Metabolic Brain Disease (2019) 34:319–329
https://doi.org/10.1007/s11011-018-0342-z

ORIGINAL ARTICLE

Allicin attenuated chronic social defeat stress induced depressive-like behaviors through suppression of NLRP3 inflammasome

Wenqi Gao[1] · Wei Wang[1] · Gang Liu[2] · Jing Zhang[1] · Jian Yang[1] · Zhifang Deng[3]

Received: 24 July 2018 / Accepted: 8 November 2018 / Published online: 4 December 2018

Abstract

Allicin, one of the main biologically active compounds derived from garlic, was previously reported to possess multiple pharmacological activities. Whether allicin protected against chronic social defeat stress (CSDS) induced depressive-like behaviors remained unknown. Thus, our present study for the first time investigated the potential antidepressant effects and the mechanisms of allicin on the CSDS mice model. Thirty minutes before social defeat stress, allicin (2, 10, 50 mg/kg) was treated by intraperitoneal injection. The duration times of CSDS model establishment and allicin intervene were 10 days. Subsequently, the force swimming test (FST), social interaction test (SIT), and sucrose preference test (SPT) were applied for behavioral assessments. The levels of inflammation mediators were determined by commercial ELISA kits. The concentration of iron was tested, and relative protein expressions were measured by western blot. Oxidative stress and apoptosis markers were also detected by commercial kits and western blot. The behavioral defects induced by social defeat stress were obviously improved by allicin. Microglia activation, as well as inflammatory cytokines elevation in the hippocampus of CSDS also down-regulated by administration of allicin. Furthermore, content of iron and protein expressions of key components in iron metabolism were remarkably aberrant changed in the CSDS mice hippocampus, meanwhile, allicin ameliorated this phenomenon. Allicin decreased the production of reactive oxygen species (ROS), malondialdehyde (MDA), and protein carbonyl, and the protein expression of NOX4, as well as up-regulated the activities of superoxide dismutase (SOD) and Nrf2/HO-1 pathway. In addition, allicin attenuated the enhanced neuronal apoptosis. Finally, allicin supplementation inhibited the Nucleotide-binding oligomerization domain containing 3 (NLRP3) inflammasome hyperactivity, and the expressions of inflammasome components, such as ACS, caspase-1, and IL-1β in the hippocampus of CSDS mice. Allicin attenuated depressive-like behaviors of CSDS mice through reducing neuroinflammation, ameliorating iron abnromal accumulation, balacing oxidative stress, and attenuation neuronal apoptosis in the hippocampus via suppression of NLRP3 inflammasome.

Keywords Allicin · Chronic social defeat stress · Depressive-like behavior · Neuroinflammation · Iron homeostasis · NLRP3

✉ Jian Yang
yangjian@ctgu.edu.cn

✉ Zhifang Deng
dengzhifang@ctgu.edu.cn

[1] Department of Central Experimental Laboratory & Yichang Key Laboratory of Ischemic Cardiovascular and Cerebrovascular Disease Translational Medicine, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, Yichang 443003, China

[2] Department of Pharmacology, School of Basic Medical Sciences, Guizhou Medical University, Guiyang, Guizhou, China

[3] Department of Pharmacy, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, Yichang 443000, China

Abbreviations

CSDS	Chronic social defeat stress
SPT	Sucrose preference test
SIT	Social interaction test
FST	Force swimming test
Fpn1	Ferroportin
DMT-1	Divalent metal trasporter-1
Iba-1	Ionized calcium-binding adapter molecular 1
ROS	Reactive oxygen species
MDA	Malondialdehyde
SOD	Superoxide dismutase
NOX	NADPH oxidase
Nrf2	Nuclear factor erythroid 2-related factor 2
HO-1	Heme oxygenase-1

NLRP3 Nucleotide-binding oligomerization domain containing 3
IL-1β Interleukin-1β

Introduction

Depression is a common and devastating illness, which is a leading cause of disability throughout the world (Whiteford et al. 2013). Over 15% of the world population have been affected, it has become a personal suffering and social problem (Kessler et al. 2003). At present, more than twenty different antidepressant medications are used for the treatment of depression, however, there are high rates of partial- or non-response, furthermore, a substantial proportion of patients fail to achieve a sustained remission (Gaynes et al. 2009). The ambiguity of molecular mechanisms in pathophysiological process of the depression and in the therapeutic actions of the antidepressants are the primary contributors to the poor outcomes of antidepressant treatment. Therefore, it is critical to understand its aetiology and to innovate therapeutic strategies.

Mounting investigations gradually reveal there is a close linkage between major depressive disorder and inflammation. In clinical investigations, the release of pro-inflammatory cytokines, in particular, tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), are higher in depressed patients than those of normal people, suggesting the important role of inflammation in the pathophysiology of depression (Al-Hakeim et al. 2015; Dowlati et al. 2010). Furthermore, it is demonstrated, in the depressed patients, treatment of antidepressant medication decreased serum levels of inflammatory cytokines (Hannestad et al. 2011). In addition, higher levels of proinflammatory cytokines and activation of microglia are also similarly detected in the depressed animal models (Yamawaki et al. 2018; Jiang et al. 2017). Thus, these research findings indicate that it is necessary to search for antidepressants by focusing on anti-inflammatory properties.

Iron is crucial factor for living cells and various biological processes (Brittenham et al. 2000; Todorich et al. 2009). Normal level of iron is essential to maintain regular physiological brain function, including oxygen transportation, DNA synthesis, mitochondrial respiration, neurotransmitter synthesis and metabolism (Boelaert and Crichton 2001). Numerous studies report that neuroinflammation and activation of glial cells disturb iron homoeostasis and lead to elevation store of iron (Lee et al. 2004; Nemeth et al. 2004). Neurotoxicity might occur during excess concentration of iron which triggering oxidative stress by generating reactive oxygen species (ROS) and leading to the oxidation and modification of proteins, lipids, and DNA, finally, resulting in various downstream effects including gene expression changes and cell apoptosis (Ward and Crichton 2013; Ward and Crichton 2006).

NLRP3, an intracellular multi-protein complex, primarily appears in inflammatory cells, such as monocytes, macrophages, and splenic neutrophils (Guarda et al. 2011). NLRP3 is composed of an apoptosis-associated speck-like protein containing a card (ASC) and an effector molecule (caspase-1). The abnormal activation of NLRP3 inflammasome is tightly related to the pathogenesis of neuropsychiatric diseases, especially depression (Li et al. 2017). Clinical evidences show the concentration of NLRP3 inflammasome and IL-1β are significantly increase in blood of depressed patients. In addition, in NLRP3-deficient mice, the depressive-like behaviors induced by stress are markedly ameliorated (Alcocer-Gomez et al. 2016). Overall, all the above evidences imply that NLRP3 may be an outstanding therapeutic target for treat neuroinflammation related depression.

There is an increasing interest in exploring the new antidepressants based on anti-inflammation regulation, and the food-medicine, natural extracts and plant preparations to cure depression are considered and popularly in using. Allicin, main biologically active compounds derived from garlic, can freely permeate through the blood-brain barrier and accumulate up to high level in the brain (Chung 2006). Recently, an increasing number of studies have reported that allicin possessed outstanding neuroprotective effects in various animal disease models (Chen et al. 2014; Zhang et al. 2018; Kong et al. 2017; Xiang et al. 2017). Nevertheless, there are no reports demonstrated whether allicin possesses functions in protection CSDS mice against depressive-like behaviors. Thus, we performed the present investigation. Our results showed for the first time that allicin improved depressive-like behaviors by ameliorating the neuroinflammation, iron aberrant accumulation, oxidative stress, and neuronal apoptosis through inhibiting the NLRP3 pathway in the hippocampus.

Method

Animals

The male CD-1 mice weighing 23–25 g and 8-week-old male C57BL/6 J mice were obtained from the Experimental Animal Center of China Three Gorges University. All mice were housed in a 12-h dark/light cycle (20 ± 2 °C) and humidity controlled environment with free access to water and food for 1 week. All experimental procedures were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Ethics Committee of the China Three Gorges University.

Drug treatments and experimental design

The allicin dosage was dependent on the previous publications (Chen et al. 2014; Kong et al. 2017; Zhu et al. 2012; Zhang et al. 2015), which investigated the protective effects of allicin on brain diseases. The mechanisms of allicin in quoting publications were based on inflammation, oxidative stress and apoptosis in brain. Therefore, 2, 10, 50 mg/kg allicin were chose for our present study.

The C57BL/6 J mice were divided into five groups ($n = 10$ each group): Control, CSDS, CSDS + allicin (2, 10, 50 mg/kg). The establishment for CSDS model continued for 10 days. During each day, the experimental C57BL/6 J mice were exposed to different aggressive CD1 mice for 10 min, and then, plastic dividers containing holes were used to separate them. The allicin was treated once day by intraperitoneal injection at 30 min before social defeat stress. Allicin was obtained from MeilunBio Co., Ltd. (MB5783, Meilun, China) and dissolved in DMSO and diluted in saline. The final concentration of DMSO used was less than 1 ‰ (*V*/V). Control and CSDS groups were administered an equipotent volume of vehicle. All behavioral tests were performed during the light period (between 8:00 and 17:00) under dim light. The SPT, SIT, and FST were performed from the eleventh day. After all the behavioral tests, all animals were sacrificed by CO_2. Hippocampus tissue were collected, isolated and stored at −80 °C.

Sucrose preference test

The sucrose preference test was performed on the eleventh day. During the first 2 days, each C57BL/6 J mouse was individually exposed to two bottles containing pure water and 1% sucrose solution, respectively. In order to avoid place preference, bottles of the 1% sucrose solution and pure water were changed daily. On the 3rd day, both the food and two bottles were deprived for 18 h. On the 4th days, the test began and lasted for 6 h. At the end of test, the two bottles were weighed and calculated sucrose preference by the equation: Sucrose preference (%) = sucrose intake / (sucrose intake + water intake) × 100%.

Force swimming test

This test was performed after SPT according to previously study (Porsolt et al. 1977). A clear plastic cylinder (height 30 cm; diameter 15 cm) containing 10 cm of water at 23 ± 2 °C was used in this behavioral test. The behaviors that the C57BL/6 J mice floating in the water without struggling and only moving to keep their head or nose above the water were identified as immobility. The total test time was 6 min. Immobility time was recorded during the final 4 min.

Social interaction test

This test was performed according to previous reports (Berton et al. 2006). The social interaction test contained two trials ("target absent" trial, "target present" trial). In the "target absent" trial, the C57BL/6 J mouse was placed in an empty cage. In the "target present" trial, an unfamiliar CD-1 mouse was placed in the cage to stay with the C57BL/6 J mouse. After each trial, the equipment was cleaned with 70% ethanol to remove olfactory cues. Each trial lasted for 5 min, and the duration time in the interaction zone spent by the test mouse was individually recorded.

Immunofluorescent staining

For immunofluorescent staining, anti-Iba-1 was used as primary antibody to incubate with paraffin sections (Abcam, 1:200). Cy3-Labeled goat anti-rabbit IgG antibodies (Beyotime, 1:1000) was served as secondary reagent to apply. Immunofluorescent images were obtained with an inverted fluorescence microscope (Leica, Germany). The results of immunofluorescent staining were selected from three randomly sections of each mouse.

Measurement of pro-inflammatory cytokines release by ELISA

The mice were sacrificed and hippocampal tissues were immediately dissected. The RIPA (Servicebio, China) was used to extract the hippocampal protein. The lysates were centrifuged (12,000×g, 15 min, 4 °C), and the supernatants were collected. One part of supernatants were used for western blot, the other part were reserved to detect the levels of pro-inflammatory factors, TNF-α, IL-6, and IL-1β. The commercial kits were used following the manufacturer's instructions (Boster, China).

Iron accumulation assay

The level of iron in the hippocampus were measured by the commercial kit (BioVision, USA) according to the manufacturer's instruction. The optical density at 590 nm was measured with a microplate reader (Thermo, USA). The concentration of iron was calculated following the standard curve obtained and presented as mean fold change.

Oxidative factors assay

The activity of SOD and contents of ROS, MDA and protein carbonylation in the hippocampus were determined by commercial kits (Nanjing Jiancheng, China). All the experiment procedures followed the instructions of commercial kits.

Nissl staining

The paraffin blocks were sliced into 5 μm thick sections, which were deparaffinized, rehydrated and stained with 0.5% cresyl violet for 10 min at room temperature. The stained sections were washed by double distilled water and 75% ethanol two times. Then, light microscope (Leica, Germany) was used to observe the Nissl staining of hippocampus. The results of Nissl staining were selected from three randomly sections of each mouse.

TUNEL staining

An in situ cell death detection kit (Roche, Germany) was used for TUNEL staining according to the manufacturer's instructions. The results of TUNEL staining were selected from three randomly sections of each mouse.

Immunostaining

The hippocampus paraffin was cut into 6 μm thickness sections, which then incubated with NLRP3 antibody (Abcam, 1:200) overnight at 4 °C and followed by the secondary goat anti-rabbit IgG (Servicebio; 1:300) for 2 h at room temperature. Leica microscope was used to obtain the images. The results of immunostaining were selected from three randomly sections of each mouse.

Western blot

The 100 μg protein were separated by 8% polyacrylamide gel electrophoresis and transferred onto PVDF membranes (0.45 μm, Millipore, USA). Then, the membranes were blocked with 5% (*w*/*v*) nonfat milk for 2 h at room temperature and subsequently incubated with the following primary antibodies at 4 °C overnight: Iba-1(Abcam,1:400), CD-11b (Abcam, 1:500), Fpn1 (Proteintech, 1:500), Hepcidin (Proteintech, 1:500), DMT1 (Proteintech, 1:500), Ferritin (Proteintech, 1:500), NOX4 (Proteintech,1:800), Nrf2 (Proteintech, 1:800), HO-1 (Proteintech, 1:800), BCL-2 (Proteintech, 1:500), BAX (Proteintech, 1:500), Caspase3 (Cell Signaling Technology, 1:600), Cleaved Caspase3 (Cell Signaling Technology, 1:600), NLRP3(Abcam, 1:500), ACS (Abcam, 1:500), Caspase1 (Abcam, 1:500), IL-1β (Abcam, 1:500). After incubation with the horseradish peroxidase-conjugated secondary antibodies (Servicebio, China) for 2 h at room temperature, the membranes were visualized by the chemiluminescence system (Clinx Science Instruments Co. China).

Statistical analysis

The data were shown as means ± SD. The comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by post hoc LSD test or two-way ANOVA followed by post hoc Bonferroni's test. A value of $P < 0.05$ was considered as significant.

Results

Effects of allicin on depressive behaviors of CSDS mice

The experimental procedure of present study was exhibited in Fig. 1. After the CSDS model was built, behavioral tests, including sucrose preference, social interaction and force swim, were performed. As shown in Fig. 2, mice exposed to social defeat stress showed less bodyweights compared with control mice (Fig. 2a). The CSDS mice showed significantly decreased ratio of sucrose consumption (Fig. 2a), reduced interaction time in SIT (Fig. 2d), and increased immobility time in FST (Fig. 2c). However, allicin (10, 50 mg/kg) treatment significantly improved the depressive-like behaviors in CSDS mice, including increase bodyweight, ratio of sucrose

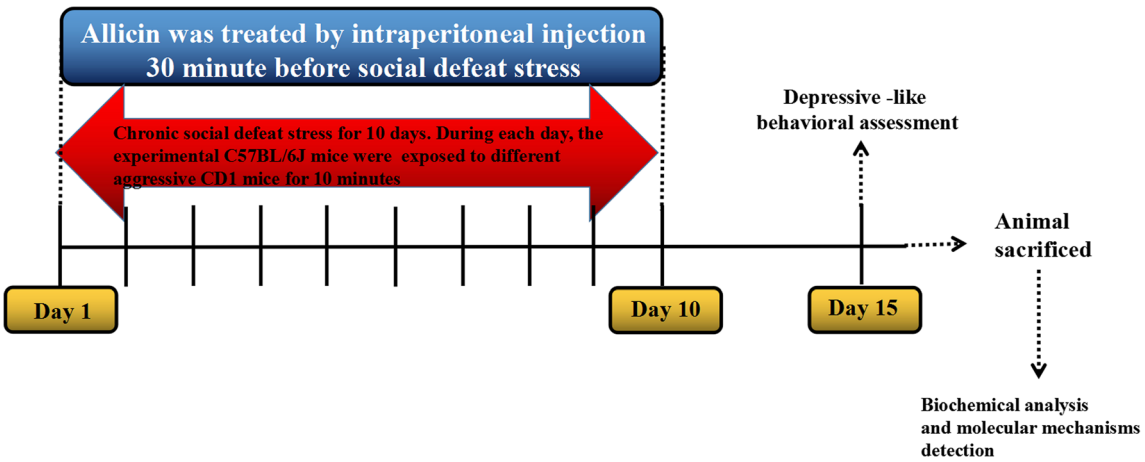

Fig. 1 The experimental procedure of present study

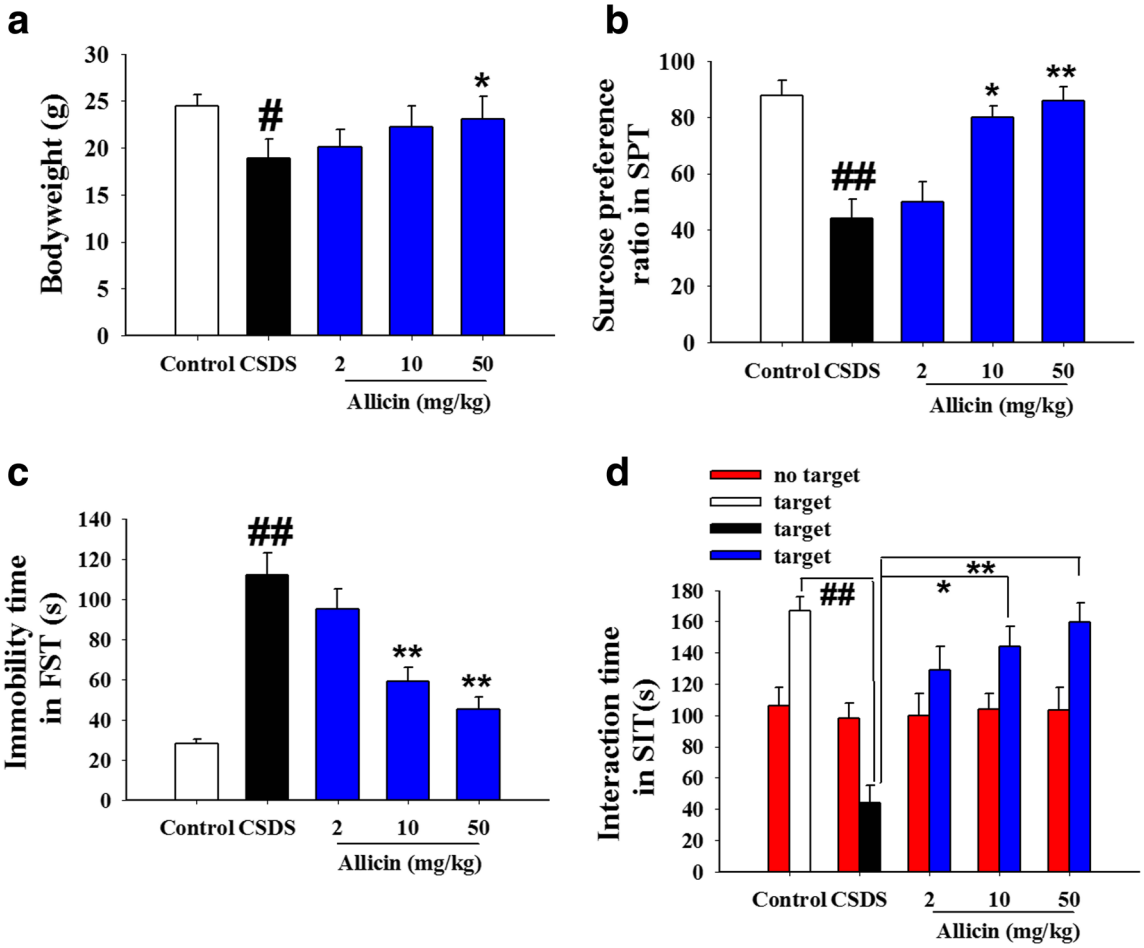

Fig. 2 The effects of allicin on bodyweights and behavioral tests. **a** Bodyweight. **b** Surcose preference ratio. **c** Immobility time in force swim test. **d** Interaction time in social interaction test. Data are expressed as mean ± SD, $n = 10$. #$p < 0.05$, ##$p < 0.01$, versus the control group, *$p < 0.05$, **$p < 0.01$, versus the CSDS group

consumption, social interaction time, and mobility time in FST. The present results suggested that chronic social defeat stress successfully induced depression-like behaviors and allicin possessed anti-depressive effects in CSDS mice. The beneficial effects of 50 mg/kg dose was more remarkable than the others, thus, we chose 50 mg/kg dose to investigate the potential mechanisms.

Effects of allicin on neuroinflammation in the hippocampus of CSDS mice

To determine the role of neuroinflammation in depressive-like behaviors and the functions of allicin, the assays of immunofluorescence, western blot, and QPCR were used. As shown in Fig. 3, the accumulation and amount of Iba-1-labeled microglial cells were significantly larger in the hippocampus of CSDS mice than normal animal (Fig. 3a, b). The protein expressions of Iba-1 (Fig. 3c) and CD-11b (Fig. 3d), another microglial marker, were also increased significantly in the CSDS mice hippocampus. Allicin-treated mice exhibited remarkable inhibition in neuroinflammation by improvement in staining of Iba-1, as well as reduction in protein expressions of Iba-1 and CD-11b. In addition, social defeat stress dramatically up-regulated the levels of proinflammatory cytokines in the hippocampus, IL-1β (Fig. 3e), IL-6 (Fig. 3f), TNF-α (Fig. 3g) which were also substantially corrected by allicin. Taken together, the present results demonstrated the social defeat stress induced neuroinflammation and microglial activation in the hippocampus, which was conspicuously inhibited by allicin.

Effects of allicin on iron homeostasis in the hippocampus of CSDS mice

Iron homeostasis might be perturbed after glial cells activated during neuroinflammation (Lee et al. 2004; Nemeth et al. 2004). Moreover, iron imbalance and aberrant accumulation in the brain was involved in the pathophysiological process of several neurodegenerative disorders (Ward et al. 2014). In order to verify whether this phenomenon induced by social

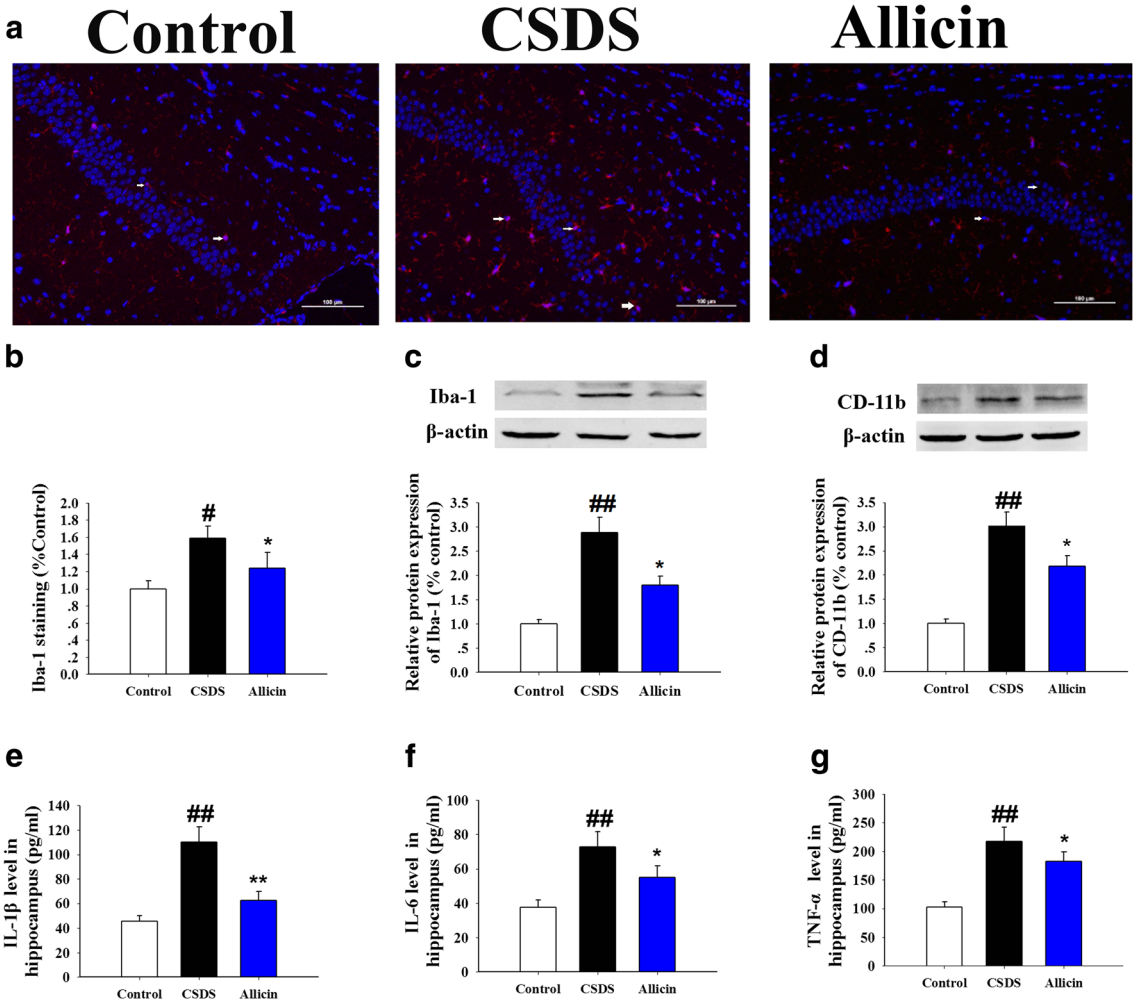

Fig. 3 The effects of allicin on neuroinflammation markers in the hippocampus. **a** Iba-1 immunofluorescence. **b** Quantitation of Iba-1 staining. **c** Protein expression of Iba-1. (D) Protein expression of CD-11b. (E-G) Levels of IL-1β, IL-6, TNF-α. Data are expressed as mean ± SD, $n = 5$. ##$p < 0.01$, versus the control group, *$p < 0.05$, **$p < 0.01$, versus the CSDS group

defeat stress and improved by allicin administration, the iron content and protein expressions of key components in iron regulation were measured. As shown in Fig. 4, compared to control group, iron excessively accumulated in the hippocampus of CSDS mice (Fig. 4a). Furthermore, social defeat stress for 10 days caused protein expressions change, including Fpn-1 reduction (Fig. 4c), hepcidin (Fig. 4d), DMT1 (Fig. 4e) and ferritin (Fig. 4f) elevation. However, pretreatment of allicin significantly improved iron aberrant deposition and corrected protein expressions, suggesting allicin possessed protective effects on iron regulation.

Effects of allicin on oxidative stress in the hippocampus of CSDS mice

The iron concentration exceeds the cellular iron sequestration capacity of storage proteins or other molecules might trigger oxidative stress (Kruszewski 2003), therefore, the key components and pathway related to the oxidative stress were also examined. The present results found that levels of ROS (Fig. 5a), MDA (Fig. 5b), and protein carbonyl (Fig. 5c) elevated in the hippocampus of CSDS mice, which significantly reduced after allicin treatment. It was proved previously that NOX4 contributed to ROS generation (Richter et al. 2015). Our results suggested the protein expression of NOX4 increased in the CSDS group, and the allicin treatment significantly corrected the abnormal protein expression of NOX4 (Fig. 5f). Moreover, Nrf2/HO-1 pathway, function as cellular defense and contributing to antioxidation, was also investigated in our study. The Nrf2/HO-1 pathway and SOD activity were both found to decrease in the hippocampus of CSDS mice, but allicin have significantly increased protein expressions of Nrf2/HO-1 pathway (Fig. 5f, g) and SOD (Fig. 5d) activity. In all, allicin treated-CSDS mice improved oxidative stress by adjust the balance of oxidative stress markers and antioxidants.

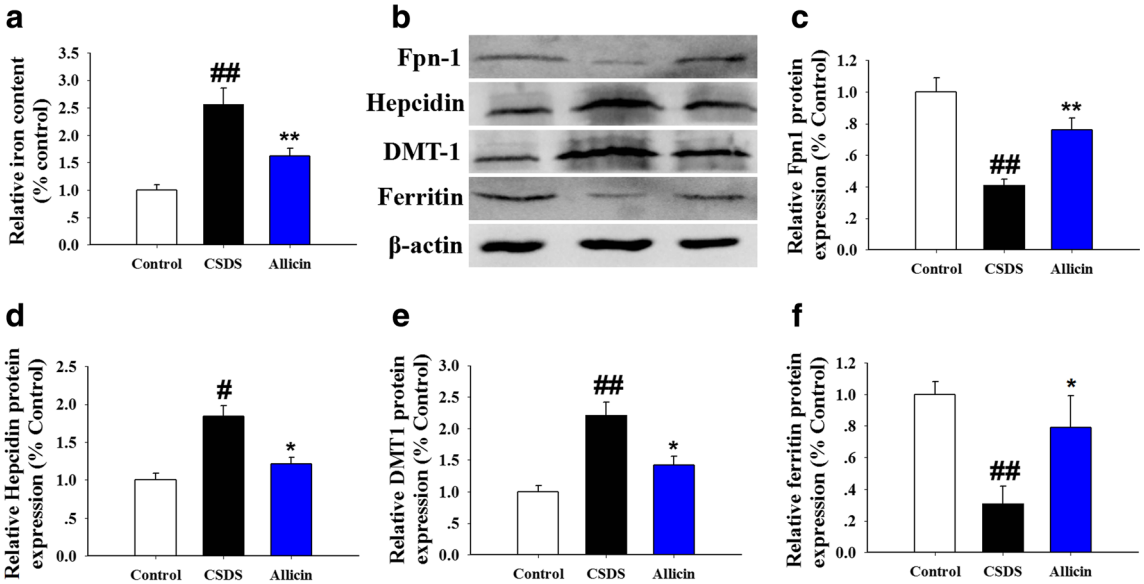

Fig. 4 The effects of allicin on the iron metabolism. **a** Relative iron concentration. **b**–**f** Protein expressions of Fpn-1, Hepcidin, DMT1, Ferritin. Data are expressed as mean ± SD, $n = 5$. #$p < 0.05$, ##$p < 0.01$, versus the control group, *$p < 0.05$, **$p < 0.01$, versus the CSDS group

Effects of allicin on neuronal damage in the hippocampus of CSDS

Neuronal damage and apoptosis were evaluated by Nissl and TUNEL staining and expressions of key proteins in apoptosis. In control group, Nissl bodies in the neurons were abundant and the neuronal morphology was integrity. However, CSDS mice were found that apparent cell losses and less nuclear integrity. In addition, administration of allicin significantly increased the mean number of surviving cells in the CA1 region (Fig. 6g). In TUNEL staining, the number of TUNEL-positive neuronal cells in the CSDS mice CA1 region was obviously increased, indicating a significant increment of cell apoptosis. Nevertheless, these changes were effectively restored by the allicin treatment (Fig. 6f). ki67 immunofluorescence staining showed that allicin elevated neurogenesis (Fig. 6h).

The expression of cleaved caspase-3 and ratio of Bcl-2/Bax in the hippocampus of CSDS mice were increased, while allicin significantly inhibited the activation of caspase-3 (Fig. 6d) and decreased Bcl-2/Bax ratio (Fig. 6b, c).

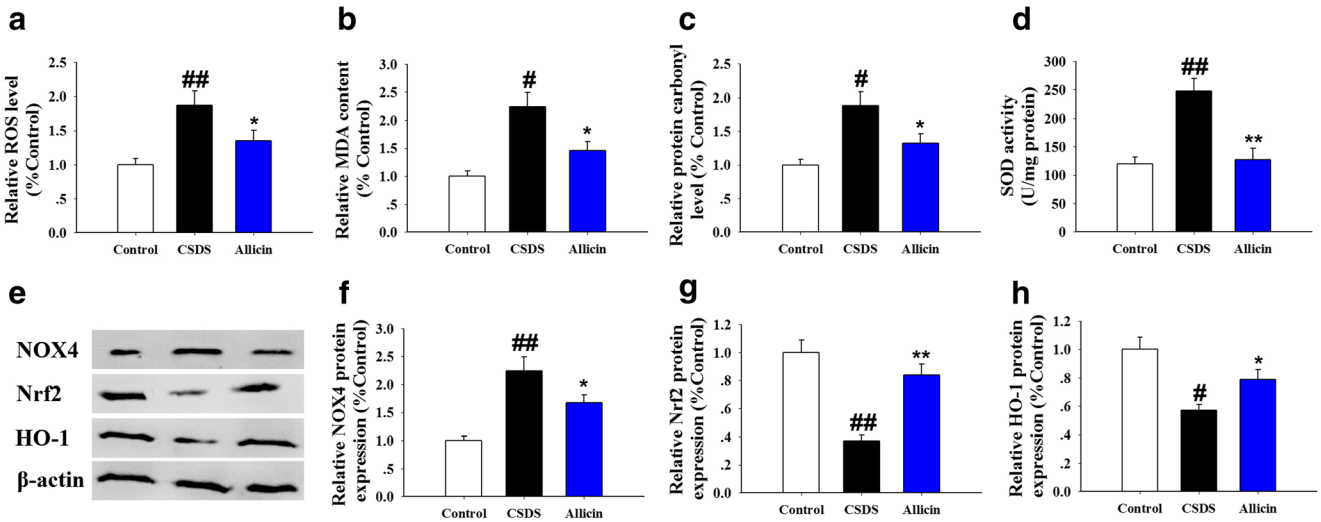

Fig. 5 The effects of allicin on the oxidative stress and antioxidant markers. **a**–**c** Relative ROS, MDA and protein carbonylation levels. **d** SOD activity. (E-H) Protein expressions of NOX4, Nrf2, and HO-1. Data are expressed as mean ± SD, $n = 5$. #$p < 0.05$, ##$p < 0.01$, versus the control group, *$p < 0.05$, **$p < 0.01$, versus the CSDS group

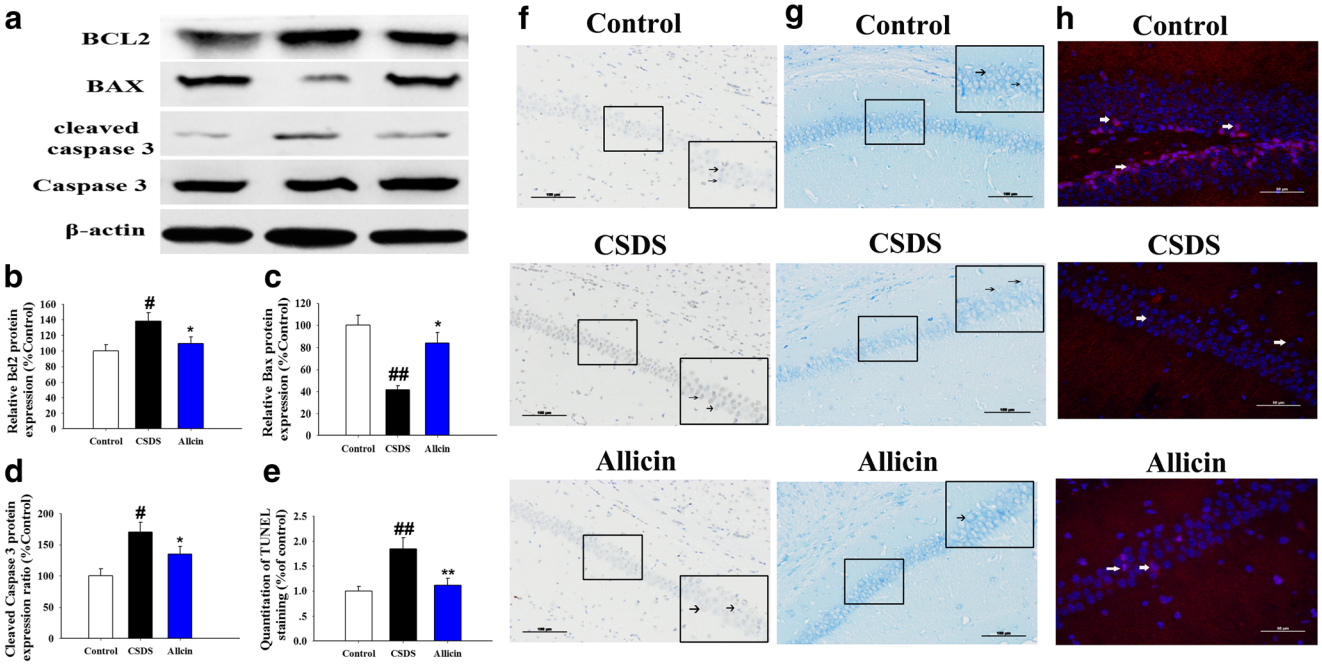

Fig. 6 The effects of allicin on neuronal death in hippocampus of CSDS mice. **a**–**d** Protein expressions of Bcl2, BAX and caspase 3. **e** TUNEL staining of hippocampal neurons. **f** Quantitation of TUNEL staining. **g** Nissl staining of hippocampal neurons. (H) Ki67 immunofluorescence staining. Data are expressed as mean ± SD, n = 5. #$p < 0.05$, ##$p < 0.01$, versus the control group, *$p < 0.05$, versus the CSDS group

Effects of allicin on NLRP3 expression in the hippocampus of CSDS mice

To determine whether hyperactivation of NLRP3 inflammasome was related to the behavioral changes in CSDS mice, the protein expressions of NLRP3 pathway were measured in the hippocampus. Our data found, the protein expression of NLRP3 in CSDS mice was significantly higher than that of the control mice (Fig. 7a–d). Similarly, social defeat stress up-regulated the protein expression of ACS (Fig. 7e), elevated the activation degree of caspase-1 (Fig. 7f), and enhanced the protein expression of mature IL-1β (Fig. 7g). However, allicin administration attenuated the activation level of NLRP3 inflammasome, corrected the protein levels of NLRP3, ASC, and caspase-1, and finally, reduced the IL-1β protein expression in the hippocampus of CSDS mice.

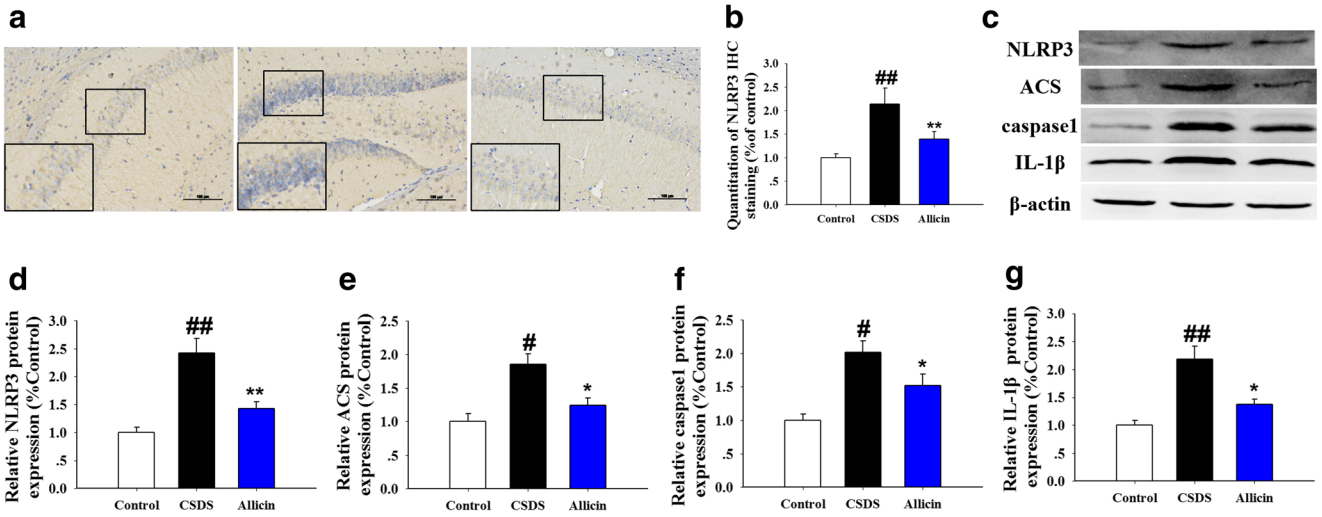

Fig. 7 The effects of allicin on the NLRP3 inflammasome and corresponding components. **a** NLRP3 immunohistochemical. **b** Quantitation of NLRP3 IHC staining. **c**–**g**) Protein expressions of NLRP3, ACS, caspase1, and IL-1β. Data are expressed as mean ± SD, $n = 5$. #$p < 0.05$, ##$p < 0.01$, versus the control group, *$p < 0.05$, versus the CSDS group

Discussion

Recently, the researches of pathogenesis mechanisms under depression have moved beyond the monoamine hypothesis. Multiple evidences support that neuroinflammation might be involved in the pathophysiology of depression and, moreover, might be served as effective therapeutical target (Uher et al. 2014; Berk et al. 2011). Products derived from a variety of natural food have been reported to possess the anti-inflammation and neuroprotective activities, such as allicin. Allicin could across the blood-brain barrier, causing neuroprotection activities in the brain. However, the ability of allicin attenuated depressive-like behaviors induced by social defeat stress have not yet been studied. Hence, in this study, we demonstrated for the first time that the protective activities of allicin against depressive-like behaviors of CSDS mice and inhibitive effects on NLRP3 inflammasome hyperactivation, which mechanisms might be attributed to attenuation of neuroinflammation, regulation iron homeostasis, improvement of oxidative stress, and reduction of neuronal damage.

Inflammation, increasingly attracting more and more attentions, was considered as a key and core feature in mood disorders. Depression is a inflammation state accompanied with higher levels of pro-inflammatory markers (Schiepers et al. 2005). Inflammatory imbalance was observed in mood disorders in pre-clinical and animal model studies (Howren et al. 2009; Dowlati et al. 2010; Lu et al. 2018; Tang et al. 2018). Furthermore, antidepressant medication could reduce the levels of pro-inflammatory cytokines (Baumeister et al. 2016). In line with these study, CSDS mice in our study also exhibited remarkable neuroinflammation status in the hippocampus, as evidenced by increase levels of pro-inflammation cytokines, elevation protein expressions of microglial markers, Iba-1 and CD-11b.

NLRP3 inflammasome was comprised of Nucleotide-binding oligomerization domain containing 3 (NLRP3), apoptosis-associated speck-like protein (ASC) and pro-caspase-1. The NLRP3 inflammasome complex triggers maturation of caspase-1, which subsequently proteolytically processes its substrates pro-IL-1β into mature IL-1β. Normal activation level of the NLRP3 inflammasome functioned as the host defense (Jo et al. 2016). However, the occurrence of multiple diseases might increase when abnormal and excessive extent activation of NLRP3 inflammasome. Several lines of evidences showed that the NLRP3 inflammasome was closely linked the pathophysiology of depression and chronic neuroinflammation statu (Kaufmann et al. 2017). In depressed patients, NLRP3 inflammasome found to be activate (Alcocer-Gomez et al. 2014). The depression-like behaviors triggered by either stress or LPS in animals accompanied with activation of NLRP3 inflammasomes, which mediating depressed animal model via neuroinflammation. Furthermore, knockout of NLRP3 blocked depressive-like behaviors in animals, increased the stress tolerance and attenuated inflammatory responses (Su et al. 2017). Antidepressant medicines improved depressive-like behaviors via inhibiting NLRP3 inflammasome activation (Du et al. 2016). Similarly, our evidences indicated that higher activation of NLRP3 inflammasome appeared in the hippocamous of CSDS mice, as well as the key components-ASC, caspase-1, and IL-1β. Therefore, all these evidences demonstrated that NLRP3 inflammasome might be an applicable therapeutic target and pathway for depression treatmen.

Inflammation and iron homeostasis are tightly regulated and interplayed with each other. Iron accumulation in neurons and microglia were increased after pro-inflammation factors stimulation (Rathore et al. 2012; Zhang et al. 2012). Moreover, elevated iron store might aggravate disease vulnerability and the response to infection and inflammation (Murray et al. 1978). Additionally, psychological stress could also lead to impaired iron regulation in the brain (Wang et al. 2008). The protein concentration of DMT1, the iron transporter, was elevated in hippocampal neurons associated with the iron abnormal concentration during chronic inflammation. Hepcidin rapidly increased consistent with the inflammatory stimuli triggering iron overload. In order to ravel out whether there was imbalance iron accumulation in the hippocampus of CSDS mice accompanied with chronic neuroinflammation, iron concentration and iron transporter signaling were determined. The increase of iron concentration and abnormal protein expressions of core factors in iron transporter signaling were presented in the hippocampus of CSDS mice, suggesting iron overload emerged. However, although the phenomenon of iron overload was finally appeared in the CSDS mice, whether the inflammatory condition was the unique adverse factor needed to be further investigated because of the complexity of the depression pathogenesis.

Excessive iron concentration and aberrant expressions of iron transporters could induce ROS production, which subsequently triggering oxidative stress and finally leading to neuronal death. In consistence with these studies, our data showed a significant increase level in oxidative stress in the hippocampus, as demonstrated by the obviously elevation levels of ROS, MDA and protein carbonylation and the decrease of SOD activity in the hippocampus of CSDS mice. Similarly, the ratio of ROS producer NOX4 and cellular defense system Nrf2/HO-1 signaling pathway was dysregulation. In addition, the degrees of neurological deficit and apoptotic neuronal death were also elevated in the hippocampus after social defeat stress, as evidenced by the lost of Nissl bodies, the increase of positive apoptotic cells in TUNEL staining, and the increase protein expression of cleaved caspase-3 and Bcl-2/Bax ratio suggested the phenomenon of neuronal loss and apoptosis appears in hippocampus of CSDS mice.

In order to investigate the effects and mechanisms of allicin on depression, a suitable animal model is needed. According

to the previous studies, chronic social defeat stress procedure can induce many behavioral changes compared to the control, such as decreased social interaction (Yan et al. 2010; Berton et al. 2006; Tsankova et al. 2006) and anhedonia (Von Frijtag et al. 2000), accompanied with physiological, neuroendocrinal and neurobiological consequences of social stress. These changes are interpreted as signs mimicking certain aspects of human depression (Meerlo et al. 1996). In addition, there are two reasons to illustrate the why male rodents were chose for the present study. Firstly, women exhibit a higher susceptibility to stress-related illnesses, such as mood and anxiety disorders, than men in general (Kessler 2003), which is particularly true during their reproductive years (Gutiérrez-Lobos 2002). Despite this, most basic studies assessing MDD and BD are performed in males. This is due, in part, to the complexity of studying females given the differences in a number of factors that are observed across the oestrus cycle, for example OXT-R expression and HPA axis activity (Bale et al. 1995). The other one is that male rodents are more appropriate for this model than female, since female rats or mice do not fight each other in a resident–intruder confrontation (Björkqvist 2001).

Recently, allicin has reported to possess several biological activities and beneficial effects, including anti-inflammatory, anti-oxidative stress activities (Chung 2006).

Our present results for the first time added another therapeutic action of allicin—anti-depressive like behaviors. Furthermore, allicin ameliorated excessive inflammation status, deceased inflammation marker level of microglial cell in the hippocampus. Meanwhile, we also found that allicin attenuated iron overload and corrected protein expressions of factors in iron transporter signaling, markedly ameliorated oxidative stress, decreased the neuronal apoptosis. Additionally, down-regulation activation of NLRP3 and corresponding components also participated in the beneficial effects of allicin.

In conclusion, our data showed for the first time that the antidepressant action of allicin was accomplished through the prevention of neuroinflammation, regulation of iron overload and oxidative stress, and interruption of cell apoptosis in the hippocampus. Moreover, the inhibitive effects on NLRP3 inflammasome of allicin was first demonstrated in our results. All these findings supported the potential for allicin as a possible food-medicine agent for depression treatment in future.

Acknowledgements We gratefully acknowledge the National Natural Science Foundation of China (81470387) for the generous financial support.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

References

Alcocer-Gomez E et al (2014) NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder. Brain Behav Immun 36:111–117

Alcocer-Gomez E et al (2016) Stress-induced depressive behaviors require a functional NLRP3 Inflammasome. Mol Neurobiol 53(7): 4874–4882

Al-Hakeim HK, Al-Rammahi DA, Al-Dujaili AH (2015) IL-6, IL-18, sIL-2R, and TNFalpha proinflammatory markers in depression and schizophrenia patients who are free of overt inflammation. J Affect Disord 182:106–114

Bale TL, Dorsa DM, Johnston CA (1995) Oxytocin receptor mRNA expression in the ventromedial hypothalamus during the estrous cycle. J Neurosci 15(1):5058–5064

Baumeister D, Ciufolini S, Mondelli V (2016) Effects of psychotropic drugs on inflammation: consequence or mediator of therapeutic effects in psychiatric treatment? Psychopharmacology 233(9):1575–1589

Berk M, Kapczinski F, Andreazza AC, Dean OM, Giorlando F, Maes M, Yücel M, Gama CS, Dodd S, Dean B, Magalhães PVS, Amminger P, McGorry P, Malhi GS (2011) Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neurosci Biobehav Rev 35(3): 804–817

Berton O, McClung C, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ (2006) Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311(5762):864–868

Björkqvist K (2001) Social defeat as a stressor in humans. Physiol Behav 73(3):435–442

Boelaert JR, Crichton RR (2001) Inorganic biochemistry of iron metabolism :from molecular mechanisms to clinical consequences, 2nd edn. Wiley, Chichester, p 326

Brittenham GM et al (2000) Clinical consequences of new insights in the pathophysiology of disorders of iron and heme metabolism. Hematology Am Soc Hematol Educ Program:39–50

Chen W, Qi J, Feng F, Wang MD, Bao G, Wang T, Xiang M, Xie WF (2014) Neuroprotective effect of allicin against traumatic brain injury via Akt/endothelial nitric oxide synthase pathway-mediated anti-inflammatory and anti-oxidative activities. Neurochem Int 68:28–37

Chung LY (2006) The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide. J Med Food 9(2):205–213

Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL (2010) A meta-analysis of cytokines in major depression. Biol Psychiatry 67(5):446–457

Du RH, et al. (2016) Fluoxetine inhibits NLRP3 inflammasome activation: implication in depression. Int J Neuropsychopharmacol 19(9)

Gaynes BN et al (2009) What did STAR*D teach us? Results from a large-scale, practical, clinical trial for patients with depression. Psychiatr Serv 60(11):1439–1445

Guarda G, Zenger M, Yazdi AS, Schroder K, Ferrero I, Menu P, Tardivel A, Mattmann C, Tschopp J (2011) Differential expression of NLRP3 among hematopoietic cells. J Immunol 186(4):2529–2534

Gutiérrez-Lobos, Karin, et al. "The influence of age on the female/male ratio of treated incidence rates in depression." BMC Psychiatry 2.1(2002):3

Hannestad J, DellaGioia N, Bloch M (2011) The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. Neuropsychopharmacology 36(12):2452–2459

Howren MB, Lamkin DM, Suls J (2009) Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med 71(2):171–186

Jiang P, Guo Y, Dang R, Yang M, Liao D, Li H, Sun Z, Feng Q, Xu P (2017) Salvianolic acid B protects against lipopolysaccharide-induced behavioral deficits and neuroinflammatory response: involvement of autophagy and NLRP3 inflammasome. J Neuroinflammation 14(1):239

Jo EK, Kim JK, Shin DM, Sasakawa C (2016) Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 13(2):148–159

Kaufmann FN, Costa AP, Ghisleni G, Diaz AP, Rodrigues ALS, Peluffo H, Kaster MP (2017) NLRP3 inflammasome-driven pathways in depression: clinical and preclinical findings. Brain Behav Immun 64:367–383

Kessler RC (2003) Epidemiology of women and depression. J Affect Disord 74(1):5–13

Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS, National Comorbidity Survey Replication (2003) The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 289(23):3095–3105

Kong X, Gong S, Su L, Li C, Kong Y (2017) Neuroprotective effects of allicin on ischemia-reperfusion brain injury. Oncotarget 8(61): 104492–104507

Kruszewski M (2003) Labile iron pool: the main determinant of cellular response to oxidative stress. Mutat Res 531(1–2):81–92

Lee P, Peng H, Gelbart T, Beutler E (2004) The IL-6- and lipopolysaccharide-induced transcription of hepcidin in HFE-, transferrin receptor 2-, and beta 2-microglobulin-deficient hepatocytes. Proc Natl Acad Sci U S A 101(25):9263–9265

Li M, Li C, Yu H, Cai X, Shen X, Sun X, Wang J, Zhang Y, Wang C (2017) Lentivirus-mediated interleukin-1beta (IL-1beta) knockdown in the hippocampus alleviates lipopolysaccharide (LPS)-induced memory deficits and anxiety- and depression-like behaviors in mice. J Neuroinflammation 14(1):190

Lu X, Yang RR, Zhang JL, Wang P, Gong Y, Hu WF, Wu Y, Gao MH, Huang C (2018) Tauroursodeoxycholic acid produces antidepressant-like effects in a chronic unpredictable stress model of depression via attenuation of neuroinflammation, oxido-nitrosative stress, and endoplasmic reticulum stress. Fundam Clin Pharmacol 32:363–377

Meerlo P et al (1996) Long-term changes in open field behaviour following a single social defeat in rats can be reversed by sleep deprivation. Physiol Behav 60(1):115–119

Murray MJ, Murray AB, Murray MB, Murray CJ (1978) The adverse effect of iron repletion on the course of certain infections. Br Med J 2(6145):1113–1115

Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T (2004) IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 113(9):1271–1276

Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229(2):327–336

Rathore KI, Redensek A, David S (2012) Iron homeostasis in astrocytes and microglia is differentially regulated by TNF-alpha and TGF-beta1. Glia 60(5):738–750

Richter K, Konzack A, Pihlajaniemi T, Heljasvaara R, Kietzmann T (2015) Redox-fibrosis: impact of TGFbeta1 on ROS generators, mediators and functional consequences. Redox Biol 6:344–352

Schiepers OJ, Wichers MC, Maes M (2005) Cytokines and major depression. Prog Neuro-Psychopharmacol Biol Psychiatry 29(2):201–217

Su WJ et al (2017) NLRP3 gene knockout blocks NF-kappaB and MAPK signaling pathway in CUMS-induced depression mouse model. Behav Brain Res 322(Pt A):1–8

Tang J, Yu W, Chen S, Gao Z, Xiao B (2018) Microglia polarization and endoplasmic reticulum stress in chronic social defeat stress induced depression mouse. Neurochem Res 43:985–994

Todorich B, Pasquini JM, Garcia CI, Paez PM, Connor JR (2009) Oligodendrocytes and myelination: the role of iron. Glia 57(5): 467–478

Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 9(4): 519–525

Uher R, Tansey KE, Dew T, Maier W, Mors O, Hauser J, Dernovsek MZ, Henigsberg N, Souery D, Farmer A, McGuffin P (2014) An inflammatory biomarker as a differential predictor of outcome of depression treatment with escitalopram and nortriptyline. Am J Psychiatry 171(12):1278–1286

Von Frijtag JC et al (2000) Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. Behav Brain Res 117(1):137–146

Wang L, Wang W, Zhao M, Ma L, Li M (2008) Psychological stress induces dysregulation of iron metabolism in rat brain. Neuroscience 155(1):24–30

Ward, R.J. and R.R. Crichton, Metal-based neurodegeneration :from molecular mechanisms to therapeutic strategies. 2006, Chichester: J. Wiley & Sons 227

Ward RJ, Crichton RR (eds) (2013) Metal-based neurodegeneration :from molecular mechanisms to therapeutic strategies, 2nd edn. John Wiley & Sons, Chichester

Ward RJ, Zucca FA, Duyn JH, Crichton RR, Zecca L (2014) The role of iron in brain ageing and neurodegenerative disorders. Lancet Neurol 13(10):1045–1060

Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N, Burstein R, Murray CJL, Vos T (2013) Global burden of disease attributable to mental and substance use disorders: findings from the global burden of disease study 2010. Lancet 382(9904):1575–1586

Xiang Q, Li XH, Yang B, Fang XX, Jia J, Ren J, Dong YC, Ou-Yang C, Wang GC (2017) Allicin attenuates tunicamycin-induced cognitive deficits in rats via its synaptic plasticity regulatory activity. Iran J Basic Med Sci 20(6):676–682

Yamawaki Y, Yoshioka N, Nozaki K, Ito H, Oda K, Harada K, Shirawachi S, Asano S, Aizawa H, Yamawaki S, Kanematsu T, Akagi H (2018) Sodium butyrate abolishes lipopolysaccharide-induced depression-like behaviors and hippocampal microglial activation in mice. Brain Res 1680:13–38

Yan HC et al (2010) Fuzi polysaccharide-1 produces antidepressant-like effects in mice. Int J Neuropsychopharmacol 13(5):623–633

Zhang Z, Zhang K, du X, Li Y (2012) Neuroprotection of desferrioxamine in lipopolysaccharide-induced nigrostriatal dopamine neuron degeneration. Mol Med Rep 5(2):562–566

Zhang B et al (2015) Protective effects of allicin against ischemic stroke in a rat model of middle cerebral artery occlusion. Mol Med Rep 12(3)

Zhang H, Wang P, Xue Y, Liu L, Li Z, Liu Y (2018) Allicin ameliorates cognitive impairment in APP/PS1 mice via suppressing oxidative stress by blocking JNK signaling pathways. Tissue Cell 50:89–95

Zhu JW et al (2012) Neuroprotective effects of allicin on spinal cord ischemia-reperfusion injury via improvement of mitochondrial function in rabbits. Neurochem Int 61(5):640–648