

## Effects of Pioglitazone and Trehalose on Cognitive Impairment in Sevoflurane Anesthetized AD Mice

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**Abstract:** Objective: To investigate the effect and mechanism of pioglitazone and trehalose on cognitive dysfunction of sevoflurane anesthetized AD mice. Methods: APP / psi double transgenic mice were divided into control group, model group, trehalose group, pioglitazone group, trehalose + pioglitazone group (n = 24). Each group was anesthetized with sevoflurane (control group was given oxygen) after the corresponding intervention, and the cognitive function of mice was evaluated. Western blot method was used to detect the expression level of Fas-FasL pathway related proteins. TUNEL method was used to calculate the neuron apoptosis rate. Results: compared with the model group, trehalose group and pioglitazone group, the escape period of water maze, the apoptosis rate and the expression level of  $\beta$  in the combined group were significantly lower, but significantly higher than that in the control group ( $P < 0.05$ ). The time of water maze exploration and the expression level of Fas, FasL and caspase-3 protein in the combined group were significantly higher than those in the other groups. Conclusion: Trehalose combined with pioglitazone can improve cognitive function, which may be related to down regulating Fas / FasL pathway.

Alzheimer's disease (AD) is a neurodegenerative disease with progressive cognitive and functional defects and behavioral changes, which is related to the accumulation of amyloid protein and tau deposition in the brain [1-3]. Cognitive symptoms of AD usually include impairment in short-term memory, execution, and visual spatial dysfunction. Some rare AD variants with relatively preserved memory are identified. Clinical evaluation, including cognitive testing, is still crucial for the diagnosis and staging of AD. Although the latest advances in amyloid imaging and genetics promote early detection and treatment, AD is the most common cause of death in neurodegenerative diseases. Although there is increasing evidence that AD pathology begins to deposit in the brain in middle age, the initial clinical symptoms usually occur after the age of 65.2 and 3. To a large extent, the incidence of AD is increasing rapidly because the proportion of people aged 65 and above is growing faster than that of any other age group in the world. Between 1997 and 2050, the number of Americans aged 65 and over will increase from 63 million to 37 million. Africa is 1838 million, Europe is 113 million to 170 million, and Asia is 172 million to 435 million. "A national representative data set, the aging, demography and memory study (Adams), estimates that 14% of the elderly aged 71 and over in the United States suffer from dementia [4-5]. AD accounted for 70% of dementia in this age group. In a subsequent publication, the Adams researchers reported that another 22% (5.4 million Americans) 71 years or older had cognitive abilities. Apoptosis related factor (FAS) is a kind of apoptosis program factor. It can activate the downstream caspase-3 protein and mediate the formation of apoptotic bodies by binding with the factor associated molecule signal surface ligand (FasL). Peroxisome proliferator activated receptor gamma (PPAR  $\gamma$ ) is related to cognitive function, and PPAR  $\gamma$  agonists may improve neurological damage. Pioglitazone is a PPAR  $\gamma$  agonist. In recent years, pioglitazone has been found to play a neuroprotective role in a variety of brain injury and nerve injury diseases.

The aim of this study was to investigate the effect of sevoflurane anesthesia on  $\beta$  deposition in the hippocampus of APP / psi double transgenic mice, Fas / fasl-caspase3 and PPAR  $\gamma$  related

pathway protein, and to observe the effect of pioglitazone and trehalose combined intervention, so as to provide theoretical basis for the effect of anesthesia on neural function and the clinical treatment of AD or other cognitive dysfunction patients.

## 1. Materials and Method

120 experimental animals and group app / PS1 double transgenic mice, 9 months old, weighing  $(23 \pm 3)$  g, were purchased from the animal research institute of the Second Affiliated Hospital of Harbin Medical University. The study has been approved by the college ethics committee. Randomly divided into 5 groups ( $n = 24$ ): control group, model group, trehalose group, pioglitazone group, trehalose pioglitazone (combined) group.

Main reagents and instruments TUNEL cell apoptosis in situ detection kit Shanghai Yisheng Biotechnology Co., Ltd., China, DAB Staining Kit (Shanghai Xinyu Biotechnology Co., Ltd., China), SP immunohistochemical staining kit (Xiamen Huijia Biotechnology Co., Ltd., China), Rabbit anti Fas monoclonal antibody (Abcam company, USA), Rabbit anti FasL monoclonal antibody (Abcam company, USA) Rabbit anti PPAR  $\gamma$  monoclonal antibody (Abcam company, USA), Rabbit anti PPAR  $\gamma$  monoclonal antibody (Abcam company, USA, cell signaling technology, USA II anti rabbit) Invitrogen company, USA II anti mouse (Invitrogen company, USA)

## 2. Intervention and Sevoflurane Anesthesia in Each Group

Intervention and administration methods sevoflurane ventilation was used in each group. Trehalose group was given  $300 \mu\text{g} \cdot \text{kg} / 1 \cdot \text{D}$  trehalose 30 minutes before anesthesia. Pioglitazone group: two hours before anesthesia,  $10 \text{ mg} / \text{kg} \cdot \text{d}$  pioglitazone was given to the stomach. In the combined group, the operation of pioglitazone group was repeated 2 hours before anesthesia and trehalose group 30 minutes before ventilation. Control group and model group: the same amount of normal saline was given to the stomach 2 hours before ventilation. Each group was intervened for 5 days.

After 5 days of intervention in each group, the control group was continuously infused with pure oxygen 2L min, and the model group, trehalose group, pioglitazone group and combination group were all infused with 2% sevoflurane.

Evaluation of cognitive impairment Morris water maze experiment was used to evaluate the degree of cognitive impairment in mice, including two parts: positioning cruise experiment and space search experiment. Morris maze experiment was carried out for 5 days, the first 4 days were cruise experiment, the fifth day was space exploration experiment.

The diameter of the positioning cruise experiment pool is 120cm. A platform is set in the fourth quadrant. The mice are put into the pool from the second quadrant with their heads facing the pool wall. The automatic camera and computer system record the time when the mice find the platform, that is, the escape latency.

In the space search experiment, the mice were put into the pool from the first, second, third and fourth quadrants respectively, and the retention time of the mice in the original platform quadrant within 120s was recorded, which was the space exploration time.

Measurement of neuron apoptosis in hippocampus 24 hours after ventilation anesthesia, the remaining 12 mice in each group was anesthetized by intraperitoneal injection of 10% chloral hydrate. The brain was cut off and the hippocampus was taken for making tissue sections. Add 50  $\mu\text{L}$  of TUNEL reaction mixture. DAB staining was 50  $\mu\text{L}$ , and the apoptotic neurons in hippocampus were observed under microscope ( $\times 400$ ).

The hippocampal area was taken from the AB deposition of hippocampal area for making tissue sections, adding 50  $\mu\text{L}$  DAB for color development, hematoxylin re staining, and sealing. One slice was selected for each mouse, and three visual fields were randomly selected under X40 objective lens. The absorbance value was analyzed by Image Pro Plus software, and the expression level of AB in hippocampus was semi quantitatively analyzed.

Western blot was used to detect the expression of Fas, FasL, caspase 3 and PPAR  $\gamma$ . Tissue protein extraction.

Slowly take out the tissue, wash it with 1 ml PBS twice or three times until the color of the culture solution is not visible. Prepare the lysate according to the ratio of Ripa to protease inhibitor 100:1, and add a proper amount of lysate to the culture bottle (generally 50  $\mu$ l lysate is needed for each bottle). Cut the tissue and vortex once every five minutes, 4 times in total. The EP tube was then placed in a 4-degree centrifuge at 13500 R / min. Take out the EP tube and carefully suck the supernatant, which is the total protein of the extracted tissue. Prepare BCA working solution according to the instructions of biyuntian kit to measure the protein concentration, and add it into 96 well plate. Add 200  $\mu$ l to each hole, and measure the concentration with enzyme standard instrument. SDS polyacrylamide gel electrophoresis (15%) was used. The electrophoresis device and gel plate were installed. The prepared electrophoresis buffer was poured in. The sample was added. The electrophoresis was run at 70V voltage. The membrane was transferred. With constant current of 300 Ma, turn 45 min. Block and label the antibody, do not add 1.5ml of the target protein FAS, FasL, caspase3pary, IDE (1:500) and GAPDH (1:1000) antibody configured with PBS, and use the image analysis software Odyssey 1.2 to analyze the optical density integral value. 1.8 Statistical method: spss19.0 statistical software was used for statistical analysis. The measurement data subject to normal distribution was expressed by  $X \pm s$ , and variance analysis was used for comparison among multiple groups. Isd4 test was used for comparison between two groups ( $P < 0.05$ ).

### 3. Results

Evaluation results of cognitive impairment Morris maze experiment results show that: ① comparison between escape latency groups (Table 1): the exploration time of the combined group is significantly longer than that of trehalose group and pioglitazone group, with a statistically significant difference ( $P < 0.05$ ); the difference between pioglitazone group and trehalose group is not statistically significant ( $P > 0.05$ ).

Table 1 Comparison of escape latency and exploration time of mice in each group (n = 24)

project	control group	model	Trehalose group	Pioglitazone group	Combined group	Fvalue	P value
incubation period							
1d	50.97 $\pm$ 6.38 <sup>1, 3, 4, 5</sup>	74.65 $\pm$ 6.13 <sup>1, 3, 4, 5</sup>	67.49 $\pm$ 5.13 <sup>1, 2, 5</sup>	62.45 $\pm$ 4.35 <sup>1, 2, 5</sup>	55.62 $\pm$ 4.95 <sup>1, 2, 3, 4</sup>	34.522	0.000
2d	42.35 $\pm$ 5.23	72.31 $\pm$ 6.25 <sup>1, 3, 4, 5</sup>	68.23 $\pm$ 5.64 <sup>1, 2, 5</sup>	59.62 $\pm$ 4.78 <sup>1, 2, 5</sup>	69.18 $\pm$ 4.98 <sup>1, 2, 3, 4</sup>	36.421	0.000
3d	41.26 $\pm$ 5.13 <sup>2, 3, 4, 5</sup>	62.59 $\pm$ 5.63 <sup>1, 3, 4, 5</sup>	59.46 $\pm$ 4.65 <sup>1, 2, 5</sup>	59.88 $\pm$ 4.63 <sup>1, 2, 5</sup>	44.12 $\pm$ 3.65 <sup>1, 2, 3, 4</sup>	42.366	0.000
4d	38.56 $\pm$ 5.36 <sup>2, 3, 4, 5</sup>	57.88 $\pm$ 6.13 <sup>1, 3, 4, 5</sup>	51.23 $\pm$ 4.99 <sup>1, 2, 5</sup>	48.55 $\pm$ 4.66 <sup>1, 2, 5</sup>	45.36 $\pm$ 4.25 <sup>1, 2, 3, 4</sup>	25.466	0.000
Exploration time	42.56 $\pm$ 5.89 <sup>2, 3, 4, 5</sup>	29.56 $\pm$ 4.33 <sup>2, 3, 4, 5</sup>	33.56 $\pm$ 4.23 <sup>1, 2, 5</sup>	36.21 $\pm$ 4.25 <sup>1, 2, 5</sup>	36.48 $\pm$ 4.26 <sup>1, 2, 5</sup>	17.255	0.000

Note: compared with the control group, 1  $p < 0.05$ ; compared with the model group, 2  $P < 0.05$ ; compared with the trehalose group, 3  $P < 0.05$ ; compared with the pioglitazone group, 4  $P < 0.05$ ; compared with the combined group, 5  $p < 0.05$ .

Neuron apoptosis in hippocampus is shown in Figure 1. The apoptotic rate of neurons in the control group, model group, trehalose group, pioglitazone group and combination group were (5.44  $\pm$  0.67)%, (37.29  $\pm$  4.64)%, (27.84  $\pm$  3.15)%, (25.53  $\pm$  4.06)% and (18.49  $\pm$  2.27)%, respectively; the apoptotic rate of the combination group was significantly lower than that of other groups ( $P < 0.05$ ), and the apoptotic rate of the combination group was significantly higher than that of the control group ( $P < 0.05$ );

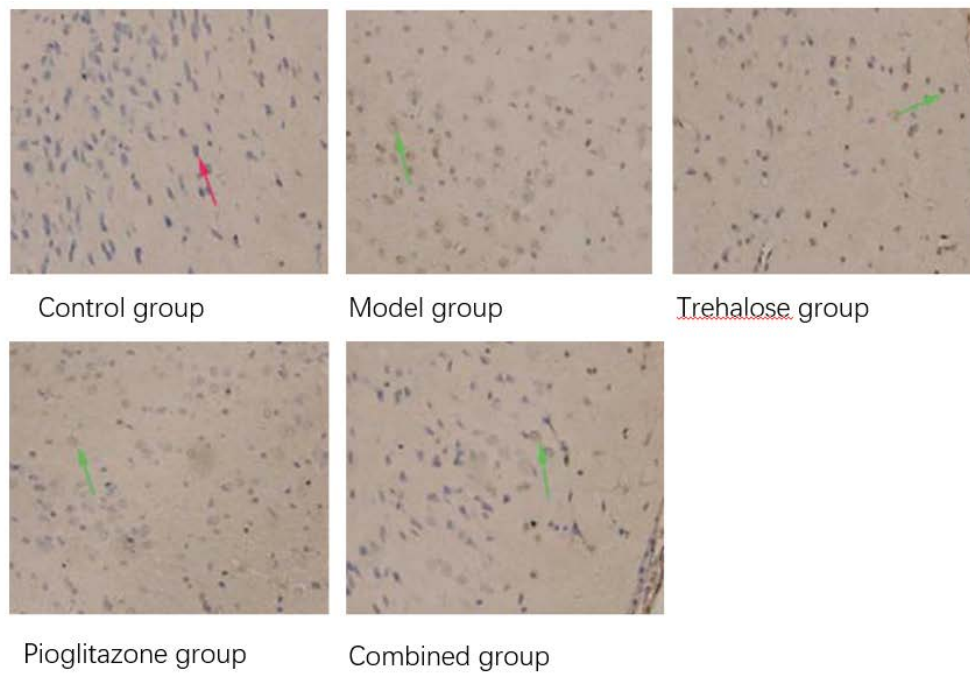


Fig. 1 Neuron apoptosis in hippocampus detected by TUNEL method ( $\times 400$ )

Immunohistochemical staining of a  $\beta$  in hippocampus showed that a  $\beta$  was yellow brown granules. The expression in each group was  $(0.086 \pm 0.012)$ ,  $(0.258 \pm 0.043)$ ,  $(0.217 \pm 0.062)$ ,  $(0.198 \pm 0.070)$  and  $(0.135 \pm 0.051)$  respectively. There was significant difference between the groups ( $F = 20.9523$ ,  $P = 0.000$ );

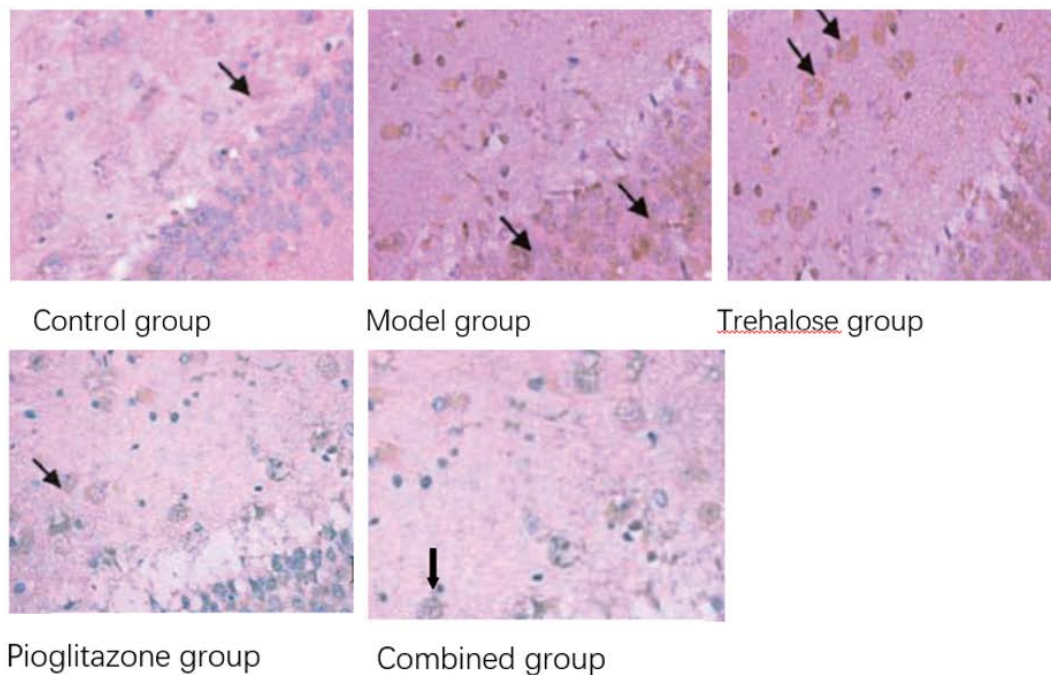


Fig. 2 Immunohistochemical staining of a  $\beta$  deposition in hippocampus of mice in each group ( $\times 400$ )

The expression level of Fas, FasL and caspase-3 protein in trehalose group was lower than that in model group ( $P < 0.05$ ), but higher than that in control group ( $P < 0.05$ ). The expression level of Fas, FasL and caspase-3 protein in trehalose group was lower than that in pioglitazone group ( $P < 0.05$ )

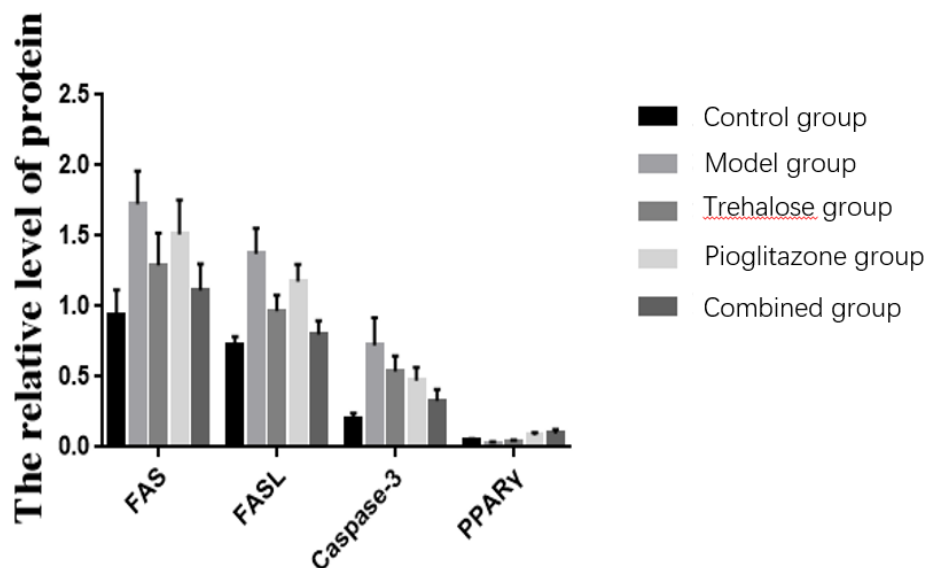


Fig. 3 statistical map of Fas, FasL, Caspase-3, PPAR  $\gamma$  and protein in hippocampus of mice in each group

#### 4. Discussion

Neurodegenerative diseases are defined as hereditary, fluctuating and age-related diseases, which are characterized by cognitive decline, especially in learning and memory. These diseases are often associated with motor or mental function (dementia). Alzheimer's disease (AD) and other dementia, brain cancer encephalitis, epilepsy, Parkinson's disease (PD), multiple sclerosis (MS) and primary diseases are the most common and neurodegenerative diseases in the elderly [7].

It accounts for 50-75% of Alzheimer's cases over 60 years old. The number of people affected by ad in the world is more than 35 million, and the number of people affected by ad in China is more than 70 million [8]. Ad is multifactorial, characterized by early neurological loss. In AD brain, two pathological features were observed: extracellular insoluble senile plaques formed by amyloid B (AB) peptide and neurofibrillar bundles (NFT) formed by tau protein 2. Other central nervous system (CNS) diseases, including chronic infections, develop with the emergence of these specific histopathological markers. In addition, recent studies have shown that AB has antibacterial effect, which suggests that infection can induce the production and deposition of Theanine in the brain. These studies show that AB has antiviral properties and regulates virus binding to phagocytes [9]. Current data show that cerebrovascular factors, oxidative stress and neuroinflammatory processes are the main factors of pathogenesis. Among many key components of ADA, the most important are immune response and inflammation [10]. Pioglitazone is a kind of thiazolidinedione antidiabetic drug. It can control blood glucose by improving the insulin sensitivity of peripheral and liver. At present, some studies have shown that pioglitazone can improve Alzheimer's disease by regulating the activity of neuron cells [12]. Trehalose is a kind of stable non reducing disaccharide obtained from corn starch by biological fermentation technology, which is very soluble in water. Trehalose can help organism to resist various harsh environments to maintain the biological activity of organism, such as dry, frozen, high temperature, high osmotic state, etc. [13]; in this study, mice were divided into different groups after anesthesia with sevoflurane. Through the design of experiments, we evaluated the intervention effect and internal mechanism of pioglitazone, trehalose, and the combination of the two on cognitive dysfunction in mice. Through consulting the literature, we found that the existing research results focused on Fas, FasL, caspase-3 proteins, which are related to cognitive function. It was found that pioglitazone and trehalose could alleviate the cognitive dysfunction of AD mice after sevoflurane anesthesia through the above-mentioned proteins, and the effect of the combination of the two was significantly better than that of the single use, but there are still some shortcomings in this study, and the mechanism discussion is not detailed enough, which will be further improved in the future research.

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