Original Paper

Cerebral Mast Cells Participate In Postoperative Cognitive Dysfunction by Promoting Astrocyte Activation

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Key Words

Mast cells • Astrocyte activation • Neuroinflammation • Postoperative cognitive dysfunction • Cromolyn

Abstract

Background: Astrocytes, the major glial cell type that has been increasingly recognized as contributing to neuroinflammation, are critical in the occurrence and development of postoperative cognitive dysfunction (POCD). Although emerging evidence showed that brain mast cells (MCs) are the “first responders” in neuroinflammation, little is known about the functional communication between MCs and astrocytes. **Methods:** In this study, we investigated the potential regulation of astrocyte activation by MCs. Rats received an intracerebroventricular injection of Cromolyn (an MC stabilizer) or sterile saline 30 min before undergoing open tibial fracture surgery, and the levels of neuroinflammation and the degree of memory dysfunction were evaluated at 1 day and 3 days after surgery. In the *in vitro* study, the effect of activated MCs on astrocytes were further clarified. **Results:** Surgery increased the number of MCs, the astrocyte activation and the production of inflammatory factors, and resulted in cognitive deficits. Site-directed pre-injection of Cromolyn can inhibit this effect. In the *in vitro* study, the conditioned medium from C48/80-stimulated mast cells (P815) could induce primary astrocyte activation and subsequent production of inflammatory cytokines, which could be inhibited by Cromolyn. **Conclusion:** These findings indicate that activated MCs could trigger astrocyte activation, be involved in neuroinflammation and possibly contribute to POCD. Interactions between MCs and astrocytes could provide potential therapeutic targets for POCD.

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Introduction

Postoperative cognitive dysfunction (POCD), a complication that occurs after surgery, has recently gained more attention. POCD may exist over long durations of time and

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may evolve into an irreversible central nervous system (CNS) disease [1]. However, the pathological mechanisms involved in POCD remain unclear. Hence, it is important to unravel the neuropathogenesis of POCD. Recent studies have demonstrated that surgery-induced neuroinflammation is involved in POCD development [2-5]. The occurrence and propagation of neuroinflammation rely on the interactions between glia and immune cells. However, glia-immune cell communication has not been comprehensively investigated.

Astrocytes, which are major glial cells, participate in all essential CNS functions, including neuronal survival and differentiation [6, 7], energy metabolism, blood flow regulation, ion and water homeostasis, neurotransmission, adult neurogenesis, immune defence, etc. [8]. Astrocytes, as one of primary responders to cellular stress, perturbation, infection and injury of the CNS, can secrete a number of important cytokines that affect the surrounding cells, such as neurons, microglia, and astrocyte, leading to neuroinflammation, neurodegeneration, and apoptosis. For instance, cytokines such as TNF- α , IL-1 β , IL-6, and transforming growth factor- β 1 (TGF- β 1) can act as pro- or anti-inflammatory mediators. Compared to moderate astrocyte activation, which is crucial in the repair of brain injury due to the secretion of neurotrophic factors, a rapid, prolonged and severe process may cause an excessive inflammatory response, ultimately contributing to neuronal damage and even neuronal apoptosis, resulting in neurodegenerative diseases [9]. Hence, the inhibition of astrocyte activation may improve surgery-induced neuroinflammation and cognitive dysfunction.

Although astrocyte activation has been recognized to contribute to neuroinflammation, proinflammatory mediators released from other immune cells, such as mast cells (MCs), are also crucial players. MCs, famous for their role in allergic responses, are also often found in the CNS, especially along the blood vessels and leptomeninges [10, 11]. Under various types of stress, MCs serve as important sources of several mediators, including proteases and vasoactive amines such as tryptase and histamine [12], which have been reported to induce astrocyte activation and cytokine production *in vitro* [13, 14]. These inflammatory mediators released by MCs participate in the pathology of several neuroinflammatory diseases [15]. Furthermore, activated brain MCs can disrupt the blood-brain barrier and can recruit neutrophils and activated T cells to enter the CNS [5, 16]. Several molecular mechanisms for potential interactions between MCs and astrocytes have been determined *in vitro* [17]. However, the exact effects of MCs on astrocytes are still unclear. Understanding the triggers of neuroinflammation is key in improving cognitive function after surgery. In this study, we hypothesized that activated brain MCs contribute to astrocyte activation and neuroinflammation, which may then cause cognitive dysfunction after surgery.

Materials and Methods

In Vivo Studies

Animals. Male adult Sprague-Dawley (S-D) rats, 8 weeks-old, weighting 200-250 g, were used in this *in vivo* experiment. All rats were housed in groups of five per cage under standard environmental conditions (12 h light/dark cycle, ambient temperature of 22.0 ± 1.0 , and 40% humidity) during the experimental period. Food and water were available *ad libitum*. The experimental process was approved by the Nanjing Medical University Animal Care and Use Committee. All experimental procedures involving animals were approved by IACUC (Institutional Animal Care and Use Committee of Nanjing Medical University).

Intracerebroventricular Cannula Implantation. Intracerebroventricular (i.c.v.) cannula implantation was used for the administration of drugs in rodents as previously described [4, 5]. In brief, rats were placed in the stereotaxic apparatus (Stoelting Instruments, USA) for the i.c.v. administration of drugs following anaesthesia. According to the atlas of Paxinos and Watson (1982), guide cannulas (Plastic One) were inserted into the lateral ventricle (0.8 mm posterior, 1.5 mm lateral, and 3.7 mm ventral to the bregma) and were secured to the skull with dental cement. The animals were allowed to recover in cages for one week. Animals with broken guide cannulas were removed from the procedure. At the time of drug administration, a corresponding injection cannula connected to a microsyringe pump by a PE-20 catheter was filled with drug

solution and was inserted into the guide cannula. The needle was maintained in this position for 5 min after the injection, and then it was slowly extracted from the brain.

Surgery and Drug Administration

The rats were randomly assigned to one of four groups with 12 rats in each group, and investigators were blinded to the experimental treatment: (A) i.c.v. injection of saline (Ctrl group); (B) i.c.v. injection of disodium cromoglycate (Cromolyn) (Cro group); (C) tibial fracture surgery following i.c.v. injection of saline (Sur group); or (D) tibial fracture surgery following i.c.v. injection of cromolyn (Cro+Sur group). Rats in the Cro group and Cro+Sur group received 200 µg of Cromolyn (100 µg/µl, 2 µl) i.c.v. 30 min before surgery, while the other animals received 2 µl of saline.

Surgery consisted of the placement of an open tibia fracture of the left hind paw in aseptic conditions under anaesthesia as described previously [3-5]. Briefly, the left hind limb of the rat was meticulously shaved and disinfected with povidone iodine after the animals were placed under anaesthesia. Followed by the insertion of a 20-G pin in the intramedullary canal, a middle incision was performed on the left hind paw; the periosteum was then stripped, and an osteotomy was performed. After the fracture, the wound was irrigated, and the skin was sutured. The animals were allowed to recover spontaneously from the anaesthesia. During the surgical process, the body temperature of the animals was monitored and maintained with the aid of warming pads. Analgesia (0.1 mg/kg buprenorphine) was given subcutaneously after the anaesthesia induction and before the skin incision.

Behavioural tests

Trace Fear Conditioning (TFC). To assess hippocampal-dependent memory, TFC was used in rodents as previously described [4, 5, 18, 19]. Rats were trained to associate an environment (context) with a conditional stimulus (tone) and an unconditional stimulus (foot shock). Performance was assessed at 1 or 3 days after surgery. Freezing behaviour was recorded for 300 s, and a decreased percentage of freezing time indicated an impairment of memory.

Y maze test. The Y maze was another test that was used to assess learning ability as previously described. Nine continuous correct responses were defined as the learning criteria. The total number of stimulations to reach the criterion was recorded. All rats reached the learning criterion in the present study.

MC staining and counting

Rats were anaesthetized with 5% chloral hydrate (0.8 ml/100 g) and perfused first with 0.9% saline and then with cold 4% paraformaldehyde. The cerebral tissues were harvested and fixed with 4% paraformaldehyde at 4 °C for 24 h. Brain sections (10-µm thick) were prepared and were processed for toluidine blue (TB) staining and immunohistochemistry as follows. Slides with sections of the hippocampus were stained in 0.05% TB. The criteria for degranulation included the following: a loss of purple staining, a fuzzy appearance, a distorted shape, or multiple granules visible in the vicinity of the cell. The entire surface area of the CA1 area of the hippocampus was scanned manually using a light microscope at 200× magnification, and MCs were quantified with Cell D software (Olympus).

In addition, immunohistochemistry was performed to label MCs with a mast cell tryptase monoclonal antibody as follows.

Immunohistochemistry

Slides were incubated with the mast cell tryptase monoclonal antibody (1:100; Abcam; USA) or the GFAP monoclonal antibody (1:200; CST, USA) at 4 °C overnight and were then incubated with secondary antibody for 2 h. Positive cells in the CA1 area of three hippocampus sections from each animal were visualized using a Leica 2500 microscope (Leica Microsystems, Wetzlar, Germany) at 200× magnification.

In Vitro Studies

P815 Cell Culture. P815 cells, an MC line derived from mouse tumour cells, were incubated as previously described [4]. Experiments were performed when the cells were in the logarithmic phase of growth.

Astrocyte-enriched cultures. Primary mouse astrocyte were prepared as described in a previous protocol, with slight modifications [20, 21]. Briefly, whole brains were isolated from neonatal rats. The meninges and blood vessels were removed in cold PBS under a microscope. Next, the tissues were minced

with sterile scissors and were digested with 0.25% Trypsin-EDTA at 37°C. Trypsinization was stopped by the addition of an equal volume of culture medium containing 10% FBS. The dissociated cells were passed through a 100- μ m pore mesh, pelleted at 1,500 rpm for 5 minutes, and re-suspended in culture medium. The cells were seeded onto poly-D-lysine pre-coated cell culture flasks and were cultured at 37°C in a humidified atmosphere of 5% CO₂. The medium was replaced every three to four days after seeding. After the glial cells formed a confluent monolayer (10–14 days), the astrocyte cells were separated from the microglia by shaking. Cultures were passaged every 2 weeks into new 10-cm dishes at least 3 times to achieve highly pure astrocyte cultures [22]. Culture purity was > 95%, as determined by the assessment of GFAP expression.

Co-culture of astrocyte and P815 cells. After treatment with Cromolyn for 30 min, the P815 cells (1 $\times$ 10⁶ cells) were stimulated with Compound 48/80 (C48/80) and were cultured. Primary astrocytes (1 $\times$ 10⁶ cells) were grown in 5 cm $\times$ 5 cm flasks until confluent and were cultured for 24 hours with conditioned medium from P815 cells that had undergone different treatments.

ELISA

The concentrations of tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in rat hippocampus tissue extracts and in the culture medium of astrocyte were measured with a commercial ELISA kit from R&D Systems. The concentrations of histamine and mast cell tryptase in the supernatant of MCs were quantified with an ELISA kit from Fitzgerald.

Western Blotting

Hippocampal tissue extracts and astrocyte cells were collected and homogenized in RIPA lysis buffer (Biyuntian, Shanghai, China), which contained 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, sodium orthovanadate, sodium fluoride, EDTA, leupeptin, etc. After an incubation for 20 min on ice, the lysate was centrifuged, and protein concentration was determined with a BCA kit. Proteins (50 μ g) were denatured with sodium dodecyl sulphate (SDS) sample buffer and separated by 10% SDS-polyacrylamide gel electrophoresis. Proteins were transferred to PVDF membranes (Millipore) with a Bio-Rad miniprotein-III wet transfer unit. The membranes were incubated with 5% BSA dissolved in Tris-buffered saline with Tween 20 (TBST) (pH 7.5, 10 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween 20) at room temperature for 1 h. This was followed by the incubation of the membranes in different antibody solutions overnight at 4°C. The following primary antibodies were used: mouse monoclonal anti-GFAP (CST, #3670), rabbit monoclonal anti-Tau (Abcam, ab32075), c-Jun N-terminal kinase (JNK, CST, #9258S), phospho-JNK (CST, #4668), Extracellular regulated protein kinases (ERK, CST, #4695S), and phospho-ERK (CST, #14227). After adding the goat-anti-mouse or goat-anti-rabbit secondary antibody (1:5000) for 1 h, the protein bands on the membranes were detected with an enhanced chemiluminescence kit.

RNA purification and Real-time PCR

Total RNA extraction and real-time PCR amplification was performed as previously described. Primers included the following: mouse GAPDH forward, AAC TTT GGC ATT GTG GAA GG reverse, GGA TGC AGG GAT GAT GTT CT; mouse GFAP forward, 5'-CGG AGA CGC ATC ACC TCT G-3', and reverse, 5'-TGG AGG AGT CAT TCG AGA CAA-3'. The relative mRNA values were normalized to the GAPDH gene control values and were calculated using the comparative cycle threshold ($\Delta\Delta$ Ct) method.

Statistical Analysis

All values are presented as the mean $\pm$ SD. The significance of the differences between control samples and samples treated with various drugs was determined by one-way ANOVA followed by the post-hoc least significant difference test. Differences were considered significant at $P < 0.05$.

Results

Cromolyn inhibited the increase of MC number in the hippocampus induced by tibial surgery.

To examine whether MCs were involved in surgery-induced neuroinflammation, we first quantified brain MCs in TB and mast cell tryptase-stained sections of the hippocampus at 1

Fig. 1. Cromolyn inhibited the surgery-induced increase in MC number in the hippocampus. (A) Immunostaining was used to detect mast cell tryptase in the CA1 area of the hippocampus. Scale bar: 50 μ m. (B) Mast cells were stained with toluidine blue (TB) in the CA1 area of the hippocampus. Scale bar: 50 μ m. (C) Quantification of tryptase-positive cells in the CA1 area of the hippocampus. (D) Quantification of mast cells stained with TB. * P < 0.05, ** P < 0.01 vs. the control group. # P < 0.05, ## P < 0.01 vs. the surgery group. Data are presented as the mean $\pm$ SD (n=6).

day and 3 days after surgery. As shown in Figures 1A and 1B, compared to the control group at 1 day and 3 days, surgery led to a significant increase in the number of MCs in the CA1 area of the hippocampus. This effect was inhibited by the i.c.v. injection of the MC stabilizer Cromolyn (200 μ g, 2 μ l). Treatment with Cromolyn alone had no effect on the number of MCs in the brain. These results suggest that surgery can induce MC activation in the hippocampus.

Cromolyn inhibited surgery-induced astrocyte activation and cytokine production in the hippocampus.

In order to explore the effects of activated MCs on astrocyte activation, immunostaining was used to detect GFAP, which is a marker for astrocytes. Surgery led to notable astrocyte activation in the hippocampus at 1 day and 3 days after surgery, as indicated by the large number of GFAP-positive cells (Figs. 2A and 2B) and the increased expression levels of GFAP protein (Fig. 4). The effect could once again be prevented through pre-treatment with the MC stabilizer Cromolyn. Cromolyn treatment alone had no effect on astrocyte activation in the brain.

Since astrocyte-mediated neuroinflammation is mainly due to the excessive secretion of proinflammatory factors from activated astrocytes and their downstream signalling cascades, the levels of the proinflammatory factors TNF- α and IL-6 were detected by ELISA.

Fig. 2. Cromolyn inhibited the hippocampal astrocyte activation and cytokine production induced by tibial surgery. (A) Immunostaining was used to detect GFAP, a marker for astrocyte, in the CA1 area of the hippocampus. Scale bar: 50 μm. (B) Quantification of GFAP-positive cells in the CA1 area of the hippocampus. (C-D) The levels of proinflammatory factors TNF-α and IL-6 were detected via ELISA. *P < 0.05, **P < 0.01 vs. the control group. #P < 0.05, ##P < 0.01 vs. the surgery group. Data are presented as the mean ± SD (n=6).

Fig. 3. Cromolyn treatment ameliorated surgery induced cognitive decline. (A) The freezing time in the Trace Fear Conditioning test and (B) the number of learning trials in the Y Maze test were recorded to analyse cognitive changes. *P < 0.05, **P < 0.01 vs. the control group. #P < 0.05, ##P < 0.01 vs. the surgery group. Data are presented as the mean ± SD (n=10).

Surgery induced significant increases in TNF-α and IL-6 production, and the injection of Cromolyn could inhibit the inflammatory response; Cromolyn alone had no effect on cytokine secretion (Figs. 2D and 2E). These results suggest that the stabilization of MCS can inhibit the activation of astrocyte and that it can reduce neuroinflammation.

Fig. 4. MAPK signalling was involved in surgery-induced astrocyte activation and neuroinflammation. (A) The phosphorylation levels of ERK and JNK, and the expression levels of GFAP and Tau in the hippocampus were detected via Western blotting using specific antibodies. Each blot is representative of three experiments. (B) The expression levels of GFAP, Tau, phosphorylated ERK and phosphorylated JNK were quantified and normalized to total levels. Each value was then expressed relative to the control, which was set to 1. * $P < 0.05$, ** $P < 0.01$ vs. the control group. # $P < 0.05$, ## $P < 0.01$ vs. the surgery group. Data are presented as the mean $\pm$ SD ($n=5$).

Cromolyn alleviated surgery-induced cognitive decline.

To further confirm whether activated mast cells participated in cognitive dysfunction, we evaluated spatial working memory with the TFC test and Y maze. Compared to the control groups, the rats that underwent surgery exhibited a significant cognitive impairment at 1 day and 3 days after surgery. Notably, treatment with Cromolyn significantly improved freezing behaviour and the number of learning trials. However, the injection of Cromolyn alone did not change the freezing time or the number of learning trials (Fig. 3A and 3B). These results suggest that Cromolyn may help protect against surgery-induced cognitive dysfunction.

Cromolyn inhibited surgery-induced MAPK activation in the hippocampus.

To confirm the signalling pathways involved in neuroinflammation, Tau and MAPK related proteins were evaluated with Western blot. Surgery led to increased levels of Tau and rapid phosphorylation of ERK and JNK in the hippocampus, a change that could be prevented by Cromolyn (200 μ g) pre-treatment (Fig. 4A and 4B). Cromolyn (200 μ g) alone had no obvious effect. These results suggest that the stabilization of MCs can inhibit the proinflammatory response in the hippocampus and that MAPK signalling pathways are involved in this process.

C48/80-activated P815 cells increased astrocyte activation.

Given that the data *in vivo* clearly indicated a relationship between MCs and neuroinflammation caused by surgery, we then sought to clarify the mechanism of this correlation. Since astrocyte activation is a recognized sign that triggers neuroinflammation in neurodegenerative disorders, we explored the effect of activated MCs on astrocytes *in vitro*.

At 24 h after C48/80 stimulation, the release of histamine and tryptase reached a peak, which was referred to as MC degranulation (Fig. 5). Astrocyte activation was detected via

Fig. 5. C48/80 evoked MC degranulation. Histamine (A) and mast cell tryptase (B) concentrations in the supernatant of P815 cells were evaluated to analyse the degree of activation and degranulation. * $P < 0.05$, ** $P < 0.01$ vs. the control group. Data are presented as the mean $\pm$ SD ($n=3$).

Fig. 6. Activated P815 cells increased astrocyte activation. (A) The cells were identified with a GFAP antibody and upregulated GFAP-immunopositive expression (green) on the activated astrocyte was observed using confocal scanning. The blue staining represents DAPI. Scale bar: 50 μ m. (B) Quantification of GFAP-positive cells. * $P < 0.05$, ** $P < 0.01$ vs. the control group. # $P < 0.05$, ## $P < 0.01$ vs. the CM (P815 C48/80) group. Data are presented as the mean $\pm$ SD ($n=3$).

immunofluorescent staining, real-time PCR and Western blot for GFAP as before [21]. As shown in Figures 6 and 7A-C, similar to the effect of LPS exposure (1 μ g/ml) alone, incubation with the conditional medium (CM) from P815 cells with C48/80-stimulation (24 h) for 24 h could induce astrocyte activation. Pre-treatment with Cromolyn (10 μ g/ml) for 30 min could prevent the effect of MCs on astrocytes. These results indicated that mediators released by MC degranulation can induce astrocyte activation, which may be crucial in neuroinflammation.

MAPK signalling pathways were involved in astrocyte activation and cytokine production.

Since changes in the MAPK signalling pathways were observed during MC-induced astrocyte activation *in vivo*; we then investigated these signalling pathways *in vitro* for further

Fig. 7. MAPK signalling pathways were involved in astrocyte activation and the production of cytokines. (A) The expression levels of GFAP and the phosphorylation levels of ERK and JNK were detected via Western blotting using specific antibodies. Each blot is representative of three experiments. (B) Expression levels of GFAP, phosphorylated levels of ERK and phosphorylated levels of JNK were quantified and normalized to total levels. Each value was then expressed relative to the control, which was set to 1. (C) GFAP mRNA expression was detected via Real-Time PCR. (D-E) The levels of the proinflammatory factors TNF- α and IL-6 were detected via ELISA. * $P < 0.05$, ** $P < 0.01$ vs. the control group. # $P < 0.05$, ## $P < 0.01$ vs. the CM (P815 C48/80) group. Data are presented as the mean $\pm$ SD ($n=3$).

verification. C48/80-stimulation (24 h) of MCs *in vitro* increased the phosphorylation of ERK and JNK in astrocytes, which was ameliorated by Cromolyn (10 μ g/ml) pre-treatment (Fig. 7A and 7B). To observe the inflammatory effects of MC-astrocyte co-cultures, TNF- α and IL-6 in the supernatant were detected via ELISA. As shown in Figures 7D and 7E, cytokine production was sharply upregulated by both the direct application of LPS and exposure to CM from P815 stimulated with C48/80; Cromolyn (10 μ g/ml) pre-treatment could reduce the inflammatory response. Collectively, from this data, we concluded that MAPK signalling pathways were involved in MC-induced astrocyte activation and the subsequent production of cytokines.

Discussion

Pathological mechanisms underlying neuroinflammation have not been well characterized, even though neuroinflammation may contribute to the occurrence and development of POCD. Previous studies evaluated neuroinflammation by exploring glia and MCs in isolation from each other, ignoring the important role of the communication between them [23]. In this study, we explored the effects of activated MCs on astrocyte activation and the role of this interaction in POCD. We found that activated brain MCs can induce astrocyte activation and that it can aggravate neuroinflammation, resulting in cognitive dysfunction. These results may provide a novel therapeutic target for the treatment of POCD.

POCD has gained increasing attention due to the long-term adverse outcomes associated with the disorder, including a heavy economic burden, a low quality of life and an increasing mortality rate [24, 25]. In rodents, surgery may induce deficits in exploratory behaviour and spatially-based working memory. TFC is widely used to assess learning

and memory in rodents [4, 5, 26]. In the present study, we found that surgery can induce cognitive dysfunction in SD rats by decreasing the rate of freezing time on both 1 day and 3 days after surgery, which can be prevented by the i.c.v. administration of the MC stabilizer Cromolyn. These results were then confirmed with the Y maze test, one of the most widely-used paradigms for testing spatial based working memory in rodents [3-5, 27]. It is very sensitive for the measurement of early-period postoperative cognitive impairment and is usually used to assess hippocampus-dependent working memory [28, 29]. Hippocampal neurogenesis-related cognition is found to decline when animals are exposed to the electric shocks in the Y maze [30]. The results of the Y maze assessment were approximately the same as the results of the TFC. As observed in the results of our previous study, activated brain mast cells contributed to neuroinflammation and cognitive dysfunction by the promotion of BBB disruption [5], the activation of microglia and the induction of neuronal apoptosis [4]. Thus, we propose that activated MCs play a crucial role in the formation of cognitive deficits observed after tibial surgery. Brain MCs were implicated in the modulation of anxiety-like behaviour and provided the evidence for the behavioural importance of neuroimmune links [31]. The inhibition of the survival, migration and activity of MCs by masitinib can improve cognitive impairment in ADs [32]. In addition, approximately one-third of patients with mastocytosis display neuropsychological symptoms [33]. All of these findings show the closely correlation between brain MCs and cognitive function.

Astrocytes are crucial regulators of innate and adaptive immune responses in the injured central nervous system, and play primary roles in synaptic transmission and information processing by neural circuits, which are also considered to participate in neuroinflammation and CNS diseases [34-36]. Several experiments have shown that both microglia and astrocyte participate in neuroinflammation in animal models of neurodegeneration [37]. Microglial cells are mainly activated during the initial phase after minor surgery in rats, whereas the activation of astrocyte persists into the final periods [38]. Furthermore, astrocyte play a key role in the modulation of immune responses to CNS infections or disease [39]. Given that astrocyte reactivity was originally characterized by morphological changes (hypertrophy, remodelling processes) and the overexpression of the intermediate filament GFAP, we examined the level of GFAP to evaluate the activity of astrocytes. We found that surgery could induce astrocyte activation at 1 day and 3 days after surgery, which was accompanied by the secretion of proinflammatory cytokines. Therefore, the inhibition of astrocyte activation may offer clinical therapeutic benefits for neuroinflammation-related neurodegenerative disorders.

MCs are found in most tissues, including in the CNS, where they are often located adjacent to blood vessels and nerves [40] and can traverse the blood-brain barrier under pathological conditions [41]. Previous studies showed that activated brain MCs were the "first responders" in brain trauma and that they could induce microglial activation and neuronal apoptosis [4, 42]. Although other resident immune cells in the CNS also produce TNF- α (most notably microglia [43, 44], astrocyte [13, 21] and endothelial cells [45]), the presence and release of TNF- α from MCs precedes its detection in other cells. Our previous study also indicated that cerebral mast cells contribute to the pathogenesis of neuroinflammation and POCD in the rat by affecting the integrity of the BBB [5]. If the activation of brain MCs is necessary for the initiation of neuroinflammation and subsequent neurodegeneration, the inhibition of this response should be neuroprotective.

Even though astrocytes play a crucial role in neuroinflammation and chronic neurodegenerative diseases, the factors that are responsible for the over-activation of astrocytes are still unknown. Several studies have mentioned the potential crosstalk between MCs and astrocytes *in vitro*. There is evidence of the existence of H1- and H2-receptors on astrocytes in rats [14]. Patel et al reported that histamine could upregulate MMP-9 release via H1-receptor activation in astrocytes [46]. In addition, tryptase was shown to regulate the release of cytokines from astrocytes via PAR-2 signalling pathways in our previous study [13]. All of the above information notes the possibility of MC-astrocyte communication, which provides new insights for therapies targeting neuroinflammation by modulating the

activation of MCs to control the over-activation of astrocytes. In this study, we found that surgery could activate not only MCs but also astrocyte at 1 day and 3 days after surgery and that the activation could be ameliorated by Cromolyn pre-treatment. To further clarify the effect of MCs on astrocytes directly, we co-cultured P815 cells and primary astrocytes *in vitro*. We found that C48/80-stimulated P815 cells can activate astrocyte and induce TNF- α and IL-6 secretion. Pre-treatment with the “mast cell stabilizer” Cromolyn for 30 min could inhibit this phenomenon. However, the P815 cells without C48/80-stimulation or with Cromolyn alone had no effect on astrocyte activation. The MAPK signalling pathway was involved. These results further confirm that activated MCs can induce astrocyte activation and cytokine production.

Conclusion

In summary, our findings demonstrate that peripheral surgery can induce MC degranulation, following by astrocyte activation and neuroinflammation, resulting in cognitive dysfunction, which can be improved by treatment with the “MC stabilizer” Cromolyn. In an *in vitro* study, we confirmed that only the activated MCs can induce astrocyte activation and that MAPK pathways are involved. These results indicate that activated MCs could contribute to neuroinflammation by evoking astrocyte over-activation and that MC-astrocyte communication could provide a novel therapeutic target for neuroinflammation-related diseases.

Abbreviations

POCD (postoperative cognitive dysfunction); MAPK (mitogen-Activated Protein Kinase); LPS (lipopolysaccharide); TNF- α (tumour necrosis factor- α); IL-6 (interleukin-6); MCs (mast cells); Cromolyn (disodium cromoglycate); CNS (central nervous system); i.c.v. (intracerebroventricular); TFC (Trace Fear Conditioning); SDS (sodium dodecyl sulphate); TBST (Tris-buffered saline with Tween 20); JNK (c-Jun N-terminal kinase); ERK (extracellular regulated protein kinases); TB (toluidine blue).

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Disclosure Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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