Acupuncture and Herbal Medicine

Mechanistic studies of the beneficial effects of Anshen Dingzhi prescription for PTSD treatment: roles of the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway

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Abstract

Objective: Anshen Dingzhi prescription (ADP) is an effective remedy for treating post-traumatic stress disorder (PTSD); however, the mechanism underlying its beneficial effects is unclear. This study explores the roles of the neuroinflammation regulated by the FKBP prolyl isomerase 5 (FKBP5)-IκB kinase alpha (IKKα)-nuclear factor kappa-B (NF-κB)-NOD-like receptor thermal protein domain-associated protein 3 (NLRP3) signaling pathway in PTSD.

Methods: The primary components of ADP, including ginsenosides Rg1 and Rb1, were quantified using ultra-performance liquid chromatography. Twelve C57BL/6 mice were allocated to control (D0) and experimental groups on days one, seven, and 14 of single prolonged stress (SPS). Eighteen C57BL/6 mice were allocated to control, SPS, and MCC950, an NLRP3 inhibitor (5 mg/kg) groups. Finally, 24 C57BL/6 mice were allocated to control, SPS, paroxetine hydrochloride (PRX), or ADP (18.4 and 36.8 mg/kg) groups. Mice were administered MCC950, PRX, or ADP for 14 days. The open field test and elevated plus maze were used to evaluate anxiety-like behaviors, whereas fear memory extinction was evaluated using the fear memory test. Western blotting was employed to evaluate the expression levels of the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway, tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β. The expression of FKBP5 and NLRP3 was further confirmed by immunofluorescence staining.

Results: The amounts of ginsenosides Rg1 and Rb1 in ADP were (96.85 ± 1.14) and (9.04 ± 0.22) μg/g, respectively. Compared with the D0 group, the levels of the inflammatory cytokine proteins, TNF-α, IL-6, and IL-1β were elevated 1.33- to 1.51-fold and those of FKBP5-IKKα-NF-κB-NLRP3 signaling pathway were increased 1.16- to 1.41-fold in the hippocampus of the D14 group ($P < 0.05$); the fluorescence intensity of FKBP5 and NLRP3 was also markedly increased (1.33–1.79-fold) in the hippocampus of the D14 group ($P < 0.5$). Notably, injection of MCC950 (5 mg/kg) reduced the levels of FKBP5-IKKα-NF-κB-NLRP3 (0.80–0.88-fold) and inflammatory cytokines (0.74–0.83-fold), thereby improving the PTSD-like behaviors induced by SPS ($P < 0.05$). In addition, ADP (36.8 g/kg) significantly improved PTSD-like behaviors and reduced levels of hippocampal inflammatory cytokines (0.70–0.79-fold) and FKBP5-IKKα-NF-κB-NLRP3 (0.50–0.79-fold) ($P < 0.05$) in SPS mice.

Conclusion: The results suggest a potential therapeutic benefit of ADP in PTSD due to the inhibition of the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway.

Keywords: Anshen Dingzhi prescription, FKBP5-IKKα-NF-κB-NLRP3 signaling pathway, Neuroinflammation, Post-traumatic stress disorder

Introduction

Post-traumatic stress disorder (PTSD) is a psychological condition caused by exposure to a major traumatic event[1]. Notable clinical features of PTSD include traumatic re-experiencing, traumatic stimulus avoidance and numbing, and increased alertness. The quality of life of patients with PTSD is severely reduced, and patients have an increased probability of developing psychiatric disorders, such as depression and anxiety, and some individuals are potentially at risk of suicide[2]. The incidence of PTSD is increasing annually owing to the increasing global frequency of catastrophic and traumatic events. Data from the World Mental Health Survey revealed that the occurrence rate of PTSD following various disasters

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is as high as 32.15%, whereas the prevalence of PTSD over a lifetime is approximately 5%[3]. Owing to the complexity of its pathogenesis, no medication has been formally approved to treat PTSD.

The Anshen Dingzhi prescription (ADP), which consists of Ginseng Radix et Rhizoma (*Panax ginseng* C. A. Mey. [Araliaceae]), Poria (*Poria cocos* [Schw.] Wolf [Polyporaceae]), Polygalae Radix (*Polygala tenuifolia* Willd. [Polygalaceae]), Poria cum Radix Pini (*Poria cocos* [Schw.] [Polyporaceae]), Acori Tatarinowii Rhizome (*Acorus tatarinowii* Schott [Araceae]), and dragon tooth (*Dens Draconis* [mammalian Rhinocertidae Chilotherium]), is a classic traditional Chinese medicine (TCM) prescription used to treat emotional disorders, as documented in *Yixue Xinwu*. ADP is known for its ability to tranquilize the spirit and mind and to enhance *qi*. Modern pharmacological research has shown that ADP can effectively treat neurological diseases, including anxiety, depression, and memory impairment[4]. We have also demonstrated the beneficial effect of ADP in mice subjected to a single prolonged stress (SPS)[5–7]; however, the underlying mechanisms require further investigation.

The pathogenesis of PTSD is strongly associated with increased inflammation, increased release of neuroendocrine factors, and impaired brain structure and function[8–9]. We previously reported functional damage in the hippocampus of mice with PTSD[6], and found that neuroinflammation could be a potential mechanism for PTSD[10–11]. The NOD-like receptor thermal protein domain-associated protein 3 (NLRP3) plays a key role in regulating neuroinflammation and has been implicated in the development and progression of inflammatory responses and neurological diseases[12–13]. Therefore, NLRP3 is expected to be an important target for preventing neuroinflammation in PTSD[14]. FKBP prolyl isomerase 5 (FKBP5) regulates nuclear factor kappa-B (NF-κB) through its complex with IκB kinase alpha (IKKα) and participates in NLRP3 activation, thus regulating neuroinflammation[15]. Using a network pharmacology approach, our team has discovered, that inflammatory factors such as tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) may represent potential targets of ADP for treating PTSD[7]. Interestingly, ADP has been shown to decrease levels of TNF-α, IL-6, and other inflammatory factors, and improve motor behavior in rats with chronic fatigue syndrome[16]. However, whether ADP targets neuroinflammation to prevent PTSD has not been evaluated.

We used the SPS mouse model[17] to determine whether ADP could improve PTSD-like behavior by interacting with the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway in hippocampus. Our findings provide experimental support for the clinical use of ADP in the treatment of PTSD.

Materials and methods

Chemicals and reagents

The various components of ADP, including Ginseng Radix et Rhizoma, Polygalae Radix, Poria, Acori Tatarinowii Rhizome, Poria cum Radix Pini, and dragon tooth were obtained from the First Affiliated Hospital of Anhui University of Chinese Medicine in October 2022, and were authenticated by Dr. Yongfu Zhu, a physician from the First Affiliated Hospital of Anhui University of Chinese Medicine (Hefei, China). Ginsenoside Rg1 (purity ≥98%, Z18S9L70220) and Ginsenoside Rb1 (purity ≥98%, J19IB217937) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). MCC950 (S8930) was purchased from Selleck Biotechnology, Ltd. (Houston, TX, USA). Paroxetine hydrochloride (PRX, H10950043) was purchased from Tianjin Smith Kline & French Laboratories, Ltd. FKBP5 (14155-1-AP) antibody was purchased from Proteintech Group, Inc. (Wuhan, China), and the antibodies against IKKα (R381289), NF-κB (222013), NLRP3 (R381207), IL-6 (MS20021UC), TNF-α (MS20028UC), and β-actin (8F10-G10) were purchased from Zen-Bioscience (Chengdu, China); Interleukin 1β (IL-1β) (Bioss, bs-0812R) was purchased from Beijing Biosynthesis Biotechnology (Beijing, China). Horseradish peroxidase-labeled anti-rabbit IgG (ZB-2301) and anti-mouse IgG (ZB-2305) antibodies were purchased from ZSGB-BIO (Beijing, China).

Detection of the main components of ADP using ultra-performance liquid chromatography (UPLC)

The ADP solution was prepared as previously described[6]. According to *Yixue Xinwu*, ginseng is the sovereign drug of ADP. Thus, we quantified the levels of the main ginsenosides Rg1 and Rb1 using UPLC. The ADP solution was separated on an ACQUITY UPLC® HSS T3 (2.1 mm × 100 mm, 1.8 μm) column using a mobile phase of water (A)-acetonitrile (B) at a flow rate of 0.2 mL/min under gradient elution: 0 to 4 min for 18% B, 4 to 7 min for 18% to 29% B, 7 to 12 min for 29% B, 12 to 15 min for 29% B to 60% B, 15 to 25 min for 60% B, 25 to 25.1 min for 60% to 82%, 25 to 30 min for 82% B. The column temperature was 30°C, the detection wavelength 203 nm, and the injection volume 10 μL. ADP without ginseng was used as a negative control. Standard working solutions of ginsenosides Rg1 and Rb1 were prepared, and standard curves were plotted to calculate the contents of ginsenosides Rg1 and Rb1 in ADP.

Animals

Fifty-four 8-week-old healthy male C57BL/6 mice (weighing 20–25 g) were purchased from Hangzhou Ziyuan Laboratory Animal Technology Co. (Animal certificate number: SCXK[Zhe] 2019-0004). Mice were habituated to (22 ± 2)°C temperature conditions and 45% to 65% humidity levels for 7 days, with a 12-hour light-dark cycle.

Implementation of the SPS model and drug administration

The SPS mouse model has been shown to mimic specific neuroendocrine and behavioral changes associated with PTSD[5–6,17–19]. Therefore, 7 days after adaptation to their environment, mice were subjected to SPS. Briefly, mice were restrained within a centrifuge tube for 2 h, during which a small aperture in the tail facilitated ventilation.

Subsequently, mice were released from the tube and transferred to a water-filled container maintained at a temperature of 20°C to 22°C for forced swimming for 20 min. Mice were rested for 15 min and were placed following drying of the fur in an anesthesia chamber containing 3% isoflurane until they became unconscious. When mice recovered and could move freely, they were transferred to a chamber with an electric grid where they received a single 2 mA, 2-second shock. Mice were then placed back into their home cage after stimulation. After completion of the behavioral tests, mice were anesthetized with isoflurane, decapitated, and the brain tissues were collected. We referred to the ARRIVE guidelines to justify the humane endpoint by maintaining 2% isoflurane anesthesia in mice[20]. All animal experiments were approved by the Animal Ethics Committee of Anhui University of Chinese Medicine (Approval No. AHUCM-mouse-2022014). To minimize animal numbers the G*power 3.1 software was used with the parameters α set at 0.05 and power ($1 - \beta$) set at 0.8 to calculate sample size based on data from our previous study[5].

Twelve mice were randomly divided into four groups for experiment 1 ($n = 3$): control (D0), day one (D1), day seven (D7), and day 14 (D14) after SPS. A schematic representation of the experimental protocol is shown in Figure 1A.

In experiment 2, mice were randomly divided into three groups ($n = 6$ each): control, SPS, and SPS + MCC950 (CP-456773, CRID3). MCC950 (NLRP3 inhibitor, 5 mg/kg in PBS) was injected intraperitoneally (ip)[21] 24 h after SPS, and equal volumes of saline were administered to the control and SPS groups for 14 days. After the final day, mice behaviors were evaluated using the open field and the elevated plus maze tests (Figure 1B).

In experiment 3, mice were randomly divided into five groups ($n = 8$ each): control, SPS, SPS + PRX, and SPS + ADP (SPS + ADP-H and SPS + ADP-L) groups. The ADP doses of 18.4 mg/kg/day (L) and 36.8 mg/kg/day (H) were selected based on our previous publication[6]. Therefore, the SPS + ADP groups were treated with 18.4 or 36.8 mg/kg/day of ADP by gavage 24 h after SPS treatment, respectively. The positive drug group received 10 mg/kg/day of PXR by gavage[22], and the control and SPS groups were administered an equal volume of saline. After 14 consecutive days of administration, open field, elevated plus maze, and fear memory tests were conducted (Figure 1C).

Open field test

Before the start of the experiment, mice were allowed to acclimatize for 20 min in the experimental environment. At the beginning of the test, each mouse was placed in the central area of the device and allowed to explore the box quietly and freely for 5 min. The open field was divided into 16 squares (4 × 4; 4 squares in the central area and 12 squares around the perimeter) using the SuperMaze software, and the trajectories of the mice were analyzed. After each experiment, the surfaces of the box were cleaned with alcohol.

Elevated plus maze test

The elevated plus maze was constructed as an X-shaped apparatus measuring 25 cm × 5 cm × 5 cm. It featured two opposing open arms, each 25 cm × 5 cm in size, two opposing closed arms, and a central platform measuring 5 cm × 5 cm. The height of the entire structure was 50 cm. Each mouse was placed in an open area facing the cross arms, and a 5-minute random exploration time was set on the elevated platform. The SuperMaze System recorded movements and calculated the percentage of time spent on open-arm exploration relative to the total time. After each trial, the interior and floor of the platform were disinfected with alcohol.

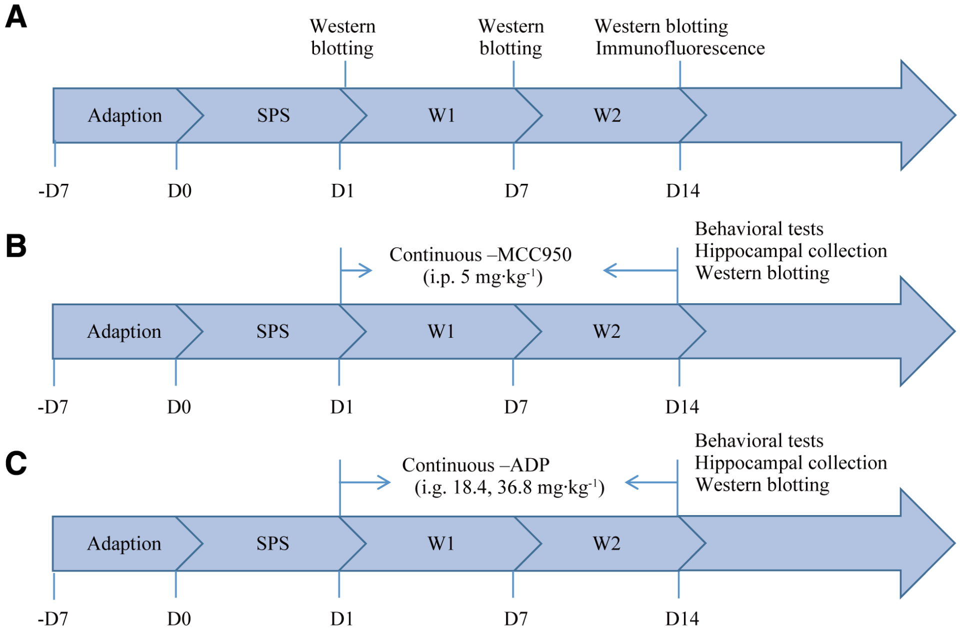

Figure 1. Schematic diagram of experimental protocols (A) experiment 1, (B) experiment 2, and (C) experiment 3. ADP: Anshen Dingzhi prescription; MCC950: CP-456773, CRID3; SPS: Single prolonged stress.

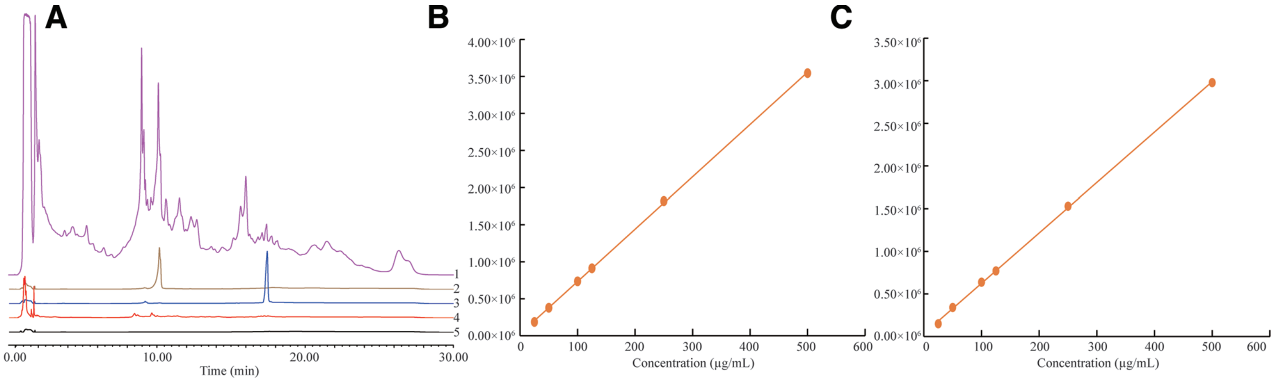

Figure 2. Quantifications of the major components in ADP by UPLC. (A) Representation chromatograms of ADP (1), Rg1 (2), Rb1 (3), ADP without ginseng (4), and blank solvent (5). (B–C) linear ranges of Ginsenosides Rg1 and Rb1. ADP: Anshen Dingzhi prescription; UPLC: Ultra performance liquid chromatography.

Fear memory test

The fear memory test consisted of adaptation, training, re-exposure, and extinction phases. The adaptation phase occurred on the first day, during which each mouse was acclimatized to the fear chamber for 5 min. The training period was on the second day. Mice were subjected to three electric shocks (1.0 mA, 2 seconds each, with 73–74-second intervals) after being placed in the box for 5 min. The re-exposure period was on day 3. Mice were repositioned in the box for 15 min without electric shocks. The freezing time of each mouse was recorded for 15 min, and mice behavior was analyzed every 3 min. The extinction period was on day 4. Mice were repositioned in the box for 5 min without shocks, and freezing behavior was recorded during the initial 3 min. The box was cleaned with 75% ethanol to eliminate odors left by the previous experimental mouse.

Western blotting

Brains were immediately placed on ice after decapitation. The hippocampus was rapidly separated on ice and homogenized in radio immunoprecipitation assay lysate and phenylmethanesulfonyl fluoride under ice-cold conditions. Homogenates were centrifuged at 12,000 rpm/min for 5 min at 4°C, and the supernatant was collected. The collected supernatant was divided and added to loading buffer in a 1:1 ratio and heat-denatured at 100°C for 10 min. Equal amounts of proteins were separated using sodium dodecyl sulfate -polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride membrane. After blocking with 5% skim milk for 2 h, membranes were incubated with the various antibodies against TNF-α (1:1,000), IL-6 (1:1,000), IL-1β (1:1,000), FKBP5 (1:1,000), IKKα (1:1,000), NF-κB (1:1,000), NLRP3 (1:1,000), and β-actin (1:1,000) for 14 to 16 hours at 4°C. Membranes were washed three times for 10 min using PBS with Tween-20 and incubated with a peroxidase-labeled secondary antibody for 2 h at room temperature (20°C–25°C). Finally, protein bands were visualized using enhanced chemiluminescence and analyzed using the ImageJ software.

Immunofluorescence

Briefly, mouse brains were immersed in 4% paraformaldehyde for fixation, followed by dehydration in 30% sucrose solution. Frozen, fixed brains were sectioned (20 μm thick), and brain slices were subjected to immunofluorescence staining. Sections were incubated with primary antibodies against FKBP5 (1:200) or NLRP3 (1:200) for 16 h. Sections were then washed three times with PBS for 15 min each, and incubated with the secondary antibody for another 2 h. Images were captured using an Olympus confocal microscope (F3000; Olympus, Tokyo, Japan). Mean fluorescence intensity for FKBP5 and NLRP3 staining in CA1 and dentate gyrus (DG) was quantified.

Statistical analysis

All data analysis was performed using the GraphPad Prism 8.0 statistical software and data are expressed as means ± standard error of the mean (SEM). An unpaired two-tailed Student t test was used to compare two independent experimental groups. One-way analysis of variance (ANOVA) followed by the Tukey test was performed to compare the differences among three or more groups. Statistical significance was set at $P < 0.05$.

Results

Content analysis of the main components in ADP

The levels of ginsenosides Rg1 and Rb1 in ADP were determined using UPLC. As shown in Figure 2A, ginsenosides Rg1 and Rb1 were well separated with UPLC. The standard curves for ginsenoside Rg1 ($y = 7{,}064.6x + 25{,}672$; $R^2 = 0.9999$; linear range: 25–500 μg/mL) and Rb1 ($y = 5{,}912x + 33{,}171$; $R^2 = 0.9997$; linear range: 25–500 μg/mL) were generated by plotting peak area as a function of concentration (Figure 2B–C). These data indicate that the specificity and linearity of the method satisfied the analytical requirements. The contents of ginsenosides Rg1 and Rb1 in ADP were (96.85 ± 1.14) and (9.04 ± 0.22) μg/g, respectively (means ± SEM of three experiments).

Increased expression of the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway in hippocampus of SPS mice

Using network pharmacology, we proposed that inflammatory factors could be potential targets in PTSD[7]. Western blot analysis was performed to evaluate the expression of inflammation-related proteins in hippocampus at various time points. On days one, seven, and 14 after SPS, the relative levels of IL-6 [(1.08 ± 0.10), (1.23 ± 0.08), and (1.41 ± 0.08), respectively], TNF-α [(1.20 ± 0.08), (1.26 ± 0.11), and (1.33 ± 0.04), respectively], and IL-1β [(1.09 ± 0.05), (1.13 ± 0.04), and (1.51 ± 0.07), respectively] were increased, and significant differences were observed for IL-6 ($P < 0.05$; $F[3, 16] = 5.62$), TNF-α ($P < 0.05$; $F[3, 16] = 4.49$), and IL-1β ($P < 0.05$, $F[3, 8] = 22.30$) in the D14 group, as compared with that in the D0 group (Figure 3A–D). These results point to IL-6, TNF-α, and IL-1β as potential contributors to the development of PTSD.

The FKBP5-IKKα-NF-κB-NLRP3 signaling pathway regulates neuroinflammation[15], and in this study, the protein expression of FKBP5-IKKα-NF-κB-NLRP3 signaling pathway after SPS was further explored by western blotting. Compared to the D0 group, the relative levels of FKBP5 [(1.04 ± 0.05), (1.17 ± 0.03), and (1.21 ± 0.04), respectively], IKKα [(1.01 ± 0.01), (1.10 ± 0.03), and (1.16 ± 0.02), respectively], NF-κB [(1.02 ± 0.09), (1.16 ± 0.09), and (1.41 ± 0.13), respectively], and NLRP3 [(1.18 ± 0.11), (1.17 ± 0.13), and (1.37 ± 0.02), respectively] gradually increased in the hippocampus of SPS mice, and significant differences were observed for FKBP5 ($P < 0.05$; $F[3, 12] = 7.57$), IKKα ($P < 0.05$, $F[3, 12] = 14.21$), NF-κB ($P < 0.05$; $F[3, 12] = 4.33$), and NLRP3 ($P < 0.05$, $F[3, 12] = 3.20$) in the D14 group, as compared to the D0 group (Figure 3E–I). Immunofluorescence analysis revealed a marked elevation in FKBP5 in CA1 ($P < 0.01$, t value = 10) and DG ($P < 0.01$, t value = 17) as well as in NLRP3 in CA1 ($P < 0.05$, t value = 3) and DG ($P < 0.05$, t value = 4.58) in the SPS group on day 14 relative to the control group. This result was consistent with the western blot results (Figure 3J–Q). These results suggest that the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway may participate in the development of PTSD by regulating inflammatory factors.

Prevention of PTSD-like behavior in SPS mice by NLRP3 inhibition

NLRP3 is a key target of FKBP5-IKKα-NF-κB-NLRP3 signaling pathway and directly regulates inflammatory factors[14]. We used the NLRP3 inhibitor MCC950 (ip injection, 5 mg/kg) to reduce FKBP5-IKKα-NF-κB-NLRP3 signaling pathway. As the results of experiment 1 revealed significant changes in inflammatory factors and FKBP5-IKKα-NF-κB-NLRP3 signaling pathway after 14 days of SPS, we tested the effects of MCC950 at 14 days after SPS. Compared with the SPS alone group, mice in the SPS + MCC950 group showed significant increases in the open field test in movement distance in the central area [SPS + MCC950 (3,889 ± 648.8) mm *vs.* SPS (1,401 ± 210.6); $P < 0.05$; $F[2, 15] = 7.42$] and time spent in the central region [SPS + MCC950 (28.50 ± 2.72) seconds *vs.* SPS (19.67 ± 1.94) seconds; $P < 0.05$; $F[2, 15] = 7.60$]; however, no significant differences were observed (*vs.* SPS; $P > 0.05$; $F[2, 15] = 0.97$) in total distance between the SPS + MCC950 (31,308 ± 3,292) mm and the SPS groups (25,591 ± 2,527) mm (Figure 4A–C). The elevated plus maze test results demonstrated that the time to enter the open arm was significantly elevated in the SPS + MCC950 (5 mg/kg) group (86.67 ± 9.32 seconds) as compared to the SPS group (43.50 ± 7.43 seconds) (*vs.* SPS; $P < 0.05$; $F[2, 15] = 14.97$) (Figure 4D–E). These findings indicate that NLRP3 inhibition improves PTSD-like behavior in SPS mice.

The results revealed that MCC950 (5 mg/kg) not only reduced the level of inflammatory cytokines, including IL-6 (*vs.* SPS; $P < 0.05$; $F[2, 15] = 9.98$; relative expression decreased from 1.20 ± 0.04 to 0.89 ± 0.08), TNF-α (*vs.* SPS; $P < 0.05$; $F[2, 6] = 15.03$; relative expression decreased from 1.19 ± 0.03 to 0.96 ± 0.05), and IL-1β (*vs.* SPS; $P < 0.05$; $F[2, 9] = 10.71$; relative expression decreased from 1.36 ± 0.06 to 1.13 ± 0.08), but also inhibited protein expressions of several upstream molecules, including FKBP5 (*vs.* SPS; $P < 0.05$; $F[2, 6] = 18.01$;

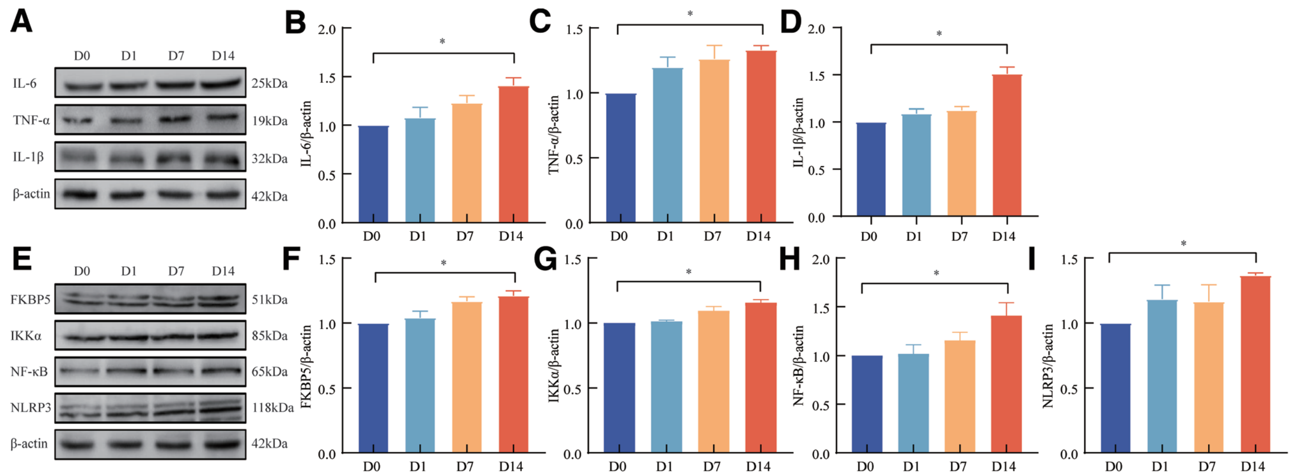

Figure 3. Aberrant expression of FKBP5-IKKα-NF-κB-NLRP3 signaling pathway was observable in the hippocampus of SPS mice. (A–D) The expression of inflammatory proteins IL-6, TNF-α, and IL-1β in the hippocampi was examined using western blotting. (E–I) The expression of FKBP5, IKKα, NF-κB, and NLRP3 proteins in the hippocampi was examined using western blotting. The expression of (J–M) FKBP5 and (N–Q) NLRP3 in the CA1 and DG regions of the hippocampi in control and D14 groups using immunofluorescence (shown by the orange arrows). Data are presented as mean ± SEM ($n = 3$). *$P < 0.05$ and **$P < 0.01$ indicates significant differences. CA1: Cornu ammonis 1; DG: Dentate gyrus; FKBP5-IKKα-NF-κB-NLRP3: Prolyl isomerase 5-IκB kinase alpha-nuclear factor kappa-B-NOD-like receptor thermal protein domain-associated protein 3; IL: Interleukin; SEM: Standard error of the mean; SPS: Single prolonged stress; TNF-α: Tumor necrosis factor-α.

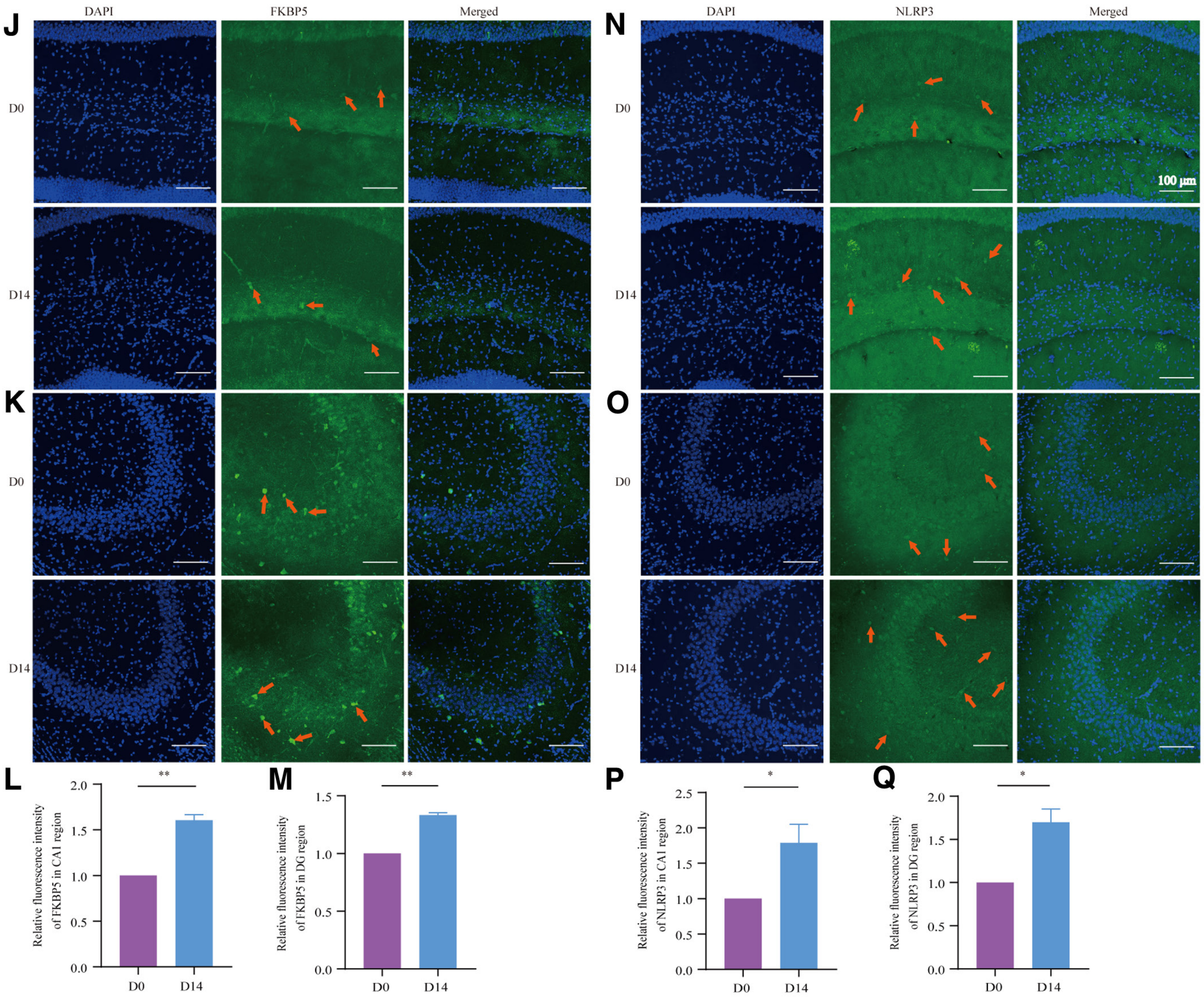

Figure 3. Continued

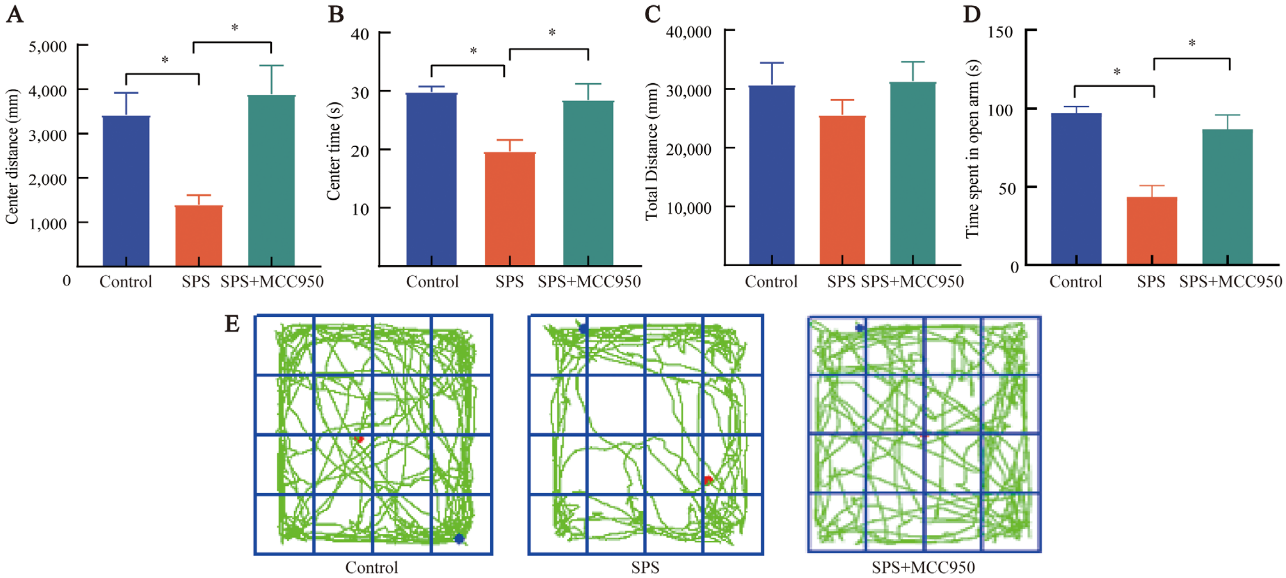

Figure 4. Inhibition of NLRP3 prevented PTSD-like behavior. (A) The central distance in open field test. (B) The time spent in the central area. (C) The total distance in open field test. (D) The time to enter the open arm. (E) The representative movement traces in open field test. Data are presented as mean ± SEM (n = 6). *P < 0.05 indicates a significant difference. MCC950: CP-456773, CRID3; NLRP3: NOD-like receptor thermal protein domain-associated protein 3; PTSD: Post-traumatic stress disorder; SEM: Standard error of the mean; SPS: Single prolonged stress.

relative expression decreased from [(1.25 ± 0.04) to (1.02 ± 0.05)], IKKα (*vs.* SPS; $P < 0.05$; $F[2, 9] = 9.98$; relative expression decreased from 1.20 ± 0.04 to 1.06 ± 0.04), NF-κB (*vs.* SPS; $P < 0.05$; $F[2, 12] = 5.54$; relative expression decreased from [(1.21 ± 0.05) to (0.97 ± 0.08)], and NLRP3 (*vs.* SPS; $P < 0.05$; $F[2, 9] = 8.67$); relative expression decreased from [(1.20 ± 0.04) to (1.03 ± 0.05)] (Figure 5A–I). The above results indicate that inhibition of the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway effectively reduces inflammation and prevents PTSD-like behavior.

Improved anxiety-like behavior and enhanced fear extinction in SPS mice by ADP

We used the open field and elevated plus maze tests to evaluate anxiety-like behaviors in mice. Compared to the control group, time spent in the central area by mice of the SPS group was significantly reduced [$P < 0.05$; $F[2, 21] = 7.08$; (27.38 ± 1.71) seconds *vs.* (18.75 ± 1.87) seconds], as was the distance traveled within the central region [$P < 0.05$; $F[2, 21] = 7.52$; (2,353.87 ± 201.80) mm *vs.* (1,349.92 ± 139.50) mm] (Figure 6A–B). Mice administered with ADP-H (36.8 mg/kg) exhibited significant increases in time spent in the central region [$P < 0.05$; $F[2, 21] = 7.08$; (18.75 ± 1.87) *vs.* to (25.25 ± 1.46) seconds] and distance traveled within the central area [$P < 0.05$; $F[2, 21] = 7.52$; (1,349.92 ± 139.50) mm *vs.* (2,022.02 ± 210.10) mm $P < 0.05$], as compared with mice in the SPS group; the PRX group was comparable to the ADP-H group while the ADP-L group was not statistically significant (Figure 5A–B). Notably, the total distance traveled by the mice in the open field test showed no significant difference across groups [SPS + ADP-H (13,872 ± 856.8) mm *vs.* SPS group (14,132 ± 958.3) mm; $P > 0.05$; $F[2, 21] = 0.19$] (Figure 6C–D), suggesting that the anxiolytic effect of ADP (36.8 mg/kg) was not related to the spontaneous activity of the mice. In the elevated plus maze test the time to enter the open arm was reduced by 37% (123.57 ± 7.53) *vs.* (77.67 ± 9.06) seconds in SPS mice as compared with control mice, while the time to enter the open arm was significantly increased by 44% (77.67 ± 9.06) *vs.* (111.68 ± 7.48) seconds in the SPS + ADP-H; it was also significantly elevated in the SPS + PRX group, as compared to the SPS group ($P < 0.05$; $F[2, 21] = 8.75$) (Figure 6E). These results suggest that ADP improves anxiety-like behavior in SPS mice and is more effective at high doses than at low doses.

The fear memory test indicated that during the re-exposure period, the freezing time of mice in each group gradually decreased with an increase in exposure time; however, no significant difference was observed between groups (Figure 6F). At day 1, the SPS group exhibited a prolonged freezing time (58.95 ± 7.75) seconds as compared to the control group (34.83 ± 3.89) seconds. Notably, the freezing time for mice in the SPS + ADP-H (36.8 mg/kg group) and the SPS + PRX (34.16 ± 5.08) seconds group were significantly reduced relative to the SPS group (SPS + ADP-H *vs.* SPS group; $P < 0.05$; $F[2, 21] = 5.93$). However, the lower dose of ADP (ADP-L) did not result in a statistically significant effect (Figure 6G). These results indicated that ADP promoted fear memory extinction in SPS mice.

Downregulation of hippocampal inflammatory protein levels and FKBP5-IKKα-NF-κB-NLRP3 signaling pathway in SPS mice by ADP

Downregulation of inflammation may be one of the main mechanisms by which ADP could improve PTSD. We assessed the expression of inflammation-related proteins in hippocampus using western blotting. ADP-H (36.8 mg/kg) significantly reversed the relative increase in IL-6 [(1.13 ± 0.03) to (0.89 ± 0.04); $P < 0.05$; $F[2, 9] = 17.63$], TNF-α [(1.27 ± 0.04) to (0.89 ± 0.08); $P < 0.05$; $F[2, 9] = 12.26$], and IL-1β [(1.22 ± 0.07) to (0.96 ± 0.03); $P < 0.05$; $F[2, 6] = 10.74$] in hippocampus

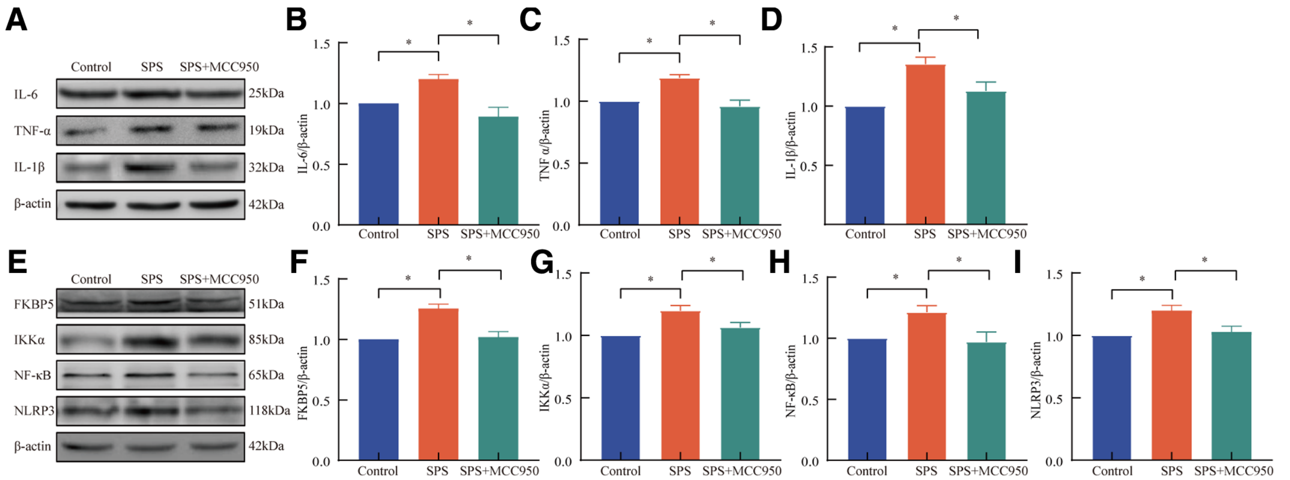

Figure 5. Inhibition of NLRP3 reversed the aberrant expression of inflammatory proteins and FKBP5-IKKα-NF-κB-NLRP3 signaling pathway. (A–D) The expression of inflammatory proteins IL-6, TNF-α, and IL-1β in the hippocampi was examined using western blotting. (E–I) The expression of FKBP5, IKKα, NF-κB, and NLRP3 proteins in the hippocampi was examined using western blotting. Data are presented as mean ± SEM ($n = 3$). *$P < 0.05$ indicates a significant difference. FKBP5-IKKα-NF-κB-NLRP3: Prolyl isomerase 5-IκB kinase alpha-nuclear factor kappa-B-NOD-like receptor thermal protein domain-associated protein 3; IL: Interleukin; MCC950: CP-456773, CRID3; SEM: Standard error of the mean; SPS: Single prolonged stress; TNF-α: Tumor necrosis factor-α.

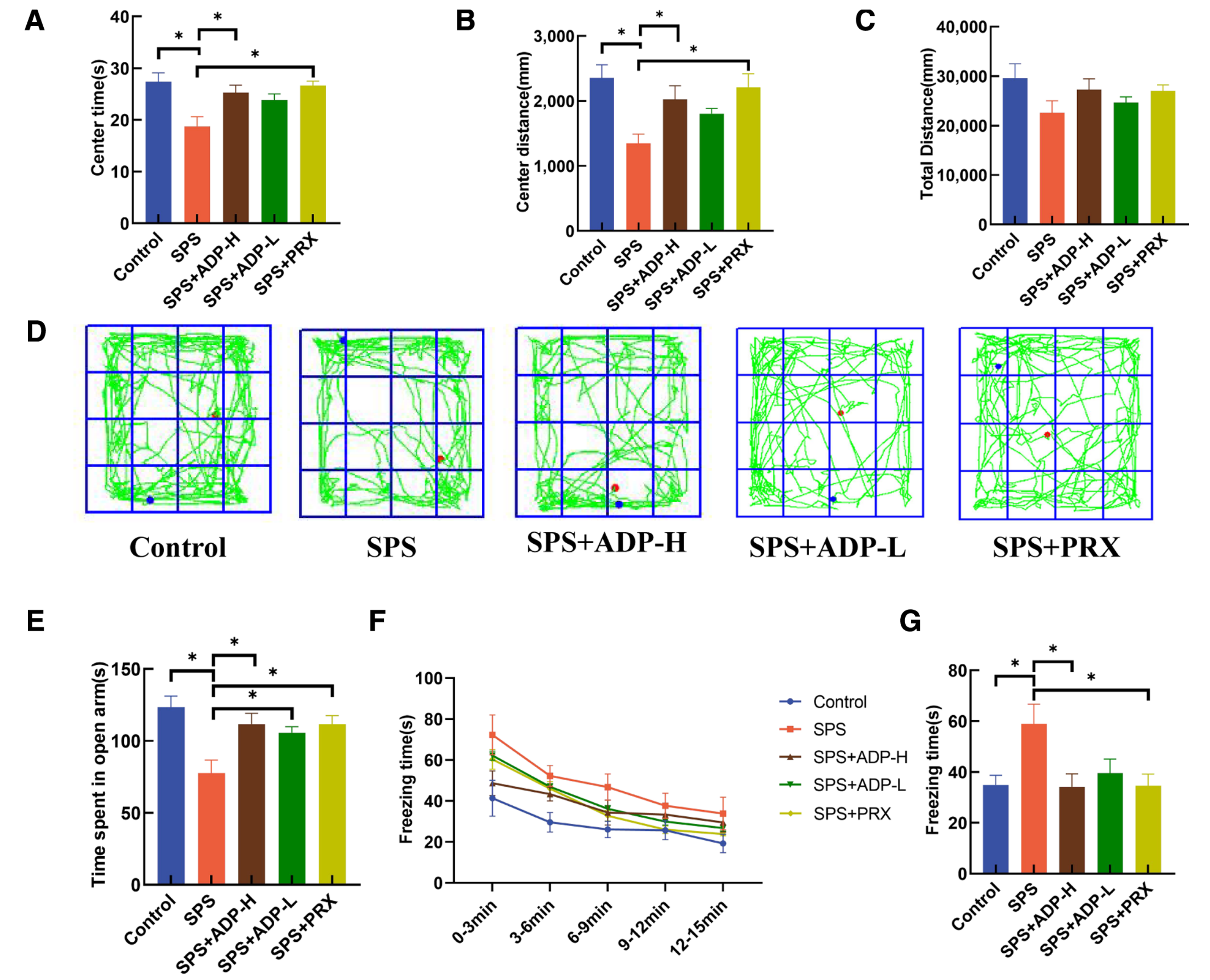

Figure 6. ADP improved anxiety-like behavior and promoted fear memory extinction in SPS mice. (A) The central distance in the open field test. (B) The movement time in the central region. (C) The total distance of movement in the open field test. (D) The representative movement traces in the open field test. (E) The time to enter the open arm. (F) The freezing time of 15 min of exposure. (G) The freezing time of 24 h after exposure. Data are presented as mean ± SEM ($n = 8$). *$P < 0.05$ indicates a significant difference. ADP: Anshen Dingzhi prescription; PRX: Paroxetine hydrochloride; SEM: Standard error of the mean; SPS: Single prolonged stress.

of mice with PTSD (Figure 7A–D). These results indicate that ADP can downregulate the PTSD-induced increased expression of IL-6, TNF-α, and IL-1β in hippocampus, thereby resulting in reducing negative symptoms of PTSD.

We also analyzed by western blots the levels of proteins in the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway that regulates inflammation. Protein levels of the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway were markedly increased in SPS mice as compared to the control group (Figure 7E–I). ADP-H significantly reversed the relative increase in FKBP5 [(1.15 ± 0.02) to (0.86 ± 0.04); $P < 0.05$; $F[2, 6] = 34.05$], IKKα [(1.19 ± 0.07) to (0.94 ± 0.03); $P < 0.05$; $F[2, 9] = 8.82$], NF-κB [(1.48 ± 0.15) to (0.74 ± 0.13); $P < 0.05$; $F[2, 12] = 10.34$], and NLRP3 [(1.20 ± 0.02) to (0.94 ± 0.06); $P < 0.05$; $F[2, 9] = 13.08$] in the hippocampus. The above results indicate that ADP reverses SPS-induced increased expression of proteins of the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway, thereby reducing inflammatory responses, and improving PTSD symptoms.

Discussion

This study investigated the changes in FKBP5-IKKα-NF-κB-NLRP3 signaling pathway in hippocampus of SPS mice and tested the effects of ADP treatment. Our data indicate that ADP treatment reversed SPS-induced increased expression of FKBP5-IKKα-NF-κB-NLRP3 signaling pathway and inflammation-related proteins and improved PTSD-like behaviors. Results of this study provide support for the use of ADP for PTSD treatment and its translational potential in clinical applications.

Neuroinflammation is an important mechanism underlying the development of PTSD. Pro-inflammatory markers increased after the trauma experienced by individual subjects, as levels of inflammatory biomarkers in the blood of patients with PTSD are significantly elevated compared with those in healthy participants[23–24]. In a previous study, we found that inflammatory factors were increased in the serum of SPS mice[11]. Additionally, levels of inflammatory factors are significantly increased across various brain regions in animals displaying PTSD-like behaviors[25]. Our prior research pinpointed several inflammatory factors

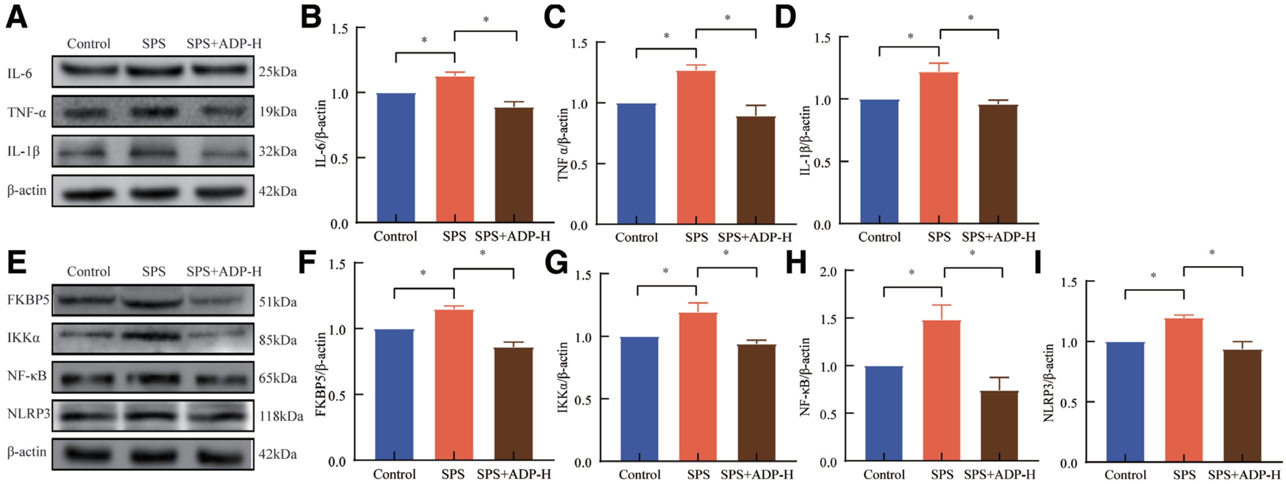

Figure 7. ADP suppressed expression of inflammatory proteins and FKBP5-IKKα-NF-κB-NLRP3 signaling pathway in the hippocampi of SPS mice. (A–D) The expression of inflammatory proteins IL-6, TNF-α, and IL-1β in the hippocampi was examined using western blotting. (E–I) The expression of FKBP5, IKKα, NF-κB, and NLRP3 proteins in the hippocampi was examined using western blotting. Data are presented as mean ± SEM (n = 3). *$P < 0.05$ indicates a significant difference. ADP: Anshen Dingzhi prescription; FKBP5-IKKα-NF-κB-NLRP3: Prolyl isomerase 5-IκB kinase alpha-nuclear factor kappa-B-NOD-like receptor thermal protein domain-associated protein 3; IL: Interleukin; SEM: Standard error of the mean; SPS: Single prolonged stress; TNF-α: Tumor necrosis factor-α.

that could serve as PTSD treatment targets, and our current findings indicate significantly elevated hippocampal levels of IL-6, TNF-α, and IL-1β in SPS *versus* control mice. Notably, expression of IL-6, TNF-α, and IL-1β increased with time following SPS. We also found that the immunoreactivity of the pro-inflammatory marker (IL-1β) increased in hippocampus following SPS in a time-dependent manner. Moreover, blocking IL-1β was previously found to be effective in improving stress-enhanced fear learning[26]. Previous studies have also shown that neuroinflammation was involved in the initiation and development of PTSD, and that pro-inflammatory activity signaling may play a key role in the development of PTSD.

NLRP3 is a potential target for regulating neuroinflammation, and its inhibition has been shown to alleviate anxiety-like behavior in mice with PTSD[27–28]. Activation of NLRP3 promotes IL-1β maturation and release[29]. In addition, NLRP3 regulates the expression of IL-1β, TNFα, and IL-6[30], which are involved in neuroinflammation onset and development. Furthermore, FKBP5 inhibits the assembly of the IKKα complex and the activation of NF-κB, is involved in NLRP3 activation, and thus regulates the pro-inflammatory factors IL-1β, IL-6, and TNF-α[31–32]. The findings of the present study indicate that NLRP3 protein levels progressively increased following SPS, and this is accompanied by an increase in protein expression in the FKBP5-IKKα-NF-κB signaling pathway. Treatment with MCC950, an NLRP3 inhibitor, reduced hippocampal inflammatory protein levels and alleviated anxiety-like behaviors in mice, which is consistent with prior findings[14]. These results suggest that reducing the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway can be effective for PTSD treatment.

Patients with PTSD are susceptible to stress, and FKBP5, an important stress-sensitive gene, is involved in the regulation of the stress process in patients with PTSD[33]. Notably, the FKBP5 protein complex is elevated in blood of patients with PTSD, and disruption of the FKBP5 complex reverses behavioral and molecular changes induced by fear conditioning in mice[32]. This fits well with our recent results showing that FKBP5 expression continued to increase after SPS[34]. In addition, FKBP5 activates NF-κB by interacting with IKKα[32]. The increase in FKBP5 protein was accompanied by an increase in levels of IKKα and NF-κB. NF-κB, a key regulator of inflammation, further regulates the expression of NLRP3. Our results showed that MCC950 treatment could inhibit the expression of FKBP5, IKKα, and NF-κB, the upstream molecules of NLRP3. The mechanism by which regulating downstream NLRP3 affects upstream FKBP5 is not yet clear. However, chronic inhibition of downstream molecules may cause compensatory reactions in upstream molecules. Similar results have been reported in previous studies. For example, NF-κB is a key transcription factor for the upregulation of NLRP3, yet there is a negative feedback regulation of NLRP3 with NF-κB[35]. Nevertheless, the potential underlying mechanisms require further investigation.

ADP is a TCM used to treat emotional disorders, which exhibit PTSD-like symptoms, such as anxiety, depression, and insomnia. Therefore, ADP could be a promising therapeutic agent for the treatment of PTSD[36]. The hippocampus is critical for encoding and accessing fear memories[37]. Our previous study found that ADP at a dose of 36.8 mg/kg/day alleviated hippocampal damage and improved PTSD-like behavior in mice[6]. In the present study, doses of 36.8 and 18.4 mg/kg/day of ADP were chosen to evaluate their impact on PTSD-like behaviors in SPS mice using the open field, elevated plus maze, and fear memory tests. The results demonstrated that ADP ameliorated PTSD-like behaviors in mice, with the 36.8 mg/kg/day dose proving particularly effective. This finding is consistent with prior findings[6], thus supporting the notion that a 36.8 mg/kg/day dose of ADP significantly improves PTSD-like behaviors in SPS mice.

ADP may also target hippocampal injury as a potential therapeutic approach for PTSD[6]. However, whether ADP modulates hippocampal inflammation to exert its

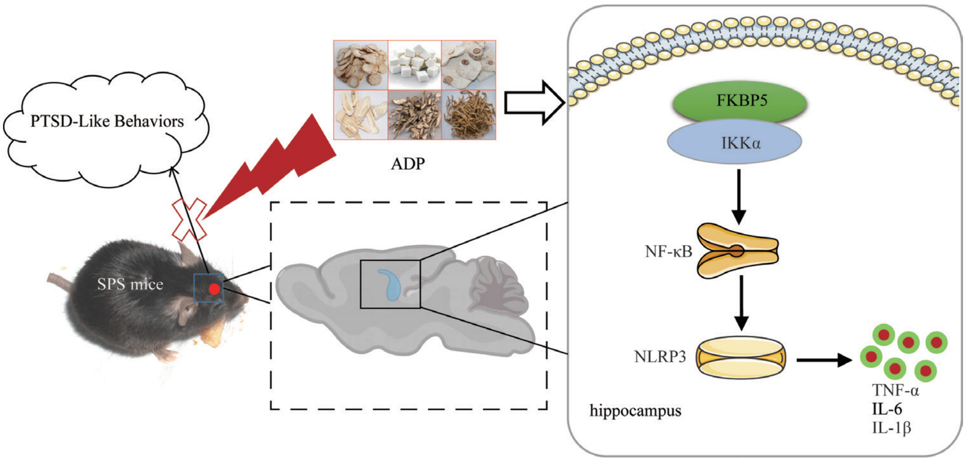

Figure 8. ADP regulates neuroinflammation *via* FKBP5-IKKα-NF-κB-NLRP3 signaling pathway to improve PTSD-like behavior. ADP: Anshen Dingzhi prescription; FKBP5-IKKα-NF-κB-NLRP3: Prolyl isomerase 5-IκB kinase alpha-nuclear factor kappa-B-NOD-like receptor thermal protein domain-associated protein 3; IL: Interleukin; PTSD: Post-traumatic stress disorder; SPS: Single prolonged stress; TNF-α: Tumor necrosis factor-α.

therapeutic effects in PTSD remains unclear. In the present study, ADP inhibited SPS-induced increased expression of inflammatory factors IL-6, TNF-α, and IL-1β in hippocampus, which may be due to the action of ADP active ingredients in hippocampus. Various ADP components, including tenuifolin, ginsenoside Rg1, poricoic acid B, ginsenoside Rb1, and α-asarone, were detected by UPLC-Q-TOF/MS in our previous study[6], and they all have anti-inflammatory activities. Ginsenosides Rg1 and Rb1, the major components of the main herb Ginseng Radix et Rhizoma in ADP, exert neuroprotective effects by suppressing inflammatory mediators in the striatum[18,38]. In our study, levels of Rg1 and Rb1 were found to be (96.85 ± 1.14) and (9.04 ± 0.22) μg/g, respectively, by using UPLC, which is in accordance with a previous study[6] and demonstrates the stability and reliability of the ADP preparation. Tenuifolin, a natural neuroprotective compound extracted from Polygalae Radix, also inhibits inflammatory factors and is considered a potentially effective neuroprotective agent[39]. Further studies are needed to explore the mechanism and material basis of ADP intervention in neuroinflammation.

The FKBP5-IKKα-NF-κB-NLRP3 signaling pathway has been found to regulate neuroinflammation in mice with PTSD, and investigating whether ADP inhibits neuroinflammation through FKBP5-IKKα-NF-κB-NLRP3 signaling pathway is important. Our studies demonstrated that ADP inhibited protein expression in the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway. These results indicate that ADP inhibits the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway to exert an anti-inflammatory effect, thereby alleviating PTSD-like behaviors in mice. We propose that the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway is involved in PTSD, and that ADP, by acting on the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway improves PTSD-like behaviors caused by SPS (Figure 8). In future studies, we will identify the exact components that may directly target this signaling pathway.

Nonetheless, this study had some limitations. First, this study only used male animals, because estrogen is known to affect behavior, and estrogen levels in female animals vary during the estrus cycle[40–41]. Thus, it remains unclear whether the mechanism we are proposing is applicable to female animals. Second, additional processes, including neuronal cell death and synaptic function, are likely to be regulated by the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway, and therefore, the contribution of these processes in the beneficial effects of ADP needs to be further explored.

Conclusion

Our study demonstrated that ADP improves SPS-induced PTSD-like behaviors and suppresses neuroinflammation in mice through the regulation of the FKBP5-IKKα-NF-κB-NLRP3 signaling pathway. This study provides an important basis for the clinical treatment of PTSD with ADP.

Conflict of interest statement

Xiaohe Xiao is editorial board members of this journal. The other authors declare no conflict of interest.

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Author contributions

Daokang Chen, Jiamin Hu, and Shaojie Yang performed the experiments and analyzed the data. Daokang Chen and Jiamin Hu drafted and revised the manuscript. Manman Ji, Pan Xie, and Zhengrong Zhang contributed to the discussion of this study and the revision of this manuscript. Michel Baudry helped to revise the manuscript. Sheng Zhang and Guoqi Zhu conceived and designed this study, wrote and revised the manuscript, conducted the project administration, and acquired the funding grants.

Ethical approval of studies and informed consent

The animal study was reviewed and approved by the Experimental Animal Ethics Committee of the Anhui University of Chinese Medicine (Approval No. AHUCM-mouse-2022014).

Acknowledgments

None.

Data availability

All data generated or analyzed in this study are included in this published article; further inquiries can be directed to the corresponding authors.

References

[1] He M, Wei JX, Mao M, et al. Synaptic plasticity in PTSD and associated comorbidities: the function and mechanism for diagnostics and therapy. *Curr Pharm Des* 2018;24(34):4051–4059.
[2] Chou PH, Ito M, Horikoshi M. Associations between PTSD symptoms and suicide risk: a comparison of 4-factor and 7-factor models. *J Psychiatr Res* 2020;129:47–52.
[3] Kessler RC, Aguilar-Gaxiola S, Alonso J, et al. Trauma and PTSD in the WHO World Mental Health Surveys. *Eur J Psychotraumatol* 2017;8(5):1353383.
[4] Qu Y, Wu H, Zhu G, et al. The improving effect and mechanisms of Anshen Dingzhi Prescription on Alzheimer's disease-like behavior induced by D-galactose combined with AβO in mice. *Acta Pharm Sin* 2024;59(1):119–134.
[5] Wang J, Zhao P, Cheng P, et al. Exploring the effect of Anshen Dingzhi prescription on hippocampal mitochondrial signals in single prolonged stress mouse model. *J Ethnopharmacol* 2024;323:117713.
[6] Yang S, Qu Y, Wang J, et al. Anshen Dingzhi prescription in the treatment of PTSD in mice: Investigation of the underlying mechanism from the perspective of hippocampal synaptic function. *Phytomedicine* 2022;101:154139.
[7] Zhang Z, Gao F, Yang S, et al. Mechanism of Anshen Dingzhi prescription intervening against anxiety-like behavior in post-traumatic stress disorder: an analysis based on network pharmacology and molecular docking. *J Anhui Univ Chinese Med* 2023;42(4):74–80.
[8] Florido A, Velasco ER, Monari S, et al. Glucocorticoid-based pharmacotherapies preventing PTSD. *Neuropharmacology* 2023;224:109344.
[9] Peruzzolo TL, Pinto JV, Roza TH, et al. Inflammatory and oxidative stress markers in post-traumatic stress disorder: a systematic review and meta-analysis. *Mol Psychiatry* 2022;27(8):3150–3163.
[10] Holmes SE, Scheinost D, Finnema SJ, et al. Lower synaptic density is associated with depression severity and network alterations. *Nat Commun* 2019;10(1):1529.
[11] Ji M, Zhang ZR, Zhu GQ. Mechanism of cuculigoside against PTSD-induced inflammatory response in mouse hippocampus. *J Biol* 2022;39(6):14–19.
[12] He W, Hu Z, Zhong Y, et al. The potential of NLRP3 inflammasome as a therapeutic target in neurological diseases. *Mol Neurobiol* 2023;60(5):2520–2538.
[13] Yao H, Zhang D, Yu H, et al. Gut microbiota regulates chronic ethanol exposure-induced depressive-like behavior through hippocampal NLRP3-mediated neuroinflammation. *Mol Psychiatry* 2023;28(2):919–930.
[14] Dong Y, Li S, Lu Y, et al. Stress-induced NLRP3 inflammasome activation negatively regulates fear memory in mice. *J Neuroinflammation* 2020;17(1):205.
[15] Hao W, Wang L, Li S. FKBP5 regulates RIG-I-mediated NF-kappaB activation and influenza a virus infection. *Viruses* 2020;12(6):672.
[16] Han L, Yan ZL, Liu CZ. Effects of Anshen Dingzhi prescription on tryptophan metabolic pathway and exercise capacity in rats with chronic fatigue syndrome. *Chinese Trad Patent Med* 2021;43(12):3493–3497.
[17] Wang J, Gao F, Cui S, et al. Utility of 7,8-dihydroxyflavone in preventing astrocytic and synaptic deficits in the hippocampus elicited by PTSD. *Pharmacol Res* 2022;176:106079.
[18] Gao F, Wang J, Yang S, et al. Fear extinction induced by activation of PKA ameliorates anxiety-like behavior in PTSD mice. *Neuropharmacology* 2023;222:109306.
[19] Xie P, Chen L, Wang J, et al. Polysaccharides from Polygonatum cyrtonema Hua prevent post-traumatic stress disorder behaviors in mice: mechanisms from the perspective of synaptic injury, oxidative stress, and neuroinflammation. *J Ethnopharmacol* 2024;319(1):117165.
[20] Leary S, Underwood W, Anthony R, et al. AVMA guidelines for the euthanasia of animals: 2013 edition. 2013. Available from: https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx. Accessed July 2023.
[21] Hoffmann J, Luxan G, Abplanalp WT, et al. Post-myocardial infarction heart failure dysregulates the bone vascular niche. *Nat Commun* 2021;12(1):3964.
[22] Huang ZL, Liu R, Bai XY, et al. Protective effects of the novel adenosine derivative WS0701 in a mouse model of posttraumatic stress disorder. *Acta Pharmacol Sin* 2014;35(1):24–32.
[23] Speer K, Upton D, Semple S, et al. Systemic low-grade inflammation in post-traumatic stress disorder: a systematic review. *J Inflamm Res* 2018;11:111–121.
[24] Tursich M, Neufeld RW, Frewen PA, et al. Association of trauma exposure with proinflammatory activity: a transdiagnostic meta-analysis. *Transl Psychiatry* 2014;4(7):e413.
[25] Levkovitz Y, Fenchel D, Kaplan Z, et al. Early post-stressor intervention with minocycline, a second-generation tetracycline, attenuates post-traumatic stress response in an animal model of PTSD. *Eur Neuropsychopharmacol* 2015;25(1):124–132.
[26] Jones ME, Lebonville CL, Barrus D, et al. The role of brain interleukin-1 in stress-enhanced fear learning. *Neuropsychopharmacology* 2015;40(5):1289–1296.
[27] Guan Y, Han F. Key mechanisms and potential targets of the NLRP3 inflammasome in neurodegenerative diseases. *Front Integr Neurosci* 2020;14:37.
[28] Nie Y, Huang MY, Yang TY, et al. Ferulic Acid Reduces Inflammatory Response Induced by Radiation through Sirt1-NLRP3 Pathway. *Acupunct Herb Med*. 2024;4(3):367–374.
[29] Guan X, Zhu S, Song J, et al. Microglial CMPK2 promotes neuroinflammation and brain injury after ischemic stroke. *Cell Rep Med* 2024;5(5):101522.
[30] Yang L, Xu HH, Hong Q, et al. Crocus sativus L. produces anti-inflammatory effects and regulates NLRP3–NF-κB pathway.. *Acupunct Herb Med*. 2024;4(3):375–385.
[31] Wang X, Lin C, Jin S, et al. Cannabidiol alleviates neuroinflammation and attenuates neuropathic pain via targeting FKBP5. *Brain Behav Immun* 2023;111:365–375.
[32] Li H, Su P, Lai TK, et al. The glucocorticoid receptor-FKBP51 complex contributes to fear conditioning and posttraumatic stress disorder. *J Clin Invest* 2020;130(2):877–889.
[33] Li G, Wang L, Zhang K, et al. FKBP5 genotype linked to combined PTSD-depression symptom in Chinese earthquake survivors. *Can J Psychiatry* 2019;64(12):863–871.
[34] Zannas AS, Jia M, Hafner K, et al. Epigenetic upregulation of FKBP5 by aging and stress contributes to NF-kappaB-driven inflammation and cardiovascular risk. *Proc Natl Acad Sci U S A* 2019;116(23):11370–11379.
[35] Afonina IS, Zhong Z, Karin M, et al. Limiting inflammation-the negative regulation of NF-kappaB and the NLRP3 inflammasome. *Nat Immunol* 2017;18(8):861–869.
[36] Liu X, Xie DJ, Wang X, et al. Clinical observation on Anshen Dingzhi Pill combined with escitalopram in the treatment of panic disorder. *Clin J Trad Chinese Med* 2019;31(12):2281–2284.
[37] Jimenez JC, Berry JE, Lim SC, et al. Contextual fear memory retrieval by correlated ensembles of ventral CA1 neurons. *Nat Commun* 2020;11(1):3492.

[38] Lin J, Gao S, Wang T, et al. Ginsenoside Rb1 improves learning and memory ability through its anti-inflammatory effect in Aβ1-40 induced Alzheimer's disease of rats. *Am J Transl Res* 2019;11(5):2955–2968.
[39] Jiang N, Zhang Y, Yao C, et al. Tenuifolin ameliorates the sleep deprivation-induced cognitive deficits. *Phytother Res* 2023;37(2):464–476.
[40] Gegenhuber B, Wu MV, Bronstein R, et al. Gene regulation by gonadal hormone receptors underlies brain sex differences. *Nature* 2022;606(7912):153–159.
[41] Krause WC, Rodriguez R, Gegenhuber B, et al. Oestrogen engages brain MC4R signalling to drive physical activity in female mice. *Nature* 2021;599(7883):131–135.