Advances in Engineering Research, volume 197
Proceedings of the 2020 9th International Conference on Applied Science, Engineering and Technology (ICASET 2020)

Effects of Antioxidant Compounds on Methamphetamine-Induced Learning and Memory Impairment in Mice

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ABSTRACT
Purpose: To observe effects of antioxidant compounds on the learning and memory impairment induced by methamphetamine (MA) in mice. **Methods:** Fifty 6 weeks old male C57bl/6 mice were randomly divided into 5 groups: group A was control group, group B was model group, group C was 50 mg/kg group, group D was 100 mg/kg group and group E was 200 mg/kg group. Groups B, C, D, and E were intraperitoneally injected with methamphetamine to prepare a model of learning and memory impairment in mice, and group A was injected with saline. Groups C, D, and E were intragastrically pre-administered with 50, 100, and 200 mg/kg of antioxidant compound for 2 weeks before modeling, and continued to be administered during modeling and behavioral testing, and groups A and B were administered with saline. The Morris water maze was used to test the short-term learning and memory ability of mice (the swimming speed, escape latency, percentage of time spent in the target quadrant). Determination of superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and malondialdehyde (MDA) expression levels in serum, hippocampus and striatum of mice by enzyme-linked immunosorbent assay (ELISA). **Results:** Behavioral test results: Compared with group A, there was no significant difference in the average speed between days 1-5 of group B, the escape latency was significantly increased on days 3 and 5 ($P<0.05$), and the percentage of time spent in target quadrant decreased significantly ($P<0.05$); compared with group B, there was no significant difference in the average speed and escape latency between days 1-5 of groups C, D and E, but the percentage of time spent in target quadrant of group D increased significantly ($P<0.05$). ELISA results: in serum, compared with group A, SOD and T-AOC of group B were significantly decreased, and MDA of group B was significantly increased ($P<0.05$); compared with group B, SOD of groups D and E were significantly increased, T-AOC of groups C and D were significantly increased, and MDA of group D was significantly decreased ($P<0.05$). In the hippocampus, compared with group A, SOD and T-AOC of group B were significantly decreased, and MDA of group B was significantly increased ($P<0.05$); compared with group B, SOD and T-AOC of group D were significantly increased, and MDA of group D was significantly decreased ($P<0.05$). In the striatum, compared with group A, the SOD of group B was significantly decreased, and MDA of group B was significantly increased ($P<0.05$); compared with group B, SOD of groups C and D were significantly increased, and MDA of groups C, D and E were significantly decreased ($P<0.05$). **Conclusion:** The 100mg/kg antioxidant compounds could improve the oxidative stress of mice induced by MA, which could alleviate the memory damage in mice.

Keywords: *antioxidant compounds, methamphetamine, T-AOC, SOD, MDA*

1. INTRODUCTION

Methamphetamine (MA) is an amphetamine-type stimulant. Its appearance is pure white and crystal. Imaging medical research data showed that: Neurons in the frontal cortex, hippocampus, and striatum of MA abusers were damaged and could be produced pathological changes similar to Alzheimer's disease and Parkison's disease, resulting in impaired learning, memory, and motor functions [1-3]. At present, the results of domestic and international researchs have not fully defined the neurotoxic mechanism of MA. The current research results showed that a variety of mechanisms were involved in the neurotoxicity of MA, including oxidative stress, neuronal apoptosis, excitotoxicity, mitochondrial dysfunction,

etc.[4-6]. Oxidative stress was one of the important mechanisms of action of neurotoxic damage caused by MA [7, 8]. At present, the prevention and treatment of nerve damage caused by MA is still unclear. Studies have shown that traditional chinese medicine compound could induce the expression of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) of antioxidant system enzymes by regulating Nrf2 signaling pathway to reduce cell damage caused by ROS and maintain the body's redox dynamic balance [9]. Sun et al believe that antioxidants and some related Chinese medicine compounds are expected to become effective drugs for the treatment of neurodegenerative diseases [10]. Therefore, this experiment explored the effect of antioxidant compounds on methamphetamine (MA)-induced learning and memory impairment in mice, and had a certain inspiration for the study of the mechanism of neurotoxicity of MA, which provided a basis for further exploration of MA intervention and treatment.

2. MATERIALS AND METHODS

2.1 Animals

SPF 2 month old male C57bl/6 mice were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd.

2.2 Main reagents and instruments

Antioxidant compounds (Ginsenoside, Tea Polyphenols, Resveratrol=1:1:2); MA (purity 98%) was provided by the Anti-drug Corps of Hubei Provincial Public Security Department; SOD, MDA, T-AOC kits were purchased from Nanjing Jiancheng Bioengineering Institute. Full-wavelength microplate reader (Thermo Scientific Co., Ltd); constant temperature water bath (Shanghai Xinmiao Medical Device Manufacturing Co., Ltd.); benchtop low speed centrifuge (Thermo Scientific Co., Ltd); Morris water maze and analysis software were purchased from Shanghai Xinruan Information Technology Co., Ltd.

2.3 Model preparation and grouping

Fifty 6 weeks old male C57bl/6 mice were randomly divided into 5 groups: group A was control group, group B was model group, group C was 50 mg/kg group, group D was 100 mg/kg group and group E was 200 mg/kg group, and were free to drink water and eat throughout the experiment. Groups C, D, and E were intragastrically administered with 50, 100, and 200 mg/kg of antioxidant compounds for 2 weeks before model preparation, and groups A and B were administered with saline. Then, groups B, C, D, and E were intraperitoneally injected with 10 mg/ml MA per day for model preparation, and group A was intraperitoneally injected with saline for seven days. After the model was prepared, the Morris water maze experiment was performed. During the model preparation and water maze experiment, groups C, D, and E were continuously intragastrically administered with different concentrations of antioxidant compounds, and groups A and B were administered with saline.

2.4 Evaluation of learning and memory ability of mice

Morris water maze was used to determine the learning and memory ability of each group of mice, which consists of a pool with a platform, an automatic camera and Morris water maze data processing software. A mixture of water and titanium dioxide was injected into the tank before the test, the water depth was about 30 cm (the water surface was 1 cm above the surface of the platform, and the mice could not see the platform), and the water temperature was (20 ± 1) °C. The experimental environment was required in a room with sound insulation and week lighting, and remained unchanged in each experiment. The test period was 8 days, the first 2 days was the pre-adaptation phase, then the 5d is the training phase, and the 8th is the test phase. During the training phase, each mouse was trained 4 times a day for 60 s each time, and entered the pool from the four quadrants of the pool respectively. Then, the camera started recording when the mouse entered the water and the Morris water maze data processing software started recording and calculation: ① escape latency: the time of each mouse entering the platform during the training phase. If the mouse still has not found the platform within 60 s, it can be placed on the platform at the 60th s and stayed on the platform for 10 s. At this time, the escape latency is recorded as 60 s; ② average speed: average swimming speed of the mouse per day during the training phase; ③percentage of time spent in the target quadrant: the platform was removed during the test phase on the 8th day, and the mouse was free to swim for 60 s. The time spent in the quadrant of the original station, divided by 60 s equal to the percentage of time spent in the target quadrant [11].

2.5 Sample collection and preservation

All mice were sacrificed after the Morris water maze test. After anesthesia with 4% chloral hydrate, the eyeballs were taken for blood collection. The blood was allowed to settle at room temperature and centrifuged at 4 °C after 24 hours, and then the supernatant was stored in a refrigerator at -80 °C. The brain was quickly decapitated after blood was taken, and then the striatum and hippocampus were separated on ice and stored in a refrigerator at -80 °C.

2.6 Detection of indicators

The SOD activity and MDA content in 10% tissue homogenate were determined by ELISA. The specific

methods were strictly in accordance with the reagent instructions.

2.7 Statistical analysis

All data were presented as means and standard error of the mean (±SEM). SPSS17.0 statistical software was used. The comparison between groups was analyzed by ANOVA. P<0.05 was considered statistically significant.

3. RESULTS

3.1 Escape latency and average speed

There was no significant difference in the average speed of mice in each group during the training period (Figure 1A). On the 3rd and 5th day of training, compared with group A, the escape latency of group B mice increased significantly (P<0.05). Compared with group B, there was no significant difference between the other three groups (Figure 1B).

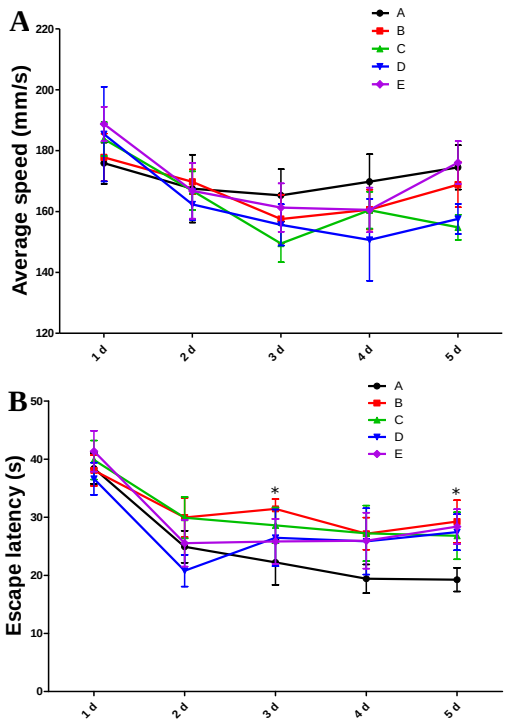

Figure 1 Comparison of escape latency and average speed of mice in each group on the 1st to 5th day of training (mean±SEM). A: control group, B: model group, C: 50 mg/kg group, D: 100 mg/kg group and E: 200 mg/kg group. Compared with group A, * P<0.05.

3.2 The percentage of time spent in the target quadrant

In the test phase, the percentage of time spent in the target quadrant of group B mice was significantly reduced compared to group A (P<0.05). Compared with group B, the percentage of time spent in the target quadrant of group D was significantly increased (P<0.05) (Table 1).

3.3 Escape latency and average speed

In serum, compared with group A, SOD and T-AOC in group B were significantly decreased, and MDA was significantly increased (P<0.05). Compared with group B, SOD in groups D and E were significantly increased; T-AOC in groups C and D were significantly increased, and MDA in group D was significantly decreased (P<0.05) (Table 2).

3.4 T-AOC, SOD and MDA levels in hippocampus

In the hippocampus, compared with group A, SOD and T-AOC in group B were significantly decreased, and MDA was significantly increased (P<0.05). Compared with group B, SOD and T-AOC in group D were significantly increased, and MDA was significantly decreased (P<0.05) (Table 3).

3.5 T-AOC, SOD and MDA levels in striatum

In the striatum, compared with group A, SOD in group B was significantly decreased, and MDA was significantly increased (P<0.05). Compared with group B, SOD in groups C and D were significantly increased, and the MDA in groups C, D and E were significantly decreased (P<0.05) (Table 4).

4. CONCLUSION

MDA is an important product of lipid peroxidation. SOD can directly scavenge free radicals in the body, which is an antioxidant enzyme that protects the body from metabolism and the damage coused by oxygen free radical [12, 13] .The results of this study showed that intraperitoneal injection of MA resulted in impaired learning and memory, the antioxidant activity of the hippocampus and striatum of mice was weakened, and lipid peroxidation was enhanced. Although, the learning function damage of the mice was not significantly improved by administering the antioxidant compounds, the memory function damage of the mice was significantly improved, and the antioxidant activity of the hippocampus and striatum of the mice was significantly increased, the lipid peroxidation was significantly decreased. It is indicated that the antioxidant compounds could improve the memory damage induced by MA and repair the

oxidative stress damage of the brain. However, this experiment did not directly demonstrate how antioxidant compounds interacted with these factors because that MA-induced learning and memory impairments could create a complex cascade reaction, which involves a variety of signaling pathways. Further research is needed from the perspectives of cell level, molecular metabolism and signal transduction pathways, and then provides a theoretical basis for the prevention and treatment of cognitive dysfunction caused by MA.

Table 1 Comparison of the percentage of time spent in the target quadrant of the Morris water maze in the test phase (mean±SEM)

Group	Percentage of time spent in the target quadrant (%)
A	20.25±2.53
B	13.11±1.60 *
C	17.50±2.37
D	20.60±3.00 #
E	12.99±1.65 *

A: control group, B: model group, C: 50 mg/kg group, D: 100 mg/kg group and E: 200 mg/kg group. Compared with group A, *P<0.05; compared with group B, #P<0.05.

Table 2 Comparison of T-AOC, SOD and MDA levels in serum of each group of mice (mean±SEM)

Group	T-AOC（mmol/L）	SOD（U/ml）	MDA（nmol/ml）
A	0.92±0.040	63.57±2.33	6.31±0.66
B	0.78±0.034 *	48.98±2.90 *	9.35±0.76 *
C	0.96±0.033 #	54.51±2.69	7.87±1.79
D	0.93±0.045 #	58.22±2.62 #	7.02±0.69 #
E	0.97±0.083	58.52±2.12 #	7.90±0.47

A: control group, B: model group, C: 50 mg/kg group, D: 100 mg/kg group and E: 200 mg/kg group. Compared with group A, *P<0.05; compared with group B, #P<0.05.

Table 3 Comparison of T-AOC, SOD and MDA levels in hippocampus of each group of mice (mean±SEM)

Group	T-AOC（mmol/gprot）	SOD（U/mgprot）	MDA（nmol/mgprot）
A	0.14±0.012	65.70±2.37	4.57±0.30

B	0.10±0.0055 *	57.24±2.62 *	5.82±0.48 *
C	0.10±0.013	65.62±6.59	5.24±0.82
D	0.12±0.0058 #	67.38±3.30 #	4.53±0.31 #
E	0.13±0.017	60.98±3.24	4.65±0.42

A: control group, B: model group, C: 50 mg/kg group, D: 100 mg/kg group and E: 200 mg/kg group. Compared with group A, *P<0.05; compared with group B, #P<0.05.

Table 4 Comparison of T-AOC, SOD and MDA levels in striatum of each group of mice (mean±SEM)

Group	T-AOC（mmol/gprot）	SOD（U/mgprot）	MDA（nmol/mgprot）
A	0.15±0.013	87.40±7.59	3.41±0.26
B	0.11±0.0083	61.86±5.96 *	4.86±0.37 *
C	0.14±0.014	87.91±10.46 #	3.52±0.40 #
D	0.15±0.012 #	90.14±5.91 #	3.18±0.27 #
E	0.14±0.016	92.05±17.28	3.19±0.39 #

A: control group, B: model group, C: 50 mg/kg group, D: 100 mg/kg group and E: 200 mg/kg group. Compared with group A, *P<0.05; compared with group B, #P<0.05.

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