European Review for Medical and Pharmacological Sciences 2019; 23: 2263-2272

Study on ceramide modulates EAAT-2 participation in the immunoinflammatory response in schizophrenia

W.-M. LING, M.-C. CHEN, Y.-C. ZHANG, M.-M. OU, L.-Z. GU

Department of Laboratory Medicine, The Affiliated Wuxi Mental Health Center of Nanjing Medical University, Wuxi, P. R. China

Weiming Ling and Minchi Chen contributed equally to this study

Abstract. – OBJECTIVE: Abnormal immunoinflammatory responses play important roles in the pathogenesis of schizophrenia, but the underlying molecular mechanisms are still unclear.

MATERIALS AND METHODS: In this study, the ceramide agonist daunorubicin (DNR) was injected into the lateral ventricles to induce ceramide accumulation. The behavioral tests were used to observe schizophrenia-like behavioral changes. Changes in the mRNA levels of the proinflammatory cytokines and the protein levels of the glutamate transporter excitatory amino acid transporter-2 (EAAT-2) were detected. After inhibition of nuclear factor-κB (NF-κB), the above indices were detected again. Nissl staining was used to assess neuronal damage.

RESULTS: After intracerebroventricular injection of DNR, ceramide significantly accumulated in the hippocampus, and behavioral tests revealed negative schizophrenia symptoms accompanied by induced learning and memory dysfunction. Furthermore, the hippocampus demonstrated increased mRNA levels of the proinflammatory cytokines including interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) and significantly decreased EAAT-2 protein levels. Nissl staining revealed neuronal damage after ceramide accumulation. The NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) reduced the behavioral abnormalities caused by ceramide accumulation, downregulated

CONCLUSIONS: The experimental results suggest that ceramide reduces EAAT-2 expression through the NF-κB/TNF-α pathway and causes neuronal excitotoxicity in the pathogenesis of schizophrenia, leading to neuronal damage.

Key Words

Schizophrenia, Inflammation, Ceramide, Cytokine, Glutamate transporter.

Abbreviations

CA: Cornu ammonis; DNR: Daunorubicin; IL: Interleukin; TNF: Tumor necrosis factor; EAAT-2: excitatory amino acid transporter-2; NF-Kb: nuclear factor-κB; PDTC: pyrrolidine dithiocarbamate; MCP-1: monocyte chemotactic protein-1; COX-2: cyclooxygenase-2; SAPK: stress-activated protein kinase; PKCδ: kinase protein kinase C delta; JNK: c-Jun N-terminal kinase; KSR: kinase suppressor of Ras; PP2A: protein phosphatase 2A.

Introduction

The current diagnosis of schizophrenia primarily depends on the subjective judgment of the patient's clinical manifestations[1], and there is no accurate and sensitive biomarker-assisted diagnosis. Therefore, the in-depth exploration of schizophrenia pathogenesis and the identification of effective and reliable diagnostic markers and therapeutic targets have important scientific significance.

Schizophrenia is one of the most serious mental illnesses, frequently occurring in young adults with slow onset. The characteristic symptoms include basic personality changes and discoordination between mental activity and the environment[2,3]. However, the pathogenesis is not yet clear. The current theories include the dopamine hyperactivity hypothesis, serotonin hypothesis and glutamate hypothesis. In addition, an increasing number of scientists[4,5] have identified immune metabolism disruptions in the peripheral blood and cerebrospinal fluid of patients with schizophrenia, while other studies[6,7] have revealed that inflammation is involved in the regulation of many aspects of physiological and pathological processes, such as monoamine metabolism, neuroendocrine function, synaptic plasticity and neurodevelopment and is closely related to schizophrenia etiology and pathogenesis[8]. Recent studies[9,10] have shown that multiple inflammatory cytokines, including interleukin-1β (IL-1β), IL-2, IL-6, IL-8, tumor necrosis factor-α (TNF-α) and monocyte chemotactic pro-

Corresponding Author: Lize Gu, MD; e-mail: mayamaha@163.com

tein-1 (MCP-1), are significantly increased in the peripheral blood serum and cerebrospinal fluid of schizophrenia patients and that the immunoregulatory drug cyclooxygenase-2 (COX-2) inhibitor can improve some clinical schizophrenia symptoms. These data suggest that abnormal immunoinflammatory reactions play an important role in schizophrenia pathogenesis.

It has been reported that patients with first-episode schizophrenia had increased levels of IL-6, TNFα, IL-8, and MCP-1 in the peripheral blood. In particular, TNF-α levels showed significant increases. However, the TNF-α upstream regulatory mediators and downstream targets involved in schizophrenia pathogenesis have not been identified[11]. A recent work[12,13] found that the ceramide content in the white matter of autopsy brain tissue from schizophrenia patients was higher than those in the normal control group. Based on the above study, researchers[14] found that first-episode schizophrenia patients had a higher ratio of ceramide among total lipids in the stratum corneum of the epidermis compared with the normal control group. Our previous work found that ceramide concentrations in the peripheral blood of first-episode schizophrenia patients were higher than those in the normal control group.

Ceramide is a second messenger in many processes, including cell proliferation, differentiation and apoptosis[15,16]. Significant ceramide accumulation activates various signaling molecules, such as stress-activated protein kinase (SAPK), kinase protein kinase C delta (PKCδ), c-Jun N-terminal kinase (JNK), kinase suppressor of Ras (KSR) and protein phosphatase 2A (PP2A)[17]. The ceramide signaling pathway is closely related to intracellular inflammatory stress, and its downstream effector PKCδ is involved in the expression of inflammatory cytokines regulated by nuclear factor-κB (NF-κB)[18].

Previously, the glutamate receptor and transporter systems have both been shown to play very important roles in the pathogenesis of schizophrenia. Excitatory amino acid transporter-2 (EAAT-2), as an important glutamate transporter on glial cells, can take up and clear 90% of glutamate in the synaptic cleft, and it has an important role in maintaining a low glutamate concentration in the synaptic cleft, thereby preventing neuro-excitotoxicity[19]. Scholars have found that NF-κB in glial cells can indirectly bind to the EAAT-2 promoter, thereby inhibiting its transcription. Based on the literature and previous studies, we suggested that ceramide reduces EAAT-2 expression in schizophrenia patients through the NF-κB/TNF-α pathway, resulting in excitotoxicity in neurons, ultimately leading to neuronal damage.

Materials and Methods

Animal Model

Adult male Sprague-Dawley rats weighing 250 to 300 g were used in the study. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Institutional Animal Care and Use Committee of Nanjing Medical University. All surgery was performed under anesthesia with 10% chloral hydrate (350 mg/kg intraperitoneal injection) and treated with an injection of DNR (0.5 μM, 4 μl, Wanle, Shenzhen, China). The animals in the sham group were treated with the same surgical procedure.

Injection and Drug Treatment

The ceramide agonist daunorubicin (DNR; 0.5 μM, 4 μl, Wanle, Shenzhen, China), the nuclear factor κB (NF-κB) inhibitor pyrrolidine dithiocarbamate (PDTC; 25 μM, 4 μl, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or their vehicle were injected into the lateral ventricles (0.8 mm posterior and 1.5 mm lateral to the bregma, 3.5 mm deep) using a microinjector as described in previous reports[20,21]. The micro-injector was kept in place for 5 min after injection.

Open-Field Experiment

The experimental system primarily consisted of the open-field test box, tracking and monitoring system, and automatic data acquisition device and analysis system. The open field box is a 40 cm 40 cm 30 cm (length width height) glass box, and the bottom of the box is made of black polyethylene plastic. Each open field is divided into a central region and the surrounding area. The characteristics of spontaneous activity in rats are evaluated based on the distance traveled in the central region, the number of standing-up events and the total distance traveled during spontaneous activity. The research was performed in a relatively quiet and dim environment. After each test, the animal feces were cleaned, and the bottom and walls of the box were wiped with 10% ethanol to avoid affecting the next experimental subject. The spontaneous activity and stereotyped movements of the rats were observed.

Morris Water Maze Test

The black round pool of MWM was 190 cm in diameter, filled with water (26°C) and divided into four quadrants. The water entry points of four quadrants were marked with high contrast lighted geometric images (90 90 cm) on the curtain wall. A 15 cm diameter hidden platform was placed in the center of the fourth quadrant 1 cm below the water. Animal positional tracking and digitizing were recorded as path length and escape latency by water maze acquisition software. During the first five days, all animals were trained three times a day. Each training lasted for 60 s. If the rats failed to climb on the platform within 60 s, they were guided to the platform. The sixth day was the space exploration experiment (reversal memory probe) in which the platform was removed and the rats were allowed to swim for 60 s. The passing time in the target quadrant was measured. Data were recorded and analyzed by Top View Animal Behavior Analyzing System.

Real Time-Polymerase Chain Reaction Examination

Total RNA was isolated from hippocampal tissue using the TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The extracted total RNA was reverse-transcribed into cDNA using the PrimeScript RT Reagent Kit (TaKaRa Biotechnology, Dalian, China) for quantitative Polymerase Chain Reaction (PCR). Real Time-PCR was analyzed using the SYBR Real Time-quantitative PCR kit (TaKaRa Biotechnology, Dalian, China) and performed using the Eppendorf MasterCycler RealPlex Sequence Detection System (Life Technologies, Gaithersburg, MD, USA). Data were analyzed and quantified using the $2^{-\Delta\Delta CT}$ method.

Protein Extraction and Western Blot Assay

The hippocampi were dissected and harvested from the unilateral hemisphere and the proteins were extracted with sample buffer containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). Then, they were stored at -80°C. The sample proteins were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred onto a nitrocellulose membrane. The membranes were incubated with primary antibodies EAAT-2 (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and β-actin (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C, and reacted with secondary antibodies horseradish peroxidase (HRP)-conjugated Immunoglobulin G (IgG). Finally, the bands were scanned and analyzed using the Jieda image analyzer (Jieda, Nanjing, China).

Immunohistochemistry and Nissl Staining

The rats were perfused with 0.9% saline and 4% paraformaldehyde from the left ventricle. The brains were embedded in paraffin and cut into 4 μm sections. The sections were mounted on poly-L-lysine-coated slides, and immersed in ethanol. After that, they were treated with xylene for five minutes twice. Then, the sections were rinsed in double-distilled water, and submerged in 1% cresyl violet solution (Sigma-Aldrich, St. Louis, MO, USA) for 5 min at 50°C. Finally, the tissue was dehydrated with ethanol and covered with resinous medium. The immunohistochemistry sections were incubated for 10 min with 1% H_2O_2 and then blocked with 3% bovine serum albumin (BSA). The sections were incubated with the antibody (ceramide 1:200; Sigma-Aldrich, St. Louis, MO, USA), for 24 h at 4°C. Finally, the immunohistochemistry stained sections were incubated with 3, 3'-diaminobenzidine (DAB) reagent.

Statistical Analysis

Statistical analysis was performed with SPSS 16.0 and carried out by one-way analysis of variance (ANOVA) and SNK test. The escape latency of the Morris water maze test was analyzed by 2-way repeated-measures analysis. All the results were expressed as the mean ± SD values from at least four animals. $p < 0.05$ was taken to be a statistically significant difference.

Results

Ceramide Accumulation Induces Behavioral Abnormalities

Intracerebroventricular injection of the ceramide agonist DNR resulted in significant ceramide accumulation in the CA1 region of the hippocampus of rats (Figure 1A). In addition, open-field experiments demonstrated that the total distance traveled during spontaneous activity and the number of standing-up events in the DNR-treated group was less than those in the sham group (Figure 1B). The Morris water maze test showed that compared with the sham group, the DNR-treated group had an increased latency

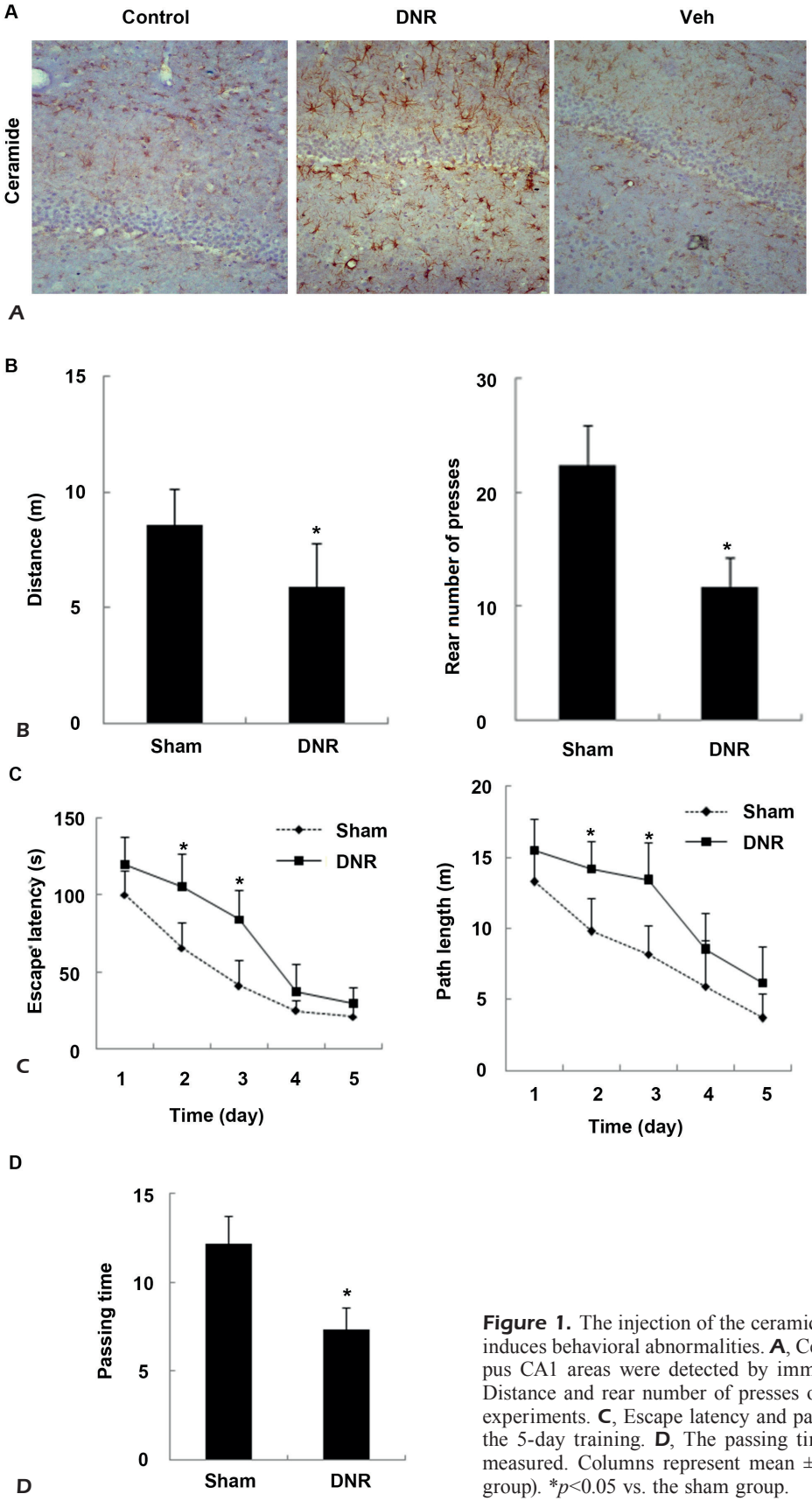

Figure 1. The injection of the ceramide agonist daunorubicin (DNR) induces behavioral abnormalities. **A**, Ceramide levels in rat hippocampus CA1 areas were detected by immunohistochemistry (x 100). **B**, Distance and rear number of presses of each group in the open-field experiments. **C**, Escape latency and path length of each group during the 5-day training. **D**, The passing time in the target quadrant was measured. Columns represent mean ± standard deviation (n=12 per group). *$p<0.05$ vs. the sham group.

reaching the platform and swimming distance (Figure 1C), while the number of crossings over the target quadrant in exploratory experiments decreased (Figure 1D). These results suggest that increased ceramide production leads to decreased spontaneous activity and manifestation of negative symptomatic features of schizophrenia, while inducing impaired learning and memory function.

Ceramide Accumulation Regulates the Inflammatory Response

Abnormal immunoinflammatory response plays an important role in schizophrenia pathogenesis, but its molecular mechanism is not fully understood. In this study, Real Time-PCR was used to detect the effect of ceramide on IL-1β, IL-6, and TNFα mRNA levels. IL-6 mRNA levels started to increase from 1 h after DNR administration, peaked at 12 h and gradually decreased at 24 h (p <0.05). IL-1β and TNFα mRNA levels began to increase from 12 h after administration to 24 h after administration, followed by a gradual decrease (p <0.05; Figure 2A). In addition, we administered the NF-κB inhibitor PDTC after ceramide accumulation and examined the mRNA levels of inflammatory cytokines by Real Time-PCR. The results showed that IL-1β, IL-6, and TNFα mRNA levels significantly decreased after NF-κB inhibition (Figure 2B). The above findings indicate that ceramide regulates the immunoinflammatory responses in schizophrenia through the NF-κB/TNF-α pathway.

Ceramide Accumulation Decreases EAAT-2 Expression and Induces Secondary Inflammatory Lesions in Adjacent Neurons

It has previously been found that the glutamate receptor and transporter systems both play very important roles in the pathogenesis of schizophrenia. High TNF-α concentrations over a long time can inhibit EAAT-2 expression and induce neuronal damage. To determine whether ceramide accumulation regulates EAAT-2 expression and neuronal damage through the inflammatory reaction in schizophrenia, we performed Western blotting to detect EAAT-2 protein levels after ceramide accumulation. We found that EAAT-2 expression markedly decreased upon ceramide accumulation (Figure 3A). Nissl staining revealed that ceramide accumulation led to neuronal damage and significantly decreased the number of Nissl bodies in rat hippocampal CA1 neurons compared with the sham operation group and solvent group (Figure 3B). Treatment with the NF-κB inhibitor PDTC increased EAAT-2 protein expression (Figure 3C) and alleviated the ceramide-induced neuronal damage (Figure 3D), suggesting that ceramide accumulation induces neuronal damage in schizophrenia by inhibiting EAAT-2 protein expression via the regulation of the immunoinflammatory response.

Ceramide Modulates Schizophrenia-Related Behavioral Changes Through the NF-κB/TNF-α Pathway

To determine whether ceramide works through the NF-κB/TNF-α pathway, we administered the NF-κB inhibitor PDTC after ceramide accumulation. The results of the open-field test showed that the total distance traveled during spontaneous activity and the number of standing-up events in the PDTC-treated group were higher than those in the control group (Figure 4A). Morris water maze results showed that, compared with the sham group, the PDTC treatment group showed remarkably reduced latency to reach the platform and swimming distance on the second day, while there was no significant difference in the learning process on the third, fourth and fifth days (Figure 4B). Furthermore, there was no significant difference in the number of crossings over the target quadrant in exploratory experiments (Figure 4C). These results suggest that ceramide regulates schizophrenia-like behavioral changes through the NF-κB/TNF-α pathway.

Discussion

Researchers[22] have shown that the levels of a variety of inflammatory cytokines are markedly increased in the peripheral blood serum and cerebrospinal fluid of schizophrenia patients. It has been reported that patients with first-episode schizophrenia had increased levels of IL-6, TNFα, IL-8, and MCP-1 in the peripheral blood, of which TNF-α level showed particularly significant increase[23]. However, the underlying regulatory mechanism is unclear. Additionally, the white matter of autopsy brain tissues from schizophrenia patients has been reported to have higher ceramide levels than normal control group brains[12]. As a class of lipid signaling molecules, ceramide plays an important role in cell proliferation, apoptosis, differentiation and growth inhibition[24,25].

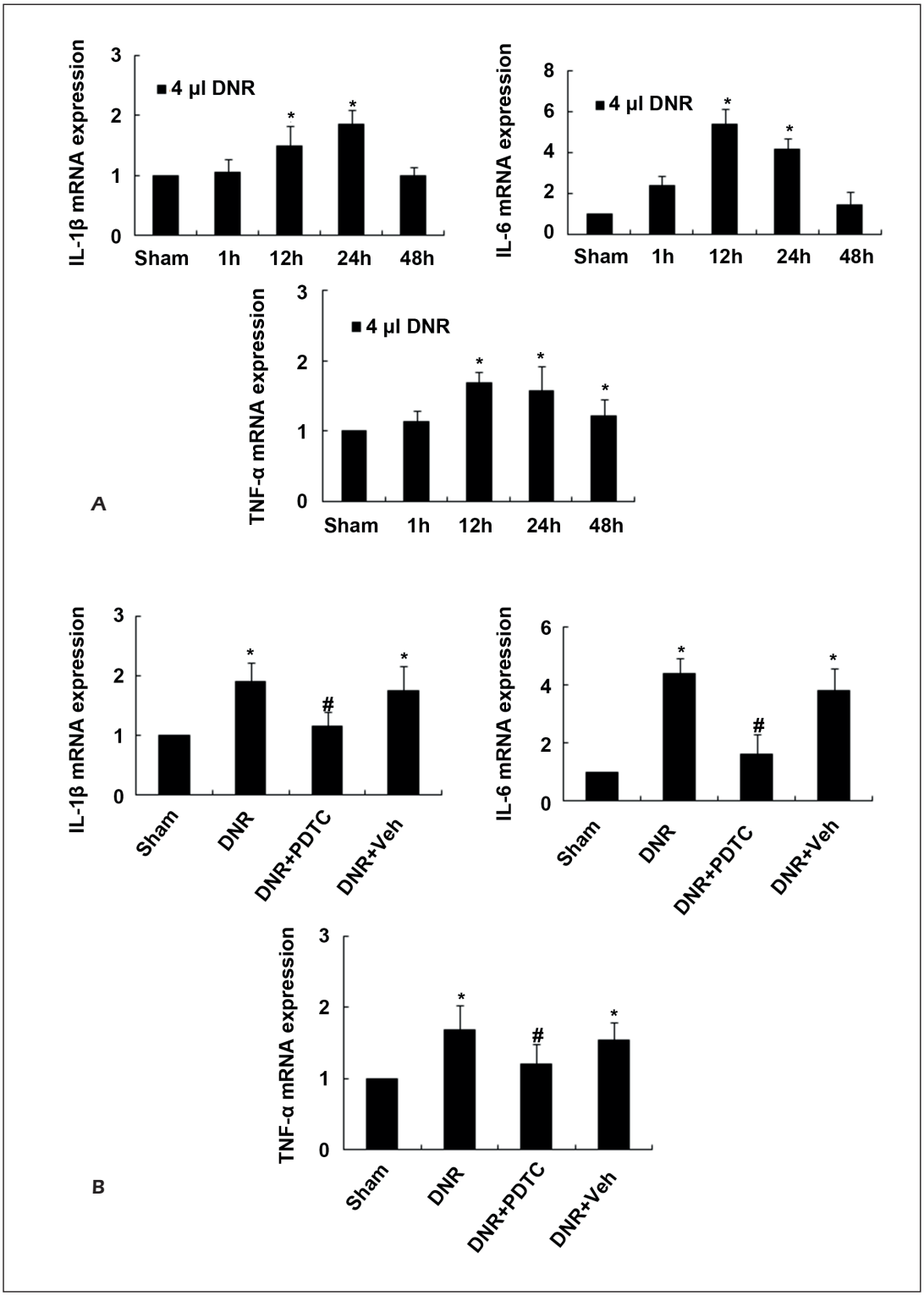

Figure 2. Ceramide accumulation regulates the inflammatory response. Real Time-PCR was performed to detect the mRNA levels of interleukin 1β (IL-1β), IL-6 and tumor necrosis factor α (TNF-α). **A**, The treatment of DNR (0.5 mM, 4 μl) stimuli of different durations (1 h, 12 h, 24 h and 48 h). **B**, Injection with pyrrolidine dithiocarbamate (PDTC, 25 mM, 4 μl) into the lateral ventricle after the treatment of DNR. Data are presented as the means ± standard deviation of at least four separate experiments (n=3). *p<0.05 vs. sham control. #p<0.05 vs. DNR treatment control.

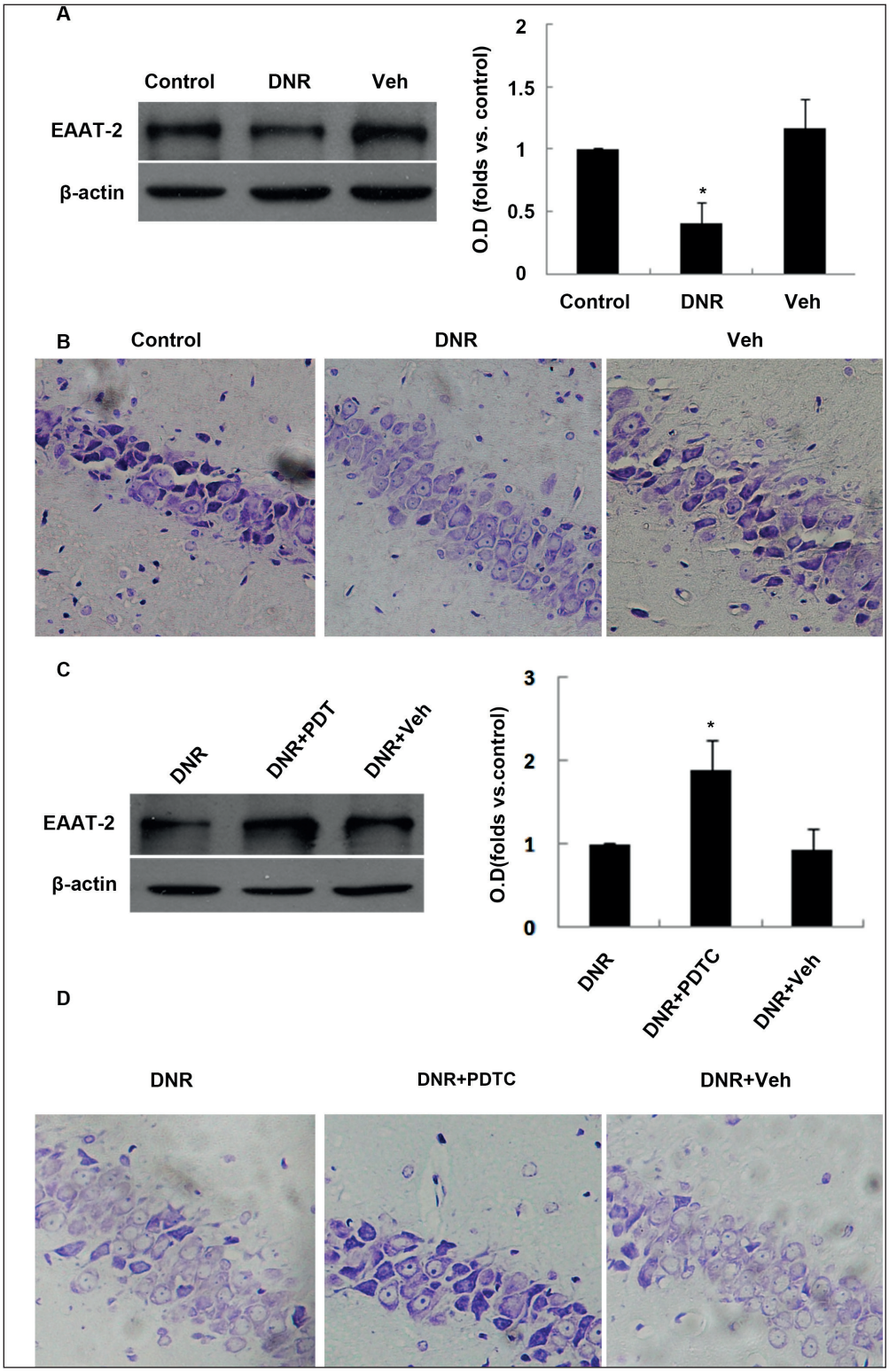

Figure 3. Ceramide accumulation decreases excitatory amino acid transporter-2 (EAAT-2) expression and induces secondary inflammatory lesions in adjacent neurons. **A**, The protein expression of EAAT-2 in the rat hippocampus were detected in the different groups. Neuronal damage in the rat CA1 hippocampal area was detected in the different groups that received different treatments using Nissl staining. **B**, Normal group with DNR stimulus for 24 h. **C-D**, Injection with DNR, PDTC and their solvent into the lateral ventricle. Data are presented as the means ± standard deviation of at least three separate experiments (n=3). *p<0.05 vs. sham control (x 200).

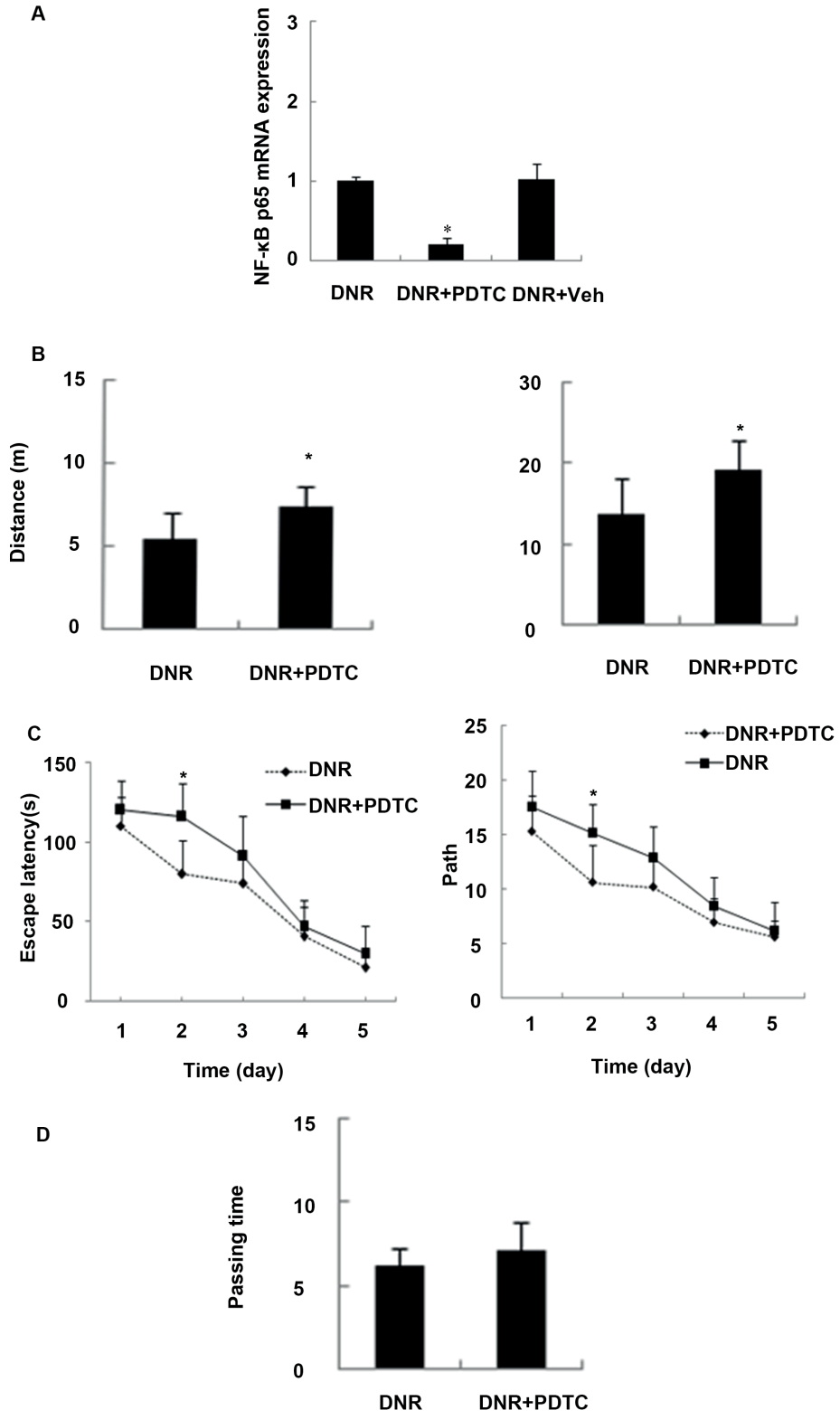

Figure 4. Through the NF-κB/TNF-α pathway ceramide modulates schizophrenia-related behavioral changes. **A**, Real Time-PCR was performed to detect the mRNA levels of NF-kB P65. **B**, Escape latency and path length of each group during the 5-day training. **C**, The passing time in the target quadrant were measured on the sixth day. Columns represent mean ± standard deviation (n=12 per group). **p*<0.05 *vs.* the sham group. **D,** The passing time in the target quadrant were measured on the sixth day. Columns represent mean ± standard deviation (n=12 per group). **p*<0.05 *vs.* the sham group.

According to a new study[26,27], an increase in ceramide levels in hippocampal astrocytes was observed seven days later in mice injected with A β polypeptide, and ceramide expression was also detected in subjects with human Alzheimer undefined's disease. In addition, *in vitro* stimulation of hippocampal neurons with A β 1-42 could increase the expression of ceramide, and may mediate the cytotoxicity induced by A β at several levels and participate in the regulation of neuronal damage[28,29]. Ceramide can induce astrocyte overactivation and NF-κB pathway activation to upregulate pro-inflammatory cytokine production, including IL-1β, IL-6, and TNF-α[30]. TNF-α is an important downstream molecule regulated by NF-κB and is involved in regulating the inflammatory response. During *Aspergillus fumigatus* infection, TNF-α induces the immune response by promoting the production of IL-1, IL-6, and interferon-γ (IFN-γ). In cerebral ischemia-reperfusion injury, the p38 MAPK pathway and PKCδ regulate TNF-α expression through NF-κB[23,24,30]. It has been suggested that ceramide is involved in regulating immunoinflammation in schizophrenia through the NF-κB/TNF-α pathway.

The proper amount of glutamate is necessary to maintain normal cellular physiological activity, whereas excessively high concentrations of extracellular glutamate can lead to toxic effects and neuronal damage, and are thus the source of neuronal excitotoxicity[31]. Glutamate concentrations in the central nervous system are primarily regulated by glutamate transporters and receptors. Glutamate over-release and uptake dysfunction are two major causes of excessively high extracellular glutamate levels[32,33]. Glutamate transporters are capable of taking up extracellular glutamate into neurons and glial cells against the concentration gradient, thereby terminating the glutamate excitatory signals[32]. EAAT-2, which is an important glutamate transporter on glial cells, can take up and clear 90% of glutamate in the synaptic cleft. It plays an important role in maintaining low glutamate concentrations in the synaptic cleft, thereby preventing its neuro-excitotoxicity. However, studies[34] of ischemia found that high TNF-α concentrations over a long duration can inhibit EAAT-2 expression and cause neuronal excitotoxicity. These findings suggest that in the pathogenesis of schizophrenia, abnormal immunoinflammatory reactions may induce glutamate transporter EAAT-2 dysfunction, thus leading to glutamate accumulation in the synaptic cleft, neuro-excitotoxicity and neuronal damage.

Related studies in China and abroad have been limited to the detection of ceramide in autopsy brain tissues and epidermal lipids, with few reports on the role of ceramide in schizophrenia pathogenesis, and the molecular mechanism of the inflammatory response in schizophrenia is not comprehensively understood.

Conclusions

Our work enriches the understanding of the molecular mechanisms of ceramide and inflammatory responses in schizophrenia pathogenesis by exploring the interactions between ceramide, inflammation, EAAT-2 expression and neuronal damage.

Acknowledgments

The present study was supported by the National Natural Science Foundation of China (No. 81501157).

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Schultz SK, Andreasen NC. Schizophrenia. Lancet 1999; 353: 1425-1430.
2) Luo X, Jin C, Zhou Z, Zhang F, Yuan J, Liu X, Cheng Z. Association study of DISC1 genetic variants with the risk of schizophrenia. Psychiatr Genet 2016; 26: 132-135.
3) Gu LZ, Jiang T, Cheng ZH, Zhang YC, Ou MM, Chen MC, Ling WM. TSNARE1 polymorphisms are associated with schizophrenia susceptibility in Han Chinese. J Neural Transm (Vienna) 2015; 122: 929-932.
4) Watanabe Y, Someya T, Nawa H. Cytokine hypothesis of schizophrenia pathogenesis: evidence from human studies and animal models. Psychiatry Clin Neurosci 2010; 64: 217-230.
5) Kelly DL, Demyanovich HK, Eaton WW, Cascella N, Jackson J, Fasano A, Carpenter WT. Anti gliadin antibodies (AGA IgG) related to peripheral inflammation in schizophrenia. Brain Behav Immun 2018; 69: 57-59.
6) Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 2012; 37: 137-162.
7) Song X, Fan X, Li X, Zhang W, Gao J, Zhao J, Harrington A, Ziedonis D, Lv L. Changes in pro-inflammatory cytokines and body weight during 6-month risperidone treatment in drug naive, first-episode schizophrenia. Psychopharmacology (Berl) 2014; 231: 319-325.

8) Khandaker GM, Zimbron J, Lewis G, Jones PB. Prenatal maternal infection, neurodevelopment and adult schizophrenia: a systematic review of population-based studies. Psychol Med 2013; 43: 239-257.
9) Mondelli V, Cattaneo A, Murri MB, Di Forti M, Handley R, Hepgul N, Miorelli A, Navari S, Papadopoulos AS, Aitchison KJ, Morgan C, Murray RM, Dazzan P, Pariante CM. Stress and inflammation reduce brain-derived neurotrophic factor expression in first-episode psychosis: a pathway to smaller hippocampal volume. J Clin Psychiatry 2011; 72: 1677-1684.
10) Prata J, Santos SG, Almeida MI, Coelho R, Barbosa MA. Bridging autism spectrum disorders and schizophrenia through inflammation and biomarkers - pre-clinical and clinical investigations. J Neuroinflammation 2017; 14: 179.
11) Muller N. Neuroprogression in schizophrenia and psychotic disorders: the possible role of inflammation. Mod Trends Pharmacopsychiatry 2017; 31: 1-9.
12) Schwarz E, Prabakaran S, Whitfield P, Major H, Leweke FM, Koethe D, McKenna P. Bahn S. High throughput lipidomic profiling of schizophrenia and bipolar disorder brain tissue reveals alterations of free fatty acids, phosphatidylcholines, and ceramides. J Proteome Res 2008; 7: 4266-4277.
13) Brodowicz J, Przegalinski E, Muller CP, Filip M. Ceramide and its related neurochemical networks as targets for some brain disorder therapies. Neurotox Res 2018; 33: 474-484.
14) Smesny S, Schmelzer CE, Hinder A, Kohler A, Schneider C, Rudzok M, Schmidt U, Milleit B, Milleit C, Nenadic I, Sauer H, Neubert RH, Fluhr JW. Skin ceramide alterations in first-episode schizophrenia indicate abnormal sphingolipid metabolism. Schizophr Bull 2013; 39: 933-941.
15) Brann AB, Scott R, Neuberger Y, Abulafia D, Boldin S, Fainzilber M, Futerman AH. Ceramide signaling downstream of the p75 neurotrophin receptor mediates the effects of nerve growth factor on outgrowth of cultured hippocampal neurons. J Neurosci 1999; 19: 8199-8206.
16) Schwarz A, Futerman AH. Distinct roles for ceramide and glucosylceramide at different stages of neuronal growth. J Neurosci 1997; 17: 2929-2938.
17) Garcia-Alvarez L, Caso JR, Garcia-Portilla MP, de la Fuente-Tomas L, Gonzalez-Blanco L, Saiz Martinez P, Leza JC, Bobes J. Regulation of inflammatory pathways in schizophrenia: a comparative study with bipolar disorder and healthy controls. Eur Psychiatry 2018; 47: 50-59.
18) Novgorodov SA, Gudz TI. Ceramide and mitochondria in ischemia/reperfusion. J Cardiovasc Pharmacol 2009; 53: 198-208.
19) Shan D, Mount D, Moore S, Haroutunian V, Meador-Woodruff JH, McCullumsmith RE. Abnormal partitioning of hexokinase 1 suggests disruption of a glutamate transport protein complex in schizophrenia. Schizophr Res 2014; 154: 1-13.
20) Guo J, Wu HW, Hu G, Han X, De W, Sun YJ. Sustained activation of Src-family tyrosine kinases by ischemia: a potential mechanism mediating extracellular signal-regulated kinase cascades in hippocampal dentate gyrus. Neuroscience 2006; 143: 827-836.
21) Zhu J, Shen W, Gao L, Gu H, Shen S, Wang Y, Wu H, Guo J. PI3K/Akt-independent negative regulation of JNK signaling by MKP-7 after cerebral ischemia in rat hippocampus. BMC Neurosci 2013; 14: 1.
22) Lu XB, Zhang Y, Yang DY, Yang YZ, Wu FC, Ning YP, Wu K. Analysis of first-episode and chronic schizophrenia using multi-modal magnetic resonance imaging. Eur Rev Med Pharmacol Sci 2018; 22: 6422-6435.
23) Di Nicola M, Cattaneo A, Hepgul N, Di Forti M, Aitchison KJ, Janiri L, Murray RM, Dazzan P, Pariante CM, Mondelli V. Serum and gene expression profile of cytokines in first-episode psychosis. Brain Behav Immun 2013; 31: 90-95.
24) Mencarelli C, Martinez-Martinez P. Ceramide function in the brain: when a slight tilt is enough. Cell Mol Life Sci 2013; 70: 181-203.
25) Ballou LR, Laulederkind SJ, Rosloniec EF, Raghow R. Ceramide signalling and the immune response. Biochim Biophys Acta 1996; 1301: 273-287.
26) Van Overloop H, Denizot Y, Baes M, Van Veldhoven PP. On the presence of C2-ceramide in mammalian tissues: possible relationship to etherphospholipids and phosphorylation by ceramide kinase. Biol Chem 2007; 388: 315-324.
27) Zhang Y, Li X, Becker KA, Gulbins E. Ceramide-enriched membrane domains--structure and function. Biochim Biophys Acta 2009; 1788: 178-183.
28) Sot J, Bagatolli LA, Goni FM, Alonso A. Detergent-resistant, ceramide-enriched domains in sphingomyelin/ceramide bilayers. Biophys J 2006; 90: 903-914.
29) Hannun YA. The sphingomyelin cycle and the second messenger function of ceramide. J Biol Chem 1994; 269: 3125-3128.
30) Gu L, Huang B, Shen W, Gao L, Ding Z, Wu H, Guo J. Early activation of nSMase2/ceramide pathway in astrocytes is involved in ischemia-associated neuronal damage via inflammation in rat hippocampi. J Neuroinflammation 2013; 10: 109.
31) Tsai MC, Tanaka K, Overstreet-Wadiche L, Wadiche JI. Neuronal glutamate transporters regulate glial excitatory transmission. J Neurosci 2012; 32: 1528-1535.
32) O'Donovan SM, Sullivan CR, McCullumsmith RE. The role of glutamate transporters in the pathophysiology of neuropsychiatric disorders. NPJ Schizophr 2017; 3: 32.
33) Piyabhan P, Wannasiri S, Naowaboot J. Bacopa monnieri (Brahmi) improved novel object recognition task and increased cerebral vesicular glutamate transporter type 3 in sub-chronic phencyclidine rat model of schizophrenia. Clin Exp Pharmacol Physiol 2016; 43: 1234-1242.
34) Lee A, Pow DV. Astrocytes: glutamate transport and alternate splicing of transporters. Int J Biochem Cell Biol 2010; 42: 1901-1906.