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Protective Effect of Melatonin on Oxidative Stress in Hippocampus of Schizophrenic Rats

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Abstract

Objective: To investigate the protective effect of melatonin (MT) on oxidative stress of hippocampal neurons in schizophrenic rats. Methods: 36 male SD rats were divided into 3 groups: control group, model group and model +MT group (10mg/kg), with 12 rats in each group. The rat model of schizophrenia was established by intraperitoneal injection of MK-801. After successful modeling, the model +MT group was intraperitoneally injected with MT, while the control group and the model group were injected with the same dose of 10% alcohol for 14 days. Morris water maze experiment was used to detect the learning and memory function of rats. Primary hippocampal cells were cultured by disconnection. The contents of ROS, SOD and MDA related factors in the culture medium of hippocampal cells were detected by kit method, and the number of apoptosis of hippocampal neurons was detected by TUNEL method. Results: Compared with the control group, the learning and memory function of the rats in the model group was impaired, the contents of ROS and MDA in the hippocampus were significantly increased, SOD activity was decreased, and the number of apoptotic neurons was significantly increased ($P < 0.05$).Compared with the model group, the learning and memory functions of the rats in the model +MT group were significantly improved, the contents of ROS and MDA in the hippocampus were decreased, SOD activity was increased, and the number of apoptotic neurons was significantly decreased ($P<0.05$). Conclusion: MT can significantly reduce the oxidative stress response and protect the neurons in the hippocampus of schizophrenic rats.

Keywords

Schizophrenia, Melatonin, hippocampal, oxidative stress.

1. TESTS

Schizophrenia is often accompanied by sensory, thinking, emotional, behavioral and other aspects of the disorder, the etiology and pathogenesis is not clear, clinical treatment is mainly to alleviate symptoms, so far there is no very effective treatment drugs. In recent years, the hypothesis of dysfunction of glutamate receptor in schizophrenia has attracted attention. It is believed that glutamate receptor deficiency is an important cause of schizophrenia. Continuous injection of MK-801 can reduce the learning and memory ability of rats, and the animal model of schizophrenia is prepared for basic research [1]. Melatonin (MT) is an endogenous free radical scavenger, which can directly remove a large number of free radicals. It is easy to penetrate the blood-brain barrier and enter the brain cells to play the role of antioxidant damage and mitochondrial protection. In recent years, the role of MT in neuroprotection has

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attracted much attention. Studies have shown that MT can repair damaged neuronal synapses, promote signal communication between neurons, and delay neurodegenerative diseases[2] . The development of schizophrenia is accompanied by oxidative stress injury of hippocampal neurons. Whether melatonin has protective effect on hippocampal neuronal apoptosis and oxidative stress injury and the protective mechanism is not clear. In this experiment, we observed the apoptosis of hippocampal neurons and the degree of oxidative stress reaction in the process of MT treatment in rats with schizophrenia, to explore the possible mechanism of MT, and to provide ideas for the research of schizophrenia drugs.

2. MATERIALS AND METHODS

2.1. Laboratory Animals and Main Reagents

SD rats born in the same litter for 7 days (provided by Jinan Pengyue experimental animal breeding Co., Ltd.) were selected. During the experiment, the rats were fed in the SPF standard environment, and the experiments were carried out for 5 consecutive times.

Main reagents: glutamate receptor antagonist dezoxipine maleate (MK-801) and deoxyribonuclease (DNase) were purchased from Worthington company; TUNEL kit was purchased from millipore company; dhr-123 Kit (AAT bioquest), SOD, MDA ELISA Kit (MSK), Triton X-100, Hoechst were purchased 33342 and normal horse serum were purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.; Neurobasal a, B27, trypsin, L-glutamine derivative (glutamax-i) and kanamycin were purchased from Invitrogen company; digestion termination fluid and poly-D-lysine (PDL) were purchased from sigma company; bFGF was purchased from peprotech company.

2.2. Modeling and Experimental Grouping

SD rats were randomly divided into three groups: control group, model group and model + MT group, with 12 rats in each group. In addition to the control group, the glutamate receptor antagonist dezoxipine maleate (MK-801, 0.6mg/kg) was intraperitoneally injected once a day for 14 days; the rats were scored by the sams-dodd stereotyped behavior score method [7], which confirmed that all rats were successfully established. From the 15th day of the experiment, the model + MT group was intraperitoneally injected with melatonin 10 mg / kg (sigma), while the control group and model group were injected with the same dose of 10% alcohol for 14 days. After administration, all rats were tested for behavior. After the test, the rats were anesthetized, and the hippocampus was taken for primary cell culture.

2.3. Evaluation of Stereotyped Behavior

Sams-dodd stereotyped behavior scale: 0: static or no activity at all; 1: normal activity with occasional forward movement; 2: activity accompanied by repeated forward exploration, circling around the cage in a stereotyped behavior; 3: continuous forward exploration; 4: repeated head, head shaking or rotation; 5: rapid head shaking, circling or head movement. Double blind method was used for evaluation. The average score of two evaluators was included in the statistics. The score was scored every 10 minutes, and the average value was observed for 60 minutes.

2.4. Morris Water Maze Experiment

Morris water maze experiment consists of two parts. ① Positioning navigation experiment: the rats were trained on the 17th day of Administration for 4 consecutive days to evaluate the learning ability of rats The Morris water maze was placed in the area where the platform was located at the bottom left. When the rats were put into the water maze, their heads were facing the wall of the pool. When they were free to swim, they were found and climbed onto the platform, and the time (escape latency) was recorded. If the rats did not find them within 1 min,

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they were pulled to the platform, stayed for 10 s and put back into the cage. The latency time was recorded as 60s. ② Space exploration experiment: after 4 consecutive days of learning, the platform was removed and the rats were put into the pool wall from the upper right side of the water maze. The times of crossing the original platform area and staying time in the original platform area (space exploration time) were recorded.

2.5. Primary Culture of Hippocampal Cells

The hippocampus of decapitated rats was isolated under the anatomic microscope. The blood vessels and meninges were removed. The tissues were digested by 0.05% trypsin in shaking water bath at 37°C for 10 min. the trypsin digestion termination solution (final concentration 0.4 g / L and containing 2% B27 Neurobasal a) was added. The round head dropper was blown several times, centrifuged, and the supernatant was filtered into another centrifuge tube. The above steps were repeated 3-4 times. The supernatant was centrifuged and discarded. Fresh culture medium (Neurobasal a of 2% B27) was added and blown into single cell suspension. Cell counting was performed. The cell density was diluted to 400-500 cells / mm2 with cell culture medium (containing 2% B27, 1 mmol / L glutamax-i, 10 μ g / L bFGF and 0.1 g / L kanamycin) After 30 min incubation in the incubator with 5% CO2 at 37°C, the cells were added to 1ml / well, and then the cells were cultured in the incubator. After that, the medium was changed once every two days, and the related experiments were carried out on the 7th day.

2.6. Detection Methods of Oxidative Stress

Dhr-123 kit was used to detect the relative level of reactive oxygen species (ROS) in primary cultured hippocampal cells. ELISA kit was used to detect the content of superoxide dismutase (SOD) and malondialdehyde (MDA) in the culture medium of primary cultured hippocampal cells.

2.7. Apoptosis Detection

The number of apoptotic cells was detected by TUNEL method and stained according to the instructions of TUNEL Kit (cat < s7165) of millipore company. The cells were treated with equilibration buffer solution in the kit for about 10 seconds. The filter paper was dried and the main effective component was deoxyribonucleotide terminal transferase (TDT) A series of 3 ′ - Oh terminals were produced by double strand breaks or one strand gap in apoptotic cells. Under the action of TDT enzyme, the derivatives of deoxyribonucleotides and digoxin were labeled on the 3 ′ - end of DNA After 30 hours, TDT enzyme blocking solution was used to terminate the reaction, and the anti digoxin fluorescent second antibody coupled with rhodamine red was incubated in dark at room temperature for 30 min, and then re stained with Hoechst 33342.

2.8. Data Collection and Statistical Processing Methods

The number of apoptotic cells and apoptotic cells labeled with Hoechst 33342 was counted by Image J software. Spss20.0 statistical software was used for data analysis. The measurement data were expressed by X (-) ± s. The comparison between groups was performed by one-way ANOVA, and the pairwise comparison between groups was performed by lsdt method. $P < 0.05$ was statistically significant.

3. RESULT

3.1. Evaluation of Stereotyped Behavior:

Compared with the model group, the score of stereotype behavior in the model + MT group was significantly lower than that in the model group ($P < 0.05$)

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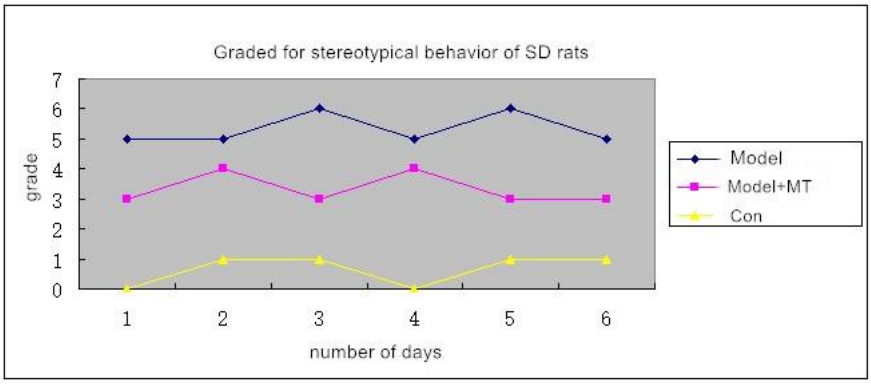

Figure 1. The effect of melatonin on the score of stereotyped behavior in rats

3.2. Morris Water Maze Test Results

In the positioning navigation experiment: compared with the control group, the escape latency time of the model group was significantly prolonged on the third and fourth days of training, and the differences were statistically significant (all $P < 0.05$); compared with the model group, the escape latency time of the model + MT group on the third and fourth days of training was significantly shorter, with statistically significant differences (all $P < 0.05$). (see Table 1)

Table 1. Comparison of escape latency time of each group in positioning navigation experiment (in each group n = 12, $\bar{X}\pm s$)

Group	training day 1	training day 2	training day 3	training day 4
control group	51.84±8.75	47.91±9.08	42.85±7.01	38.31±6.02
model group	55.72±8.56	56.82±9.02	55.84±8.57a	51.77±9.51a
Model + MT group	52.71±7.81	49.87±9.62	43.98±7.84b	41.85±6.06b

Compared with the control group: aP < 0.05; compared with the model group: b P < 0.05

In the space exploration experiment: compared with the control group, the times of crossing the platform and the stay time in the model group were significantly reduced (all $P < 0.05$); compared with the model group, the residence time in the original platform area of the model + MT group was significantly increased, with significant differences (all $P < 0.05$) (see Table 2)

Table 2. Comparison of experimental results of space exploration among groups (in each group n = 12, $\bar{X}\pm s$)

Group	crossing the original platform area Times	stay time (s)
control group	6.02±1.14	24.27±3.71
model group	3.97±0.83a	12.89±2.61a
Model + MT group	5.18±0.65	17.45±2.70b

Compared with the control group: aP < 0.05; compared with the model group: b P < 0.05

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3.3. Comparison of Oxidative Stress Factors in Hippocampus of Each Group

Compared with the control group, the contents of ROS and MDA in primary cultured hippocampal cells in the model group were significantly increased, and the activity of SOD was decreased (all $P < 0.05$); compared with the model group and the model + MT group, the contents of ROS and MDA were significantly decreased, and the activity of SOD was increased (all $P < 0.05$) (see Table 3)

Table 3. Comparison of oxidative stress factor levels in hippocampus of each group (in each group n = 12, $\bar{X}\pm s$)

Group	ROS	SOD (kU/L)	MDA (μmol/g)
control group	41.62±6.07	35.21±4.92	5.07±0.59
model group	97.07±10.56a	21.78±3.01a	11.99±2.53a
Model + MT group	70.01±9.79b	24.68±3.23	9.08±1.13

Compared with the control group: aP < 0.05; compared with the model group: b P < 0.05

3.4. Apoptosis Detection Results

The percentage of neuronal apoptosis in the model group was significantly higher than that in the control group; in the model + MT group, the percentage of neuronal apoptosis was significantly lower than that in the model group, but compared with the control group, the percentage of neuronal apoptosis was still significantly increased, and the differences were statistically significant (all $P < 0.05$) (see Table 3 and Figure 2)

Table 4. Apoptosis ratio of hippocampal neurons in each group (in each group n = 12, $\bar{X}\pm s$)

Group	Percentage of apoptotic cells (%)
control group	100%
model group	153.59%±4.86%a
Model + MT group	128.99%±8.15%b

Compared with the control group: aP < 0.05; compared with the model group: b P < 0.05

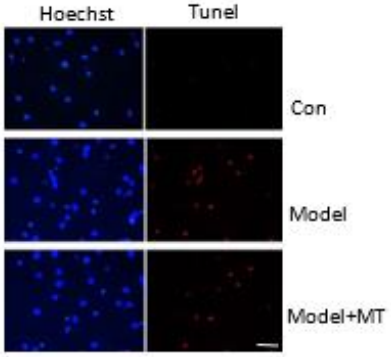

Figure 2. Apoptosis detection results of control group, model group and model + MT group (bar = 50 μ m)

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4. DISCUSSION

At present, it is believed that the occurrence of schizophrenia is due to the dysfunction of NMDA receptor. MK-801 is a specific blocker of NMDA receptor, which can significantly reduce the activity of NMDAR in neurons, inhibit the proliferation and differentiation of neurons, and lead to apoptosis. Therefore, MK-801 is widely used to establish animal models of schizophrenia and can cause cognitive dysfunction in rodents, such as slow or hyperactivity, abnormal movement, etc. In this study, the learning and memory functions of rats were significantly decreased after continuous injection of MK-801. The animal model was proved to be successful by stereotyped behavior test and Morris water maze test. Some studies have shown that Alzheimer's disease, schizophrenia, Parkinson's disease and other brain neuropsychiatric diseases are related to oxidative stress damage [4]. It may be the imbalance of oxidation antioxidant state caused by external stimulation, resulting in brain homeostasis disorder and excessive release of cytochrome c, leading to neuroinflammation and mitochondrial dysfunction, resulting in neuronal damage. In this study, we found that in the primary culture medium of hippocampal cells of schizophrenic rats, the contents of ROS and MDA were significantly increased, and the activity of SOD was significantly decreased. Under the condition of schizophrenia, the hippocampal region suffered severe oxidative stress reaction. After adding a certain dose of MT, the oxidative stress response of hippocampal cells could be reduced.

Melatonin (MT) is an endogenous hormone secreted by the pineal gland, which can correct the biological clock and regulate neuroendocrine function. It is a strong free radical scavenger with anti stress and anti-aging effects [5]. It has been proved that MT exists in anxiety disorder, depression, schizophrenia and other mental diseases related to sleep disorders The phenomenon of insufficient secretion [6]. This study found that MT can reduce the oxidative stress injury of hippocampal cells in schizophrenic rats and reduce the apoptosis of hippocampal neurons. However, the specific mechanism of MT against oxidative stress needs further study.

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