

Original Article

Analysis of the mechanism by which ketamine affects astrocytes in Parkinsonian rats through the PI3K/AKT axis

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Article Info

Abstract



Article history:

Received: January 16, 2024

Accepted: April 06, 2024

Published: May 31, 2024

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Parkinson's disease (PD) remains the most common neurodegenerative disease worldwide, seriously affecting the normal life of patients. Currently, there is no effective clinical cure for PD. In this study, the research team explored the effect of ketamine (KET) on PD, which can lay a reliable foundation for future KET treatment of PD. First, the research team established a PD rat model with 6-hydroxydopamine (6-OHDA). The detection showed that the maximum angle of the inclined plate stay, the number of times of grid crossings and standing, and the ATPase activity in brain tissue were significantly lower in PD rats than in control rats, while the positive rate of α -synuclein in brain tissue was increased, showing typical pathological manifestations of PD. After using KET to intervene in PD rats, the behavioral and brain pathological changes were significantly alleviated, and the inflammation and oxidative stress damage of brain tissue were effectively reduced, suggesting the potential therapeutic effects of KET on PD. Furthermore, the use of KET inhibited the PI3K/AKT axis in the brain tissue of PD rats and promoted autophagy. Moreover, the significant suppression of the PI3K/AKT axis by KET was also demonstrated in the PD cell model established through lipopolysaccharide (LPS) inducement of astrocyte cell line HA1800. It is suggested that the mechanism of KET on PD is related to the inhibition of the PI3K/AKT axis.

Keywords: Parkinson's disease, Ketamine, PI3K/AKT, Astrocyte, Autophagy

1. Introduction

Parkinson's disease (PD) is an extremely common neurodegenerative disease in the elderly worldwide, with characteristic motor (e.g., tremor, bradykinesia, and myotonia) and non-motor symptoms (sleep disorders, neurological dysfunction, etc.) [1]. The incidence of PD in people over 65 years old is approximately 1700 per 100,000, showing an increasing trend with age [2]. At present, PD has been clinically considered as a major disease affecting the normal life of the elderly [3], which must be taken seriously by patients and clinical professionals. However, there is currently no clinical cure for PD, and patients need lifelong conservative treatment to alleviate disease progression once they develop the disease [4]. Therefore, the search for the diagnosis and treatment of PD has always been a hotspot and Gordian knot in clinical research.

In fact, neurodegenerative diseases such as PD and Alzheimer's disease are associated with dysfunction of N-methyl-D-aspartate (NMDA) receptors, with overactivated NMDA receptors inducing excitotoxicity, leading to the damage and loss of related neurons [5]. In rodent studies, NMDA receptor inhibitors have been shown to

reduce the degree of brain injury during craniocerebral injury and ischemic brain injury [6]. Therefore, the use of NMDA inhibitors could theoretically alleviate and ameliorate the progression of neurodegenerative changes. Ketamine (KET), as a non-competitive NMDA receptor inhibitor, can inhibit the release of lipopolysaccharide (LPS)-induced inflammatory mediators such as interleukins at a subanesthetic dose of 7 mg/kg [7]. It has also been shown that intraperitoneally administered subanesthetic doses (10-30mg/kg) of KET can produce a rapid, long-lasting, and dose-dependent antidepressant effect in mice [8]. However, the effects of KET on PD are still rarely reported, and its mechanism of action remains to be defined.

By analyzing the influence of KET on PD, this study confirms the future clinical therapeutic significance of KET in PD and provides a more reliable guarantee for the health of PD patients.

2. Materials and methods

2.1. Animal data

We purchased 30 SPF SD rats 6 weeks old and weighing (200±25) g from Jiangxi Biomerit Biotechnology

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Co., Ltd. (SYXK (Jiangxi) 2023-0001), and raised them with food and water available *ad libitum* at (23±2)°C room temperature and (60±5)% relative humidity.

2.2. Grouping and modeling

The 30 rats were randomized into control, model, and intervention groups. Among them, the control group was not treated, and the other two groups were injected with 6-hydroxydopamine (6-OHDA) into the left striatum at two points to prepare a PD model [9]. Twenty-one days later, apomorphine (APO) was dissolved in 0.9% normal saline for intraperitoneal injection into the rats (0.5 mg/kg). The animals were then placed in a plexiglass cylinder (diameter: 25 cm, height: 30 cm) with clean hardwood underneath. When the rats exhibited head-to-tail rotation behavior with the opposite front or rear limb of the damaged side as the fulcrum, the number of rotations within 30 min was recorded. The modeling was considered successful if the rotation conditions were met and the rotation speed was greater than 7 rpm/min [10]. Subsequently, rats in the intervention group received intraperitoneal injection of 20 mg/kg KET, while those in the model and control groups were injected with the same amount of normal saline, all for 7 days.

2.3. Behavioral testing

Behavioral tests were performed 2 h after the last injection. Inclined-plane test: A flat wooden board was prepared with a rubber pad (about 0.5cm thick) placed on it. Rats were placed in the center of the rubber pad in turn, ensuring that they did not scratch or adhere to the edges of the wooden board, and the inclination angle of the wooden board was then slowly raised to 30°. The inclination angle was gradually increased and was further raised if the rat could stay on the board for more than 5s, until the stay on board was less than 5s, and the inclination angle at this moment was recorded. Open field-test: A special open-field box was used for the test. The bottom area of the box was five rows of squares horizontally and vertically, and the size of each square was 25cm×25cm. The rats were placed in the central grid at the bottom of the box, and the number of times of grid crossing (valid if the rat had more than three claws stepping into the grid) and standing (valid if the forelimbs were more than 1cm off the ground) within 3 min were recorded.

2.4. Pathological testing

After the completion of behavioral testing, all rats were sacrificed with their necks severed under anesthesia. The brain was dissected and the brain tissue was separated into two parts. One aliquot was polished to a homogenized state using a homogenizer and tested for ATPase activity by colorimetry, with the kit purchased from Shanghai Guyan Industrial Co., Ltd. In addition, enzyme-linked immunosorbent assays were carried out to measure tumor necrosis factor- α (TNF- α), interleukin-1 β /6 (IL-1 β /6), superoxide dismutase (SOD), and malondialdehyde(MDA), using kits all ordered from Zhejiang Yuxiang Biotechnology Co., Ltd. The other aliquot was subjected to isolation of the substantia nigra, some of which were cut off and ground into powder for cryopreservation after liquid nitrogen treatment, and some were fixed in 4% paraformaldehyde. After fixation for 24 hours, paraffin sections were prepared by conventional dehydration and embedding, followed by

deparaffinization to water and tissue antigen retrieval by heat treatment with citric acid antigen retrieval solution. Next, H₂O₂ was dripped into the sample for incubation (5 min) at room temperature, goat serum blocking solution was used for blocking (10 min), and α -synuclein primary antibody (1:200) was added dropwise for overnight cultivation (4°C). Then, a biotin-labeled goat anti-rabbit secondary antibody was added for incubation (15 min) at an ambient temperature, and hematoxylin was used for restaining after color development with DAB solution. The positive rate of α -synuclein was observed under a microscope after alcohol gradient dehydration and mounting.

2.5. Western Blot

After being isolated from the substantia nigra of the rat brain, the total protein was tested by BCA for its concentration. It was transferred to a polyvinylidene fluoride (PVDF) membrane following sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), blocked with skimmed milk powder (5%), and immersed in PI3K, p-PI3K, AKT, p-AKT, Beclin1, LC3-II, and GAPDH antibodies (1:1, 000) overnight (4°C). The corresponding secondary antibody (1:2, 000) was added for room temperature incubation for 1h, after which development with ECL luminescent solution was carried out. Image-Pro Plus software analyzed the gray values of the bands.

2.6. PD cell grouping and modeling

Astrocytes HA1800 (Shanghai Guan&Dao Biological Engineering Co., Ltd.) were routinely cultured using Dulbecco's Modified Eagle's medium (DMEM). Referring to the research of Booth HDE et al. [11], HA1800 cells were treated with 2.0 μ g/mL lipopolysaccharide (LPS) for 12 h to establish a PD cell model. Subsequently, the successfully modeled HA1800 cells were randomized into two groups: one group (intervention group) was cultured in KET (50 μ mol/mL)-containing medium, and the other (model group) was incubated in a normal medium. Both groups of cells were collected 48 h later for follow-up detection.

2.7. Cell activity assay

Cells in each group were inoculated into a 96-well plate (3×10³ cells/well), and CCK-8 solution was added at 10 μ L/well at 24, 48, 72, and 96 h of culture, respectively. Two hours later, the optical density (OD) at 450nm wavelength was read by a microplate reader, and the cell growth curve was plotted. In addition, the cells were trypsinized and rinsed with precooled PBS, followed by supernatant removal. The apoptosis rate was detected by flow cytometry as per the apoptosis assay kit instructions.

2.8. Cell protein detection

PI3K/AKT pathway expression and autophagy-associated protein levels in cells were measured by the above method after cell lysis with a radioimmunoprecipitation assay buffer.

2.9. Statistical methods

All tests in this study were run in triplicate, and the results were presented in the form of (\bar{x} ±s). Multi-group comparisons employed the repeated measures ANOVA and LSD intra-group test, and inter-group comparisons used the independent sample t-test. Statistical significance

was indicated by $P < 0.05$.

3. Results

3.1. Comparison of behavioral test results

Compared with the control group, the maximum angle of the inclined plate stay and the times of grid crossing and standing in the model group were significantly reduced ($P < 0.05$). However, the maximum angle inclined plate stay and the times of grid crossing and standing were lower in the model group and higher in the control group than in the intervention group ($P < 0.05$) (Fig. 1).

3.2. Comparison of pathological test results

Subsequently, the model group was found to have lower ATPase activity in the brain tissue and a higher positive rate of α -synuclein than the control group ($P < 0.05$). In contrast, the ATPase activity of brain tissue was lower in the model group and higher in the control group than in the intervention group; while the positive rate of α -synuclein in the intervention group was lower compared with the model group and higher versus the control group ($P < 0.05$) (Fig. 2).

3.3. Comparison between inflammatory reaction and oxidative stress

The detection results of inflammatory factors showed that the concentrations of TNF- α , IL-1 β , and IL-6 in the brain tissue of the intervention group were (117.31 ± 11.70) ng/L, (10.36 ± 3.09) ng/L, and (13.50 ± 2.74) ng/L, respectively, which were reduced compared with the model group and elevated compared to the control group ($P < 0.05$). In terms of oxidative stress, the model group showed lower SOD and higher MDA than the control group ($P < 0.05$);

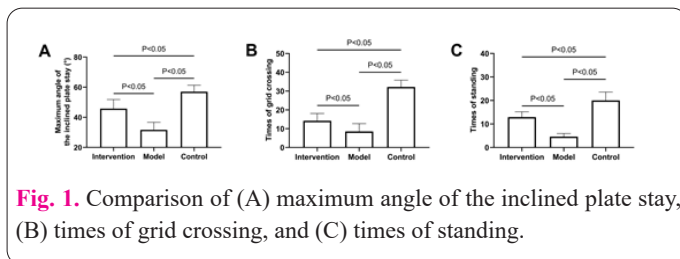


Fig. 1. Comparison of (A) maximum angle of the inclined plate stay, (B) times of grid crossing, and (C) times of standing.

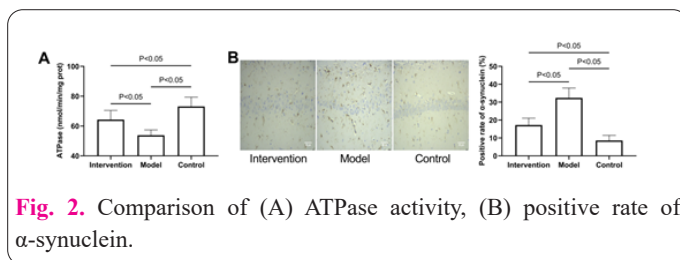


Fig. 2. Comparison of (A) ATPase activity, (B) positive rate of α -synuclein.

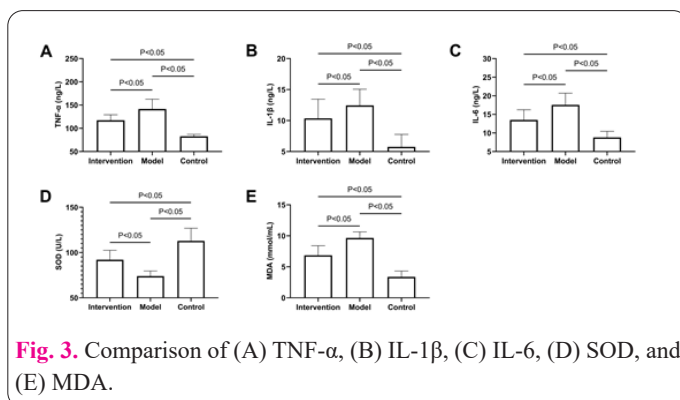


Fig. 3. Comparison of (A) TNF- α , (B) IL-1 β , (C) IL-6, (D) SOD, and (E) MDA.

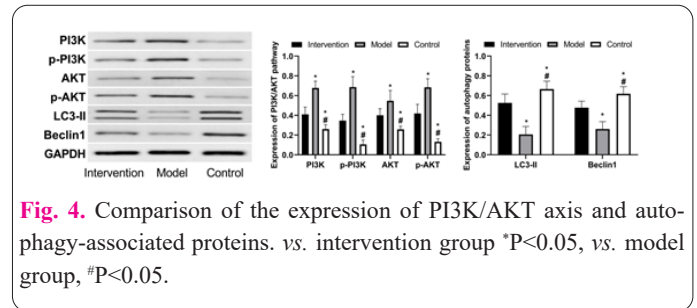


Fig. 4. Comparison of the expression of PI3K/AKT axis and autophagy-associated proteins. vs. intervention group * $P < 0.05$, vs. model group, # $P < 0.05$.

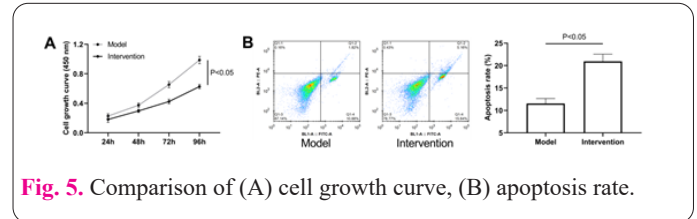


Fig. 5. Comparison of (A) cell growth curve, (B) apoptosis rate.

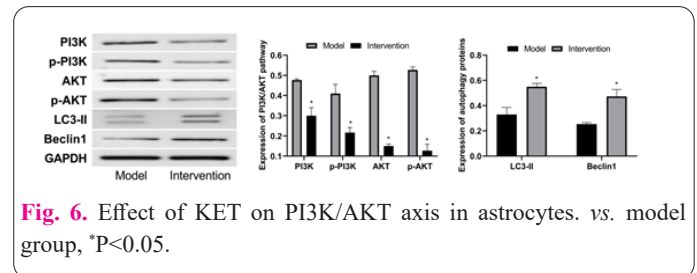


Fig. 6. Effect of KET on PI3K/AKT axis in astrocytes. vs. model group, * $P < 0.05$.

compared with the model group, SOD was elevated and MDA was reduced in the intervention group ($P < 0.05$) (Fig. 3).

3.4. Comparison of the expression of PI3K/AKT axis and autophagy-associated proteins

According to the detection results, the model group had higher levels of PI3K, AKT protein, p-PI3K, and p-AKT protein in the brain tissue than the control group, with p-PI3K/PI3K and p-AKT/AKT being the highest among the three groups ($P < 0.05$); while the intervention group showed lower p-PI3K/PI3K and p-AKT/AKT than the model group ($P < 0.05$). In addition, LC3-II and Beclin1 protein levels were lower in the model group and higher in the control group than in the intervention group ($P < 0.05$) (Fig. 4).

3.5. Effect of KET on astrocyte activity

In the cell experiment, we can see that the cell growth ability was obviously lower in the intervention group than in the model group ($P < 0.05$), and the apoptosis rate was higher ($P < 0.05$) (Fig. 5).

3.6. Effect of KET on PI3K/AKT axis in astrocytes

The detection of PI3K/AKT axis expression in cells showed that the intervention group had a significant decrease in p-PI3K/PI3K and p-AKT/AKT compared to the model group ($P < 0.05$), as well as an increase in LC3-II and Beclin1 protein expression ($P < 0.05$) (Fig. 6).

4. Discussion

This study found that the use of KET can reverse the pathological progression of PD in rats, and its mechanism may be related to the inhibition of the PI3K/AKT axis, which lays a reliable foundation for the future treatment of PD with KET.

First, we used 6-OHDA, a very classical way of mode-

ling PD, to build a rat model of PD. 6-OHDA is a neurotoxic agent that selectively destroys dopamine (DA)-containing neurons. DA and acetylcholine (ACh) are two very important neurotransmitters in the substantia nigra-striatum neural pathway. When the number of dopaminergic neurons decreases, the inhibitory effect on postsynaptic structure decreases accordingly, which leads to the overactivation of ACh function, the imbalance between ACh and DA, and consequently PD symptoms [12]. The results of behavioral and pathological tests showed that the maximum angle of the inclined plate stay, the times of grid crossing and standing, and the ATPase activity in brain tissue were lower in the model group compared with the control group, and the positive rate of α -synuclein was higher, which was consistent with the behavior of PD rats [13], confirming the success of the modeling. After KET intervention, the behavior and pathological manifestations of the intervention group rats were significantly improved compared to the model group, suggesting the potential of KET to alleviate PD. KET, whose full name is 2-o-chlorophenyl-2-methylaminocyclohexanone, is a derivative of phencyclidine (PCP), commonly known as ketamine hydrochloride. As an important NMDA receptor blocker of the glutamate-gated ion channel in the brain, it can participate in the regulation of synaptic transmission and synaptic plasticity axis by inhibiting the activity of NMDA receptor channels, thus restoring the synaptic damage in the cortex and hippocampus caused by chronic stress [14]. KET produces a distinctive anesthetic state, characterized by stupor, sedation, amnesia, and significant analgesia [15]. This state is considered to be the result of the separation of the limbic system from thalamus-neocortex system, which has been referred to in previous studies as "Dissociative anesthesia" [16]. Electroencephalogram (EEG) research has shown that KET inhibits the thalamic-cortical system, selectively blocks the transmission of pain impulses to the thalamus and cortex, and has an exciting effect on the thalamus and the cerebral limbic system, mainly as a result of inhibiting excitatory neurotransmitters (including ACh and L-glutamic acid) and interacting with NMDA receptors [17].

Meanwhile, we also observed elevated inflammatory factors and MDA and reduced SOD in the model group versus the control group, which also confirms the presence of serious inflammation and oxidative stress damage in the brain tissue, consistent with previous studies [18]. In contrast, although the inflammatory response and oxidative stress damage in the intervention group were still higher than those in the control group, they were significantly reduced compared with the model group, demonstrating the excellent inhibitory effect of KET on the inflammation and oxidative stress in the brain tissue of PD rats, which is related to the inhibition of nitric oxide synthase (NOS) activity and endotoxin protein expression by KET [19]. Wang L et al. also reported that KET not only inhibited the release of inflammatory mediators such as IL-1 and TNF- α , but also reduced the mortality of mice with endotoxin-induced shock [20], further validating the anti-inflammatory effect of KET.

On the other hand, the PI3K/AKT axis is an extremely important signaling pathway in the pathological changes of PD, and activation of its expression can accelerate cytokine growth and inhibit autophagy [21]. For PD, studies have repeatedly confirmed the activation of the PI3K/AKT

axis in the disease, resulting in abnormal proliferation of astrocytes and promoting the malignant progression of PD [22, 23]. In this study, higher p-PI3K/PI3K and p-AKT/AKT and lower LC3-II and Beclin1 protein levels were also determined in the model group versus the control group. The use of KET effectively inhibited p-PI3K/PI3K and p-AKT/AKT expression and promoted autophagy, which may be the pathway through which KET acts on PD.

Subsequently, to confirm our point of view, we conducted a validation test with HA1800 cells. In the LPS-induced PD cell model, KET inhibited HA1800 activity and promoted apoptosis, while significantly inhibiting PI3K/AKT pathway expression and elevating autophagy-associated protein levels. These results fully indicate that KET can promote astrocyte apoptosis and slow down the progression of PD by inhibiting PI3K/AKT signaling.

However, KET is a controlled drug in China and is only used in clinical anesthesia. Although more and more evidence has indicated the great potential of KET in relieving neurodegenerative diseases, there are also studies suggesting that KET should be contraindicated in PD patients, as KET-induced over-activation or over-inhibition may cause more serious neurological complications [24, 25]. Therefore, a lot of research is needed to validate the clinical application value of KET, and we will conduct a more in-depth and comprehensive analysis later.

5. Conclusion

KET is effective in reversing the pathological progression of PD and relieving inflammation and oxidative stress injury, and its mechanism is related to promoting autophagy and apoptosis of astrocytes by inhibiting the PI3K/AKT axis. KET may potentially become a new treatment option for PD in the future, but achieving its clinical application still requires extensive research and analysis.

Conflicts of Interest

The authors report no conflict of interest.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

Not applicable.

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