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Original Article

Activation of aryl hydrocarbon receptor (AhR) alleviates depressive-like behaviors in LPS-induced mice

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Abstract: The role of the aryl hydrocarbon receptor (AhR) in regulating oxidative stress and immune responses has been increasingly recognized. However, its involvement in depression and the underlying mechanisms remain poorly understood. This study aimed to investigate the effect of 6-formylindolo[3,2-b]carbazole (FICZ), an endogenous AhR ligand, on a lipopolysaccharide (LPS)-induced depression model and the underlying mechanism. After being treated with FICZ (50 mg/kg), male C57BL/6J mice received intraperitoneal injection of LPS and underwent behavioral tests 24 h later. The levels of inflammatory cytokines, including IL-1β, IL-6, and TNF-α, were measured in the hippocampus and serum using enzyme-linked immunosorbent assay (ELISA). The expression levels of CYP1A1, AhR and NLRP3 were analyzed using qPCR and Western blot. The results showed that, compared with control group, LPS alone significantly down-regulated the expression levels of CYP1A1 mRNA and AhR protein in the hippocampus of mice, reduced glucose preference, prolonged immobility time in forced swimming test, increased IL-6 and IL-1β levels in the hippocampus, increased serum IL-1β level, and up-regulated NLRP3 mRNA and protein expression levels in mouse hippocampus, while FICZ significantly reversed the aforementioned effects of LPS. These findings suggest that AhR activation attenuates the inflammatory response associated with depression and modulates the expression of NLRP3. The present study provides novel insights into the role of AhR in the development of depression, and presents AhR as a potential therapeutic target for the treatment of depression.

Key words: aryl hydrocarbon receptor; NLRP3 inflammasome; IL-1β; IL-6; depression; inflammation

芳香烃受体(AhR)激活改善LPS诱导的小鼠抑郁样行为

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摘 要：芳香烃受体(aryl hydrocarbon receptor, AhR)在调节氧化应激和免疫反应中的作用越来越被人们所认识，但它在抑郁症中的作用和潜在机制仍不明了。本研究旨在研究内源性AhR配体6-甲酰基吲哚并[3,2-b]咔唑(6-formylindolo[3,2-b]carbazole, FICZ)对脂多糖(lipopolysaccharide, LPS)诱导的小鼠抑郁症模型的作用及其机制。FICZ (50 mg/kg)处理后，雄性C57BL/6J小鼠腹膜内注射LPS，并在24 h后进行行为学试验。用酶联免疫吸附试验(enzyme-linked immunosorbent assay, ELISA)检测小鼠海马和血清中炎症细胞因子(IL-6、IL-1β和TNF-α)浓度，用qPCR和Western blot检测小鼠海马CYP1A1、AhR和NLRP3表达变化。结果显示，相比对照组，单独LPS处理显著下调小鼠海马CYP1A1 mRNA和AhR蛋白表达水平，降低小鼠糖水偏好，延长强迫游泳试验中静止不动时间，提高小鼠海马IL-6和IL-1β水平，提高小鼠血清IL-1β水平，上调小鼠海马NLRP3 mRNA和蛋白表达水平，而FICZ可显著逆转LPS的上述作用。以上结果提示，AhR激活可减弱与抑郁症相关的炎症反应，其机制是下调炎症小体关键成分NLRP3的表达。本研究为AhR在抑郁症发病机制中的作用提供了新的见解，提示AhR是治疗抑郁症的潜在靶点。

关键词：芳香烃受体；NLRP3炎性小体；IL-1β；IL-6；抑郁症；炎症

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Depression has grown into an illness with a huge socio-economic impact, as indexed by disability-adjusted life years (DALYs), and it's on track to become the world's second most serious disease (WHO, 2006). The Diagnostic and Statistical Manual of Mental Disorders (DSM-5, 2013) defines major depressive episodes based on a specific set of signs and symptoms. Individuals with major depression frequently encounter alterations in sleep patterns, appetite, psychomotor activity, cognition, and mood. It is estimated that major depressive disorder affects approximately 3.8% of the global population, equating to around 280 million people living with this disorder (WHO, 2017) [1]. Currently, selective serotonin reuptake inhibitors (SSRIs) are commonly employed as antidepressants, however, their therapeutic efficacy is constrained, and the development of a novel class of antidepressants continues to present a challenge. Several hypotheses have elucidated the nature of depression, encompassing the monoamine hypothesis, hypothalamic-pituitary-adrenal (HPA) axis hypothesis, inflammation hypothesis, neuroplasticity, and neurogenesis [2]. It is suggested that individuals with depression have elevated inflammatory markers and meanwhile those with autoimmune diseases have a heightened susceptibility to developing depression. The occurrence of treatment- resistant depression has been associated with immune system dysfunction, characterized by an imbalance between pro-inflammatory and anti- inflammatory cytokines. Studies have demonstrated that patients with treatment-resistant depression exhibit a decrease in inflammatory cytokine levels in their peripheral blood, in comparison to their levels prior to treatment [3, 4]. Elevated plasma levels of inflammatory cytokines (most commonly IL-1β, IL-6, and TNF-α) are associated with more severe depressive symptoms [5]. These cytokines sensitize the HPA axis, disrupt negative feedback loops, and further exacerbate the inflammatory response, leading to impairment in the plasticity and adaptability of neural circuits [6]. Consistent findings from magnetic resonance imaging (MRI) studies show a reduction in hippocampal volume in patients with depression [7]. The hippocampus plays an important role in stress-induced depression [8, 9]. The hippocampus is crucial for learning and memory, which are known to be impaired during depression, and also possesses a distinctive ability to produce new neurons from neural stem cells [10]. Hippocampal neurogenesis deficiencies have been observed in animal models of depression and individuals diagnosed with depressive disorders [11]. Therefore, the hippocampus is being recognized as a brain structure that significantly contributes to the development of depressive disorders. Stress-susceptible mice exhibit depression-like behavior associated with adrenocortical activation, accompanied by an increase in IL-1β levels and reduced neurogenesis in the hippocampus [12]. Studies conducted on animal models have confirmed that hippocampal volume decreases in severely depressed individuals compared to non-depressed individuals [13]. Postmortem investigations have revealed that the volume of the dentate gyrus (DG) in untreated depressed patients is approximately half that of non- depressed controls and treated depressed patients [14]. Aryl hydrocarbon receptor (AhR) is a transcription factor that is activated by certain molecules and belongs to the basic helix-loop-helix family of transcription factors [15]. In inactive state, AhR exists in the cytoplasm, forming a complex with various partner proteins. When it binds to a ligand, it becomes activated and dissociates from the cytoplasmic protein complex, entering the nucleus through the nuclear pore. It then forms a dimer with the AhR nuclear translocator (ARNT). This dimer can bind to xenobiotic response elements (XRE) in DNA, promoting the transcription and expression of target genes. At the same time, the induced transcription of target genes can promote the metabolic clearance of AhR ligands through oxidation, thereby maintaining homeostasis. Binding of an agonist enhances the AhR's affinity for its cognate response element, leading to the expression of AhR-responsive genes, primarily those belonging to the CYP1 P450 family, such as CYP1A1, CYP1A2, and CYP1B1 [16–20]. Several studies have presented evidence of the important role played by AhR in the modulation of oxidative stress and immune responses [21–23]. AhR is expressed not only in immune barriers such as the skin and intestine, but also in various regions of the brain, including the nucleus of the lateral olfactory tract, islands of Calleja, hippocampus, medial geniculate nucleus, dorsolateral geniculate nucleus, and raphe nucleus [24]. Indeed, a common characteristic of the majority of AhR ligands is their lipophilic nature, enabling them to cross the blood-brain barrier and exert an impact on brain physiology [25].

AhR interacts with diverse array of endogenous and exogenous ligands, includes microbial metabolites, tryptophan metabolites, indole-3-carbinol (I3C),

6-formylindolo[3,2-b]carbazole (FICZ), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Studies have shown that FICZ, an AhR agonist, enhances hippocampal- dependent memory and learning tasks, suggesting its positive effects on short- and long-term memory, as well as learning skills [26, 27]. Additionally, the oral intake of Diosmin, another AhR agonist, activates AhR, resulting in significant expression of neprilysin and enhanced Aβ degradation, thereby potentially mitigating Alzheimer's disease and positioning it as a therapeutic target [28]. Furthermore, in a chronic unforeseeable mild stress female mice model, the oral administration of 3,3'-diindolylmethane (DIM) and 1,4-dihydroxy-2-naphthoic acid (1,4-DHNA) has been shown to prevent mood disorders [29]. Based on the aforementioned studies, it can be inferred that AhR activation has positive effects on neuropsychiatric diseases.

As a sensor for cell perception of the external environment, AhR has been most extensively studied in connection with the environmental pollutant TCDD [30, 31], which is closely associated with environmental and atmospheric pollution. Such substances typically originate from outside organisms, and prolonged exposure can lead to toxic reactions, tissue damage, and highly carcinogenic effects. Research indicates that long-term exposure to TCDD can result in neurotoxicity, affecting the morphology of the brain [30]. In addition to exogenous activators, there are also numerous endogenous activators of AhR. Tryptophan can be metabolized by indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) to produce kynurenine, which itself exhibits a certain level of AhR agonist activity [32, 33]. Furthermore, kynurenine can be further metabolized into kynurenic acid, which possesses a higher potency as an AhR agonist. Tryptophan can also generate a highly potent AhR agonist, ITE, 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester, through oxidative reactions, or generate FICZ through UV irradiation [23]. Different AhR ligands may exhibit different biological responses under specific conditions, increasing the complexity of research and also expanding the potential for immune regulation of the AhR signaling pathway. Additionally, other two classes of receptor agonists, selective AhR modulators (SAhRMs) and rapidly metabolized AhR ligands (RMAhRLs), are rapidly degradable and lack TCDD-like toxicity. They can bypass toxicity effect, thereby achieving beneficial therapeutic effects [16].

FICZ, the potent endogenous agonist and SAhRM, has been identified as a regulator of both anti-inflammatory and pro-inflammatory cytokines in various disease models [20, 34–37]. AhR plays an important role in modulating the inflammatory response mediated by lipopolysaccharide (LPS), as evidenced by increased susceptibility to LPS-induced endotoxin shock in mice lacking the AhR gene compared to normal mice [38]. Additionally, AhR is involved in IDO1 phosphorylation, which is closely associated with depression [39]. Moreover, AhR plays a pivotal role in regulating the function of various enzymes, including IDO, TDO, kynureninase (KYNU), and kynurenine 3-monooxygenase (KMO), which are responsible for modulating tryptophan metabolism, thereby impacting immune function and serotonin (5-HT) synthesis [32, 40]. Activation of AhR by FICZ leads to sustained inflammation of the mucosal tissue by directly suppressing Th1 cell responses and increasing the expression of IL-22 [36, 41]. In dextran sulfate sodium (DSS)-induced model, FICZ administration increases IL-10 levels and decreases interferon γ (IFN-γ) levels [42]. Previous studies have shown that AhR knockout macrophages exhibit increased expression of pro-inflammatory cytokines, such as IL-6 and TNF-α, and facilitate caspase-3 activation [38, 43]. Furthermore, FICZ has been found to induce the production of IL-13, TNF-α, and regulatory IL-17 cytokines, in addition to IL-10 and IL-6 [44, 45]. Activation of AhR inhibits NLRP3 expression, caspase-1 activation, and subsequent secretion of IL-1β, as demonstrated in studies by Khan and Langmann [46, 47]. Consequently, Khan and Langmann have proposed that AhR may mitigate depression by down-regulating NLRP3.

Although the protective effect of activating AhR on neuropsychiatric diseases in the brain has been found, there has been limited discourse on its underlying mechanism. Therefore, our objective was to investigate the mechanism through which AhR activation exerts its anti-depressant effects, based on the theory of inflammation. The study aimed to explore the potential of the AhR agonist FICZ in preventing and reversing depressive-like symptoms induced by LPS in mice. To test this hypothesis, a series of behavioral tests were conducted on adult C57BL/6J mice, while various cytokine levels and NLRP3 expression were detected in their hippocampus and serum. By exploring the relationship between AhR and depression, we would provide exper-

imental basis and ideas for the development of new targets for antidepressant treatment.

1 MATERIALS AND METHODS

1.1 *Animal experiment*

Male C57BL/6J mice, weighing 21–23 g and aged 6–7 weeks, were obtained from the Experimental Animal Center of Naval Medical University in Shanghai, China. They were kept in a controlled environment at a constant ambient temperature of (22 ± 2) °C, under a 12-hour light/dark cycle, and had unrestricted access to food and tap water throughout the experiment. This research was approved by the Ethics Committee of PLA Naval Medical University. All experimental protocols adhered to the guidelines set by the Ethics Committee of the Naval Medical University, with the aim of minimizing any potential suffering experienced by the animals.

1.2 *Experimental design for drug treatment*

Prior to formal experiments, the mice were randomly divided into three groups, each consisting of an average of 8–10 mice: control (CON) group (treated with normal saline), LPS (0.83 mg/kg) treated group (LPS group), and LPS + FICZ (50 mg/kg) treated group (FICZ group). The experimental design is illustrated in Fig. 1. The LPS and FICZ were purchased from Sigma-Aldrich, USA. FICZ was dissolved in olive oil at a concentration of 1 mg/mL and administered at a dosage of 50 mg/kg body weight every other day for one week by intraperitoneal injection [23, 26]. FICZ treatment began one week prior to LPS injection. 24 h after the intraperitoneal injection of LPS, the mice underwent behavioral tests, including sucrose preference test (SPT), tail suspension test (TST) and forced swimming test (FST). Upon completion of all behavioral tests, the mice were anesthetized with isoflurane, and blood and tissue samples were collected and promptly stored at −80 °C.

1.3 *Behavioral tests*

1.3.1 *SPT*

This test aimed to gauge the mice's preference for sweetness, as it could serve as an indicator of anhedonia [48]. Prior to the experiment, mice were acclimatized to two bottles of sucrose solution with a concentration of 1% (weight/volume) for one week. Following the familiarization period, the mice were housed individually to prevent any potential interference or competition for the bottles. To avoid any bias towards either side, the bottles were switched every two days. During the test (18:00–18:00+), the mice were deprived of food, but had access to two bottles of tap water and sucrose solution [49].

1.3.2 *TST*

Previous studies have extensively utilized the TST to assess despair behavior in rodent models [50]. The duration of immobility of mice was measured using the Tail Suspension PHM-300 equipment (Med Associates Inc., USA). The TST procedure involved affixing a piece of medical tape around the tail of the mouse, approximately 1 cm from the tip. Subsequently, the tape was secured to the instrument's hook, enabling the mice to hang upside down within a dark enclosure. To prevent the mice from climbing, a 1.5 cm diameter plastic tube was attached to their tails. The duration time recorded was 6 min, and immobility time during the last 5 min was counted, with a minimum threshold value of 0.5. To mitigate the impact of odor between each trial, 75% ethanol was employed.

1.3.3 *FST*

FST was utilized to evaluate signs of despair associated with depressive-like behaviors [51, 52]. During the experiment, the mice were carefully introduced into a transparent cylindrical container, which had a diameter of 10 cm and a height of 25 cm. The container was filled with warm water maintained at a temperature of (25 ± 1) °C. The water depth was approximately 16–18 cm, allowing the mice to freely move and swim. To ensure accurate monitoring of the mice's activities, video tracking equipment was utilized (Shanghai Xinruan Info Technology Co., Ltd., China). The whole process lasted 6 min and the immobility time of the final 5 min was recorded.

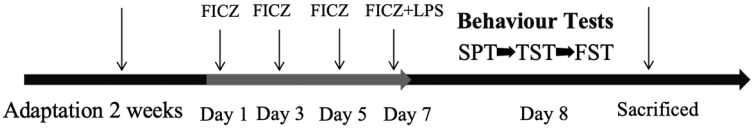

Fig. 1. Experiment time of injections and behavior tests. FICZ, 6-formylindolo[3,2-b]carbazole; SPT, sucrose preference test; TST, tail suspension test; FST, forced swimming test.

1.4 *Sample collection*

Following the completion of the behavioral tests, the animals underwent anesthesia induction using isoflurane (RWD, Shenzhen, China). Once the mice were under anaesthesia, blood and tissue samples were collected and promptly stored at −80 °C for further analysis. The hippocampi were carefully and rapidly isolated from the brain on ice. After isolation, the tissue samples were temporarily frozen in liquid nitrogen. Finally, the frozen tissue samples were stored at a temperature of −80 ℃ for long-term preservation.

1.5 *RNA isolation and qPCR analysis*

Total RNA was isolated from tissues using TRIzol Reagent according to the manufacturer's protocol. TRIzol reagent and PrimeScript^{TM} RT Master Mix (Perfect Real Time) were from Takara Bio, Japan; Taq Pro Universal SYBR qPCR Master Mix was from Vazyme Biotech, Nanjing. Then mRNA was reverse-transcribed into cDNA using 5 × All-In-One RT MasterMix. The qPCR analysis was performed in a QuantStudio Real-Time PCR Detection System (Thermo Fisher, USA) using SYBR qPCR Master Mix. The CYP1A1 and NLRP3 mRNA expression levels were calculated using $2^{-\Delta\Delta Ct}$ method. The primers used in this study were all synthesized by Shangon Biotech (Table 1).

1.6 *Enzyme-linked immunosorbent assay (ELISA)*

The levels of IL-6, TNF-α, and IL-1β in the serum and hippocampus were measured by ELISA kits (Shanghai Westang Biotech) according to the manufacturer's instructions. After being left at room temperature for 25 min, the collected blood samples were centrifuged at 4 000 r/min for 25 min, and then transferred into new sterile tubes. The hippocampus protein extracts were diluted with PBS buffer and centrifuged at 12 000 r/min at 4 °C for 30 min. Cytokines detection utilized the double antibody sandwich method. Following the addition of standards and samples, each well of the 96-well coated plate was carefully filled with 100 μL of the respective solution. After the completion of the immunoreactions, the absorbance values at 450 nm were promptly measured within a 30-minute timeframe. By correlating the optical density (OD) values obtained from the absorbance readings with the known concentrations of the standards, the concentration of IL-6, TNF-α, and IL-1β in the samples could be accurately calculated.

1.7 *Western blotting*

RIPA lysis buffer (Beyotime, Shanghai, China) was used for hippocampi. After lysis, the protein concentrations in the lysates were determined using a BCA protein assay kit (Beyotime, Shanghai, China). To separate the proteins based on their molecular weight, the lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% polyacrylamide gel. Following electrophoresis, the proteins were transferred from the gel onto nitrocellulose membranes. The blotting bands were subsequently incubated with rabbit anti-NLRP3 (1∶1 000, Affinity Biosciences, China), rabbit anti-β-actin (1∶1 000, Protein Tech, USA), mouse anti-AhR (1∶1 000, Protein Tech, USA), or mouse anti-GAPDH (1∶1 000, Protein Tech, USA) primary antibodies at 4 °C overnight followed by secondary antibodies (1∶5 000, LI-COR Biosciences) incubation for 1 h. Near-infrared fluorescence technique (Odyssey Infrared Imaging System, LI-COR, Inc., USA) and ImageJ semi-quantification were utilized for blotting bands.

1.8 *Data analysis*

Data were expressed as the mean ± SEM. GraphPad Prism 7 (GraphPad Software, Inc.) was utilized for statistical analysis. One-way analysis of variance (ANOVA) was performed for comparison between multiple groups, followed by Turkey's multiple comparison tests. A level of $P < 0.05$ was considered statistical significance.

Table 1. qPCR Primers sequences

Gene	Primers sequences	Product length (bp)
NLRP3	F: 5'-ATTACCCGCCCGAGAAAGG-3'	2 141
	R: 5'-TCGCAGCAAAGATCCACACAG-3'	
CYP1A1	F: 5'-TGGAGCCTCATGTACCTGGTAACC-3'	356
	R: 5'-CTGCCGATCTCTGCCAATCACTG-3'	
GAPDH	F: 5'-TCAACGACCCCTTCATTGACC-3'	209
	R: 5'-CTTCCCGTTGATGACAAGCTTC-3'	

2 RESULTS

2.1 FICZ activated the expression of AhR

To confirm the activation of AhR, we assessed the expression level of AhR and CYP1A1, a well-known target gene of AhR. The qPCR results showed a significant upregulation of CYP1A1 mRNA expression in the FICZ group compared to the LPS group. Additionally, we conducted Western blotting to evaluate the protein level of AhR. Consistent with the CYP1A1 mRNA expression results, the Western blot analysis showed a significant increase in AhR protein levels in the FICZ group. This confirmed the translocation and activation of AhR by FICZ, as increased AhR protein and CYP1A1 mRNA expression levels were indicative of AhR activation by FICZ.

2.2 FICZ alleviated depressive-like behaviors in LPS-induced mice

To evaluate the depressive conditions of LPS-induced mice, three commonly used behavioral tests were conducted. The outcomes of these tests are depicted in Fig. 3. It was noted that the sucrose preference performance of mice in the LPS group significantly decreased in comparison to the control group. When compared to the LPS group, the administration of FICZ resulted in an elevation in sucrose preference, signifying an amelioration in the mice's inclination towards for sweetness. The reduced intake of sucrose solution in the LPS group indicated decreased sensitivity to reward and anhedonia (Fig. 3*A*). In the FST, the immobility time of mice in the LPS group was significantly prolonged. However, this performance was ameliorated by the injection of FICZ (Fig. 3*B*), suggesting its potential to alleviate depressive-like behavior. Similarly, TST also showed a similar trend in the immobility time of the FICZ group, however, there was no statistical difference between LPS and FICZ groups (Fig. 3*C*). Both FST and SPT results indicated that FICZ had an antidepressant effect on the experimental mice.

2.3 FICZ regulated cytokine levels in LPS-induced mice

The present study assessed the effects of FICZ on IL-6, IL-1β, and TNF-α in the peripheral and central systems by analyzing serum and hippocampus samples. Initial findings revealed that FICZ led to a reduction in IL-1β levels in both the serum and hippocampus, indicating potential anti-inflammatory properties. Furthermore, the FICZ group exhibited a decrease in IL-6 levels in the hippocampus compared to the LPS group. On the other hand, the lack of statistical distinction in TNF-α levels between the FICZ and LPS groups suggests that FICZ may not significantly affect the expression of this specific pro-inflammatory cytokine in the context of this study.

2.4 FICZ inhibited activation of NLRP3

The NLRP3 inflammasome is known to play a crucial role in the pathogenesis of depression by modulating the production of IL-1β, a critical pro-inflammatory cytokine implicated in neuroinflammation. To explore the potential influence of FICZ on NLRP3 activation, we assessed the mRNA and protein expression levels of NLRP3. The findings illustrated in Fig. 5 offer insights into the effect of FICZ on NLRP3 expression. We noted a substantial decrease in the mRNA level of NLRP3 following FICZ treatment, indicating a potential down-regulation of NLRP3 gene expression. Additionally, Western blot results showed a corresponding reduction

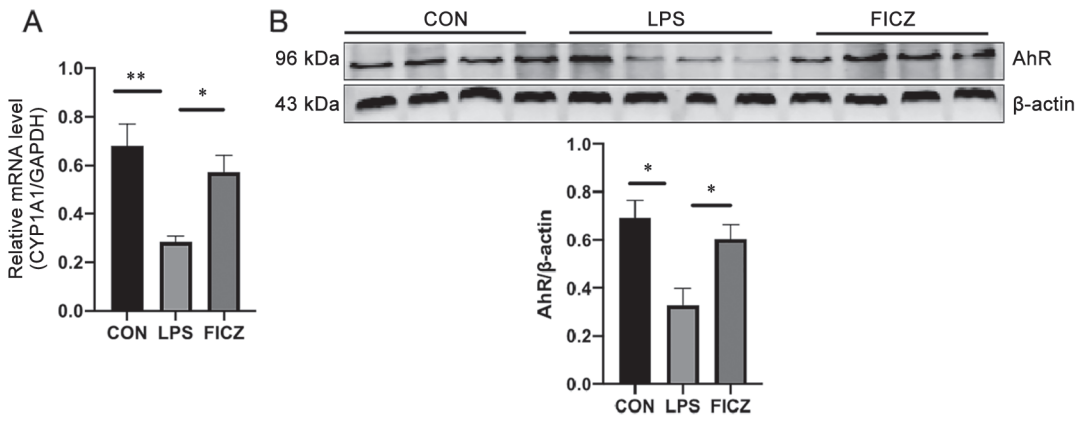

Fig. 2. Effects of FICZ on mRNA expression level of CYP1A1 (*A*) and protein expression level of aryl hydrocarbon receptor (AhR) (*B*) in hippocampus. Mean ± SEM, $n = 4–6$. $^{*}P < 0.05$, $^{**}P < 0.01$.

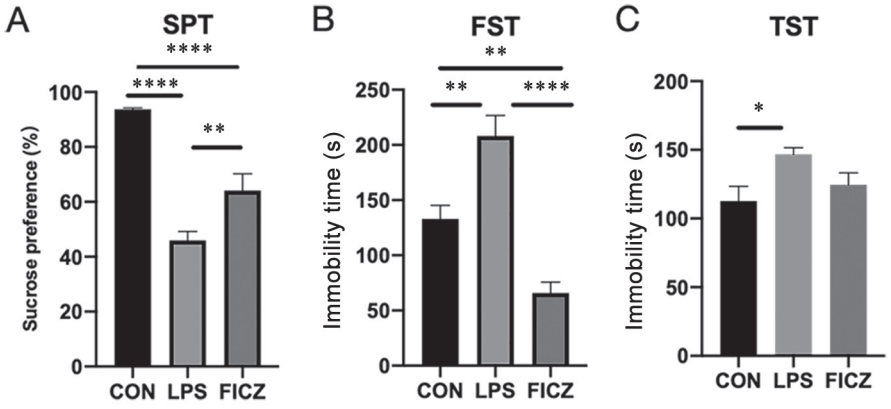

Fig. 3. FICZ alleviates depressive-like behaviors in LPS-induced mice. *A*: Sucrose preference test (SPT). *B*: Forced swimming test (FST). *C*: Tail suspension test (TST). Mean ± SEM, $n = 8–10$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.000\ 1$.

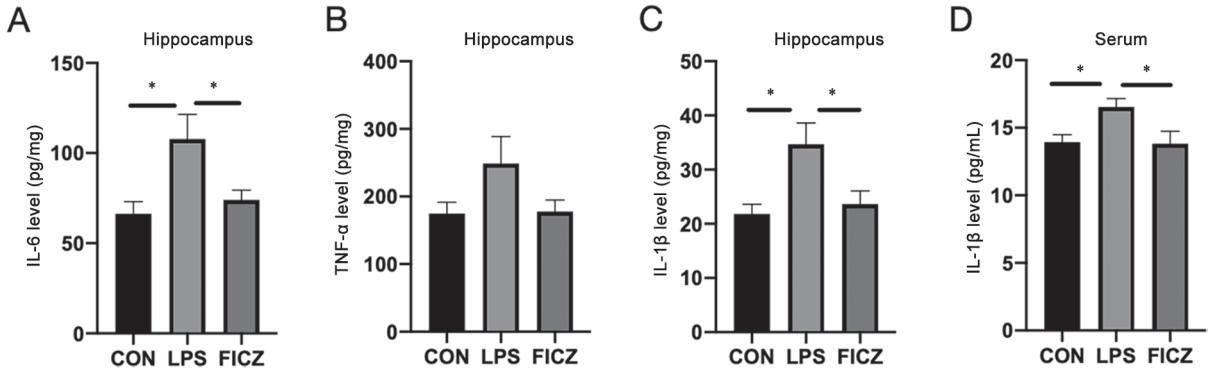

Fig. 4. Effects of FICZ on the cytokine levels in the hippocampus and serum detected by ELISA. *A*: The level of IL-6 in the hippocampus. *B*: The level of TNF-α in the hippocampus. *C*: The level of IL-1β in the hippocampus. *D*: The level of IL-1β in the serum. Mean ± SEM, $n = 8–10$. $^{*}P < 0.05$.

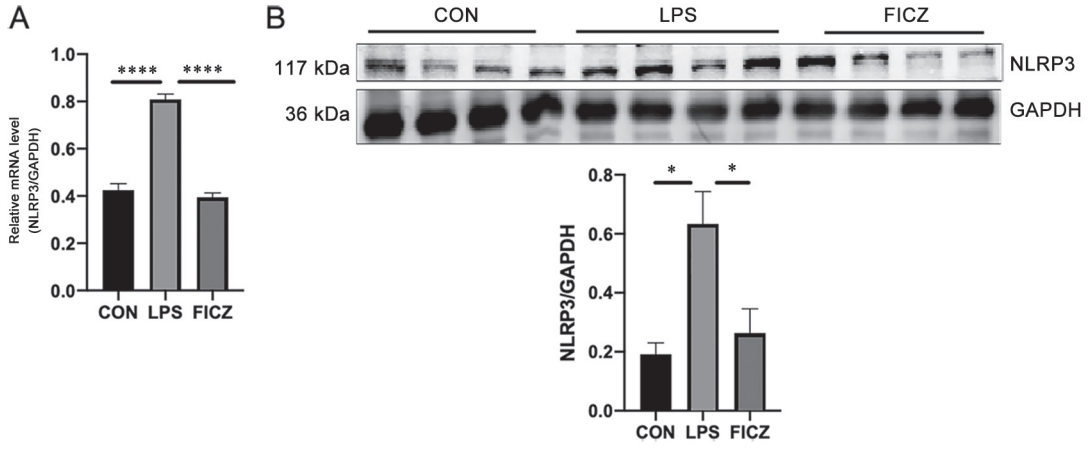

Fig. 5. Effects of FICZ on NLRP3 mRNA (*A*) and protein expression (*B*) levels in the hippocampus. Mean ± SEM, $n = 3–6$. $^{*}P < 0.05$, $^{****}P < 0.000\ 1$.

in the protein expression of NLRP3 in the FICZ group, compared with that in the LPS group. This suggests that FICZ not only affects the transcriptional regulation of NLRP3, but also down-regualtes its protein levels, potentially resulting in decreased NLRP3 inflammasome activation.

3 DISCUSSION

3.1 FICZ relieved depressive-like behaviors

The activation of AhR by FICZ was consistent with findings from prior research [24, 38], which have established the expression of AhR in the hippocampus, notably in the CA2 and CA3 pyramidal layers. The most studied ligand is TCDD. Studies have shown that long-term exposure to TCDD can influence brain morphology [53] and result in neurotoxicity [54]. FICZ, classified as RMAhRL, distinguishes itself through swift degradation and the absence of TCDD-like toxicity. Its rapid metabolism into inactive substances creates a regulatory negative feedback loop, mitigating the potentially harmful bioaccumulation of this ligand by maintaining low endogenous concentrations of the ligand [55]. Traditionally, the expression of CPY1A1 and its associated enzyme activity have served as a biomarker for AhR binding and activation [56, 57]. Downstream signaling cascades can be triggered by the interaction of pattern recognition receptors (PRRs) with pathogen-associated molecular patterns (PAMPs). This, in turn, induces the production of inflammatory or infection mediators, including IL-1β, IL-6, TNFα, and IFNs [55]. Consequently, the expression and/or activity of CYP1A1 are suppressed, resulting in a marginal reduction in CYP1A1 expression compared to control groups. In the FICZ group, the mRNA level of the AhR-target gene, CYP1A1, was elevated in the hippocampus (Fig. 2), suggesting that AhR translocated to the nucleus following FICZ injection. From the behavioral results, our study demonstrated that FICZ reversed the depressive-like behaviors induced by LPS, which aligned with a previous study that found DIM, another AhR ligand, prevented anhedonia behavior induced by unpredictable chronic mild stress (UCMS) [29]. Although research on AhR in the context of depression is limited, insights from other major brain neuropsychiatric disorders suggest our results align with a prior study demonstrating that I3C, an alternative AhR ligand, effectively mitigated despair behaviors induced by clonidine and exhibited neuroprotective properties [58]. Additionally, FICZ exhibited favorable impacts on both short- and long-term memory and was linked to the restoration of neurogenesis in hippocampal neurons [26]. The activation of AhR has also been reported to alleviate cognitive deficits in a mouse model of Alzheimer's disease [28].

3.2 AhR alleviated inflammatory disturbances and modulated NLRP3-mediated inflammation

Multiple studies have demonstrated that LPS elicits an inflammatory response in the central nervous system, leading to the synthesis of pro-inflammatory cytokines. This response is mediated by the activation of microglia by LPS, which subsequently amplifies the production of pro-inflammatory cytokines within the brain [38, 43]. Treatment with FICZ has been observed to attenuate LPS-induced inflammation by diminishing the production of pro-inflammatory cytokines, such as IL-6 and IL-1β, in both the hippocampus and blood serum. This result is consistent with other literature results [19, 34, 58–60]. Notably, in contrast to certain studies where TNF-α exhibited a marked reduction at the protein level [20, 36], our results did not demonstrate a statistically significant difference. The reduction in the levels of these inflammatory factors may hold promise for enhancing the efficacy of antidepressant treatment. NLRP3, as an integral component of PRRs, assumes a pivotal role not only in the innate immune system's response to psychological stress, but also serves as a crucial initiator in instigating downstream inflammatory cascades. The over-activation of NLRP3 culminates in the generation of a substantial quantity of inflammatory mediators, including IL-1β and IL-18 [61]. Numerous pre-clinical and clinical studies have substantiated that the inflammasome orchestrates both peripheral and central inflammatory responses, as well as depression-like behavior, in various animal models of depression [3–5]. Additionally, compared to the group treated with LPS alone, FICZ treatment resulted in a down-regulation of NLRP3 expression at both the mRNA and protein levels. FICZ may exert a suppressive effect on the transcription of NLRP3, potentially leading to reduced activation of the NLRP3 inflammasome. These findings align with previous studies conducted on different disease models [20, 34, 35]. Taken together, these results suggest that FICZ possesses the potential to mitigate inflammation and ameliorates depression by down-regulating NLRP3. At present, AhR/NF-κB [19, 60, 62] and AhR/Nrf2/NQO1 [20, 63] pathways have been explored to regulate NLRP3. We needs to continue to explore the mechanism and its function pathway of NLRP3 upon AhR activation in the depression model, building upon insights gleaned from prior research. In addition, the administration of FICZ in CUMS animal model also needs to be explored. Considering that AhR is expressed

in both astrocytes [22, 64, 65] and microglia [66–68], it is imperative to conduct cellular experiments to explore the cell types and inflammatory mechanisms associated with AhR. In summary, AhR transcription factors demonstrate a potential capacity for negatively regulatory effect on NLRP3 transcription, thereby inhibiting inflammation in LPS-induced models.

While several studies have discussed AhR as a potential therapeutic target for depression [29, 69, 70], the mechanism remains unknown. Therefore, from the perspective of inflammation, we endeavor to explore the mechanisms linking AhR to depression. Our laboratory pioneered the identification of the relationship between NLRP3 and depression globally. This research represents a continuation and expansion of the previous experiment. Although the association between AhR and NLRP3 in the context of depression has not been established previously, it has been investigated in other disease models [46, 47]. Given the presence of AhR in the brain, we postulated the link between AhR and NLRP3 in the depression model induced by LPS. The present study uncovered novel insights into the involvement of AhR in the development of depression through its interaction with NLRP3. It was found that activating AhR alleviated the depression-like behavior induced by LPS, inhibited the activation of NLRP3, and reduced the levels of IL-1β and IL-6. These findings enhance our understanding of the relationship between AhR and depression, elucidate the possible mechanism of AhR activation affecting depression, and may offer a potential therapeutic target for the treatment of depression.

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