Dihydrolipoic acid protects against lipopolysaccharide-induced behavioral deficits and neuroinflammation via regulation of Nrf2/HO-1/ NLRP3 signaling in rat

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Abstract

Background: Recently, depression has been identified as prevalent and severe mental disorder. However, the mechanisms underlying the depression risk remain elusive. The neuroinflammation and NLRP3 inflammasome activation are known to be involved in the pathology of depression. Dihydrolipoic acid (DHLA) has been reported as a strong antioxidant and exhibits anti-inflammatory properties in various diseases, albeit the direct relevance between DHLA and depression is yet unknown. The present study aimed to investigate the anti-depressant effects and potential mechanism of DHLA in the Lipopolysaccharide (LPS)-induced depression animal model.

Methods:

Adult male Sprague–Dawley rats were utilized. LPS and DHLA were injected intraperitoneally every 2 days and daily, respectively. Fluoxetine(Flu) was injected

intraperitoneally daily. PD98059, an inhibitor of ERK, was injected intraperitoneally one hour before DHLA injection daily. Small interfering ribonucleic acid (siRNA) for nuclear factor erythroid 2-like (Nrf2) was injected into the bilateral hippocampus 14 days before the DHLA injection. Depression-like behavior tests were performed. Western blot and immunofluorescence staining detected the ERK/Nrf2/HO-1/ROS/NLRP3 pathway-related proteins.

Results:

The DHLA and fluoxetine treatment exerted anti-depressant effects in LPS-induced depression rats. The DHLA treatment increased the expression of ERK, Nrf2, and HO-1 but decreased the ROS generation levels and reduced the expression of NLRP3, caspase-1, and IL-1b in LPS-induced depression rats. PD98059 abolished the effects of DHLA on anti-depression as well as the levels of Nrf2 and HO-1 proteins. Similarly, Nrf2 siRNA reversed the anti-depressant effect of DHLA administration via the decreased expression of HO-1.

Conclusions:

These findings suggested that DHLA exerted anti-depressant-like effects via ERK/Nrf2/HO-1/ROS/NLRP3

pathway in LPS-induced depression rats. Thus, DHLA may serve as a potential therapeutic strategy for depression.

Keywords: dihydrolipoic acid, neuroinflammation, NLRP3, lipopolysaccharide, depression

Background

Depression is the most common psychiatric mood disorder globally. Currently, the limited therapies available for the treatment of this condition mainly target the monoamine levels. However, this approach is not efficient for many patients [1]. The mechanisms underlying depression are complicated and largely unknown. Therefore, the knowledge of this mechanism of depression would aid the development of effective treatment.

Accumulating evidence revealed a close link between inflammation and major depression disorder [2, 3]. The

nod-like receptor pyrin-containing pyrin domain 3 (NLRP3) inflammasome is one of the most widely studied inflammasomes [4]. After NLRP3 inflammasome is assembled and activated, it activates caspase-1 by proteolytic cleavage, which in turn, converts pro-IL-1b into bioactive IL-1b, leading to inflammatory responses in bodies [5, 6]. Reactive oxygen species (ROS) is a normal metabolic product of redox reactions. The excessive level of ROS would damage the integrity of cells and result in dysfunction of the tissues via peroxidation of lipids, proteins, mitochondria, and DNA of cells[7, 8]. Despite that the specific regulatory mechanism of NLRP3 inflammasome activation is unclear, ROS has been frequently reported to be correlated with NLRP3 inflammasome activation [9, 10]. Also, it regulates the expression and/or activation of NLRP3 inflammasome in several diseases, including intestinal inflammation and cardiovascular disease.

Nuclear factor erythroid 2-like (Nrf2) is a primary element of transcription and has emerged as a potential therapeutic target for inflammatory disorders [11]. Heme oxygenase-1 (HO-1) is an enzyme that exerts anti-

inflammatory and antioxidant stress effects [12]. Nrf2 is a critical modulator of the expression of HO-1 [13, 14]. Recent studies have focused on the Nrf2/HO-1 approach with respect to anti-inflammation [15, 16]. These findings indicated that the Nrf2/HO-1 signaling pathway plays a critical role in anti-inflammatory activities. Other studies have shown that the activated Nrf2/HO-1 signaling pathway may counteract the intracellular production of ROS [17].

Dihydrolipoic acid (DHLA) is a reduced form of α-lipoic acid (LA) that can decrease oxidative stress and act as a strong antioxidant. DHLA also possesses anti-inflammatory properties [18]. Hitherto, the anti-depressant effects of DHLA have not been explored. Therefore, in the present study, we sought to investigate the anti-depressant effects of DHLA in the LPS-induced depression animal model and whether ERK/Nrf2/HO-1/ROS/NLRP3 pathway is involved in the anti-depressant effects of DHLA.

Methods

Animals

Adult male Sprague–Dawley (SD) rats (weight, 200–220

g) were purchased from Hunan SJA Laboratory Animal Co., Ltd (Hunan, China) and housed in a 12 h dark and light cycle at room temperature (18–22 °C) with free access to water and food. All procedures involving animals were approved and carried out according to the guidelines of the Institutional Animals Care Committee of Renmin Hospital of Wuhan University.

Experimental design

This study consisted of four experiments as shown in Fig 1.

Experiment 1: Rats were randomly divided into six groups (n=6/group): Control, Lipopolysaccharide (LPS)+vehicle, LPS+DHLA (15mg/kg, 30mg/kg, 60mg/kg), LPS+ fluoxetine (Flu) group. The evaluation of the body weight, open field test (OFT), and forced swim test (FST) was used to assess the anti-depression effects of DHLA. Based on the body and behavioral tests, 30 mg/kg DHLA-treated group was selected for the subsequent experiments.

Experiment 2: Rats were randomly divided into four groups (n=9/group): Control, LPS, LPS+ vehicle, and LPS+DHLA (30 mg/kg). The expression of ERK, Nrf2, and HO-1 was detected by Western blot, while that of ROS was

tested by flow cytometry (n=6/group). Immunofluorescence staining assessed the expression of HO-1 (n=3/group). The test of body weight, OFT, and FST were used to assess the anti-depression effects of DHLA.

Experiment 3: Rats were randomly divided into six groups (n=6/group): Control, LPS, LPS+ vehicle, LPS+DHLA (30 mg/kg), LPS+DHLA (30 mg/kg) + DMSO, LPS+DHLA (30 mg/kg) + PD98059. The expression of ERK, Nrf2, HO-1, NLRP3, caspase-1, and IL-1β was detected by Western blot. The test of body weight, OFT, and FST were used to assess the anti-depression effects of DHLA.

Experiment 4: Rats were randomly divided into six groups (n=6/group): Control, LPS, LPS+ vehicle, LPS+DHLA (30 mg/kg), LPS+DHLA (30 mg/kg)+AAV-control-siRNA, LPS+DHLA (30 mg/kg)+AAV-Nrf2-siRNA. The expression of ERK, Nrf2, HO-1, NLRP3, caspase-1, and IL-1β was detected by Western blot. The test of body weight, OFT, and FST were used to assess the anti-depression effects of DHLA.

Drug treatment

LPS was solubilized in dimethyl sulfoxide (DMSO) and phosphate-buffered saline (PBS) and administered

intraperitoneally (i.p.) at a dosage of 500 mg/kg every 2 days as described previously [19]. DHLA (Sigma) was diluted in DMSO and PBS and administered intraperitoneally (i.p.) daily to a total of 14 injections. The ERK antagonist PD98059 (MedChemExpress,0.3mg/kg) was diluted in DMSO and PBS and administered intraperitoneally (i.p.) daily [20]. Flu (Aladdin Reagent Shanghai) was solubilized in sterile distilled water and administered intraperitoneally (i.p.) at a dosage of 10 mg/kg/day [21].

Hippocampal administration

Adeno-associated virus-mediated small interfering RNA against Nrf2 (AAV-Nrf2-siRNA) or control vector (AAV-Control-siRNA) with an enhanced green fluorescent protein (eGFP) was purchased from Genechem Co., Ltd. (Shanghai, China). The siRNA sequence for Nrf2 is 5’-GTCTTCAGCATGTTACGTGATGAGGATGG-3’ [22]. Hippocampal AAV virus administration was performed as described previously[23]. Briefly, rats were anesthetized with 10% chloral hydrate (0.35 mL/100 g, i.p.) and placed in a stereotaxic apparatus. Rats were infused bilaterally with 1 mL of purified and concentrated AAV virus

(1.08×10^{13} v.g/mL) into the hippocampus region (coordinates from the bregma: −3.5 mm posterior, ±2.3 mm lateral, −3.0 mm ventral) using an electric microinjection pump (Stoelting, USA) at a rate of 100 nL/min. The needle was kept in place for 5 min after infusion and then removed slowly. Subsequently, the incision was closed with interrupted silk sutures, and the animal was placed in a heated cage (35 °C) and monitored carefully.

FST

FST was performed as described previously. The rats were singly placed in glass cylinders (40 cm height and 28 cm diameter) filled with 30 cm of water (25 ± 1 °C) for 15 min for training, and after 24 h, the animals were placed again in the cylinders for 6 min. The immobile time was recorded during the final 4 min. The immobility was defined as floating with only minimal movements to maintain their head above water.

OFT

OFT was performed to measure spontaneous activity, as described previously [24]. Briefly, each test rat was placed in the apparatus consisting of a black square 100 cm × 100

cm. The evaluations of every rat were recorded for 5 min, and the rat movements were recorded by a video tracking system (Ethovision XT 11.5). The frequencies of rearing, total distance, and total speed were analyzed using the video tracking system. The apparatus was cleaned with 70% alcohol after each test.

Flow cytometry

Intracellular oxidative stress was determined by flow cytometry using a ROS assay kit (Jiancheng Biotechnology, Nanjing, China) according to the manufacturer's protocol [25]. 2,7-dichlorofluorescein-diacetate (DCFH-DA) was utilized as a sensitive nonfluorescent precursor dye. It permeates the cells and is hydrolyzed by intracellular esterase to the nonfluorescent DCFH, which is rapidly oxidized to the highly fluorescent 2,7-dichlorofluorescein (DCF) in the presence of ROS. The fluorescence intensity of DCF was proportional to the level of intracellular ROS as measured by flow cytometry.

Western blot analysis

Western blot analysis was performed as described previously [26].One day after the behavioral tests, the rats were anesthetized with 10% chloral hydrate (3.5 mL/100

g), and the brains were collected. Total protein was prepared from the hippocampus, and the BCA assay was used to analyze the concentration of proteins (BCA Protein Assay, Thermo, 23228). The protein sample was resolved on SDS-PAGE and then transferred to a PVDF membrane. The membranes were probed overnight with the following primary antibodies at 4 °C: Nrf2 (Abcam, ab137550; 1:1000), HO-1 (Abcam, ab13243; 1:1000), ERK (Abcam, ab17942; 1:1000), p-ERK (Abcam, ab50011; 1:1000), NLRP3 (Abcam, ab214185; 1:1000), caspase-1 (Abcam, ab179515; 1:1000), and IL-1β (Abcam, ab9722; 1:1000). Subsequently, the membranes were washed with TBST (3 times for 5 min each) and incubated with goat anti-rabbit IgG (1:2000; Abcam, ab205718) and goat anti-mouse IgG (1:2000; Abcam, ab205719), as appropriate, for 1 h at room temperature. The immunoreactive bands were developed by chemiluminescence using Chemi Doc XRS System (Bio-rad, USA), and the intensities were normalized to that of GAPDH (1:10000; Servicebio, GB11002) used as an internal standard and quantified using ImageJ software.

Immunofluorescence

Immunofluorescence staining was conducted as described previously [27]. A series of 30 mm slices were blocked in 2% BSA and incubated overnight with the primary antibody anti-HO-1 (Abcam, ab13243; 1:1000) at 4 °C. The secondary antibody was probed on the slide for 1 h in the dark. Subsequently, the sections were washed three times with PBS before the images were acquired using a Nikon upright fluorescence microscope.

Statistical analysis

Prism software (GraphPad Prism 8.0, CA, USA) was used for all the analyses. The data are presented as mean ± standard error of mean (SEM). The normality of the distribution was assessed by the Shapiro-Wilk test. Statistical comparisons were made using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical significance was indicated by p-value < 0.05.

Results

DHLA treatment reversed the LPS-induced depression-like behavior

Body weight gain and behavioral tests, including OFT and FST were performed to investigate the effects of DHLA on LPS-induced depression-like behaviors in rats.

As shown in Fig. 2A, rats exposed to LPS showed less body weight gain as compared to the control group ($F(5,30) = 19.83$, $p < 0.0001$). However, treatment with DHLA (30 mg/kg, $p < 0.0001$; 60 mg/kg, $p < 0.01$) and Flu ($p < 0.0001$) improved the body weight gain as compared to the LPS group.

The FST is mainly used to measure the depression-like behavior. As shown in Fig. 2B, rats exposed to LPS showed less immobility time in FST as compared to the control group ($F(5,30) = 17.49$, $p < 0.0001$), whereas compared to the LPS group, DHLA (30 mg/kg, $p < 0.001$; 60 mg/kg, $p < 0.01$) and Flu ($p < 0.0001$) treatment markedly decreased the immobility time in FST.

The performance of rats in OFT is shown in Fig. 2C–E. The total distance, rearing frequencies, and total velocity were significantly decreased in the LPS group as compared to the control group (total distance ($F(5,30) = 19.93$, $p < 0.0001$); rearing frequencies ($F(5,30) = 11.66$, $p < 0.0001$); velocity ($F(5,30) = 8.132$, $p < 0.0001$)). Compared to the LPS group, the total distance in the DHLA (30 mg/kg, $p < 0.001$; 60 mg/kg, $p < 0.01$) and Flu ($p < 0.0001$) groups was significantly increased, the rearing frequencies in the

DHLA (30mg/kg, $p < 0.01$) and Flu ($p < 0.01$) groups were significantly increased, and the total velocity in the DHLA (30 mg/kg, $p < 0.01$; 60 mg/kg, $p < 0.05$) and Flu ($p < 0.01$) groups were significantly increased.

Since DHLA (30 mg/kg) was superior to the other doses in measurements (Fig. 2A-E), we selected 30 mg/kg as the optimal dose of DHLA and used it in the following experiments.

DHLA reversed the LPS-induced depression-like behavior through ERK/Nrf2/HO-1/ROS/ NLRP3-dependent inflammation pathway

Western blot and immunofluorescence staining were used to test the expression of ERK/Nrf2/HO-1/ROS/ NLRP3 signaling pathway in response to DHLA against LPS-induced depression-like behavior in rats.

As shown in Fig. 3A–D, a statistically significant difference between groups for p-ERK ($F(3,20) = 13.97$, $p < 0.0001$), Nrf2 ($F(3,20) = 9.48$, $p < 0.0001$), and HO-1 ($F(3,20) = 10.90$, $p < 0.0001$) was determined by one-way. A Tukey's post-hoc analysis revealed that expression levels of p-ERK ($p < 0.01$), Nrf2 ($p < 0.01$), and HO-1 ($p < 0.01$) were significantly higher in DHLA-treated mice as

compared to the LPS groups. Immunofluorescence staining revealed that the expression of HO-1 in the DHLA group was increased than that in the control group (Fig. 3E).

Fig. 4A–D revealed a statistically significant difference in NLRP3 ($F(3,20) = 21.62$, $p < 0.0001$), caspase-1 ($F(3,20) = 17.37$, $p < 0.0001$), and IL-1β($F(3,20) = 11.93$, $p < 0.0001$) between the groups as determined by one-way ANOVA. Tukey's post-hoc analysis revealed that the expression levels of NLRP3 ($p < 0.0001$), caspase-1 ($p < 0.0001$), and IL-1β ($p < 0.001$) were significantly lower in LPS mice as compared to the control groups. However, these effects were significantly reversed by the treatment with DHLA (NLRP3, $p < 0.01$; caspase-1, $p < 0.01$; IL-1β, $p < 0.05$).

The concentration of intracellular ROS was evaluated by the changes in DCF fluorescence intensity using flow cytometry. As shown in Fig. 4E, the ROS expression was distinctly higher in the LPS mice as compared to the control group ($F(3,20) = 25.80$, $p < 0.0001$). Conversely, the intracellular ROS level induced by LPS was markedly ameliorated by DHLA ($p < 0.001$).

Body weight gain and behavioral tests, including OFT and FST, were performed to further investigate the effects of DHLA on LPS-induced depression-like behaviors in rats. As shown in Fig. 5A, rats exposed to LPS showed less body weight gain than the control group ($F(5,30) = 20.84$, $p < 0.0001$). However, treatment with DHLA (30 mg/kg, $p < 0.01$) improved the body weight gain as compared to the LPS group. Fig. 5B showed that rats exposed to LPS showed less immobility time in FST as compared to the control group ($F(5,30) = 27.11$, $p < 0.0001$). On the other hand, compared to the LPS group, DHLA (30 mg/kg, $p < 0.001$) treatment markedly decreased the immobility time in FST. As shown in Fig. 5C–E, the total distance, rearing frequencies, and total velocity were significantly decreased in the LPS group as compared to the control group (total distance ($F(5,30) = 30.48$, $p < 0.0001$); rearing frequencies ($F(5,30) = 15.22$, $p < 0.0001$); velocity ($F(5,30) = 12.16$, $p < 0.0001$)). Compared to the LPS group, the total distance, the rearing frequencies, and total velocity in the DHLA (30 mg/kg, all $p < 0.01$) group were significantly increased.

Blockade of ERK abolished the anti-depression and

anti-inflammation effect of DHLA

Previous studies have suggested that the ERK pathway is related to the regulation of Nrf2 [28]. PD98059 (an ERK pathway inhibitor) was administered in LPS-induced depression rats 1 h before the administration of DHLA to investigate whether DHLA upregulated the expression of Nrf2/HO-1/ROS/NLRP3 in LPS-induced mice via the ERK pathway.

As shown in Fig. 6A, rats exposed to DHLA showed high body weight gain as compared to the LPS group ($F(5,30) = 22.61$, $p < 0.0001$). However, treatment with PD98059 ($p < 0.01$) improved body weight gain than the DHLA group.

As shown in Fig. 6B–E, the DHLA administration greatly attenuated the depression-like behavior in LPS-induced rats. As shown in Fig. 6B, rats exposed to DHLA showed less immobility time in FST as compared to the LPS group ($F(5,30) = 26.79$, $p < 0.0001$), whereas compared to the DHLA group, the PD98059 ($p < 0.05$) treatment markedly decreased the immobility time in FST. As shown in Fig. 6C–E, the total distance, rearing frequencies, and total velocity were significantly decreased in the DHLA group as compared to the LPS group (total distance $F(5,30) = 22.78$,

$p < 0.0001$; rearing frequencies $F(5,30) = 18.71$, $p < 0.0001$; velocity $F(5,30) = 15.17$, $p < 0.0001$). However, compared to the DHLA group, the total distance in the PD98059 ($p < 0.01$) group and rearing frequencies and total velocity in the DHLA (both $p < 0.05$) group were significantly increased. These data indicated that the anti-depression effect of DHLA in the LPS-induced mice was blocked by PD98059.

Western blot data (Fig. 7A–F) showed a statistically significant difference between the study groups as determined by one-way ANOVA with respect to Nrf2 ($F(5,30) = 35.07$, $p < 0.0001$), HO-1 ($F(5,30) = 25.19$, $p < 0.0001$), NLRP3($F(5,30) = 50.79$, $p < 0.0001$), caspase-1 ($F(5,30) = 15.64$, $p < 0.0001$), and IL-1β ($F(5,30) = 34.71$, $p < 0.0001$). Tukey's post-hoc analysis revealed that expression levels of Nrf2 ($p < 0.0001$) and HO-1 ($p < 0.0001$) were significantly higher in DHLA mice as compared to the LPS groups. In addition, the expression of NLRP3 ($p < 0.0001$), caspase-1 ($p < 0.01$), and IL-1β ($p < 0.0001$) was significantly lower in DHLA mice as compared to the LPS groups. However, inhibition of ERK with PD98059 abolished the effects of DHLA, which led to

decreased Nrf2 ($p < 0.01$) and HO-1 ($p < 0.01$), while increase in the inflammation-related proteins, such as NLRP3 ($p < 0.01$), caspase-1 ($p < 0.05$), and IL-1β ($p < 0.01$) as compared to the DHLA group.

As shown in Fig. 7G, the ROS expression was distinctly higher in the DHLA as compared to the LPS group ($F(5,30) = 23.09$, $p < 0.0001$). Conversely, the ROS level induced by DHLA was markedly ameliorated by PD98059 ($p < 0.01$).

Blockade of Nrf2 abolished the anti-depression and anti-inflammation effect of DHLA

Body weight gain and behavioral tests showed that AAV-Nrf2-siRNA completely abolished the treatment effects of DHLA in the LPS-induced depression mice.

As shown in Fig. 8A, rats exposed to DHLA showed high body weight gain as compared to the LPS group ($F(5,30) = 17.46$, $p < 0.0001$). However, treatment with AAV-Nrf2-siRNA ($p < 0.05$) improved body weight gain as compared to the DHLA group.

As shown in Fig. 8B–E, the DHLA administration greatly attenuated the depression-like behaviors observed in LPS-induced rats. As shown in Fig. 8B, rats exposed to DHLA showed less immobility time in FST as compared to the LPS

group ($F(5,30) = 24.1$, $p < 0.0001$). On the other hand, compared to the DHLA group, the AAV-Nrf2-siRNA ($p < 0.05$) treatment markedly decreased the immobility time in FST. As shown in Fig. 8C–E, the total distance, rearing frequencies, and total velocity were significantly decreased in the DHLA group that in the LPS group (total distance $F(5,30) = 17.84$, $p < 0.0001$; rearing frequencies $F(5,30) = 17.87$, $p < 0.0001$; velocity $F(5,30) = 13.86$, $p < 0.0001$). However, compared to the DHLA group, the total distance, the rearing frequencies and total velocity in the DHLA ($p < 0.05$, $p < 0.01$, $p < 0.05$, respectively) group were significantly increased. These data indicated that the anti-depression effect of DHLA in LPS-induced mice was blocked by AAV-Nrf2-siRNA.

Western blot data (Fig. 9A–E) showed a statistically significant difference between the study groups as determined by one-way ANOVA regarding Nrf2 ($F(5,30) = 11.77$, $p < 0.0001$), HO-1 ($F(5,30) = 41.88$, $p < 0.0001$), NLRP3 ($F(5,30) = 23.01$, $p < 0.0001$), caspase-1 ($F(5,30) = 20.75$, $p < 0.0001$), and IL-1β ($F(5,30) = 25.14$, $p < 0.0001$). Tukey's post-hoc analysis revealed that the expression levels of Nrf2 ($p < 0.01$) and HO-1 ($p < 0.0001$)

were significantly higher in DHLA mice as compared to that in the LPS groups. Moreover, the expression of NLRP3 ($p < 0.001$), caspase-1 ($p < 0.001$), and IL-1β ($p < 0.0001$) was significantly lower in DHLA mice as compared to the LPS groups. However, inhibition of Nrf2 with AAV-Nrf2-siRNA abolished the effects of DHLA, which led to a decrease in Nrf2 ($p < 0.05$) and HO-1 ($p < 0.01$) expression and increase in the expression of inflammation-related proteins NLRP3 ($p < 0.01$), caspase-1 ($p < 0.01$), and IL-1β ($p < 0.001$) as compared to the DHLA group.

Fig. 9G shows that the ROS expression was distinctly higher in the DHLA group as compared to the LPS group ($F(5,30) = 14.89$, $p < 0.0001$). In contrast, the ROS level induced by DHLA was markedly ameliorated by AAV-Nrf2-siRNA ($p < 0.05$).

Discussion

The present study revealed that the depression-like behaviors induced by LPS in rats are reversed by DHLA treatment with the anti-depressant Flu, and is associated with the upregulation of Nrf2 and HO-1. The inhibition of ERK abolished the anti-depressant effect of DHLA, which was related to the low expression of Nrf2 and HO-1 and

upregulation of NLRP3, caspase-1, and IL-1β. Moreover, the knockdown of Nrf2 using Nrf2 siRNA abolished the effect of the DHLA treatment effect similarly, which was associated with a decrease in HO-1 and upregulation of NLRP3, caspase-1, and IL-1β. Taken together, our findings suggested that DHLA protects against LPS-induced behavioral deficits and neuroinflammation through ERK/Nrf2/HO-1/ROS/NLRP3 signaling pathway in rats.

Lipoic acid is a natural lipophilic antioxidant that has beneficial effects on heart diseases, diabetes, and neuroinflammatory disorders [29-32]. Lipoic acid occurs in two forms: α-lipoic acid and DHLA [33]. DHLA is the reduced form of lipoic acid with a more specific and useful outcome than α-lipoic acid [34]. DHLA is known as an effective antioxidant molecule [35]. Moreover, the neuroprotective effects of DHLA have been shown in several central nervous system diseases, such as ischemic stroke, traumatic brain injury. Recently, DHLA was indicated as an effective treatment for subarachnoid hemorrhage [18]. However, no study has been published, wherein DHLA was used as a therapeutic method for depression. To the best of our knowledge, this is the first

study evaluating the anti-depression effect of DHLA.

Herein, we first evaluated the effect of three different dosages of DHLA on LPS-induced depression-like behaviors in rats. In the current study, 30 mg/kg DHLA is the most effective dose for the treatment of depression. In line with our results, a previous study has suggested that 30 mg/kg DHLA contributes to the neuroprotective effects [18]. Thus, this dose may be optimal for the treatment of nervous system disease.

Nrf2 is a major transcription factor that regulates cell antioxidant response. Nrf2 is transported to the nucleus under oxidative stress and binds to the antioxidant response elements (ARE) to regulate the expression of antioxidant enzymes such as HO-1 [36]. Previous studies indicated that upregulated HO-1 expression exerts a protective effect against increased levels of ROS [37]. In the present study, the ROS level increased significantly in the LPS-induced groups as compared to the control groups but was restored in the DHLA treatment group. Consecutively, we found that DHLA treatment increased the expression of Nrf2 and HO-1, which was abrogated when Nrf2 was downregulated by AAV-Nrf2-siRNA.

Therefore, we speculated that DHLA regulates the ROS expression by activating the Nrf2/HO-1 signaling pathway. However, the mechanisms underlying the DHLA-mediated and Nrf2 activation after LPS-induced depression have not yet been elucidated. Previous studies have shown that Nrf2 activation is regulated by the MAPK/ERK pathway [38]. In the present study, we found that DHLA promotes the expression of ERK and Nrf2. Strikingly, the expression of Nrf2 could be inhibited by the ERK inhibitor PD98059. Thus, we speculated that DHLA activates Nrf2 through the ERK signaling pathway. These findings indicated that DHLA improves the depression-like behavior induced by LPS in rats, primarily through the ERK/Nrf2/HO-1/ROS pathway.

In recent years, many studies have shown that the pathogenesis and progression of depression are related to the overreaction of inflammation and immune responses [4, 39]. The inflammasome is an inducer of immune response with the function of identifying and targeting multiple pathogens [40]. Hitherto, several forms of inflammasomes have been reported, including NLRP1, NLRP2, NLRP3, and NLRC4 inflammasomes [41]. Among these, NLRP3

inflammasome is involved in the onset and progression of several diseases [42]. It is composed of NLRP3 protein, adaptor protein apoptosis-related speckles (ASC), and procaspase-1 [43, 44]. The activation of NLRP3 inflammasome triggers the transformation of procaspase-1 to caspase-1 and catalyzes the exudation of mature IL-1β and IL-18 from pro-IL-1β and pro-IL-18, causing an inflammatory response [45, 46]. Previous studies showed that ROS, especially from mitochondria, activated the NLRP3 inflammasome [47, 48]. The current findings proposed that the expression of ROS, NLRP3, caspase-1, and IL-1β in the LPS group was increased as compared to that in the control groups; however, after the administration of DHLA, the level of NLRP3, caspase-1, and IL-1β decreased markedly.

Thus, we hypothesized that DHLA prevents the development of LPS-induced neuroinflammation by decreasing the ROS-mediated IL-1β expression through the ERK/Nrf2/HO-1 pathway in rats.

Furthermore, these results supported DHLA treatment to attenuate the oxidative stress-related neuroinflammation for LPS-induced depression.

Nevertheless, the present study. First, the downstream mechanism of Nrf2 to NLRP3 is complex; hence, we targeted only one portion of the pathway. Thus, the probability of other factors involved in DHLA treatment needs further investigation. Second, the protective effects of DHLA might be observed in other cell types such as neurons or microglia, and hence, these factors also need to be explored further.

Conclusions

In conclusion, our study suggests that DHLA has an antidepressant effect in rats with LPS-induced, and these effects are exerted via regulation of Nrf2 signaling and the NLRP3 inflammasome pathway. These results provide a theoretical basis for DHLA as a promising method in the treatment of depression.

Abbreviations

DHLA: Dihydrolipoic acid; FST: Forced swim test; OFT: open field test; LPS: Lipopolysaccharide; ROS: Reactive oxygen species; NLRP3: Nod-like receptor pyrin containing 3 inflammasome; Nrf2: Nuclear factor erythroid 2-like; HO-1: Heme oxygenase-1

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Availability of data and materials

All the necessary data are included within the article. Further data will be shared by request.

Authors' contributions

Authors G-HW, H-TB designed the study and wrote the protocol. Authors H-TB, J-JH, LL, Y-GZ, Y-YW, and HW performed the experiments and analyzed the data. Author H-TB, J-JH and LX managed the literature searches and figure drawing. Authors H-TB wrote the manuscript. LX , H-LW and G-HW revised the manuscript. All authors contributed to have approved the final manuscript.

Ethics approval

All procedures involving animals were approved and carried out according to the guidelines of the Institutional Animals Care Committee of Renmin Hospital of Wuhan University.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Reference

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Figure legends:

Fig. 1 Experimental procedures and timeline.

Experiment 1: Effects of different doses of DHLA on treatment of depression-like rats.

Experiment 2: Mechanism study of DHLA on depression-like rats.

Experiment 3: ERK inhibitor are used to test the mechanism of DHLA on depression-like behaviors rats.

Experiment 4: Nrf2 inhibitor are used to test the mechanism of DHLA on depression-like behaviors rats.

Fig. 2 Effect of different doses of DHLA on treatment of depression-like rats.

a Effect of DHLA and Flu on body weight changes. **b-e** Depression-like behaviors was assessed by forced swimming test (**b**) and open field test (**c, d, e**). The data were expressed as means ± SEM (n=6). $^{****}P < 0.0001$, versus the control group . $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$, $^{\#\#\#\#}P < 0.0001$ versus the LPS group.

Fig. 3. Effect of DHLA on ERK/Nrf2/HO-1 signaling pathway in rats.

a Representative Western blot bands in the hippocampal region. **b-d** Statistical graphs of relative protein expression of p-ERK/ERK (**b**), Nrf2 (**c**), HO-1 (**d**). The data were expressed as means ± SEM (n=6). **e** Representative images of immunofluorescence assays of HO-1 in the hippocampal. Scale bars represent 50 μm. The data were expressed as means ± SEM (n=3). $^{**}P < 0.01$; $^{****}P < 0.0001$, versus the control group. $^{\#\#}P < 0.01$, versus the LPS group.

Fig. 4. Effect of DHLA on ROS/NLRP3/Caspase-1/IL-1b

signaling pathway in rats.

a Representative Western blot bands in the hippocampal region. **b-d** Statistical graphs of relative protein expression of p-ERK/ERK (**b**), Nrf2 (**c**), HO-1 (**d**). **e** ROS expression in the hippocampal. Scale bars represent 50 μm. The data were expressed as means ± SEM (n=6). ***$P < 0.001$; ****$P < 0.0001$, versus the control group . $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.01$,versus the LPS group.

Fig. 5. Effect of optimal dose of DHLA on treatment of depression-like rats. **a** Effect of DHLA on body weight changes. **b-e** Depression-like behaviors was assessed by forced swimming test (**b**) and open field test (**c, d, e**). The data were expressed as means ± SEM (n=6). ****$P < 0.0001$, versus the control group . $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ versus the LPS group.

Fig. 6. Blockade of ERK abolished the anti-depression effect of DHLA

a Effect of DHLA on body weight changes. **b-e** Depression-like behaviors was assessed by forced swimming test (**b**) and open field test (**c, d, e**). The data were expressed as

means ± SEM (n=6). $^{**}P < 0.01$; $^{****}P < 0.0001$, versus the control group. $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$; $^{\#\#\#\#}P < 0.0001$ versus the LPS group. $^{\&}P < 0.05$; $^{\&\&}P < 0.01$ versus the LPS+DHLA group.

Fig. 7. Blockade of ERK abolished the anti-Inflammation effect of DHLA

a Representative Western blot bands in the hippocampal region. **b-f** Statistical graphs of relative protein expression of Nrf2 (**b**), HO-1 (**c**), NLRP3 (**d**), Caspase-1 (**e**), IL-1b (**f**). **g** ROS expression in the hippocampal. The data were expressed as means ± SEM (n=6). $^{****}P < 0.0001$, versus the control group . $^{\#}P < 0.05$; $^{\#\#\#\#}P < 0.0001$,versus the LPS group. $^{\&}P < 0.05$; $^{\&\&}P < 0.01$ versus the LPS+DHLA group.

Fig. 8. Blockade of Nrf2 abolished the anti-depression effect of DHLA

a Effect of DHLA on body weight changes. **b-e** Depression-like behaviors was assessed by forced swimming test (**b**) and open field test (**c, d, e**). The data were expressed as means ± SEM (n=6). $^{****}P < 0.0001$, versus the control

group. $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ versus the LPS group. $^{\&}P < 0.05$; $^{\&\&}P < 0.01$ versus the LPS+DHLA group.

Fig. 9. Blockade of Nrf2 abolished the anti-Inflammation effect of DHLA

a Representative Western blot bands in the hippocampal region. **b-f** Statistical graphs of relative protein expression of Nrf2 (**b**), HO-1 (**c**), NLRP3 (**d**), Caspase-1 (**e**), IL-1b (**f**). **g** ROS expression in the hippocampal. The data were expressed as means ± SEM (n=6). $^{**}P < 0.0001$; $^{****}P < 0.0001$, versus the control group . $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$; $^{\#\#\#\#}P < 0.0001$,versus the LPS group. $^{\&}P < 0.05$; $^{\&\&}P < 0.01$ versus the LPS+DHLA group.