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Effect of ketamine combined with DHA on lipopolysaccharide-induced depression-like behavior in rats

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ABSTRACT

Depression has become a common mental illness, and studies have shown that neuroinflammation is associated with depression. Ketamine is a rapid antidepressant. In order to obtain better antidepressant effects, it is necessary to explore the efficacy of combination therapy with ketamine and other antidepressants. DHA is an unsaturated fatty acid with excellent application prospects due to its safety and antidepressant effects. This study was designed to investigate the effect of ketamine combined with DHA on lipopolysaccharide-induced depression-like behavior. In behavioral experiments, lipopolysaccharide prolongs the immobility time of the forced swimming and tail suspension tests in rats and reduces the sucrose preference. The combination of ketamine and DHA can reverse these changes and work better than the single application. Nissl staining showed that ketamine combined with DHA can reverse the nerve damage caused by lipopolysaccharide. Cell morphology observation the combination of ketamine and DHA group was more complete than that of LPS group. The combination of ketamine and DHA significantly decreased the levels of IL-1, IL-6 and TNF-αin hippocampus and PC12 cells and increased the content of BDNF. Immunofluorescence results showed that ketamine combined with DHA can effectively inhibit P—P65 nuclear translocation. Western blot results showed that ketamine combined with DHA can effectively inhibit the expression of NF-KB in hippocampus and PC12 cells, and increase the expression of P-CREB and BDNF. In summary, the combination of ketamine with DHA may be a more effective treatment for depression caused by inflammation and is mediated by inhibition of the inflammatory pathway.

1. Introduction

Depression is a serious disease that has a serious impact on today's society. Many studies have shown that neuroinflammation plays an important role in the pathophysiology of depression [1,2]. Recently, studies have shown an increase in the concentration of pro-inflammatory cytokines in the blood of patients with depression, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) [3]. In addition, studies have shown that depression model rats will exhibit activation of NF-KB expression , leading to an increase in the body's inflammatory factors [4]. These findings suggest that inflammation is an important factor in the development of depression [5]. LPS-induced cytokine IL-1, IL-6 secretion can cause behavioral changes, neuroendocrine and neurotransmitter changes. These results disrupt the balance between neurotransmitters directly associated with depression, leading to the emergence of depressive behavior [6,7]. The inflammatory pathway has recently become an important topic in the development of new antidepressants. Therefore, antidepressants with few side effects and good comprehensive effects should be developed.

Ketamine, as an NMDA receptor antagonist, is a rapid antidepressant. The discovery of the antidepressant effect of ketamine is a very important development in the field of antidepressant drugs [8]. The efficacy of ketamine as a rapid antidepressant in improving core depressive symptoms, including depressed mood, anhedonia and bipolar depressed patients when administered at sub-anesthetic doses [9]. The association of ketamine with inflammatory pathway depression has also recently been proposed, and studies have shown that ketamine can eliminate lipopolysaccharide-induced depression-like behavior in rats [10]. However, the application of ketamine has certain side effects. The application of ketamine increased motor incoordination, so we need to find more effective ways to use ketamine [11]. Clinical trial data show that most traditional antidepressants can be combined with ketamine without affecting the efficacy or increasing the burden of adverse reactions [12]. In order to achieve better antidepressant effects, it is necessary to explore the therapeutic effects of ketamine combined with

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other antidepressants.

DHA is a polyunsaturated fatty acid that is an important component of biofilms and plays an important role in homeostasis and brain development [13]. Studies have shown that DHA can play a therapeutic role in neurological diseases through anti-inflammatory and neurotrophic processes [14]. DHA-rich fish oils on the prevention of depressive symptoms among pregnant women at an increased risk of depression has been demonstrated [15]. In addition, DHA can prevent interferon-α-induced depression, and DHA can play a role in preventing inflammatory depression [16,17]. Therefore, due to the safety of DHA and the effect on the treatment of depression, it has a good prospect in the treatment of depression.

In general, in order to explore a more effective treatment of ketamine, this study investigated the therapeutic effect of combined ketamine combined with DHA on lipopolysaccharide-induced depression-like behavior in rats and explored its therapeutic mechanism. To study the effects of combined application on PC12 nerve cells, further illustrate the effects and mechanisms of combined application at the cellular level.

2. Materials and methods

2.1. Animals

60 healthy adult male Wistar rats (250 g–300 g) were obtained from Harbin Medical University of Harbin. Animals were placed in standard cages and placed in humidity control (55% ± 15%) and temperature controlled rooms (22 ± 2 °C), 12 h light/dark cycle, and free access to the same amount of standard food. All experiments were conducted in accordance with the guidelines of the Chinese Animal Experimental Ethics Committee.

2.2. Grouping and drug administration

Sixty rats were randomly divided into 6 groups: NC group (control group), LPS group (lipopolysaccharide group), LPS + KET group (lipopolysaccharide + ketamine group), LPS + DHA group (lipopolysaccharide + DHA group), LPS + KET + L-DHA group (lipopolysaccharide + ketamine + low dose DHA group), LPS + KET + H-DHA (lipopolysaccharide + ketamine + high dose DHA group) group and put them into 12 cages. After 14 days of adaptive feeding, the rats were gavaged with 100 mg/kg/400 mg/kg DHA (Nanjing, China) or saline for seven days. The rats were then intraperitoneally injected with 1 mg/kg LPS (Sigma, L3129) or saline. After 24 h, 10 mg/kg ketamine or saline was intraperitoneally injected, and behavioral tests were performed 2 h later (Fig. 1).

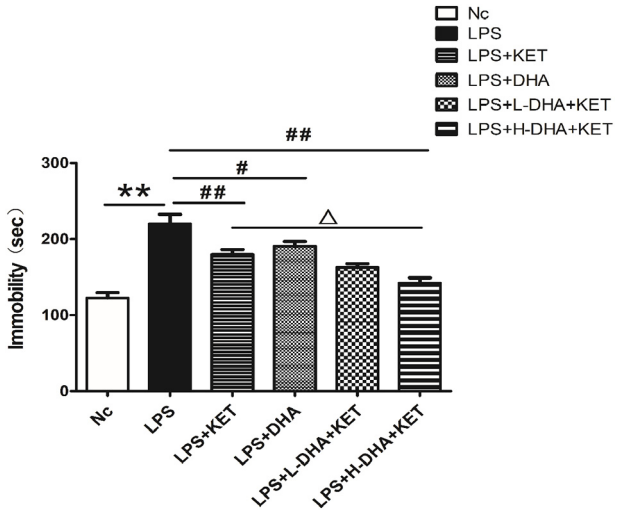

Fig. 2. Forced swimming test. The combined use of ketamine and DHA achieves better intervention in depression-like behavior. Values were the mean ± S.E.M. with 10 mice in each group. The difference with NC group is denoted by *, ** means $P < 0.01$, the difference with LPS group is denoted by #,# means $P < 0.05$,## means $P < 0.01$, and the difference with LPS + KET group is denoted by △, △ means $P < 0.05$.

2.3. Forced swimming test

Forced swimming experiments (FST) were used to determine the depressive behavior of animals [18]. The rats were placed in a 20 cm diameter glass cylinders filled with tap water (24 ± 2 °C). Mice were unable to escape or touch the bottom of the cylinder. The rats were subjected to a 15-min swimming stress pre-experiment. After each test, the animal body was wiped dry and placed in the home cage. After a period of time, the rats were again placed in the cylinder. Animals were recorded for a period of inactivity within 6 min for FST test. When the rats stopped struggling and remained floating in the water, they were considered to be stationary.

2.4. Tail suspension test

The Tail suspension test (TST) is another experiment to assess the behavior of depression in animals [19]. The main reflection is the desperate state of animal behavior. The rat tail end 3 cm was fixed with a clip and hung on a crossbar 50 cm from the ground. The animal struggles to overcome an abnormal position and appears to be stationary after a certain period of time. The immobility time of the rats was recorded within 6 min.

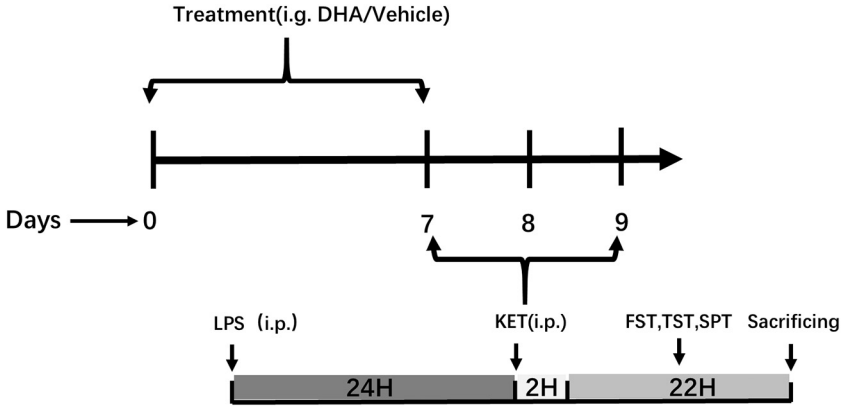

Fig. 1. Experimental arrangement. Rats were given DHA(100 mg,400 mg/kg) or saline for 7 days. After intraperitoneal injection of LPS (1 mg/kg) or saline for 24 h, ketamine (10 mg/kg) or saline was intraperitoneally injected. Behavioral testing was performed 2 h later.

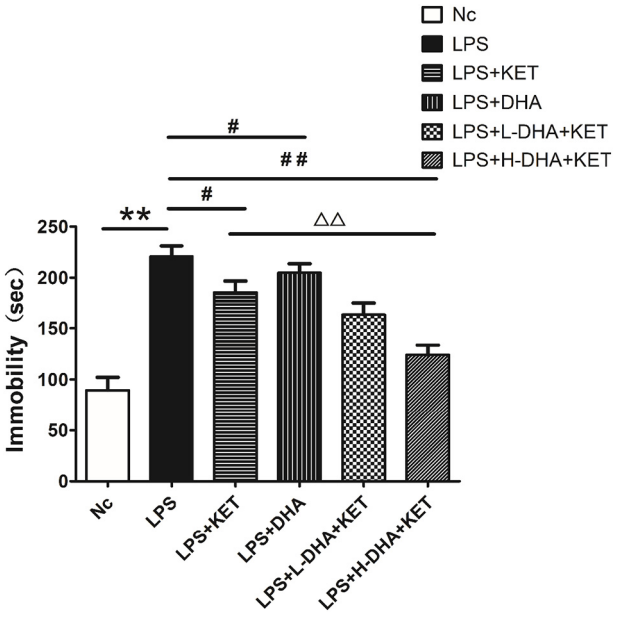

Fig. 3. Tail suspension test. Effect of combined application of ketamine and DHA on TST test in rats. Values were the mean ± S.E.M. with 10 mice in each group. The difference with NC group is denoted by *, ** means $P < 0.01$, the difference with LPS group is denoted by #,# means $P < 0.05$,## means $P < 0.01$, and the difference with LPS + KET group is denoted by △, △ means $P < 0.01$.

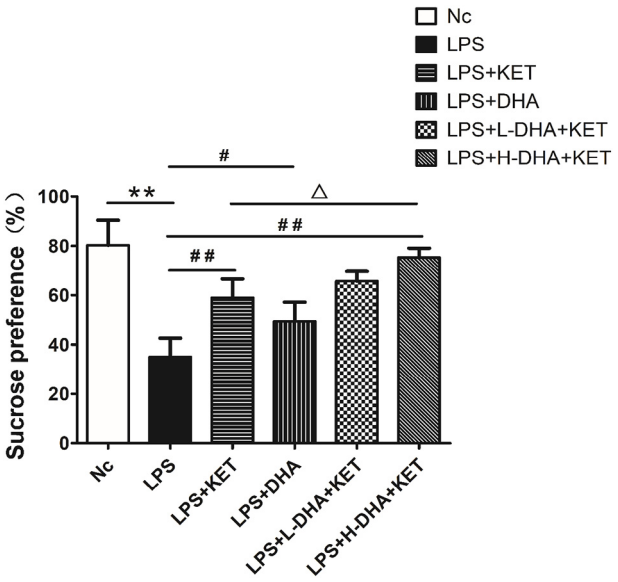

Fig. 4. Sucrose preference test. Effect of combined application of ketamine and DHA on sucrose preference rate in rats. Values were the mean ± S.E.M. with 10 mice in each group. The difference with NC group is denoted by *, ** means $P < 0.01$, the difference with LPS group is denoted by #,# means $P < 0.05$,## means $P < 0.01$, and the difference with LPS + KET group is denoted by △, △ means $P < 0.05$.

2.5. Sucrose preference test

The Sucrose preference test is used to evaluate anhedonia in animals [20]. The sucrose preference experiment consists of two parts, the adaptive training part and the test part. In the rat training section, two 1% sucrose water bottles were placed in each cage, and one of them was changed into water 24 h later. The animals are then fasted for Sucrose preference test. The 1% sucrose solution bottle and clear water bottle after weighing were placed on the rat cage, and Weigh the water bottle after 12 h. The total fluid consumption, sucrose consumption and pure water consumption of the rats were recorded. Sucrose preference index % = sugar water consumption/ (sucrose water consumption + pure water consumption) × 100%.

2.6. Sample collections

Rats were sacrificed by decapitation and hippocampal tissue was taken. Part of hippocampal tissue was stored in liquid nitrogen for Elisa and western blot experiments, and the other part was stored in 4% formalin for slicing.

2.7. Nissl's staining

The brain was fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 4 mm thick sections for Nissl staining. The staining was performed by a conventional Nissl staining method, and the hippocampal tissue lesion in the CA1 region was observed by an optical microscope (Leica DFC420, Germany) at a magnification of 400×. The three fields of view were randomly selected to observe the pathological changes of the hippocampus.

2.8. Cell culture and drug treatment

PC12 cells(poorly-differentiated) were obtained from the Northeast Agricultural University, Harbin, China. The cells were cultured in DMEM medium (Gibco), supplemented with 10% (v/v) FBS, penicillin/streptomycin (100 U/mL; 100 μg/mL) at 37 °C under an atmosphere of 5% CO2 and 95% air. The cells were seeded in cell culture flask or 96-well plates with 5–9 × 10^4 cells/well. The optimal concentration of LPS, KET and DHA (Sigma) on PC12 cells was determined using the CCK8 kit. The final screening concentrations based on cell viability, the LPS concentration was 0.25 μg/mL for 24H, the ketamine concentration was 10 μg/mL for 2H, and the DHA concentration was 15 μg/mL for 24H. Therefore, the cell experiments were divided into five groups: NC group (blank control group), LPS group (lipopolysaccharide group), LPS + KET group (lipopolysaccharide + ketamine group), LPS + DHA group (lipopolysaccharide + DHA group), LPS + KET + DHA group (lipopolysaccharide + ketamine group + DHA group).

2.9. Cell morphological observation

PC12 cells were cultured and seeded in a 6-well plate, and the cells were subjected to modeling. The culture solution was poured out and added to PBS, and the morphology of the cells was observed under an inverted microscope (Sdptop, Shanghai, China). The microscope magnification was 100×, and three fields of view were randomly selected for observation.

2.10. Immunofluorescence staining

PC12 cells were cultured and seeded on cell plates. First, 4% paraformaldehyde was fixed at 4 °C for 30 min, then goat serum (1:10) was blocked at room temperature for 20 min, and primary antibody Phospho-NF-κB p65 (1:100, Cell Signaling Technology, USA) was incubated overnight at 4 °C. Secondary antibody (1:100, Beijing, China), incubated at 37 °C for 30 min, DAPI staining for 15 min. Observations were performed using a confocal microscope (Leica, Germany), and three fields of view were randomly selected for observation.

2.11. Elisa kit detection

Hippocampus, PC12 cells were lysed with lysis RIPA buffer, Centrifuge for 10 min (12,000 r/min) in a 4 °C centrifuge, and extract the supernatant for determination of the elisa kit (Jiancheng, Nanjing,

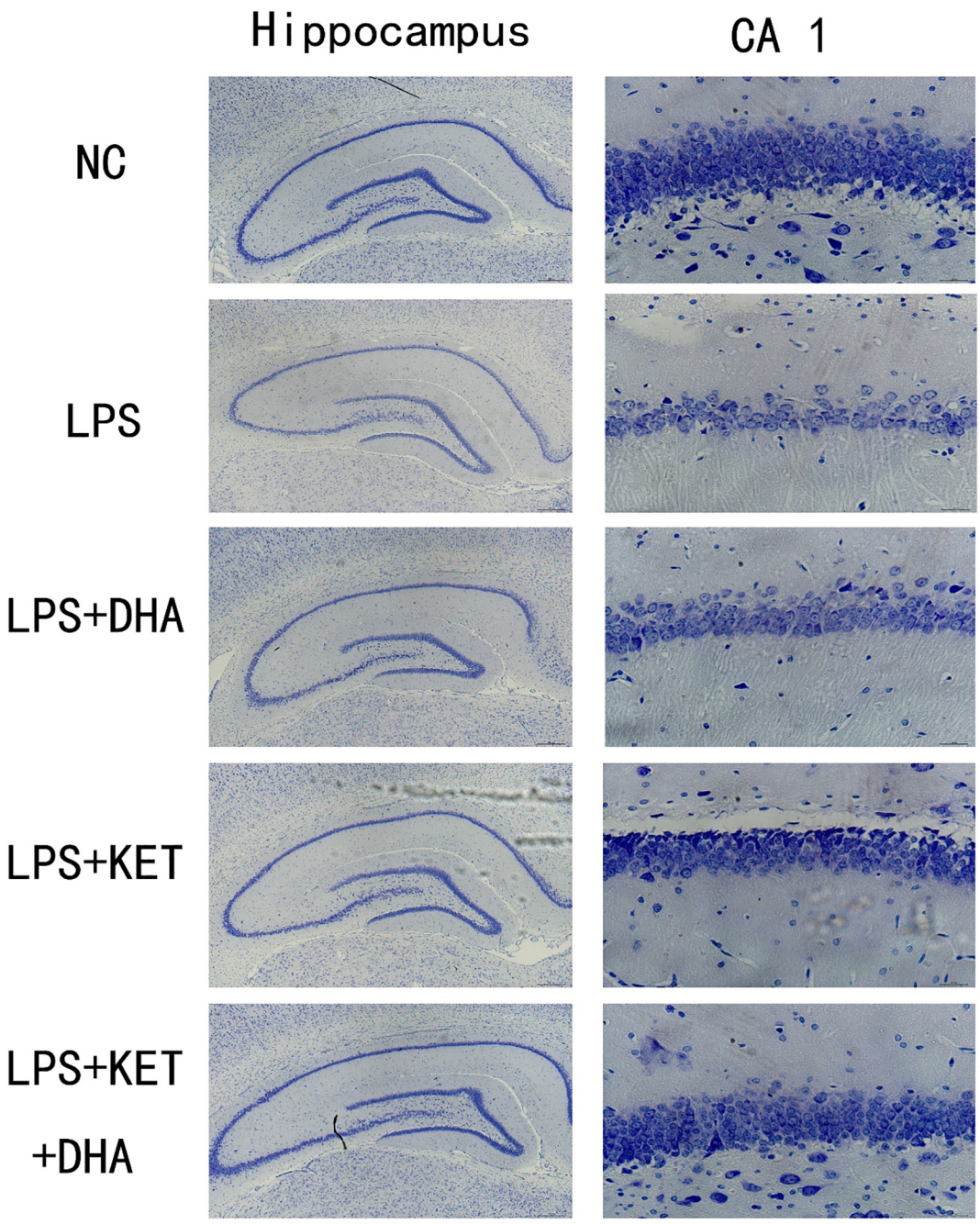

Fig. 5. Nissl's staining was utilized to observe neuronal cells. The figure shows the hippocampal area (100×) and CA1 area (400×) of the rats with nissl's staining.

China). The detection indexes are IL-1, IL-6, TNF-, BDNF. According to the kit steps and statistical data Analyze.

2.12. WB

Total protein was extracted from hippocampus and PC12 cells, and proteins were separated on SDS-PAGE gel and transferred to nitro membrane under reducing conditions (HybondTM-C Extra, GE Healthcare). After washing, carried out at room temperature for blocking treatment 2 h, after incubation anti-P-P65 (1: 500, Cell Signaling Technology), anti-P-Creb (1: 1000, EnoGene), anti-BDNF (1: 1000, Abcam) for 12 h at 4 °C. The membrane was washed three times with PBS containing 0.1% Tween 20 and then incubated with horseradish peroxidase-labeled secondary antibody (goat anti-rabbit IgG antibody HRP (ABIN) or goat anti-mouse IgG antibody HRP (Sigma) at room temperature for 1 h. The bands were observed with an enhanced chemiluminescence (ECL) solution (Beyotime, China) and quantified using a chemiluminescence detector (Tanon, China) and ImageJ (NIH, USA) image analysis software.

2.13. Data analysis

Data are presented as means ± standard deviation. Statistical analysis was performed using GraphPad Prism 7(GraphPad Software Inc., USA). The data were analyzed using a One-Way analysis of variance (ANOVA), followed by post hoc Tukey test. Differences were considered statistically significant if $p < 0.05$.

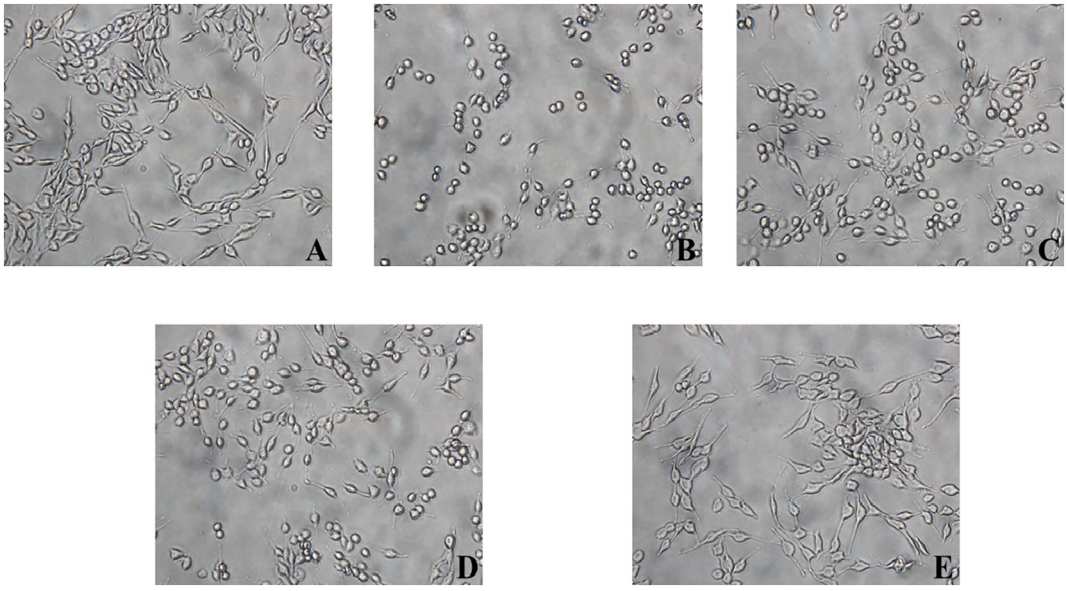

Fig. 6. Optical microscope cell morphology observation. (A)stands for NC group; (B) stands for LPS group; (C) stands for LPS + KET group; (D) stands for LPS + KET group; (E) stands for LPS + KET + DHA. Microscope magnification is 200×.

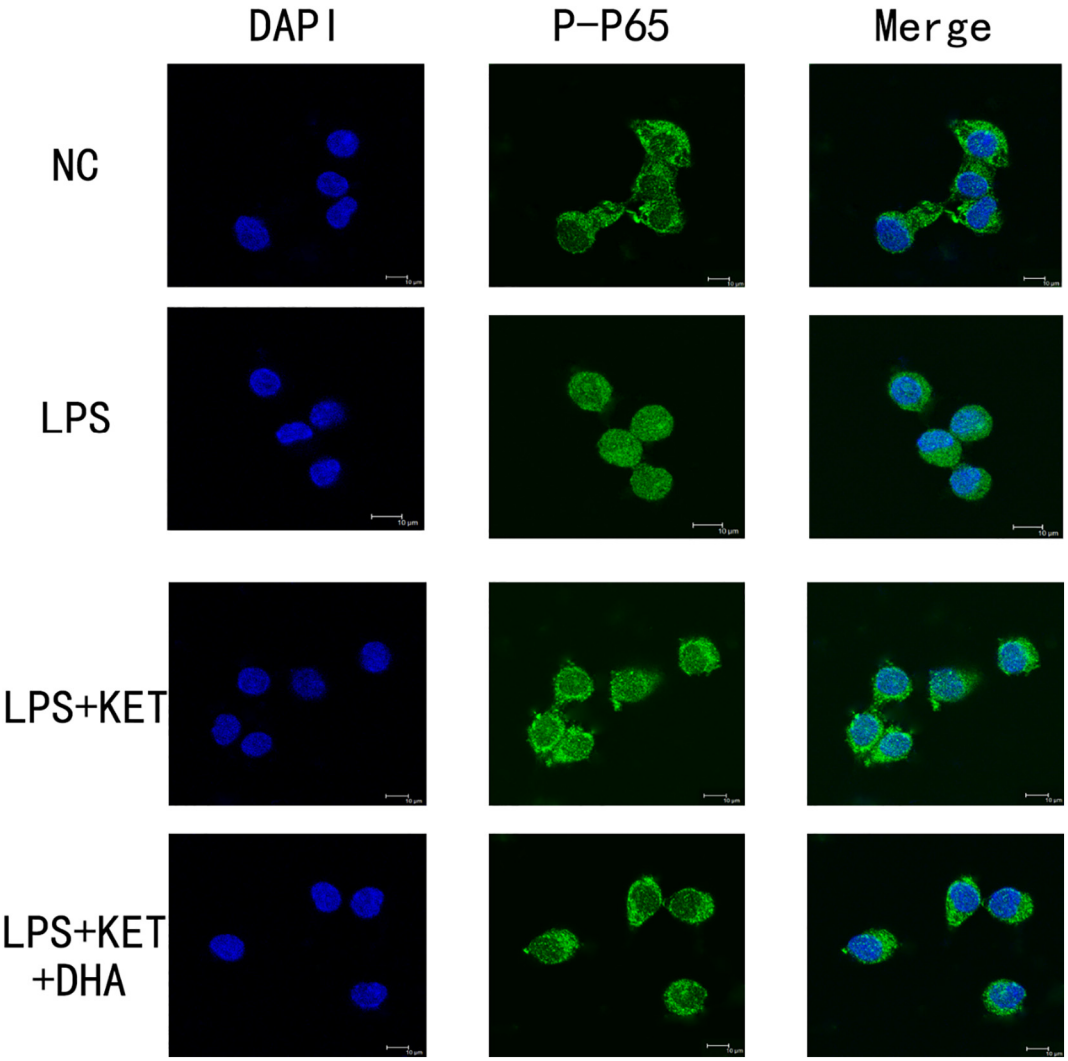

Fig. 7. Confocal microscopy observation of P−P65 staining in PC12 cells. Green fluorescence represents fluorescent staining of P−P65 and blue fluorescence represents DAPI stained nuclei. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

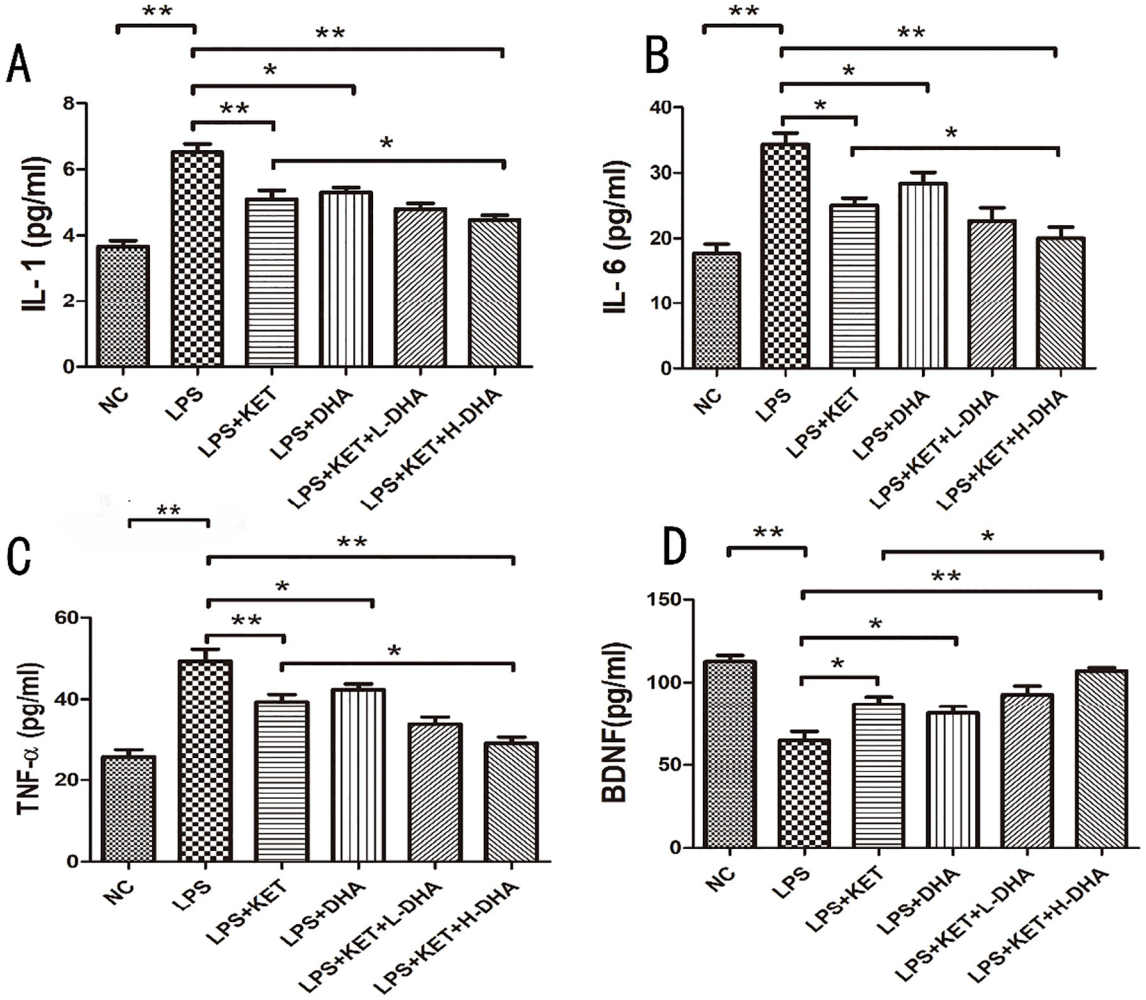

Fig. 8. Effect of ketamine combined with DHA on depression-related cytokines in hippocampus. (A) shows the results of IL-1 measurement (B) shows the results of IL-6 measurement (C) shows the results of TNF-α measurement (D) shows the results of BDNF. The above results were detected by ELISA. Values are means of three independent experiments, with standard deviations represented by vertical bars.* $P < 0.05$,**$P < 0.01$.

3. Result

3.1. Effect of ketamine combined with DHA on lipopolysaccharide-induced depression-like behavior in rats

As can be seen from the behavioral experiments of the FST (Fig. 2), the immobility time of the FST experiment of the LPS group was significantly increased compared with the NC group. In the LPS + KET group($P < 0.01$), LPS + DHA group($P < 0.05$), LPS + KET + H-DHA($P < 0.01$) group FST experiments were reduced in immobility time. The immobility time of LPS + KET + H-DHA group was significantly reduced, and it was significantly different from LPS + KET group ($P < 0.05$).

From the Tail suspension test we can see (Fig. 3) that the LPS group has the longest static time. The immobility time of rats In the LPS + KET group ($P < 0.05$), LPS + DHA group ($P < 0.05$), LPS + KET + H-DHA group ($P < 0.01$) was significantly reduced. In addition, the static immobility time of the LPS + KET + H-DHA group was significantly different from that of the LPS + KET group ($P < 0.01$).

From the sucrose preference experiment (Fig. 4), we can see that the sucrose consumption rate of the LPS group was significantly lower than that of the NC group ($P < 0.05$). The sucrose water consumption rate of LPS + KET group, LPS + DHA group and LPS + KET + H-DHA group was significantly different from that of LPS group. In addition, it can be seen from Fig. 4 that LPS + KET + H-DHA has a higher sucrose preference rate than the LPS + KET group($P < 0.05$).

3.2. Nissl's staining

The comparison between the NC group and the LPS group showed that the hippocampal area of the NC group was stained more clearly, and there were more positive cells in the NC group, the CA1 area shows a rich Nissl body. The staining of the LPS group was blurred, the Nissl body was seriously lost, and the nerve damage in the CA1 area was severe. The LPS + KET group and the LPS + KET + DHA group were well stained compared with the LPS group, the hippocampus was relatively intact, and the CA1 region was rich in nerve cells. In the CA1 region, the LPS + KET + DHA group is more abundant nerve cell than the LPS + KET group (Fig. 5).

3.3. Effect of ketamine combined with DHA on the morphology of PC12 neurons

It can be seen from Fig. 6 that the cells in the NC group have strong refractive index, clear axons, and good cell adherence. In the LPS group, the refractive index decreased, the cells contracted, the cell morphology became round, and the adherence was poor. The LPS + KET group and the LPS + KET + DHA group significantly improved cell morphology and reduced the loss of cell synapses, and the LPS + KET + DHA group

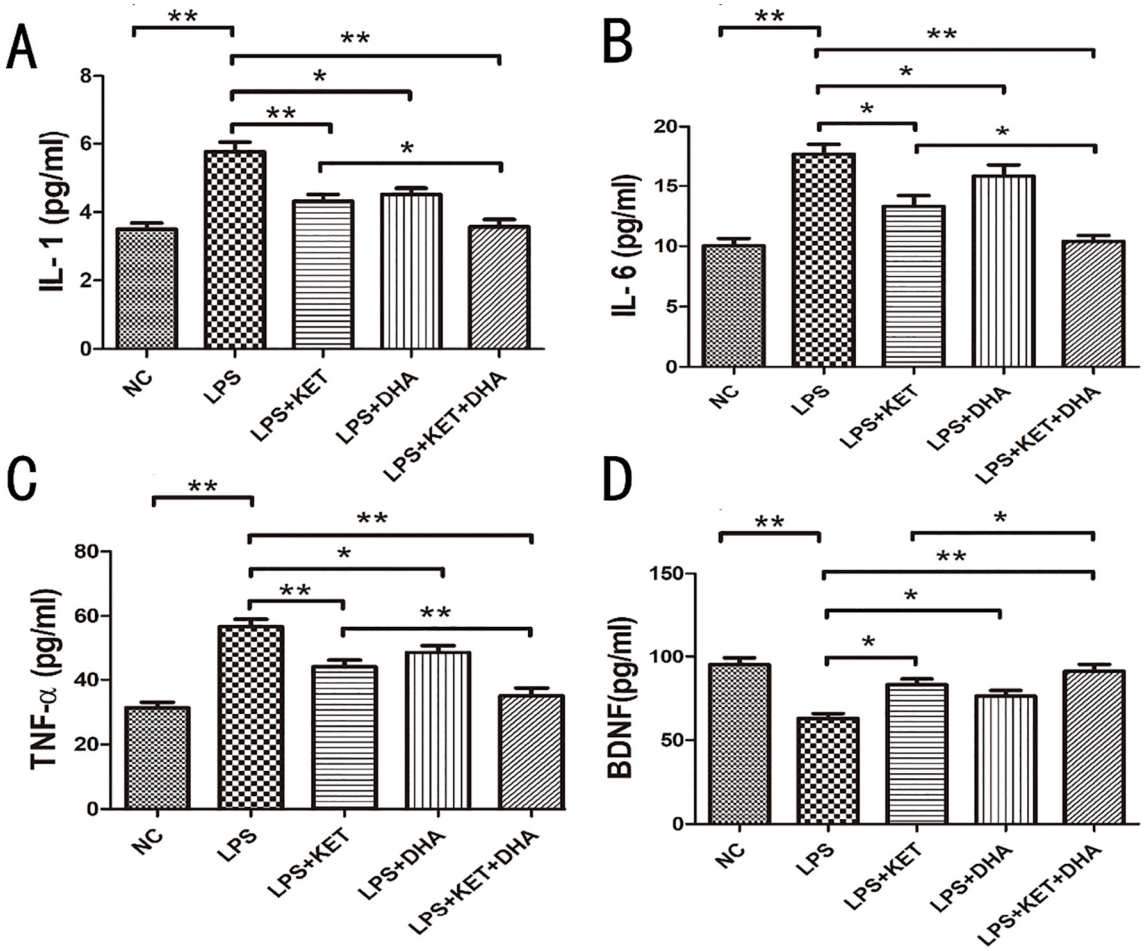

Fig. 9. Effect of ketamine combined with DHA on depression-related cytokines in PC12 cells. (A) shows the results of IL-1 measurement (B) shows the results of IL-6 measurement (C) shows the results of TNF-α measurement (D) shows the results of BDNF. The above results were detected by ELISA. Values are means of three independent experiments, with standard deviations represented by vertical bars.* $P < 0.05$,**$P < 0.01$.

had better therapeutic effect, which was closer to the cell morphology of the NC group.

3.4. Immunofluorescence detection of the effect of ketamine combined with DHA on P65 nuclear translocation in PC12 cells

As shown in Fig. 7, nerve cells in the NC group showed P—P65 labeling only in the cytoplasm, showing green fluorescence. P—P65 staining markers were observed in the cytoplasm and nucleus of the LPS group. The staining markers of P—P65 in the nucleus of LPS + KET group and LPS + KET + DHA group were significantly reduced. There was almost no staining marker of P—P65 in the nucleus of LPS + KET + DHA group.

3.5. Effect of ketamine combined with DHA on depression-associated cytokines

According to the results of the hippocampal tissue kit (Fig. 8), the LPS group significantly increased the levels of the inflammatory factors IL-1, IL-6 and TNF-α. The levels of IL-1, IL-6 and TNF-α in the LPS + KET group, LPS + DHA group, and LPS + KET + H-DHA group were significantly lower than those in the LPS group ($P < 0.05$). The BDNF content in the LPS group was significantly reduced. The BDNF levels in the LPS + KET group, LPS + DHA group and LPS + KET + H-DHA group were significantly higher than those in the LPS group ($P < 0.05$). Compared with the LPS + KET group, the LPS + KET + H-DHA group inhibited the inflammatory factors IL-1, IL-6 and TNF-α more significantly ($P < 0.05$), and the BDNF increased better ($P < 0.05$).

It can be seen from the cell Elisa kit experiment (Fig. 9) that IL-1, IL-6 and TNF-α were significantly increased in PC12 cells of LPS group, and the content of BDNF was significantly decreased. In the LPS + KET group, LPS + DHA group and LPS + KET + H-DHA group significantly inhibited the increase of inflammatory factors IL-1, IL-6 and TNF-α ($P < 0.05$). In addition, LPS + KET group, LPS + DHA group and LPS + KET + H-DHA group increased the BDNF content of PC12 cells ($P < 0.05$). Compared with the LPS + KET group, the LPS + KET + H-DHA group reduced the inflammatory factors IL-1, IL-6 and TNF-α, and the effect of increasing BDNF content was more obvious ($P < 0.05$).

3.6. Effects of ketamine combined with DHA on the expression of NF-kb, P-CREB and BDNF in hippocampus and PC12 cells

As shown in Fig. 10, LPS significantly increased P—P65 expression in hippocampus ($P < 0.001$), and significantly decreased P-CREB ($P < 0.001$), BDNF ($P < 0.001$) expression. LPS + KET group and LPS + KET + H-DHA significantly decreased the expression of P—P65 ($P < 0.001$), and increased the expression of P-CREB and BDNF ($P < 0.001$). LPS + KET + H-DHA had a higher inhibitory effect on P—P65 expression than the LPS + KET group ($P < 0.001$). LPS + KET + H-DHA had a higher inhibitory effect on P—P65 expression than the LPS + KET group ($P < 0.001$). The expression of P-CREB and BDNF was also significantly increased in LPS + KET + H-DHA compared with LPS + KET group ($P < 0.001$).

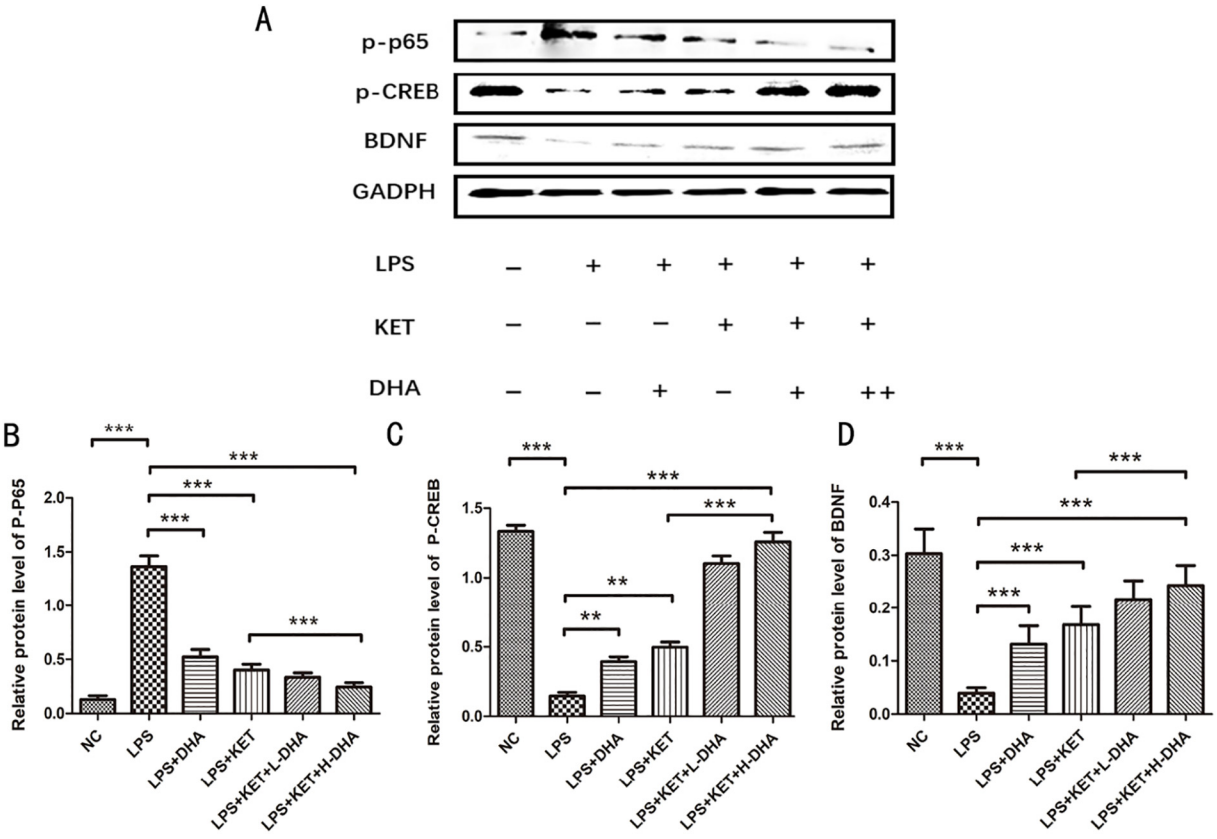

Fig. 10. Protein expression of P—P65, P-CREB, and BDNF in hippocampus.(A) Western blotting of P—P65, P-CREB, BDNF protein expression.(B) The relative protein of P-P65·(C) The relative protein of P-CREB.(D) The relative protein of BDNF. ** represents $P < 0.01$, *** represents $P < 0.001$.

As shown in Fig. 11, As shown in Fig. 11, the LPS group significantly inhibited the expression of P-CREB and BDNF ($P < 0.05$) compared with the NC group. The expression of P—P65 was significantly increased in the LPS group ($P < 0.05$), but the LPS + KET + DHA group significantly inhibited the expression of P—P65 ($P < 0.001$). The LPS + KET + DHA group inhibited the expression of P—P65 better than the LPS + KET group ($P < 0.001$). The LPS + KET + DHA group increased the expression of P-CREB and BNDF compared with the LPS group ($P < 0.01$).

4. Discussion

The development of depression includes many factors, such as cytokines, glucocorticoids and neurosecretion. A growing number of related studies have shown that inflammatory cytokines may be central factors influencing multiple neural pathways and play a role in the development of depression [21]. Therefore, the current study used LPS to induce depression-like behavior in rats [22]. This study explored the therapeutic effect of ketamine combined with DHA on LPS-induced depression and the treatment mechanism. After intraperitoneal injection of LPS for 24 h, the rats showed an increase in immobility time in the FST and RST experiments, and the sucrose preference rate decreased in the SPT experiment [23,24]. This is consistent with our experimental results. In this experiment, we used FST, TST, and SPT to evaluate lipopolysaccharide-induced depression-like behavior. In the FST and TST experiments, the immobility time of the LPS group increased significantly, and the sucrose preference rate of the LPS group decreased significantly. The depressive behaviors of theLPS + DHA group, the LPS + KET group and the LPS + LET + H-DHA group were significantly reduced, showing a decrease in immobility time and an increase in sucrose preference. Studies have shown that ketamine can inhibit lipopolysaccharide-induced depression-like behavior, and DHA can also inhibit the occurrence of depression-like behavior [25]. This is consistent with our findings. In addition, ketamine combined with DHA (400 mg/kg) significantly inhibited lipopolysaccharide-induced depression-like behavior and was more effective than the LPS + KET group and the LPS + DHA group. In summary, the combination of ketamine and DHA has a better therapeutic effect on the lack of pleasure and behavioral despair of lipopolysaccharide-induced behavior. And it works better than ketamine and DHA alone.

The main theory of inflammatory depression is that activation of the inflammatory immune system may affect neurochemicals or damage neurons, leading to the occurrence of depressive behavior, excessive cytokines or prolonged exposure to cytokines can damage the brain [26,27]. In this experiment, it can be seen from the results of Nissl staining that the neuronal body of the hippocampus in the LPS group is lost. The use of ketamine and DHA treatment can prevent this loss. And ketamine combined with DHA can better prevent neuronal damage caused by lipopolysaccharide. Studies have shown that neuronal damage is the main pathological change of current depression, and neuronal atrophy is a pathophysiological marker of MDD (major depression) [28]. In addition, ketamine can effectively reverse the atrophy of neurons in patients with depression, resulting in an effective antidepressant effect [29]. DHA can eliminate neuronal damage caused by inflammation and regenerate nerves [30]. The current study shows that poorly differentiated PC12 cells are the most suitable cells for studying depression, so this study used PC12 cells for further research [31]. In this study, in the PC12 nerve cell morphology observation experiment, it can be seen that the lipopolysaccharide group atrophies the nerve cells and loses cell morphology. The combination of ketamine and DHA can better prevent neuronal damage caused by lipopolysaccharide. Ketamine combined with DHA achieved a good therapeutic effect and reduced the damage of inflammatory factors to hippocampal and cell neurons.

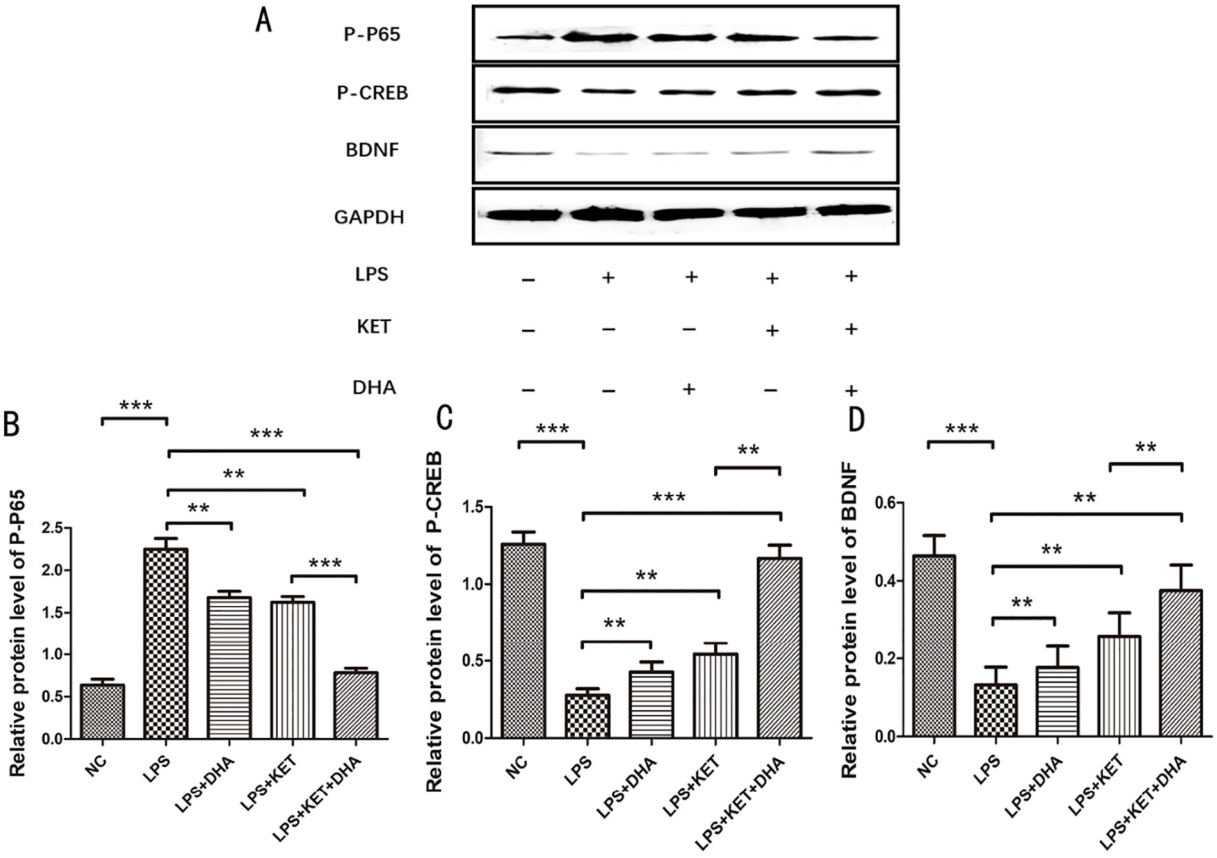

Fig. 11. Protein expression of P—P65, P-CREB, and BDNF in PC12 cells.(A) Western blotting of P—P65, P-CREB, BDNF protein expression.(B) The relative protein of P-P65·(C) The relative protein of P-CREB.(D) The relative protein of BDNF. ** represents $P < 0.01$, *** represents $P < 0.001$.

Studies have shown that elevated inflammatory cytokines in blood and brain tissue in patients with major depression, changes in inflammatory factors activate inflammatory signaling pathways in the brain, leading to changes in monoamine, glutamate and neuropeptide systems, as well as growth factors (e.g. Reduction of brain-derived neurotrophic factor) [32,33]. In this study, lipopolysaccharide significantly reduced the content of BDNF in hippocampus tissues and cells, while the application of ketamine and DHA increased the content of BDNF. The combination of ketamine and DHA has a more pronounced inhibitory effect on the reduction of BNDF content induced by lipopolysaccharide. Studies have shown that BDNF is an important determinant of the efficacy of antidepressants, thus demonstrating the excellent antidepressant efficacy of ketamine combined with DHA [34]. In addition, our study showed that ketamine and DHA can reduce the levels of IL-1, IL-6 and TNF-α in hippocampus and PC12 cells. The combination of ketamine and DHA can effectively inhibit the increase of proinflammatory factors IL-1, IL-6 and TNF-α caused by lipopolysaccharide. A Meta-Analysis shows that depression is usually accompanied by an increase in IL-1, IL-6, and TNF-α [35]. Previous evidence suggests that IL-1, IL-6 receptor blockade can reduce the occurrence of depressive behavior in animals [36,37]. From this we can conclude that ketamine combined with DHA can control the inflammatory factors IL-1, IL-6 to achieve therapeutic effects.

Activation of NF-κB has been playing an important role in the depression caused by lipopolysaccharide. Lipopolysaccharide can activate NF-κB, and the p65 subunit of NF-κB is present in the cytoplasm at rest. When NF-κB is activated, phosphorylation of p65 subunit at NF-κB is translocated into the nucleus, where the phosphorylated p65 subunit binds to the target gene and regulates gene transcription [38,39]. In this experiment, Western Blot assay showed that phosphorylated P65 was significantly increased in hippocampus and PC12 cells induced by lipopolysaccharide. The combination of ketamine and DHA inhibited the expression of phosphorylated P65 and was better than ketamine and DHA alone. In addition, in the cellular immunofluorescence experiment, ketamine combined with DHA can significantly inhibit the translocation of phosphorylated P65 into the nucleus, and inhibit the translocation is stronger than the single application. Studies have shown that inhibition of NF-κB expression can inhibit the release of the inflammatory factors IL-1, IL-6, and TNF-α [40]. Therefore, this study demonstrates that ketamine combined with DHA inhibits the expression of NF-κB and inhibits the release of inflammatory factors. Activation of NF-κB results in activation of the MAPK pathway leading to decreased expression of CREB phosphorylation leading to decreased expression of BDNF. The CREB regulatory function itself is regulated by phosphorylation, and CREB itself has no transcriptional activity and needs to be P-CREB to activate activity [41]. Studies have shown that p-CREB levels in patients with major depression are significantly lower than in the normal control group, and decreased p-CREB is also detected in the anterior frontal lobe and hippocampus in the chronic stress rat model [42,43]. Increased expression of phosphorylated CREB also increases BDNF expression, and BDNF plays a key role in the treatment of depression [44]. In this study, lipopolysaccharide inhibited the expression of P-CREB and BDNF in hippocampus and cells. The combination of ketamine and DHA significantly abolished the inhibition of P-CREB and BDNF expression. These results indicate that ketamine combined with DHA achieves therapeutic effects by inhibiting the activation of NF-κB and thereby increasing the expression of P-CREB and BDNF.

5. Conclusion

In general, lipopolysaccharide induces depression-like behavior in rats through the inflammatory pathway, and ketamine combined with

DHA has a better therapeutic effect on depression than alone. The antidepressant effect is to inhibit the expression of NF-κB, reduce the release of inflammatory factors, and increase the expression of P-CREB and BDNF. It provides a new direction for the joint application of clinical antidepressants.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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