PROTECTIVE EFFECT AND MECHANISM OF PROPOFOL ON CONDENSED AB25-35-INDUCED INJURY OF RAT HIPPOCAMPAL NEURONS

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ABSTRACT

Objective: *To investigate the protective effect and mechanism of propofol on on rat hippocampal neurons that have endured condensed Aβ25-35-induced injuries.*

Methods: *Twelve newborn rats were collected, and 12 rat hippocampal neuronal cells were taken as a control group without any treatment; the remaining rat hippocampal neuronal cells were randomly divided into an $A\beta_{25\text{-}35}$ group and a propofol group, 12 each. Both groups were induced by 10μmol/L $A\beta_{25\text{-}35}$ to establish a cell injury model. The $A\beta_{25\text{-}35}$ group was not treated and the propofol group was treated with 20μmol/L propofol After the effect was over, experiments were carried out comparing the survival rate of rat hippocampal neurons, apoptosis-related proteins (Bcl-2, Bax), Tau protein phosphorylated proteins (p-Tau Ser404, p-Tau Ser396, p-Tau Thr231) and glycogen synthase kinase 3β (glycogen synthetase kinase 3 β, GSK3β) protein expression level.*

Results: *The survival rate and apoptosis rate of hippocampal neurons in the $A\beta_{25\text{-}35}$ group were significantly lower than those in the control group ($p<0.05$). The survival rate and apoptosis rate of hippocampal neurons in the propofol group were significantly higher than those in the control group ($p<0.05$). The expression level of Bax in hippocampal neurons of the $A\beta_{25\text{-}35}$ group was significantly higher than those of the control group, and the expression of Bcl-2 and apoptosis rate were significantly lower than those of the control group ($p<0.05$). The expression level of Bax in hippocampal neuron cells of the propofol group was significantly lower than that of control group, and the expression of Bcl-2 and apoptosis rate were significantly higher than that of control group ($p<0.05$). The expression levels of p-Tau Ser404, p-Tau Ser396, and p-Tau Thr231 in hippocampal neurons of the $A\beta_{25\text{-}35}$ group were significantly higher than those in the control group ($p<0.05$). The expression levels of p-Tau Ser404, p-Tau Ser396, and p-Tau Thr231 in hippocampal neurons of the propofol group were significantly lower than those in the $A\beta_{25\text{-}35}$ group ($p<0.05$). There was no statistically significant difference in the total Tau protein expression level among the groups ($P>0.05$). The expression level of GSK-3β protein in hippocampal neurons of the $A\beta_{25\text{-}35}$ group was significantly higher than that of control group ($p<0.05$). The expression level of GSK-3β protein in the propofol group was significantly lower than that in the model group ($P<0.05$).*

Conclusion: *Propofol can protect rat hippocampal neuronal cells from damage induced by condensed $A\beta_{25\text{-}35}$, and propofol's mechanism may be achieved by blocking apoptosis and inhibiting GSK-3β kinase expression and Tau phosphorylation.*

Keywords: *Propofol, Aβ25-35, hippocampal neuronal cell damage, bax.*

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Introduction

Alzheimer's disease is one of the types of Alzheimer's disease, which accounts for about 70% of the total number of cases of dementia. Related investigations show that Alzheimer's disease is currently one of the fourth leading causes of human death[1]. At present, the degree of aging in China is accelerating and Alzheimer's disease is increasing; and its main pathological feature is neurodegenerative diseases[2]. In clinical practice, the number of patients who receive general anesthesia is gradually increasing, and the relationship between anesthesia and Alzheimer's disease has attracted the attention of clinical scholars. Amyloid beta (Aβ) is a small peptide whose amyloid precursor is also present in human brain tissue, but under normal circumstances it is not deposited in the brain[3]. The role of amyloid

precursors under normal conditions has not yet been elucidated, but under pathological conditions, they can lead to neurotoxic release and Aβ deposition, which eventually lead to senile plaques and amyloid deposition and can also cause hippocampal neuronal cell damage[4].

Many studies have confirmed that Aβ occupies a key position in the progression of Alzheimer's disease[5]. Therefore, the prevention and treatment of Alzheimer's disease and the reduction of hippocampal neuronal cell damage in Alzheimer's patients are great challenges facing the world. It has been reported that isoflurane has the effect of inducing apoptosis, can further activate β-secretase and γ-secretase, and promote the degradation of amyloid precursors and the release of Aβ, and the released Aβ can also increase apoptosis[6].

Isoflurane can be used as the initiating factor of this vicious circle and will further promote cell death. The report first confirmed that the short-term application of inhaled anesthetic drugs can play a role in the pathological process of Alzheimer's disease. Propofol is one of the common intravenous anesthesia drugs in clinical practice that can play a role in brain protection and it has numerous mechanisms of action[7]. Studies have shown that propofol can significantly reduce brain damage caused by excitatory amino acid transmission[8]. At present, there are few studies on propofol and Alzheimer's disease, so this study aims to explore the protective effect and mechanism of propofol on condensed Aβ25-35-induced injury of rat hippocampal neurons.

Materials and methods

Experimental materials

12 newborn rats, body weight (14±2) g, purchased from Shanghai Sixin Biotechnology Co., Ltd.

Main reagent

Fetal bovine serum was purchased from Jinpin Chemical Technology Co., Ltd.; rabbit anti-Bcl-2 and Bax antibodies were purchased from Hefei Kangyuan Biotechnology Research Institute; GSK-3β and β-actin antibodies were purchased from Beijing Bio-Lob Technology Co., Ltd.

Methods

• $A\beta_{25-35}$ was put into sterile physiological saline for dissolution treatment, and the concentration was set to 1mg/mL. The solution was stored in a low-temperature refrigerator to wait for testing. Before the experiment, it was taken out and placed in a 37°C water bath for incubation for 4 days. It was observed that it had coagulated and aged, and at the same time, it was diluted with sterile medium until it reached the working concentration.

• Twelve rat hippocampal neuron cells were taken as a control group without any treatment; the remaining rat hippocampal neuron cells were randomly divided into an $A\beta_{25-35}$ group and a propofol group, each with 12 rats. Both groups were induced by 10μmol/L $A\beta_{25-35}$ to establish a cell injury model, in which the $A\beta_{25-35}$ group was not treated with propofol, and the propofol group was treated with 20μmol/L propofol. Experiments were carried out after the effect was over.

• After 3 days, the newborn rats were sacrificed, and their primary hippocampal neurons were isolated and cultured.

• The survival rate of hippocampal neurons in the control group, the $A\beta_{25-35}$ group, and the propofol group was detected by CCK-8 method.

• The Western blot was used to detect apoptosis-related proteins (Bcl-2, Bax) and Tau protein phosphorylated proteins (p-Tau Ser404, p in the control group, the $A\beta_{25-35}$ group, and the propofol group)-Tau Ser396, p-Tau Thr231) and glycogen synthetase kinase 3β (Glycogen synthetase kinase 3β, GSK3β) protein expression levels.

Statistical analyses

The measurement data of rat hippocampal neuron cell survival rate and apoptosis-related protein expression levels in each group was expressed by ($\bar{x}\pm s$).

The comparison between the two groups was by t test, and the comparison between multiple groups was by one-way ANOVA. All data analysis in this study used SPSS 23.0 analysis, and $p<0.05$ was considered statistically significant.

Results

Effect of propofol on rat hippocampal neurons with damage induced by condensed $A\beta_{25-35}$

The survival rate and apoptosis rate of hippocampal neurons in the $A\beta_{25-35}$ group were significantly lower than those in the control group ($p<0.05$). The survival rate and apoptosis rate of hippocampal neurons in the propofol group were significantly higher than those in the control group ($p<0.05$). These results are shown in Table 1.

Group	Cell survival rate (%)
Control	75.59±2.88
$A\beta_{25-35}$	51.96±0.09*
Propofol	65.43±0.11#

Table 1: Effect of propofol on rat hippocampal neurons damaged by condensed $A\beta_{25-35}$ ($\bar{x}\pm s$).
**Compared with the control group. #Compared with the $A\beta_{25-35}$ group.*

Effects of propofol on rat hippocampal neuronal apoptosis resulting from condensed Aβ25-35-related proteins

The expression level of Bax in hippocampal neurons of the $A\beta_{25-35}$ group was significantly higher than that of the control group, and the expression of Bcl-2 and apoptosis rate were significantly lower than those of the control group ($p<0.05$).

The expression level of Bax in hippocampal neuron cells of the propofol group was significantly lower than that of the control group, and the expression of Bcl-2 and apoptosis rate were significantly higher than that of the control group ($p<0.05$). These results are shown in Table 2 and Figure 1.

Group	Cases	Bax	Bcl-2	Apoptosis rate (%)
Control	12	0.45±0.02	1.80±0.21	95.13±5.33
$A\beta_{25-35}$	12	1.68±0.11*	0.43±0.05*	61.00±0.08*
Propofol	12	1.01±0.04#	0.98±0.11#	85.14±4.21#

Table 2: Effect of propofol on apoptosis-related proteins induced by condensed $A\beta_{25-35}$ in rat hippocampal neurons ($\bar{x}\pm s$).
**Compared with the control group. #Compared with the $A\beta_{25-35}$ group.*

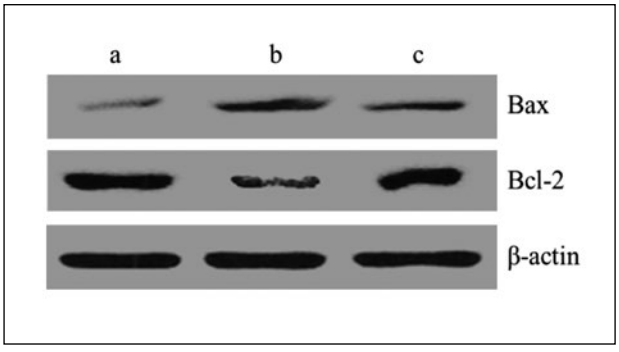

Figure 1: Effect of propofol on apoptosis-related proteins in rat hippocampal neurons induced by condensed $A\beta_{25-35}$.
Note: In the figure, a means control group, b means $A\beta_{25-35}$, and c means propofol group.

Effect of propofol on Tau protein hyperphosphorylation induced by condensed Aβ25-35 in rat hippocampal neurons

The expression levels of p-Tau Ser404, p-Tau Ser396, and p-Tau Thr231 in hippocampal neurons of the $A\beta_{25-35}$ group were significantly higher than those in the control group ($p<0.05$).

The expression levels of p-Tau Ser404, p-Tau Ser396, and p-Tau Thr231 in hippocampal neurons of the propofol group were significantly lower than those in the $A\beta_{25-35}$ group ($p<0.05$). There was no statistically significant difference in the total Tau protein expression level among the groups ($p>0.05$). These results are shown in Table 3 and Figure 2.

Group	p-Tau Ser404	p-Tau Ser396	p-Tau Thr231	Total Tau
Control	0.88±0.21	0.71±0.09	0.49±0.05	1.00±1.18
$A\beta_{25-35}$	1.34±0.45	1.36±0.22*	1.79±0.26*	1.23±0.11*
Propofol	1.11±0.19	1.18±0.12#	1.13±0.18#	0.99±0.12#

Table 3: Effect of propofol on Tau protein hyperphosphorylation induced by condensed $A\beta_{25-35}$ in rat hippocampal neurons ($\bar{x}\pm s$).
**Compared with the control group. #Compared with the $A\beta_{25-35}$ group.*

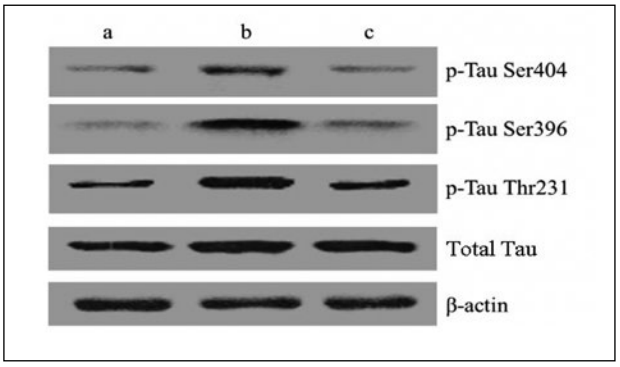

Figure 2: The effect of propofol on Tau protein hyperphosphorylation in rat hippocampal neurons induced by condensed $A\beta_{25-35}$.
Note: In the figure, a means control group, b means $A\beta_{25-35}$, and c means propofol group.

Effect of propofol on Tau protein hyperphosphorylation induced by condensed Aβ25-35 in rat hippocampal neurons

The expression level of GSK-3β protein in hippocampal neurons of the $A\beta_{25-35}$ group was significantly higher than that of the control group ($p<0.05$). The expression level of GSK-3β protein in the propofol group was significantly lower than that in the model group ($P<0.05$). These results are shown in Table 4 and Figure 3.

Group	GSK-3β
Control	1.03±0.07
$A\beta_{25-35}$	1.78±0.10*
Propofol	1.25±0.09#

Table 4: Effect of propofol on Tau protein hyperphosphorylation induced by condensed Aβ25-35 in rat hippocampal neurons ($\bar{x}\pm s$).
**Compared with the control group. #Compared with the $A\beta_{25-35}$ group.*

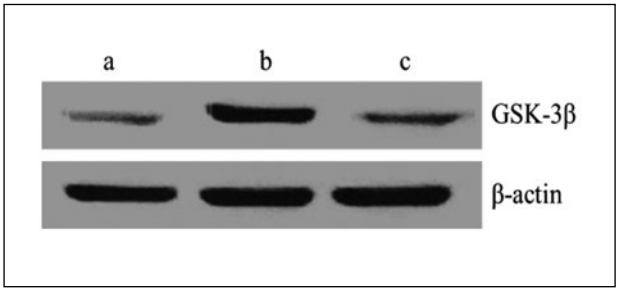

Figure 3: The effect of propofol on Tau protein hyperphosphorylation in rat hippocampal neurons induced by condensed $A\beta25_{25-35}$.
Note: In the figure, a means control group, b means $A\beta_{25-35}$, and c means propofol group.

Discussion

Most patients with Alzheimer's disease have progressive cognitive impairment: memory function disappears and daily living ability gradually declines. At the same time, it is accompanied by many neuropsychiatric and other obstacles. Relevant surveys and studies have shown that over 35 million cases of the disease occur worldwide each year, with an average of 1 person in 7 seconds. The average survival time for patients with Alzheimer's disease is no more than 6 years. It is currently known that age, genetic factors, environment, and other factors can induce Alzheimer's disease, but the key mechanism leading to the disease is not yet clear. It has been reported that general anesthesia drugs such as propofol have made remarkable achievements in improving patient health and that the social benefits outweigh the possible side effects of anesthetic drugs. These two advantages exceed the possible side effects of anesthetic drugs[9]. This study explored on the basis of determining the anesthetic effects and advantages, reducing the occurrence of neurodegenerative diseases such as Alzheimer's disease, reducing the degree of hippocampal neuronal cell damage, and understanding its mechanism of action.

Bcl-2 and Bax play an important role in controlling the process of apoptosis. Bax is a molecule that can promote cell apoptosis and can initiate the apoptosis process related to caspase, thereby triggering mitochondrial-dependent apoptosis occurrence. Bcl-2 can also regulate apoptosis through mitochondria and inhibit the production of cytochrome C, and ultimately Bcl-2 achieves the purpose of inhibiting apoptosis[10-11]. The results of this study showed that the survival rate and apoptosis rate of the $A\beta_{25-35}$ group were significantly lower than those of the control group ($p<0.05$). The survival rate and apoptosis rate of hippocampal neurons in the propofol group were significantly higher than those in the control group ($p<0.05$). It shows that $A\beta_{25-35}$ can induce damage in rat hippocampal neuron cells, while propofol can effectively protect the rat hippocampal neuron cells and promote their recovery.

The expression level of Bax in hippocampal neurons of the $A\beta_{25-35}$ group was significantly higher than that of control group, and the expression of Bcl-2 and the apoptosis rate were significantly lower than that of control group ($p<0.05$). The expression level of Bax in hippocampal neuron cells of the propofol group was significantly lower than that of control group, and the expression of Bcl-2 and the apoptosis rate were significantly higher than that of control group ($p<0.05$). The study indicates that propofol can down-regulate the expression of Bax and up-regulate the expression of Bcl-2, thereby inhibiting the apoptosis of rat hippocampal neurons induced by $A\beta_{25-35}$. Tau, as a microtubule-associated protein, can maintain the stability of microstructures and increase tube protein aggregation[12]. Many reports have confirmed that Tau hyperphosphorylation plays an important role in the progression of Alzheimer's disease. Aβ can cause Tau hyperphosphorylation and neurofibrillary tangles in neurons[13]. This is consistent with the results of this study. The expression levels of p-Tau Ser404, p-Tau Ser396, and p-Tau Thr231 in hippocampal neurons of the $A\beta_{25-35}$ group were significantly higher than those in the control group ($p<0.05$). The expression levels of p-Tau Ser404, p-Tau Ser396, and p-Tau Thr231 in hippocampal neurons of the propofol group were significantly lower than those in the $A\beta_{25-35}$ group ($p<0.05$). There was no statistically significant difference in the total Tau protein expression level among the groups ($p>0.05$). This indicates that propofol can significantly improve the hyperphosphorylation of Tau protein in rat hippocampal neurons induced

by $A\beta_{25-35}$. GSK-3β was first discovered in rabbit skeletal muscle, and studies have confirmed that it can participate in hepatic glucose metabolism and play a key role. In addition, GSK-3β also regulates cell differentiation, proliferation, and apoptosis, and it can be used as a potential target for the treatment of tumors and neurodegenerative diseases. The expression level of GSK-3β protein in the propofol group was significantly lower than that in the model group ($p<0.05$). It shows that propofol can improve the abnormal expression level of GSK-3β protein in rat hippocampal neurons caused by $A\beta_{25-35}$, and relieve Tau phosphorylation.

In conclusion, propofol can protect hippocampal neurons from damage induced by condensed $A\beta_{25-35}$, and propofol's mechanism may work by inhibiting apoptosis, GSK-3β kinase expression, and Tau phosphorylation.

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