JQ1 attenuates neuroinflammation by inhibiting the inflammasome-dependent canonical pyroptosis pathway in SAE

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Abstract

Sepsis-associated encephalopathy (SAE) manifests clinically in hyperneuroinflammation. Pyroptosis, which can induce an inflammatory cascade response, has been considered to be a causative factor of SAE. Evidence has shown that the bromo- and extraterminal (BET) proteins (including BRD2, BRD3, BRD4 and BRDT) inhibitor JQ1 can inhibit inflammation and suppress pyroptosis in various diseases. Therefore, we examined the effect of JQ1 on inflammasome-induced pyroptosis in the hippocampus in a mouse model of sepsis induced by lipopolysaccharide (LPS) injection. The results showed that JQ1 treatment alleviated sepsis-related symptoms, protected the blood–brain barrier (BBB), as indicated by upregulated expression of the tight junction proteins occludin and ZO-1, and remarkably rescued neuronal damage in SAE mice. Mechanistically, we demonstrated that JQ1 intervention inhibited the expression of BRD proteins and decreased the expression of inflammasomes by blocking nuclear factor kappa B (NFκB) signalling, attenuating the canonical pyroptosis (mediated by cleaved-Caspase1/11) pathway and the release of proinflammatory factors in the hippocampus of septic mice. Interestingly, we also found that JQ1 selectively suppressed the activation of hippocampal microglia in SAE mice. Thus, JQ1 protected the hippocampal BBB and neuronal damage through the attenuation of neuroinflammation by inhibiting the inflammasome-dependent canonical pyroptosis pathway induced by LPS injection in mice, and JQ1 may be a promising target for the prevention of SAE.

Introduction

Sepsis-associated encephalopathy (SAE) is a diffuse brain dysfunction associated with sepsis that develops a wide variety of disorders in neural functions(Gofton & Young 2012). Neuroinflammation(Xu et al. 2019), loss of blood–brain barrier (BBB) integrity, neuronal death, and microglial activation(Fu et al. 2019; Widmann & Heneka 2014)have been considered causative factors of SAE. Therefore, it is vital to search for a potential strategyto attenuate neuroinflammation, protect the BBB, and inhibitneuronal death and microglial activation in SAE. The bromodomain and extraterminal domain (BET) family, including BRD2, BRD3, BRD4, and BRDT, has been known to regulate genes involved in inflammatory processes through changes in the reading of chromatin(Singh & Sartor 2020).JQ1, a primary tool compound used to study the roles of BET proteins in brain disease models, could achieve its effect by binding to both bromodomains (BD1 and BD2) within all BET proteins and preventing BET proteins from binding to acetylated histones and proteins(Filippakopoulos et al. 2010; Singh & Sartor 2020).BET proteins can be tethered to inflammatory genes through NF-κB (which plays a critical role in inflammation) and increase their expression. In contrast, JQ1 administration disrupts the interaction of the RelA subunit of NF-κB with BRD4, leads to RelA degradation, and suppresses inflammation-associated gene transcription(J. Shi & Vakoc 2014);however, there have been few studies on the effectand mechanismof JQ1 in SAE.

Pyroptosis, characterized by cell membrane perforation and disintegration, is an inflammatory form of programmed cell death(OrningLien, & Fitzgerald 2019). This type of cell death was recently suggested to be dependent on nod-like receptor family protein 1 (NLRP1) or 3 (NLRP3) or absent in melanoma 2 (AIM2)inflammasomes, which can further trigger pyroptosis signalling pathways(Dempsey et al. 2017;

Fan et al. 2018). Pyroptosis is executed by a series of pore-forming proteins called the gasdermin superfamily (GSDMs)(Boise & Collins 2001), including gasdermin A (GSDMA), gasdermin B (GSDMB), gasdermin C (GSDMC), gasdermin D (GSDMD), gasdermin E (GSDME) and pejkakin (PJVK). Except for PJVK(Rogers et al. 2019), all members of the GSDM superfamily contain a conserved two-domain structure: the N-terminal and C-terminal domains. When the N-terminal domain of these GSDMs is released, it possesses pore-forming activity to cause inflammatory death associated with the loss of cell membrane integrity and release of inflammatory mediators(Rogers et al. 2019). Currently, GSDMD and GSDMA(Ding et al. 2016)are confirmed to be cleaved by Caspase-1/11, both of which mediate the canonical pyroptosis pathway. GSDMC is specifically cleaved by Caspase-8 and mediates the noncanonical pyroptosis pathway(Hou et al. 2020), while GSDME is similarly cleaved by Caspase-3, converting apoptosis to pyroptosis(Zhou et al. 2020). Accumulating evidence indicates that pyroptosis is induced in central nervous system diseases, including traumatic brain injury (TBI)(Ge et al. 2018), multiple sclerosis (MS)(McKenzie et al. 2018)and Alzheimer's disease (AD)(YapPickardChan, & Gan 2019).Blocking pyroptosis has been proved to be neuroprotective in rodents(Gao et al. 2020; W. Liu et al. 2018).Given the importance of BET proteins in inflammation, we hypothesized that JQ1 might be a therapeutic drug for SAE through the inhibition of pyroptosis.

We used the BET inhibitor JQ1 and analysed the effects in the SAE mouse model. We observed that JQ1 produced a protective effect in SAE mice, as indicated by improved sickness behaviour, decreased serum sepsis markers, preservation of the blood brain barrier and neuronal damage induced by LPS injection. Furthermore, JQ1 administration alleviated inflammasome-induced canonical pyroptosis and neuroinflammation through the inhibition of NF- κ B. Overall, these results indicate that JQ1 might serve as a promising prevention target for SAE.

Materials And Methods

Animals

Adult male C57BL/6 mice weighing 25-30 g (obtained from the Hunan SJA Lab Animal Center of Changsha, Hunan, China) were used in this study. Mice were group-housed (4-8 mice per cage) at a controlled temperature (22 ± 2 °C) under a 12-h light/dark cycle with free access to food and water. The mice adapted to their new environment for 14 days. The experimental protocol was approved by the Animal Care and Use Committee of the University of South China and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drugs

Lipopolysaccharide (LPS)from Escherichia coli 026:B8 (Sigma, USA) was dissolved in 0.9% NaCl. Based on a previous study(M. Wang et al. 2020), mice were treated with a single i.p. injection ofLPS(10 mg/kg) to establish the animal model of SAE, while the control mice were treated with the same amount of normal saline (NS)(F. Wu et al. 2020). One hour before LPS or NS injection, the SAE model was i.p. treated with 50 mg/kg JQ1 (dissolved in DMSO) (MCE, Shanghai, China) (JQ1+LPS group) or an equal volume of

DMSO(DMSO+LPS group), while the control mice were treated with the same amount of DMSO (DMSO+NS). n=14 mice for the DMSO+NS, DMSO+LPS and JQ1+LPS groups, among them, n=4 for IHC and Nissl staining tests, n=6 for PCR tests and n=4 for Western blot tests.

ELISA detection

Mouse (n=14 for each group) procalcitonin (PCT) and D-lactate dehydrogenase (D-LDH) ELISA kits (CUSABIO, Wuhan, China) were used to determine the plasma PCT and D-LDH levels, which are diagnostic and monitoring markers of sepsis. According to the manufacturer's instructions, 24 h after the LPS injection, mice were decapitated, and 800 µl eyeball blood was collected in 1.5 ml microtubes with heparin sodium. Then, the blood was centrifuged for 20 min at 1500 g at room temperature. The plasma supernatants were collected and used to measure the PCT and D-LDH levels of each sample. The plasma was diluted with Sample Diluent (1:30). Then, 90 µl of standard or diluted plasma was added to each well and incubated for 2 h at 37 °C, 100 µl of biotin-antibody was added to each well and incubated for 1 h at 37 °C, and 100 µl of horseradish peroxidase(HRP)-avidin was also added to each well and then incubated for 1 h at 37 °C. Each well was aspirated and washed after incubation. Wells were then developed with 3,3',5,5'-tetramethylbenzidine (TMB) substrate for 20 min at 37 °C in the dark. Stop solution was added to each well to terminate the reaction, and the absorbance was measured at 450 nm.

Nissl staining

The mice (n=4 for each group) were anaesthetized 24 h after LPS injection with an overdose of 10% sodium pentobarbital (80 mg/kg) and transcardially perfused with saline and 4% paraformaldehyde. The brains were removed and fixed in 4% paraformaldehyde for 24 hr and then cryoprotected with 30% phosphate-buffered sucrose overnight. Coronal sections (25 µm) of the brain were obtained using a cryostat at -22°C. Nissl staining was performed as previously described (Zhong et al. 2020). Free-floating sections were washed with 0.01 M PBS 3 times. Then, we mounted the sections onto gelatine-coated slides. When the sections dried, 0.5% cresyl violet (Sigma, USA) was added for thirty minutes, and then the cresyl violet solution was removed. The sections were then dehydrated through graded alcohol (70%, 80%, 90% and 100%×2), placed in xylene, and covered with a cover slip. Images were subsequently captured using a Nikon light microscope (Ming Mei, China).

Immunohistochemistry

Mice were handled as described for Nissl staining. The free-floating sections were treated with 3% H₂O₂ to remove endogenous peroxidase, which was then blocked by placing sections in 5% normal goat or rabbit serum in 0.01 M phosphate-buffered saline (PBS) containing 0.1% Triton with X-100 for 2 h. Sections were then incubated with rabbit anti-BRD4 antibody (Bethyl, A301, USA), rabbit anti-p-BRD4 (Millipore, ABE1451, USA), goat anti-IBA1 (Abcam, ab5076, Britain), rabbit anti-NeuN (Millipore, MAB377, USA) and rabbit anti-Doublecortin (DCX) (CST, 4604S, USA) at room temperature for 2 h, followed by incubation at 4 °C overnight. Secondary reagents comprised biotinylated goat anti-rabbit or rabbit anti-goat IgG (Proteintech, China). Diaminobenzidine tetrahydrochloride (DAB) (ZSGB-BIO, China) was used as

a peroxidase substrate. Washing for all procedures was accomplished in 0.01 M PBS, except for the blocking step. The slices were captured using a digital camera that was attached to the microscope (Olympus, Japan), and the average optical density (OD) of the BRD4-, p-BRD4-, IBA1-, NeuN- and DCX-immunoreactive cells was detected in regions of the dentate gyrus (DG) by using ImageJ software (NIH, USA). To avoid any bias, the researcher quantifying the number was blinded to the treatment used.

Western blotting

Mice (n=4 for each group) were anaesthetized with an overdose of 10% sodium pentobarbital (80 mg/kg), and the hippocampus (HIP) was rapidly removed following decapitation. The samples were homogenized in lysis buffer that contained phenylmethysulfonyl fluoride (PMSF) (CW BIO, China). The protein concentration was measured using a BCA Assay Kit (CW BIO, China). The sample extracts were then separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) at 90 V for 2 h and transferred onto PVDF membranes (Millipore, USA) for 2 h at 200 mA. Then, the membranes were blocked with 10% nonfat milk for 2 h at room temperature and incubated with the primary antibodies (Table 1) overnight in 5% skim milk solution at 4 °C. The next day, after washing three times in 0.02 M Tween-Tris buffered solution, the membranes were incubated with horseradish peroxidase (HRP)-conjugated donkey anti-rabbit (1:3000, Millipore, USA) or donkey anti-mouse (1:2000, CW BIO, China) polyclonal secondary antibodies for 1.5 h at room temperature. The signals were detected by an enhanced chemiluminescence (ECL) system (CW BIO, China). The protein levels were quantified by densitometry using NIH ImageJ (NIH, Bethesda, MD, USA).

Table 1. Information for the primary antibodies used in this study

Antibody	Company	Lot number	Molecular weight (KD)	Dilution ratio
BRD2	Abclonal	A16241	110	1:1000
BRD3	Abclonal	A2277	95-105	1:500
BRD4	Abclonal	WH113278	150	1:500
NF-kB	Proteintech	10745-1-AP	65	1:500
NLRP1	Abclonal	A16212	160	1:1000
NLRP3	CST	15101S	110	1:500
AIM2	Abclonal	A3356	47	1:1000
ASC	Santan Cruze	Sc-514414	24	1:500
Caspase-1	Santan Cruze	Sc-56036	45, 20-22	1:500
Caspase-11	Abclonal	A6495	30	1:500
GSDMD	Abclonal	A18281	53	1:1000
GSDMA	Abcam	Ab181027	49	1:1000
Caspase-8	Proteintech	13423-1-AP	32-45/53-57	1:500
GSDMC	Abclonal	A14550	58	1:1000
Caspase-3	CST	9662S	17,19,35	1:1000
GSDME	Proteintech	13075-1-AP	55	1:1000
CD68	Proteintech	28058-1-AP	100	1:500
IBA1	Abclonal	A1527	17	1:500
IL-1 β	Proteintech	16806-1-AP	30-35	1:500
IL-18	Proteintech	10663-1-AP	22	1:500
IL-6	Proteintech	21865-1-AP	24	1:500
occludin	Proteintech	27260-1-AP	59	1:1000
ZO-1	Proteintech	21773-1-AP	220	1:1000
NeuN	CST	24307S	46-55	1:1000
β -Actin	Proteintech	60008-1-Ig	42	1:3000

RNA Isolation and Real Time-PCR

Mouse (n=6 for each group) hippocampal total RNA was extracted by TRIzol[®] reagent (CW BIO, China) according to the manufacturer's instructions. The RNA purity was determined by the A260 nm/A280 nm

absorption ratio. cDNA synthesis was performed with the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Canada) according to the manufacturer’s instructions using 2 µg of total RNA. Gene expression was determined by an ABI-7500 real-time PCR system with TB Green™Premix Ex Taq™II(Takara, Japan). The primers were designed with Primer 3 software (Table 2).A two-step PCR protocol was used according to the manufacturer’s instructions. The PCR cycling conditions were 30 s at 95 °C followed by 40 cycles at 95 °C for 5 s and 60 °C for 45 s. The samples were processed in technical duplicates, and a melting analysis was performed for each sample at the end of PCR. The $2^{-\Delta\Delta C_t}$ method was used to determine the relative gene expression as described in our previous study(Zhong et al. 2020).

Table 2. Information for the primers used in this study

Genes	Primers	Sequence 5'-3'
BRD2	<i>forwards</i>	CAAGCGGAAGAGAGAGAAAAAG
	<i>reverse</i>	CATCTTCATCGATCCCAATTCG
BRD3	<i>forwards</i>	TAGTCAAGAAAAAGGGCGTGAA
	<i>reverse</i>	GGACAGTTTTCCCTTTTTTACCC
BRD4	<i>forwards</i>	CCCTTTTCTGCTCATATTCAGC
	<i>reverse</i>	AATGTATCATAAGCGGAGAGGG
NLRP1	<i>forwards</i>	GCCTCACATCCACATACTGCTCAC
	<i>reverse</i>	AGCTCTGCAATCACTTGGTCACTG
NLRP3	<i>forwards</i>	CGTTGCAAGCTGGCTCAGTA
	<i>reverse</i>	GGGGACTGGGATACAGCCTT
AIM2	<i>forwards</i>	CTGCCGCCATGCTTCCTTAACTAG
	<i>reverse</i>	AGCAGTCCAGTTCGGTAGTGTAGG
ASC	<i>forwards</i>	ACAATGACTGTGCTTAGAGACA
	<i>reverse</i>	CACAGCTCCAGACTCTTCTTTA
Caspase-1	<i>forwards</i>	AGAGGATTTCTTAACGGATGCA
	<i>reverse</i>	TCACAAGACCAGGCATATTCTT
Caspase-11	<i>forwards</i>	ATGCTTCTCCAGACATTCTTCA
	<i>reverse</i>	TGGCCTCCTTTATTGGGTAAAT
GSDMD	<i>forwards</i>	CTAGCTAAGGCTCTGGAGACAA
	<i>reverse</i>	GATTCTTTTCATCCCAGCAGTC
GSDMA	<i>forwards</i>	GAACTTGCACAAGGAGAGGAAA
	<i>reverse</i>	CATCACCACATAGAGGTTCTCC
Caspase-8	<i>forwards</i>	ACCAAATGAAGAACAAACCTCG
	<i>reverse</i>	CTTCATTTTTTCGGAGTTGGGTT
GSDMC	<i>forwards</i>	CCTTTCCAATGAGATTTGTGGG
	<i>reverse</i>	GGAAACTGGAGAACACAACCTTC
Caspase-3	<i>forwards</i>	GAAACTCTTCATCATTGAGGCC
	<i>reverse</i>	GCGAGTGAGAATGTGCATAAAT
GSDME	<i>forwards</i>	GAGAGTCACTCTTCGTTTGGAA

	<i>reverse</i>	CTGAAGTACCAGGTTGTCCATA
CD68	<i>forwards</i>	GAAATGTCACAGTTCACACCAG
	<i>reverse</i>	GGATCTTGGACTAGTAGCAGTG
IBA1	<i>forwards</i>	ATTATGTCCTTGAAGCGAATGC
	<i>reverse</i>	TCTCAAGATGGCAGATCTCTTG
IL-1 β	<i>forwards</i>	GCAGAGCACAAGCCTGTCTTCC
	<i>reverse</i>	ACCTGTCTTGGCCGAGGACTAAG
IL-18	<i>forwards</i>	CTGTTGGCCCAATTACTAACAG
	<i>reverse</i>	TCCCGAATTGGAAAGGGAAATA
Il-6	<i>forwards</i>	CTCCCAACAGACCTGTCTATAC
	<i>reverse</i>	CCATTGCACAACCTCTTTTCTCA
GAPDH	<i>forwards</i>	ACCACCATGGAGAAGGCTGG
	<i>reverse</i>	CTCAGTGTAGCCCAGGATGC

Statistical analyses

All statistical analyses were conducted with GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). Data are presented as the mean $\pm$ SEM. Significant differences were determined using two-way ANOVA followed by Bonferroni's post-hoc test for body weight at different time points and one-way ANOVA followed by Bonferroni's post-hoc test for other tests for the DMSO+NS, DMSO+LPS and JQ1+LPS groups. $p < 0.05$ was considered statistically significant.

Results

JQ1 treatment prevents tight junction and reverses neuronal morphological damage in the hippocampus of SAE mice exposed to LPS

High-dose lipopolysaccharide (LPS) could induce sepsis and sepsis-associated encephalopathy (M. Wang et al. 2020). In the present study, we explored the role of JQ1 administration on sepsis-related symptoms. Results revealed that 24 h after LPS injection, the body weight of mice decreased when compared with the NS group ($p < 0.01$), but no significant difference was found between the DMSO+NS groups and JQ1+LPS mice ($p > 0.05$) (Fig. 1a). And the spleen weight/body weight in DMSO+LPS mice increased when compared with those of the DMSO+NS group ($p < 0.001$), which was rescued by JQ1 treatment ($p < 0.001$) (Fig. 1b). These results suggested that JQ1 could inhibit weight loss and splenomegaly induced by LPS administration.

Procalcitonin (PCT) and D-lactate dehydrogenase (D-LDH) levels are diagnostic and monitoring markers in sepsis. Therefore, we also detected the levels of plasma PCT and D-LDH by using ELISA kits. And the results revealed that LPS caused increased PCT levels ($p < 0.01$) and D-LDH levels ($p < 0.01$) compared to NS mice, and these effects were effectively attenuated by JQ1 treatment ($p < 0.05$) (Fig. 1c, d). These results indicated that JQ1 prevented the process of sepsis induced by LPS administration.

The hippocampus is an important region of the blood-brain barrier, and a high dose of LPS can damage the blood-brain barrier (BBB) (Banks et al. 2015). In our current study, the tight junction proteins occludin and ZO-1 were detected to assess the effect of JQ1 on the blood-brain barrier of the hippocampus. The results showed that LPS stimulated downregulation of the protein levels of occludin ($p < 0.001$) and ZO-1 ($p < 0.01$) compared to NS mice, while JQ1 treatment remarkably increased the expression of occludin ($p < 0.001$) and ZO-1 ($p < 0.001$) (Fig. 1e, f).

To determine the effect of JQ1 on neurons in the hippocampus of mice induced by LPS. Nissl staining was used to estimate the number of functional neurons, and the results showed that LPS injection could decrease the number of functional neurons, while JQ1 treatment could rescue the number of functional neurons (Fig. 1g). Furthermore, western blotting was used to assess the relative expression of the NeuN protein, which is a marker of neurons, and results revealed that LPS stimulated downregulation of the protein level of NeuN ($p < 0.01$) compared to NS mice, while JQ1 treatment remarkably increased the expression of NeuN ($p < 0.05$) (Fig. 1e, f). In addition, we used immunohistochemistry to estimate the expression of NeuN and the newborn neuron marker doublecortin (DCX). Representative images of NeuN- and DCX-positive cells are shown in Fig. 1h. The results showed that LPS stimulation decreased the expression of NeuN ($p < 0.001$) and DCX ($p < 0.001$), but JQ1 administration increased the expression of NeuN ($p < 0.001$) and DCX ($p < 0.001$) (Fig. 1i). These results suggested that JQ1 administration could enhance tight junctions in the hippocampus, inhibit neuronal death, and protect against neurogenesis.

JQ1 treatment inhibits the expression of BRDs in the hippocampus of SAE mice exposed to LPS

Furthermore, we detected the expression of BRD2, BRD3, BRD4 and p-BRD4 in the hippocampus of mice, which is a main region susceptible to sepsis. Real-time PCR showed that the mRNA levels of BRD2 ($p < 0.001$), BRD3 ($p < 0.05$) and BRD4 ($p < 0.001$) in DMSO+LPS mice increased when compared with those of the DMSO+NS group, which was reversed by JQ1 treatment ($p < 0.05$) (Fig. 2a). Western blotting was used to detect the protein levels of BRD2, BRD3 and BRD4, and we found that when compared with the DMSO+NS group, the protein levels of BRD2 ($p < 0.05$), BRD3 ($p < 0.01$) and BRD4 ($p < 0.01$) in DMSO+LPS mice increased significantly, which was reversed by JQ1 treatment ($p < 0.05$) (Fig. 2b, c). Furthermore, we investigated the expression of BRD4 and p-BRD4 by immunohistochemistry, and representative images of BRD4- and p-BRD4-positive cells are shown in Fig. 2d. The analysis results showed that LPS challenge increased the expression of hippocampal BRD4 ($p < 0.001$) and p-BRD4 ($p < 0.05$), but JQ1 administration decreased the expression of BRD4 ($p < 0.05$) and p-BRD4 ($p < 0.001$) (Fig. 2e). These results suggested that JQ1 treatment not only inhibited the expression of BRD2, BRD3 and BRD4 but also repressed the phosphorylation of BRD4.

JQ1 treatment inhibits LPS-induced activation of NF- κ B and inflammasomes

Inflammasomes, which mainly include NLRP1, NLRP3 and AIM2, play an important role in triggering pyroptosis and the inflammatory response in SAE (Takeuchi & Akira 2010). To determine the role of JQ1 treatment on the activation of inflammasomes, we examined the alterations in the mRNA levels of NLRP1, NLRP3, AIM2 and ASC in the hippocampus of mice. And the results revealed that LPS increased the mRNA levels of NLRP1 ($p < 0.001$), NLRP3 ($p < 0.01$), AIM2 ($p < 0.05$) and ASC ($p < 0.01$) compared to those in NS mice, while JQ1 treatment decreased the levels of NLRP1 ($p < 0.001$), NLRP3 ($p < 0.05$), AIM2 ($p < 0.05$) and ASC ($p < 0.001$) compared to those in LPS mice (Fig. 3a).

The NF- κ B signalling pathway was found to be involved in the LPS-induced inflammatory response (Zhang et al. 2020). Furthermore, western blotting was used to determine the protein levels of NF- κ B, NLRP1, NLRP3, AIM2 and ASC in the hippocampus of mice. Results showed that LPS increased the protein levels of NF- κ B ($p < 0.05$), NLRP1 ($p < 0.01$), NLRP3 ($p < 0.01$), AIM2 ($p < 0.001$) and ASC ($p < 0.01$) compared to those in NS mice, while JQ1 treatment decreased the levels of NF- κ B ($p < 0.01$), NLRP1 ($p < 0.001$), NLRP3 ($p < 0.05$), AIM2 ($p < 0.001$) and ASC ($p < 0.05$) compared to those in LPS mice (Fig. 3b, c). These results suggested that JQ1 treatment could inhibit the activation of the NF- κ B signalling pathway and NLRP1, NLRP3 and AIM2 inflammasomes induced by LPS injection.

JQ1 treatment ameliorates pyroptosis in the hippocampus of SAE mice exposed to LPS

Accordingly, a feature of pyroptosis is pore formation in cell membranes, which can be triggered by gasdermin (GSDM) family proteins (Wree et al. 2014). Studies have shown that GSDMD and GSDMA (Ding et al. 2016) are cleaved by Caspase-1/11 upon inflammasome activation and mediate canonical pyroptosis. As displayed in the real-time PCR analysis, LPS stimulated upregulation of the mRNA levels of Caspase-1 ($p < 0.01$), Caspase-11 ($p < 0.01$), GSDMD ($p < 0.05$) and GSDMA ($p < 0.05$) compared to NS mice, and these effects were effectively attenuated by JQ1 treatment ($p < 0.05$) (Fig. 4a). Western blotting further confirmed this fluctuating expression pattern of cleaved Caspase-1, cleaved Caspase-11, GSDMD-N and GSDMA-N (Fig. 4b, c). The results indicated that JQ1 treatment inhibited LPS-induced changes in the mRNA and protein levels of the canonical pyroptosis pathway.

Caspase-8 cleaves GSDMC and then mediates the noncanonical pyroptosis pathway (Hou et al. 2020). To determine the role of JQ1 treatment on the noncanonical pyroptosis pathway, we examined the alterations in the mRNA and protein levels of Caspase-8 and GSDMC. The results of real-time PCR showed that the mRNA levels of Caspase-8 ($p = 0.7561$) and GSDMC ($p = 0.7892$) were not significantly different among the DMSO+NS, DMSO+LPS and JQ1+LPS groups (Fig. 4d). Western blotting further confirmed that there were no significant differences among these groups in the protein levels of cleaved Caspase-8 ($p = 0.9591$) and GSDMC-N ($p = 0.2205$) (Fig. 4e, f).

GSDME has been demonstrated to be cleaved by Caspase-3 and then converts apoptosis to pyroptosis (Ding et al. 2016). In our present study, we found the mRNA levels of Caspase-3 ($p = 0.7372$) and GSDME ($p = 0.9892$) (Fig. 4g), and the protein levels of cleaved Caspase-3 ($p = 0.1456$) and GSDME-N

($p=0.9982$) (Fig. 4h, i) were not significantly different among the DMSO+NS, DMSO+LPS and JQ1+LPS groups. Because GSDMB was not expressed in mice (Das et al. 2016), we did not detect the expression of GSDMB. All these results indicated that JQ1 treatment inhibited LPS-induced canonical pyroptosis but not noncanonical pyroptosis or apoptosis conversion to pyroptosis.

JQ1 suppresses the release of inflammatory cytokines in the hippocampus of SAE mice exposed to LPS

The main inflammatory cytokines produced by pyroptosis are IL-1 β and IL-18 (Kayagaki et al. 2015; F. D. Shi 2015), which can induce the release of downstream inflammatory factors, such as IL-6. Inflammatory cytokine overexpression has been associated with impairments in hippocampal-dependent memory (Rojas-Colon et al. 2021). Here, we examined alterations in the mRNA expression levels of IL-1 β , IL-18 and IL-6 in the hippocampus of SAE, and found that the mRNA levels of IL-1 β ($p<0.05$), IL-18 ($p<0.05$) and IL-6 ($p<0.001$) were significantly increased in DMSO+LPS mice compared with DMSO+NS mice, while JQ1 treatment remarkably decreased the expression of IL-1 β ($p<0.05$), IL-18 ($p<0.001$) and IL-6 ($p<0.001$) (Fig. 5a). We also examined alterations in the protein levels of IL-1 β , IL-18 and IL-6 in the hippocampus of mice. And the results revealed that LPS stimulated upregulation of the protein levels of IL-1 β ($p<0.05$), IL-18 ($p<0.05$) and IL-6 ($p<0.05$) compared to NS mice, while JQ1 treatment remarkably decreased the expression of IL-1 β , IL-18 and IL-6 ($p<0.05$) (Fig. 5b, c). These results suggested that JQ1 treatment inhibited the upregulated expression of IL-1 β , IL-18 and IL-6 induced by LPS.

JQ1 suppresses microglial activity in the hippocampus of SAE mice exposed to LPS

Microglia are innate immune cells in the brain that can be activated after LPS injection (Gu et al. 2021). Herein, we investigated the role of JQ1 treatment on the activation of microglia. And the results revealed that LPS stimulated upregulation of the mRNA levels of CD68 ($p<0.05$) and IBA1 ($p<0.05$), both are markers of activated microglia, and this effect was effectively attenuated by JQ1 treatment (Fig. 6a). Western blotting further demonstrated that the protein levels of CD68 and IBA1, and found that compared with DMSO+NS treatment, LPS stimulation upregulated the protein levels of CD68 ($p<0.05$) and IBA1 ($p<0.05$), but JQ1 treatment reversed the expression of CD68 ($p<0.05$) and IBA1 ($p<0.001$) (Fig. 6b, c). Furthermore, we investigated the expression of IBA1 by using immunohistochemistry, and representative images of IBA1-positive cells are shown in Fig. 6d. The results showed that LPS stimulation increased the expression of IBA1 ($p<0.001$), but JQ1 administration decreased the expression of IBA1 ($p<0.001$) (Fig. 6e). These results indicated that JQ1 could suppress microglial activity induced by LPS injection.

Discussion

Bromodomain and extraterminal domain (BET) proteins are epigenetic readers that regulate the expression of inflammatory genes by interacting with acetylated lysine residues. While BET inhibitors represent a promising therapeutic strategy for cancer, little is known about their implication in SAE. We observed that JQ1 produced a protective effect in SAE mice, since JQ1 not only inhibited weight loss and splenomegaly but also reduced the levels of plasma PCT and D-LDH in SAE mice. Moreover, JQ1 also

reversed the blood–brain barrier and neuronal damage, as indicated by the enhanced expression of tight junction markers and neuronal markers in the hippocampus of SAE mice. Mechanistically, we found that JQ1 administration may alleviate inflammasome-induced canonical pyroptosis and neuroinflammation through the inhibition of NF- κ B. Overall, the present study supports the hypothesis that JQ1 might serve as a promising prevention target for SAE.

Increasing studies indicate that BET might be a promising therapeutic target in multiple diseases associated with the inflammatory response, such as osteoarthritis (Jiang et al. 2017), rheumatoid arthritis (Xiao et al. 2016) and spinal cord injury (J. Wang et al. 2019). Currently, it is still unclear whether and how BET is involved in pyroptosis after SAE induced by LPS, which is a widely accepted model of endotoxin-mediated inflammation (Luo et al. 2020). LPS injected intraperitoneally damaged the blood–brain barrier (Y. Liu et al. 2021) and then induced SAE (Kikuchi et al. 2019). Our study demonstrated that JQ1 treatment could inhibit weight loss and splenomegaly and decrease the release of plasma PCT and D-LDH following LPS injection. In addition, JQ1 could block BBB disruption, as manifested by enhanced expression of tight junction proteins (occludin and ZO-1). To explore whether neuronal damage existed in SAE, we observed the expression of NeuN and DCX in the hippocampus of SAE mice. The results showed that the numbers of both mature neurons and immature neurons were significantly decreased in SAE mice compared with control mice, which could be reversed by JQ1 intervention.

As present BET inhibitors, including JQ1, could not effectively distinguish BRD2, BRD3, BRD4, and BRDT (Liang Tian, & Wu 2021), we also determined the expression of BRD2, BRD3 and BRD4, and the results showed that both the mRNA and protein levels of BRD2, BRD3 and BRD4 were upregulated by LPS injection, and JQ1 administration reduced the expression of BRD2, BRD3 and BRD4. Since BRDT is specifically expressed in the testis (Fujisawa & Filippakopoulos 2017), we did not check its expression. Although JQ1 exhibits similar affinities to all major BET proteins, the majority of studies show that dysregulation of BRD4 function is the primary effect of pharmacologic BET inhibition (Roe Mercañ Rivera Pappin, & Vakoc 2015). It is known that when BRD4 is activated by phosphorylation, it binds more readily to acetylated histones at promoters and enhancers (S. Y. Wu Lee Lai Zhang, & Chiang 2013), and interestingly, we found that BRD4 phosphorylation was enhanced in the hippocampus of SAE mice. In the future, it will be necessary to elaborate on the roles of each BET protein in the development of SAE. This could be essential to develop a new generation of BET and apply the proper BET to precisely treat diseases.

JQ1 exhibits a suppressive effect on the expression of NF- κ B, a critical transcriptional regulator of inflammatory gene expression, including inflammasome-related genes (Bao et al. 2017). Herein, we investigated the effects of JQ1 on NF- κ B-mediated inflammasome expression and found that the expression of NF- κ B, NLRP1, NLRP3, AIM2 and ASC was significantly increased in the hippocampus of SAE mice and was effectively attenuated by JQ1 treatment. As the cleavage of GSDMs by inflammasome-activated caspases is a critical step in inducing pyroptosis, we further determined pyroptosis markers in the hippocampus (Kuang et al. 2017). The results showed that the levels of GSDMD-N and GSDMA-N, which are cleaved by Caspase-1/11, were increased in the brains of SAE mice.

The expression of GSDMC-N and GSDME-N, which are cleaved by Caspase-8 or Caspase3, showed no obvious change. Importantly, we found that JQ1 administration effectively decreased the expression of GSDMD-N, GSDMA-N and cleaved Caspase-1/11, which suggested that JQ1 could prevent canonical pyroptosis. In line with the inhibited pyroptosis pathway, a series of inflammatory cytokines, such as IL-1 β and IL-18, produced during the pyroptosis process (Kayagaki et al. 2015; F. D. Shi 2015) were also prevented by JQ1 administration. Microglia, resident immune cells in the brain, showed overactivation in the hippocampus of SAE mice (Gu et al. 2021), which also recovered to normal levels after JQ1 treatment, as indicated by the reduced expression of microglial markers (CD68 and IBA1). Thus, we considered that JQ1 administration may alleviate inflammasome-induced canonical pyroptosis and neuroinflammation through the inhibition of NF- κ B.

In conclusion, our results demonstrated that JQ1 produced a protective effect in SAE mice, which might be due to its effect on alleviating inflammasome-induced canonical pyroptosis and neuroinflammation. Overall, our present study indicated that JQ1 could serve as a promising prevention target for SAE.

Declarations

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Author Contributions XLZ and ZYC performed PCR and western blotting tests, YJW and MLM performed ELISA and Immunohistochemistry tests, YCD, MMS, YX established the animal model and administered JQ1 intervention. LC performed statistical analysis. XLZ and WYC drafted the manuscript. XLZ, LC and WC performed the research design and manuscript revision.

Compliance with Ethical Standards

Conflict of interest The authors confirm that there are no conflicts of interest.

References

1. Banks W A, Gray A M, Erickson M A, Salameh T S, Damodarasamy M, Sheibani N, et al. (2015) Lipopolysaccharide-induced blood-brain barrier disruption: roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. *J Neuroinflammation* 12: 223. doi: 10.1186/s12974-015-0434-1
2. Bao Y, Wu X, Chen J, Hu X, Zeng F, Cheng J, et al. (2017) Brd4 modulates the innate immune response through Mnk2-eIF4E pathway-dependent translational control of I κ B α . *Proc Natl Acad Sci U S A* 114: E3993-E4001. doi: 10.1073/pnas.1700109114
3. Boise L H, Collins C M (2001) Salmonella-induced cell death: apoptosis, necrosis or programmed cell death? *Trends Microbiol* 9: 64-67. doi: 10.1016/s0966-842x(00)01937-5

4. Das S, Miller M, Beppu A K, Mueller J, McGeough M D, Vuong C, et al. (2016) GSDMB induces an asthma phenotype characterized by increased airway responsiveness and remodeling without lung inflammation. *Proc Natl Acad Sci U S A* 113: 13132-13137. doi: 10.1073/pnas.1610433113
5. Dempsey C, Rubio Araiz A, Bryson K J, Finucane O, Larkin C, Mills E L, et al. (2017) Inhibiting the NLRP3 inflammasome with MCC950 promotes non-phlogistic clearance of amyloid-beta and cognitive function in APP/PS1 mice. *Brain Behav Immun* 61: 306-316. doi: 10.1016/j.bbi.2016.12.014
6. Ding J, Wang K, Liu W, She Y, Sun Q, Shi J, et al. (2016) Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature* 535: 111-116. doi: 10.1038/nature18590
7. Fan Y, Du L, Fu Q, Zhou Z, Zhang J, Li G, et al. (2018) Inhibiting the NLRP3 Inflammasome With MCC950 Ameliorates Isoflurane-Induced Pyroptosis and Cognitive Impairment in Aged Mice. *Front Cell Neurosci* 12: 426. doi: 10.3389/fncel.2018.00426
8. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith W B, Fedorov O, et al. (2010) Selective inhibition of BET bromodomains. *Nature* 468: 1067-1073. doi: 10.1038/nature09504
9. Fu Q, Wu J, Zhou X Y, Ji M H, Mao Q H, Li Q, et al. (2019) NLRP3/Caspase-1 Pathway-Induced Pyroptosis Mediated Cognitive Deficits in a Mouse Model of Sepsis-Associated Encephalopathy. *Inflammation* 42: 306-318. doi: 10.1007/s10753-018-0894-4
10. Fujisawa T, Filippakopoulos P (2017) Functions of bromodomain-containing proteins and their roles in homeostasis and cancer. *Nat Rev Mol Cell Biol* 18: 246-262. doi: 10.1038/nrm.2016.143
11. Gao C, Yan Y, Chen G, Wang T, Luo C, Zhang M, et al. (2020) Autophagy Activation Represses Pyroptosis through the IL-13 and JAK1/STAT1 Pathways in a Mouse Model of Moderate Traumatic Brain Injury. *ACS Chem Neurosci* 11: 4231-4239. doi: 10.1021/acscchemneuro.0c00517
12. Ge X, Li W, Huang S, Yin Z, Xu X, Chen F, et al. (2018) The pathological role of NLRs and AIM2 inflammasome-mediated pyroptosis in damaged blood-brain barrier after traumatic brain injury. *Brain Res* 1697: 10-20. doi: 10.1016/j.brainres.2018.06.008
13. Gofton T E, Young G B (2012) Sepsis-associated encephalopathy. *Nat Rev Neurol* 8: 557-566. doi: 10.1038/nrneurol.2012.183
14. Gu E, Pan W, Chen K, Zheng Z, Chen G, Cai P (2021) LncRNA H19 Regulates Lipopolysaccharide (LPS)-Induced Apoptosis and Inflammation of BV2 Microglia Cells Through Targeting miR-325-3p/NEUROD4 Axis. *J Mol Neurosci* 71: 1256-1265. doi: 10.1007/s12031-020-01751-0
15. Hou J, Zhao R, Xia W, Chang C W, You Y, Hsu J M, et al. (2020) PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumour necrosis. *Nat Cell Biol* 22: 1264-1275. doi: 10.1038/s41556-020-0575-z
16. Jiang Y, Zhu L, Zhang T, Lu H, Wang C, Xue B, et al. (2017) BRD4 has dual effects on the HMGB1 and NF-kappaB signalling pathways and is a potential therapeutic target for osteoarthritis. *Biochim Biophys Acta Mol Basis Dis* 1863: 3001-3015. doi: 10.1016/j.bbadis.2017.08.009
17. Kayagaki N, Stowe I B, Lee B L, O'Rourke K, Anderson K, Warming S, et al. (2015) Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 526: 666-671. doi:

18. Kikuchi D S, Campos A C P, Qu H, Forrester S J, Pagano R L, Lassegue B, et al. (2019) Poldip2 mediates blood-brain barrier disruption in a model of sepsis-associated encephalopathy. *J Neuroinflammation* 16: 241. doi: 10.1186/s12974-019-1575-4
19. Kuang S, Zheng J, Yang H, Li S, Duan S, Shen Y, et al. (2017) Structure insight of GSDMD reveals the basis of GSDMD autoinhibition in cell pyroptosis. *Proc Natl Acad Sci U S A* 114: 10642-10647. doi: 10.1073/pnas.1708194114
20. Liang Y, Tian JWu T (2021) BRD4 in physiology and pathology: "BET" on its partners. *Bioessays* 43: e2100180. doi: 10.1002/bies.202100180
21. Liu W, Chen Y, Meng J, Wu M, Bi F, Chang C, et al. (2018) Ablation of caspase-1 protects against TBI-induced pyroptosis in vitro and in vivo. *J Neuroinflammation* 15: 48. doi: 10.1186/s12974-018-1083-y
22. Liu Y, Wang L, Du N, Yin X, Shao HYang L (2021) Ramelteon Ameliorates LPS-Induced Hyperpermeability of the Blood-Brain Barrier (BBB) by Activating Nrf2. *Inflammation*. doi: 10.1007/s10753-021-01451-w
23. Luo R Y, Luo C, Zhong F, Shen W Y, Li H, Hu Z L, et al. (2020) ProBDNF promotes sepsis-associated encephalopathy in mice by dampening the immune activity of meningeal CD4(+) T cells. *J Neuroinflammation* 17: 169. doi: 10.1186/s12974-020-01850-0
24. McKenzie B A, Mamik M K, Saito L B, Boghozian R, Monaco M C, Major E O, et al. (2018) Caspase-1 inhibition prevents glial inflammasome activation and pyroptosis in models of multiple sclerosis. *Proc Natl Acad Sci U S A* 115: E6065-E6074. doi: 10.1073/pnas.1722041115
25. Orning P, Lien EFitzgerald K A (2019) Gasdermins and their role in immunity and inflammation. *J Exp Med* 216: 2453-2465. doi: 10.1084/jem.20190545
26. Roe J S, Mercan F, Rivera K, Pappin D JVakoc C R (2015) BET Bromodomain Inhibition Suppresses the Function of Hematopoietic Transcription Factors in Acute Myeloid Leukemia. *Mol Cell* 58: 1028-1039. doi: 10.1016/j.molcel.2015.04.011
27. Rogers C, Erkes D A, Nardone A, Aplin A E, Fernandes-Alnemri TAlnemri E S (2019) Gasdermin pores permeabilize mitochondria to augment caspase-3 activation during apoptosis and inflammasome activation. *Nat Commun* 10: 1689. doi: 10.1038/s41467-019-09397-2
28. Rojas-Colon L A, Dash P K, Morales-Vias F A, Lebron-Davila M, Ferchmin P A, Redell J B, et al. (2021) 4R-cembranoid confers neuroprotection against LPS-induced hippocampal inflammation in mice. *J Neuroinflammation* 18: 95. doi: 10.1186/s12974-021-02136-9
29. Shi F D (2015) Neuroinflammation. *Neurosci Bull* 31: 714-716. doi: 10.1007/s12264-015-1568-y
30. Shi JVakoc C R (2014) The mechanisms behind the therapeutic activity of BET bromodomain inhibition. *Mol Cell* 54: 728-736. doi: 10.1016/j.molcel.2014.05.016
31. Singh M BSartor G C (2020) BET bromodomains as novel epigenetic targets for brain health and disease. *Neuropharmacology* 181: 108306. doi: 10.1016/j.neuropharm.2020.108306

32. Takeuchi O Akira S (2010) Pattern recognition receptors and inflammation. *Cell* 140: 805-820. doi: 10.1016/j.cell.2010.01.022
33. Wang J, Chen J, Jin H, Lin D, Chen Y, Chen X, et al. (2019) BRD4 inhibition attenuates inflammatory response in microglia and facilitates recovery after spinal cord injury in rats. *J Cell Mol Med* 23: 3214-3223. doi: 10.1111/jcmm.14196
34. Wang M, Liu M, Zhang J, Liu J, Ye J, Xu Y, et al. (2020) Resolvin D1 protects against sepsis-induced cardiac injury in mice. *Biofactors* 46: 766-776. doi: 10.1002/biof.1668
35. Widmann C N Heneka M T (2014) Long-term cerebral consequences of sepsis. *Lancet Neurol* 13: 630-636. doi: 10.1016/S1474-4422(14)70017-1
36. Wree A, Eguchi A, McGeough M D, Pena C A, Johnson C D, Canbay A, et al. (2014) NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice. *Hepatology* 59: 898-910. doi: 10.1002/hep.26592
37. Wu F, Chen X, Zhai L, Wang H, Sun M, Song C, et al. (2020) CXCR2 antagonist attenuates neutrophil transmigration into brain in a murine model of LPS induced neuroinflammation. *Biochem Biophys Res Commun* 529: 839-845. doi: 10.1016/j.bbrc.2020.05.124
38. Wu S Y, Lee A Y, Lai H T, Zhang H Chiang C M (2013) Phospho switch triggers Brd4 chromatin binding and activator recruitment for gene-specific targeting. *Mol Cell* 49: 843-857. doi: 10.1016/j.molcel.2012.12.006
39. Xiao Y, Liang L, Huang M, Qiu Q, Zeng S, Shi M, et al. (2016) Bromodomain and extra-terminal domain bromodomain inhibition prevents synovial inflammation via blocking I κ B kinase-dependent NF- κ B activation in rheumatoid fibroblast-like synoviocytes. *Rheumatology (Oxford)* 55: 173-184. doi: 10.1093/rheumatology/kev312
40. Xu X E, Liu L, Wang Y C, Wang C T, Zheng Q, Liu Q X, et al. (2019) Caspase-1 inhibitor exerts brain-protective effects against sepsis-associated encephalopathy and cognitive impairments in a mouse model of sepsis. *Brain Behav Immun* 80: 859-870. doi: 10.1016/j.bbi.2019.05.038
41. Yap J K Y, Pickard B S, Chan E W L Gan S Y (2019) The Role of Neuronal NLRP1 Inflammasome in Alzheimer's Disease: Bringing Neurons into the Neuroinflammation Game. *Mol Neurobiol* 56: 7741-7753. doi: 10.1007/s12035-019-1638-7
42. Zhang X, Guan Z, Wang X, Sun D, Wang D, Li Y, et al. (2020) Curcumin Alleviates Oxaliplatin-Induced Peripheral Neuropathic Pain through Inhibiting Oxidative Stress-Mediated Activation of NF- κ B and Mitigating Inflammation. *Biol Pharm Bull* 43: 348-355. doi: 10.1248/bpb.b19-00862
43. Zhong X, Cao W, Zhao H, Chen L, Cao J, Wei L, et al. (2020) MicroRNA-32-5p knockout eliminates lipopolysaccharide-induced depressive-like behavior in mice through inhibition of astrocyte overactivity. *Brain Behav Immun* 84: 10-22. doi: 10.1016/j.bbi.2019.11.001
44. Zhou Z, He H, Wang K, Shi X, Wang Y, Su Y, et al. (2020) Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. *Science* 368. doi: 10.1126/science.aaz7548

Figures

Figure 1

The effect of JQ1 on neuronal and tight junction proteins in the hippocampus induced by LPS injection. **a** The body weight of mice before and 8 h, 16 h and 24 h after LPS stimulation. **b** The spleen weight/body weight of mice 24 h after LPS injection. **c, d** The levels of plasma procalcitonin (PCT) and D-lactate dehydrogenase (D-LDH) by using ELISA kits. **e** Representative western blotting immunolabelling of occludin, ZO-1 and NeuN and **f** semiquantitative analysis of occludin, ZO-1 and NeuN protein relative to β -actin. **g** Representative Nissl staining images, **h** immunohistochemistry images of NeuN- and DCX-positive cells and **i** quantitative analysis of the average optical density (OD) of NeuN and DCX in the hippocampus for each group. Bar=100 μ m. $p<0.05$ was considered significant, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$. The results are shown as the mean $\pm$ SEM; n=14 animals in each group for body weight, spleen weight and ELISA test; n=6 animals in each group for PCR test, n=4 animals in each group for western blot, Nissl staining and immunohistochemistry test.

Figure 2

The effect of JQ1 on the expression of BRDs in the hippocampus induced by LPS injection. **a** Representative mRNA expression levels of BRD2, BRD3 and BRD4. **b** Representative western blotting immunolabelling of BRD2, BRD3, and BRD4 in the hippocampus and **c** semiquantitative analysis of the BRD2, BRD3, and BRD4 proteins relative to β -actin. **d** Representative immunohistochemistry images of

BRD4- and p-BRD4-positive cells and **e** quantitative analysis of the average optical density (OD) of BRD4 and p-BRD4 in the hippocampus for each group. Bar=100 μ m. $p<0.05$ was considered significant, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$. The results are shown as the mean $\pm$ SEM; n=6 animals in each group for PCR test, n=4 animals in each group for western blot test, and n=4 animals in each group for immunohistochemistry test.

Figure 3

The effect of JQ1 on the expression of NF-κB and inflammasomes in the hippocampus induced by LPS injection. **a** Representative mRNA expression levels of NLRP1, NLRP3, AIM2 and ASC. **b** Representative western blot immunolabelling of NF-κB, NLRP1, NLRP3, AIM2 and ASC. **c** Semiquantitative analysis of NF-κB, NLRP1, NLRP3, AIM2 and ASC protein relative to β-actin. $p<0.05$ was considered significant, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$. The results are shown as the mean $\pm$ SEM; n=6 animals in each group for PCR test, n=4 animals in each group for western blot test.

Figure 4

The effect of JQ1 on the expression of gasdermin family proteins in the hippocampus induced by LPS injection. **a** Representative mRNA expression levels of Caspase-1, Caspase-11, GSDMD and GSDMA. **b** Representative western blot immunolabelling of cleaved Caspase-1, cleaved Caspase-11, GSDMD-N and GSDMA-N and **c** semiquantitative analysis of cleaved Caspase-1, cleaved Caspase-11, GSDMD-N and GSDMA-N protein relative to β -Actin. **d** Representative mRNA expression levels of Caspase-8 and GSDMC. **e** Representative western blotting immunolabelling of cleaved Caspase-8 and GSDMC-N and **f** semiquantitative analysis of cleaved Caspase-8 and GSDMC-N protein relative to β -Actin. **g** Representative mRNA expression levels of Caspase-3 and GSDME. **h** Representative western blot immunolabelling of cleaved Caspase-3 and GSDME-N and **i** semiquantitative analysis of cleaved Caspase-3 and GSDME-N protein relative to β -Actin. $p < 0.05$ was considered significant, $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. The results are shown as the mean $\pm$ SEM; $n = 6$ animals in each group for PCR test, $n = 4$ animals in each group for western blot test.

Figure 5

The effect of JQ1 on the upregulated expression of inflammatory factors in the hippocampus induced by LPS injection. **a** Representative mRNA levels of IL-1 β , IL-18 and IL-6. **b** Representative western blotting immunolabelling of IL-1 β , IL-18 and IL-6 and **c** semiquantitative analysis of IL-1 β , IL-18 and IL-6 protein relative to β -actin. Bar=100 μ m. $p<0.05$ was considered significant, * $p<0.05$ and *** $p<0.001$. The results are shown as the mean $\pm$ SEM; n=6 animals in each group for PCR test, n=4 animals in each group for western blot and immunohistochemistry test.

Figure 6

Effect of JQ1 on the activation of microglia in the hippocampus induced by LPS injection. **a** Representative mRNA levels of CD68 and IBA1. **b** Representative western blotting immunolabelling of CD68 and IBA1. **c** Semiquantitative analysis of CD68 and IBA1 protein relative to β -actin. **d** Representative immunohistochemistry images of IBA1-positive cells and **e** quantitative analysis of the average optical density (OD) of IBA1 in the hippocampus for each group. Bar=100 μ m. $p<0.05$ was considered significant, * $p<0.05$ and ** $p<0.01$. The results are shown as the mean $\pm$ SEM; n=6 animals in each group for PCR test, n=4 animals in each group for western blot and Immunohistochemistry test.