1%). Treatment started in 9th month of life of the animals, overnight solutions were available to the animals and during the day they received only water.

4. Frequency and duration of seizures

Following 45 days SE onset the animals were continuously monitored 24h/day until to be established the chronic phase of this model and the frequency and duration of seizures were video-recorded (Stella system) during the 5th and 7th month of life, to observe of spontaneous seizures. After this period, the animals were divided into two groups (Epi+MEL and Epi+VEH) and started treatment with melatonin, which lasted until the end of life. These animals were video-recorded during the treatment in the 9th, 11th and 16th month of live to observe the frequency and duration of seizures.

5. Statistical Analysis

To analyze the frequency and duration of seizures it was performed a nonparametric statistical test Friedman test followed by Dunn's post for multiple comparisons. For survival analysis was performed chi-square test statistic with Yates correction. A value of $p < 0.05$ was accepted as significant in all cases. The values were expressed as a mean ± standard deviation (SD).

RESULTS

1. Seizures frequency

The animals of Epi+MEL and Epi+VEH groups were monitored before treatment at 5th and 7th months of life and during the treatment with melatonin or vehicle solution in the 9th, 11st and at 16th months of life. The Table 1 shows the frequency of seizures before and during treatment in each group.

2. Seizures Duration

Animals were video-monitored and the duration of seizures was recorded and accounted through chronometer. There was no significant difference in duration of seizure in both groups (Table 2).

Table 1: No significant differences were founded in seizure frequency before and during treatment in both groups. Friedman non-parametric statistical test followed by Dunn's post test for multiple comparisons, (Epi+MEL Fr = 3.700 p=0,448) and (Epi+VEH Fr = 1.570 p=0,814).). Values expressed as a mean ± standard deviation and considered significant at p <0.05.

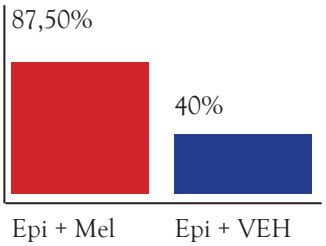
Groups	Before treatment		During treatment		
	5º month	7º month	9º month	11ºmonth	16ºmonth
EPI+MEL					
Mean	7.3	9.7	9.2	8.1	25.2
DP	9.1	8.9	10.8	11.7	33.3
EPI+VEI					
Mean	2.9	3.9	5.8	4.3	3.1
DP	2	2.1	3.3	3.7	0.3

Table 2: No significant differences in duration of seizures before and during treatment in both groups were observed. Friedman non-parametric statistical test followed by Dunn's post-test multiple comparisons test Epi+MEL (Fr = 2,189 p = 0.70) and Epi+VEH (Fr= 4.600 p=0,37). Values expressed as mean ± standard deviation and considered significant at p <0.05.

Groups	Before treatment		During treatment		
	5º month	7º month	9º month	11ºmonth	16ºmonth
EPI+MEL					
Mean	42.3	38.5	33.5	33.6	29.8
DP	15.54	10.35	8.35	10.15	10.22
EPI+VEI					
Mean	46.9	36.9	32.5	40.5	35.1
DP	15.13	5.76	1.15	13.73	6.17

3. Survival rate

Animals were kept until completed the 20th month to assess the life expectancy. In Epi + MEL group 7 animals survived until the 20th month of life while only one animal died before completing this period. In Epi+VEH group, two animals survived until the 20th month of life, while three died before completing this period. Thus, 87.50% of the animals treated with melatonin survived until the 20th month of life, while 60% of the animals treated with the vehicle died before this time.



Epi+MEL: Animals submitted to pilocarpine model and treated with melatonin. Epi+VEH: Animalssubmitted to pilocarpine model and treated with vehicle solution.

Figure 1: The chi-square test with Yates correction showed no significant difference in the survival of animals in the both groups (X^2 = 1.411 fixed, p = 0.235).

CONCLUSION

Several studies in experimental models and humans suggest that melatonin is a neuroprotective molecule, due to anticonvulsant, anti-inflammatory and scavenging properties[5,6,15], although some studies have shown that melatonin may exert proconvulsant activity in humans[16].

Our results demonstrated that the animals treated with melatonin did not present significant changes in the duration and frequency of seizures. The animals presented 2-60 seizures per month, and the duration of seizures remained similar throughout the life of the animal.

In the same context, a clinical study evaluated 11 children with epilepsy that received melatonin treatment (0.1 mg / day) in addition to their AED, and showed no change in the frequency of seizures[22].

In respect to the survival time only 40% of the Epi + VEI group animals survive until the 20th month. On the other hand, in the Epi + MEL group, 87.5% of the animals survive until the 20th month. This finding was not statistically significant, but this result leads us to believe the treatment with melatonin can extend animal survival.

Despite the beneficial effects of melatonin, treatment with melatonin was not effective in the control of the frequency and duration of seizures. Besides that, our data show no statistical differences in survival rate between the groups. However, the melatonin-treated group presented a higher survival rate compared to the control group indicating that melatonin may have a good potential to increase life expectancy.

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