High-mobility group box-1 was released actively and involved in LPS induced depressive-like behavior

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ABSTRACT

Depression disorder is a common mental illness, of which the pathogenesis is not well understood. Studies suggest that immunity imbalance and up-regulation of pro-inflammatory cytokines may be associated with the pathogenesis of depression. High-mobility group box 1 protein (HMGB1) has gained much attention as an important player in innate immune responses and a modulating factor in several inflammatory diseases. Here we sought to explore the role of HMGB1 in the development of depression. Depression model was established with low dose of lipopolysaccharide (LPS) administration. Depressive behavior was reflected with increased immobility time in tail suspension test. Accompanying with depressive-like behavior, translocation of HMGB1 from nuclei to cytoplasm was observed by immuno-fluorescence assays. Meanwhile, no significant necrosis was observed evaluated by hematoxylin-eosin staining. These data indicated that HMGB1 was released actively in the central nervous system. In addition, treating the mice with human recombinant HMGB1 (rHMGB1) could induce the development of depressive-like behavior. Blockage of HMGB1 with GZA abrogated the depressive-like behavior induced by LPS or rHMGB1. These results implicated that HMGB1 was involved in LPS-induced depressive-like behavior.

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1. Introduction

Depression disorder is a common, chronic and life debilitating mental illness characterized by low mood, low self-esteem and disrupted sleeping, eating and cognition. According to the World Health Organization (WHO), depression disorder is one of the main causes of disability, ranking the fourth on the list of the global burden of diseases (see from url: http://www.who.int/mediacentre/factsheets/fs369/en/). However, the mechanisms underlying the pathophysiology of depression disorder remain elusive. Current researches in the spectrum of antidepressant studies mostly focus on the monoamine neurotransmitters system along with their specific reuptake and receptor protein, the function of hypothalamic-pituitary-adrenal (HPA) axis and neurotropic system as well as neurogenesis (Blier and de Montigny, 1994; Duman et al., 1997; Duman and Monteggia, 2006). Clinical trial data suggest that at least one-third of depressed patients are non-responsive or resistant to all clinical antidepressants (Machado et al., 2006; Souery et al., 2006). This implicates the additional biological mechanisms involved in the pathogenesis of depression. The urgent need for new type of antidepressant with higher efficacy, and perhaps with fewer shortcomings, is also very strong. As research continues, interaction between the body and the brain through the immune system became of interest in the field of research in psychiatric disorders. Activation of the inflammatory List of abbreviations: LPS, Lipopolysaccharide; HMGB1, High-mobility group box 1 protein; GZA, Glycyrrhizic acid; TST, Tail suspension test; UCMS, Unpredictable chronic mild stress; POCD, Postoperative cognitive dysfunction; TLR, Toll-like receptor; CXCR4, Chemokine (C-X-C motif) receptor-4.

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response has been observed in patients with depression disorder and depressive animal patterns (Dantzer et al., 2011; Raison et al., 2006). Studies provided strong evidence that exogenous administration of several cytokines caused depressive symptoms in human (McDonald et al., 1987; Niiranen et al., 1988). Moreover, administration of lipopolysaccharide (LPS) to animals could induce depressive-like behavior, which resembled the symptoms of depression and could be abolished by administration of antidepressant medications (e.g. imipramine) (Dunn et al., 2005; Yirmiya, 1996). Those researches proved the contribution of inflammation to the development of depression. Inflammation and pro-inflammatory cytokines may play an important role in the pathophysiology of depression as mentioned above. Therefore, it is reasonable to infer a beneficial effect of anti-inflammatory therapy on depression-like behavior.

High-mobility group box 1 protein (HMGB1) is a ubiquitous chromatin component expressed in nucleated mammalian cells. In 1990s, it was first demonstrated that stimulated mononuclear phagocytes secreted HMGB1 (Wang et al., 1999). By far, we have known that HMGB1 can be secreted actively by various immune cells and non-immune cells (e.g. macrophages, monocytes, neutrophils and neurons) in response to exogenous and endogenous inflammatory stimuli such as endotoxin, tumor-necrosis factor alpha (TNF-α), interleukin-1 (IL-1), interferon gamma (IFN-γ) and CpG DNA (Jiang et al., 2005; Lotze and Tracey, 2005; Sun et al., 2014). Active secretion of HMGB1 is regulated via the process involving phosphorylation, acetylation, packaging into secretory lysosomes, and exocytosis (Xu et al., 2010). In addition, damaged or necrotic nonimmune cells, apoptotic cells not included, passively release HMGB1 (Klune et al., 2008). Extracellular HMGB1 has multiple activities by binding to multiple receptors, and is involved in several processes such as inflammation, immunity, migration, invasion, proliferation, differentiation, antimicrobial defense, and tissue regeneration (Kang et al., 2014; Rauvala and Rouhiainen, 2007). HMGB1 has been functionally characterized as an “alarmin” or “danger signal” whose release from cells serves to inform adjacent (or remote) cells of infection and/or injury, so that an appropriate defensive immune response can be generated. HMGB1 is an important mediator in innate immunity, inflammation and sterile injury. As reviewed by Fleur Schaper, in different disease models like sepsis, ischemia-reperfusion and arthritis, HMGB1-blocking therapies have been tested and the disease course was shown to be ameliorated (Schaper et al., 2013). Recently, several researches reported that HMGB1 was involved in some emotional and cognitive dysfunctions such as postoperative cognitive dysfunction (POCD) (He et al., 2012; Vacas et al., 2014).

In present study, we aim to explore the role of HMGB1 in depression disorder and attempt to throw more light on the relation between inflammation, especially the late-phase mediator, and depression. In addition, glycyrrhizic acid (GZA), a compound exists in liquorice root, is used as an antagonist of HMGB1.

2. Materials and methods

2.1. Reagents

Lipopolysaccharide (LPS, from Escherichia coli 0111:B4, Cat# L2830), recombinant human HMGB1 (rHMGB1, expressed in E. coli, purity≥90%, Cat# H6562), glycyrrhizic acid ammonium salt (GZA, from glycyrrhiza root, Cat# G2137), 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, Cat# D9542) were all obtained from Sigma (Sigma Aldrich, USA). Rabbit anti-HMGB1 antibody (Cat# ab75823) was obtained from Abcam (Abcam, UK). Fluorescein isothiocyanate (FITC) conjugated donkey anti-rabbit IgG antibody (Cat# A-21206) was obtained from Invitrogen (Invitrogen, USA).

2.2. Animals and treatments

Experiments were performed on male BALB/c mice weighing 20–22 g (8-week old) and in accordance with the guidelines issued by the Second Military Medical University. Mice were obtained from Animal Centre (Second Military Medical University, Shanghai, China). Before the onset of the experiments, two weeks were allowed for the mice to adapt to their new circumstances. All animals were maintained under controlled environment (temperature: 22 ± 1 °C; humidity: 52 ± 2%; 12 h day/night rhythm), and received food and water ad libitum except for during the sucrose preference test.

2.2.1. rHMGB1 or LPS administration

On the day of injection, fresh solutions were prepared by dissolving compounds in sterile endotoxin-free isotonic saline and administered intraperitoneally (i.p.).

In the experiment of LPS administration, mice were divided into four treatment groups as follows: vehicle-control (0.9% saline, i.p.); GZA treatment (20 mg/kg, i.p.); LPS treatment (0.8 mg/kg, i.p.); LPS&GZA treatments (LPS 0.8 mg/kg and GZA 20 mg/kg, i.p.). The dosage of GZA was chose referred to previous study (Yu et al., 2005). In consideration of that HMGB1 is released at 6–8 h post-stimulation and peaked at 16–18 h post-stimulation (Wang et al., 1999) (Andersson et al., 2000) and the pharmacokinetics of glycyrrhizic acid (Krahenbuhl et al., 1994), GZA was given twice at 8 and 16 h post LPS injection respectively. Thus, the total dose of GZA in LPS&GZA treatment group was 40 mg/kg. In the experiment of rHMGB1 administration, mice were divided into four treatment groups as follows: vehicle-control (0.9% saline, i.p.); GZA treatment (20 mg/kg, i.p.); rHMGB1 treatment (1 μg/mouse, i.p.); rHMGB1&GZA treatments (rHMGB1 1 μg/mouse and GZA 20 mg/kg, i.p.). In rHMGB1-GZA treatment group, GZA was administered half an hour ahead of rHMGB1 injection. The mice were sacrificed 1, 8, 16, 24 and 48 h post rHMGB1 or LPS administration respectively. In order to reduce the influence of the different administration, the control group received the same volume of saline solution as that of drugs.

2.2.2. Tail suspension test

Tail suspension test (TST) is one of the most commonly used animal behavioral tests for antidepressant screening (Bourin et al., 2005). TST is based on the measurement of time that mice spend in an immobile position. Increased immobility in the test is claimed to reflect a helpless or resignation-like state. TST was performed during the dark phase (19:00 pm–20:00 pm) and the immobility duration was detected automatically by using Tail Suspension Sof-821 (Med Associates Inc.). Briefly, a mouse was suspended by the tail with adhesive tape to a hook for 6 min. A load cell was connected to the hook to measure the mouse’s activity. When the mouse struggled, the load cell would capture and record the load’s changes and represent the activity as a voltage output. When the mouse was in an immobile position, the voltage output was set as the lower threshold. After escape-oriented struggling during the first 1 min, the mice showed increasing bouts of immobility. The duration of the voltage output lower than the lower threshold during the final 5 min was recorded and regarded as the mouse’s immobility duration.

2.3. Immunofluorescence assays and hematoxylin-eosin (HE) staining

As HMGB1 can be secreted actively by stimulated cells and passive released by necrosis cells, cell necrosis and HMGB1 protein relocation were evaluated.

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In our experiments, mice of the vehicle-control group and LPS treatment group (including 8, 16, 24 h post LPS or vehicle administration) were anaesthetized with 10% chloral hydrate and perfused transcardially with normal saline followed by ice-cold 4% paraformaldehyde. The brains were removed and fixed in 4% paraformaldehyde for at least 24 h. Brains were then embedded in paraffin and cut at 3 μm.

The HE staining could visually show the histological changes of mice brains. For hematoxylin and eosin staining, the tissue sections were deparaffinized in xylene and rehydrated in ethanol. Then, the sections were stained with haematoxylin for 20 min followed by counterstaining with eosin for 2 min, dehydrated through 95% alcohol, cleared in xylene and mounted with resinsous mounting medium for microscopic examination. To evaluate the level of necrosis, sections were examined under a light microscope (Olympus, Japan). Prior to immunohistochemistry, the tissue sections were deparaffinized in xylene and rehydrated in ethanol. For the IF assays, a monoclonal rabbit antibodies (abcam, UK) directed against HMGBI1 was used as the primary antibody and a fluorescein isothiocyanate (FITC) conjugated donkey anti-rabbit IgG antibody (Invitrogen, USA) was used as the secondary antibody. Incubation with primary antibody (dilution 1:100) overnight at 4 °C was followed by incubation with secondary antibody (dilution 1:200) for 30 min at 37 °C. Nuclei were stained by 4,6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma, USA) for 2 min at room temperature. Then, the sections were imaged on fluorescence microscope (Olympus, Japan) at ×400.

For the accumulating evidence implicating that hippocampus dysfunction in the pathophysiology of depression (Duman et al., 1997; Duman and Monteggia, 2006), we focused our attention on the changes in hippocampus histology.

2.4. Blood sample collection

After the behavioral tests, the rodents from each group were deeply anesthetized with 10% chloral hydrate. Animals were then sacrificed with sharp scissors immediately. Blood was collected, centrifuged at 3000 rpm for 15 min, and the supernatant was collected. All these samples were stored at −80 °C for further analysis.

2.5. Cytokines analysis

The protein levels of cytokines (TNF-α and IL-1β) were analyzed using a Bio-Plex Pro Mouse Cytokine 6-Plex panel in combination with the Bio-Plex Suspension Array System (Bio-Rad Laboratories Inc., Hercules, CA, USA). This assay is a bead-based suspension array system where microsphere sets are internally dyed with different ratios of fluorophores conjugated to different capture probes (cytokine specific antibodies). This assay have been validated in both blood, brain and CSF (Datta and Opp, 2008). The lower limits of detection for this procedure were 1.4 pg/ml (TNF-α), 9.4 pg/ml (IL-1β). The intra-assay coefficients of variation (%) were 3 (TNF-α), 4 (IL-1β). The inter-assay coefficients of variation (%) were 6 (TNF-α), 7 (IL-1β).

The level of HMGBI1 in serum was measured by enzyme-linked immunosorbent assay (ELISA) following manufacturer's instructions (Westong Inc., Shanghai, China; minimum detectable quantity at 2 pg/ml).

2.6. Statistical analysis

Values were expressed as the mean ± standard error (SEM) and analyzed by one-way analysis of variance (ANOVA) followed by using SPSS software; Bonferroni's Test was used in multiple comparison test and a P-value of less than 0.05 was considered significant. If the means were significantly different, nonparametric test was used. In the analysis of serum level of HMGBI1 and inflammatory cytokines, the protein content of some samples were lower than the minimum detectable quantity and the value was assigned as 2 pg/ml.

3. Results

3.1. Serum HMGBI1 level increases along with depressive behavior 24 h post LPS administration

Generally, depressive-like behavior is evaluated 16–24 h after LPS injection. This time point is chosen because previous study demonstrated that LPS could induce depressive-like behavior without inducing significant sickness behavior in mice (Capuron and Miller, 2011). As shown in Fig. 1A–B, the serum expression levels of pro-inflammatory cytokines increased significantly (A, TNF-α, 24 h vs. control, P < 0.01; B, IL-1β, 24 h vs. control P > 0.1). At this time point, these mice exhibited depressive-like behavior reflected with a significant increase in immobility duration in TST (shown in Fig. 1C, t = 5.04, P < 0.01). Along with the depressive behavior and being different from the serum levels of TNF-α and IL-1β, the serum level of HMGBI1 significantly increased 24 h post-LPS administration (shown in Fig. 2, t = 3.36, P < 0.05).

3.2. HMGBI1 was secreted actively in the brain after LPS administration

As was reported in the previous study of our group and many other studies, the central nervous system was still in an inflammatory state (e.g. increased cytokine protein concentration in the cerebrospinal fluid and hippocampus) post-LPS administration (Frenois et al., 2007; O'Connor et al., 2009; Sulakhiya et al., 2014; Zhang et al., 2014). In accord with the inflammatory state in the brain, HMGBI1 translocation was observed in the mice's brain 24 h post-LPS administration by immunofluorescence (shown in Fig. 3).

HMGBI1 is located in nucleus under normal circumstances and extracellular HMGBI1 can act as an cytokine and chemokine and induce subsequent inflammatory responses. Secretion of HMGBI1 occurs after translocation from nucleus to cytoplasm (Gardella et al., 2002). Thus, translocation of HMGBI1 can be regarded as a sign of HMGBI1 activation. As shown in Fig. 3, in the sections from control group (Fig. 3A–C), the nuclei stained with green strongly, while no cytoplasm stained with green, indicating that HMGBI1 is distributed mainly in the nuclei. As shown in Fig. 3D–F, no significant change was observed 8 h post-LPS administration compared with control group (Fig. 3A–C). As time went by, significant relocation of HMGBI1 from nuclei to cytoplasm was observed 16 h post-LPS administration (as shown in Fig. 3G–I). HMGBI1 relocation still could be observed 24 h post-LPS administration (as shown in Fig. 3J–L). As is mentioned, HMGBI1 can be passively released by damaged or necrotic cells. To verify whether the extra-nuclear HMGBI1 protein was passively released by the necrotic cells, we then tested the necrosis of mice brain slices by HE staining. Karyolysis, karyopyknosis and karyorrhexis are the pathological criteria of cell necrosis. As shown in Fig. 4, no significant necrosis could be observed 24 h post-LPS treatment. This result indicated that HMGBI1 was secreted actively by cells after LPS administration.

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3.3. rHMGB1 induces depressive-like behavior

Since the serum level of HMGB1 significantly increased and the early-phase inflammation mediator returned to normal level when the depressive-like behavior developed 24 h post-LPS administration, we speculated that there might be a close relation between HMGB1 and depressive-like behavior. In order to identify the depressive-like behavior inducible effect of HMGB1, the experiment of recombinant HMGB1 administration was designed. HMGB1 is highly conserved among species, with over 98% sequence identity between rodents and humans (Yang et al., 2005). What is more, human rHMGB1 is commercialized and economized. Therefore, we used human rHMGB1 in our study. Mice were administrated with recombinant human HMGB1. Compared with the normal control, the immobility duration showed a rising tendency 8 h post-rHMGB1 injection, but without statistical significance. With time prolonged, the immobility duration significantly increased 24 h post-rHMGB1 administration. Moreover, the immobility duration returned to baseline 48 h later (Shown in Fig. 5A, $F = 6.56, P < 0.05$; control vs. $t = 2.95, P < 0.05$; $8$ h vs. control $t = 0.81, P > 0.1$; $24$ h vs. control $t = 0.47, P > 0.1$; $24$ h vs. $48$ h $t = 3.05, P < 0.05$).

3.4. GZA abrogated rHMGB1-induced and partially abrogated LPS-induced depressive behavior

Glycyrrhizic acid (GZA), a compound exists in liquorice root, is now used as an anti-inflammatory, anti-allergic and anti-virus drug clinically (Cinatl et al., 2003; Saeedi et al., 2003; van Rossum et al., 1999). Many researches demonstrated that GZA could bind to HMGB1 directly, act as an HMGB1 direct inhibitor and inhibit its cytokine activities (Girard, 2007; Mollica et al., 2007). Thus, in the further experiment, we treated the mice with GZA 30 min pre-rHMGB1 administration to verify whether the behavior changes induced by rHMGB1 could be blocked. As shown in Fig. 5B, GZA itself had no significant effect on the animals' behavior, but GZA pre-treatment did block the depressive-like behavior induced by rHMGB1 ($F = 5.06, P < 0.01$; control vs. GZA $t = 0.70, P > 0.1$; control vs. rHMGB1 $t = 2.98, P < 0.05$; control vs. rHMGB1&GZA $t = 0.19, P > 0.1$; rHMGB1 vs. rHMGB1&GZA $t = 2.23, P < 0.05$). Since GZA could block the effect of rHMGB1, we then treated the mice with GZA 8 h and 16 h post-LPS administration. As shown in Fig. 5C, treatment with GZA could partially improve the LPS induced depressive behavior ($F = 23.06 P < 0.001$; control vs. LPS $t = 6.778, P < 0.01$; LPS vs. LPS&GZA $t = 2.707, P < 0.05$; control vs. LPS&GZA $t = 3.481, P < 0.01$).

In addition, GZA failed to abolish the increase of HMGB1 or inflammatory cytokines induced by LPS administration (Shown in Fig. 2). Although GZA is used as an anti-inflammatory drug, in our experiment, GZA treatment failed to abrogate the increase of TNF-α, IL-1β or HMGB1 induced by LPS. Thus, blocking of HMGB1 might be the principal mechanism of GZA’s protective effect in LPS induced depressive like behavior. All these results indicated that LPS induced depressive-like behavior was partly due to the increased level of HMGB1.

4. Discussion

The present study shows that HMGB1 is actively released during the LPS induced depression process and establishes the important role of HMGB1 in LPS induced depressive behavior. We provide evidence that HMGB1 acts as a novel late-phase mediator that links
inflammation and subsequent depressive-like behavior. In addition, this study provides more evidence for the inflammatory hypothesis of depression disorder.

Since Ader and Cohen’s report in 1975 (Ader and Cohen, 1975), interaction between the body and the brain through the immune system has become of interest in the field of research in psychiatric disorders. In 1992, “sickness behavior” was adopted as a term for depressive like behavior induced by peripheral immune activation based on the finding that peripheral injection of the bacterial toxin lipopolysaccharide could induce depressive like behavior (Kent et al., 1992). By far, systemic administration of low-dose LPS is a well-established model to study behavioral responses following the

Fig. 3. Translocation of HMGB1 from nuclei to cytoplasm induced by LPS administration. A–C, control group, hippocampus; D–F, 8 h post-LPS treatment, hippocampus; G–I, 16 h post-LPS treatment, hippocampus; J–L, 24 h post-LPS administration, hippocampus. White bar = 20 μm.

Fig. 4. Hematoxylin-eosin staining of brain sections. A, control group, hippocampus; B, 24 h post-LPS treatment, hippocampus.
acute activation of peripheral immune system (Dunn and Swiergiel, 2005). Generally, sickness behavior develops rapidly after administration of LPS and usually peaks at 2–6 h post-treatment, then gradually resolves and evolves into depressive-like behavior after 16–18 h (Capuron and Miller, 2011). Therefore, depressive-like behavior was evaluated at 24 h post-LPS administration in this experiment.

According to our results, the early-phase cytokines (e.g. TNF-α and IL-1β) significantly increased 1 h post-LPS administration and returned to base level 24 h post-LPS administration (Fig. 1A–B). The mice displayed depressive-like behavior-increased immobility time in TST (Fig. 1C), which is consistent with other reports (O’Connor et al., 2009).

Different from the early-phase cytokines, the late-phase inflammation mediator HMGB1 still keeps high level in the plasma at 24 h after LPS administration (Fig. 2). HMGB1 is well known as its role in mediating systemic inflammation, tissue damage, and lethality initiated by bacterial infection and trauma (Andrassy et al., 2008; Okuma et al., 2012; Ulloa and Messmer, 2006). As a late-phase inflammation mediator, HMGB1 is released at 6–8 h and peaked at 16–18 h post-stimulation in vitro (Gardella et al., 2002; Wang et al., 1999). HMGB1 serum level is increased in several (auto)-immune diseases like sepsis, lupus, arthritis and atherosclerosis, and is mostly correlated with disease activity (Kalinina et al., 2004; Taniguchi et al., 2003; Urbonaviciute et al., 2008). Much knowledge has already been amassed by studies in vitro, which investigate the pathways, receptors and post-translational modifications of HMGB1 (Gardella et al., 2002; Yu et al., 2006). Thus, many preclinical studies are attempting to alleviate the symptoms of diseases by using blocking agents or neutralizing antibodies against HMGB1. In different disease models like sepsis, ischemia-reperfusion and arthritis, HMGB1-blocking therapies have been tested and the disease course was shown to be ameliorated (Kim et al., 2012; Kokkola et al., 2003; Wang et al., 1999). In our research, we found that the plasma level of HMGB1 was increased and HMGB1 was secreted in the brain in LPS-induced depression rodent models at 24 h (Figs. 2 and 3). Increased serum level of HMGB1 through administration of recombinant HMGB1 could also produce depressive-like behavior (Fig. 5). Moreover, HMGB1-blocking with glycyrrhizic acid also showed beneficial effect in improving the depressive behavior induced by LPS or rHMGB1 (Fig. 5B and C). These results indicated that HMGB1 might be involved in LPS induced depressive behaviors.

In our study, GZA was used as the antagonist of HMGB1. Different from HMGB1 antibody, GZA interferes HMGB1 combining to its receptor but only mildly with the intranuclear association of HMGB1 with DNA. Moreover, as a clinical medication, GZA is much secure and affordable. It may have huge clinical value, if the beneficial effect of GZA is fully demonstrated. We tested the serum HMGB1 levels of the rodents treated with LPS&GZA. The levels of HMGB1 had no significant difference between the LPS treatment group and the LPS&GZA treatment group (Fig. 2). This result was reasonable because GZA is a low-molecular weight organic compound. The molecular weight of GZA is only about 840. GZA binds to a specific locus of HMGB1 through Van der Waals’ force and interferes HMGB1 combining to its receptor. However, the antibody can react with HMGB1 through other antigenic epitopes.

As HMGB1 can be passively released by necrotic cells and be actively secreted by many kinds of activated cells, cell necrosis and relocation of HMGB1 protein in the brain were evaluated. Significant relocation of HMGB1, but no significant morphological change, was observed (Figs. 3 and 4) indicating that HMGB1 was actively secreted in the brain after LPS stimulus. However, HMGB1 can be secreted actively by various cells, such as macrophages, monocytes, neutrophils and neurons. We do not have enough evidence to draw a conclusion about which kind of cells plays the major role.

Besides, little is known about the downstream signaling pathways via which extracellular HMGB1 induces the depressive-like behavior. Toll-like receptor (TLR) 2, 4 and 9 and chemokine (C-X-C motif) receptor-4 (CXCR4) are the receptors of HMGB1 (Andersson and Tracey, 2011; Schiraldi et al., 2012; Yu et al., 2006), which are also closely related to inflammatory response. Recent study showed an increase in the concentration level of CXCR4 in patients with depression disorder (Oglodek et al., 2014) and that CXCR4 antagonism was beneficial for the neurologic function recovery after stroke (Ruscher et al., 2013). In addition, many researchers reported that TLR 4 activation was also involved in depression disorder (Garate et al., 2011; Lucas and Maes, 2013). In the future, we will focus our attention on the cellular and molecular mechanism of how HMGB1 induces the cognition and emotion...
disorders. Although the inflammation inducible effect of HMGB1 has been confirmed and inflammatory response plays an important role in the pathogenesis of depression disorder, inducing inflammatory response may not be the only way that HMGB1 induces behavior disorder. Intensive study should be performed in the future.

As is known, chronic stress is one of the most important inducements for depression. By inducing neuroinflammation in stress-responsive regions of the mouse brain, which are similar to the effects of other potentially neuroinflammatory stimuli (Farooq et al., 2012; Norman et al., 2009), unpredictable chronic mild stress (UCMS) model also supports the inflammatory hypothesis of depression. Some studies found that psychological stress may compromise the intestinal barrier, and then increased gastrointestinal permeability with translocation of LPS from Gram-negative bacteria may play a role in the pathophysiology of major depression (Garate et al., 2011). Blocking Toll-like receptor 4, which is also the receptor of HMGB1 and LPS, prevents stress-induced priming of neuroinflammatory responses (Weber et al., 2013). In our preliminary experiments, along with the intestinal barrier dysfunction and depressive-like behavior, the serum level of HMGB1 increased after 4-week chronic stress (data not shown). These studies further indicated that HMGB1 might be involved in the pathogenesis of depression disorder. More studies will be performed in the future to verify whether HMGB1 blockade has beneficial effect in UCMS induced depressive-like behavior.

5. Conclusions

In conclusion, in our research we demonstrated that HMGB1, acted as a late-phase inflammation mediator, was released actively post LPS administration and was involved in LPS induced depressive-like behavior. The present study reported the depression-inducible effect of HMGB1 for the first time. Inhibition of HMGB1 may have therapeutic benefits for depression disorder.

Authors’ contributions

Teng-Yun Wu established the depression model and performed the behavioral test, with the help of Lei Liu, Yi-Zhang, Xiao-Liang Shen and Yuan–Yuan Yang. Lei Liu and Wei Zhang analyzed the results and drafted the manuscript. Teng-Yun Wu and Yun-Zi Liu performed the immunohistochemical experiments. Yun-Xia Wang and Chun-Li Jiang secured funding for the project and helped with the final version of the manuscript.

Competing interests

All authors have read and approved the final manuscript. There is no potential competing interest.

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