

RAGE antagonism by FPS-ZM1 attenuates postoperative cognitive dysfunction through inhibition of neuroinflammation in mice

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Abstract. Neuroinflammation triggered by surgical trauma contributes to postoperative cognitive dysfunction (POCD). The receptor for advanced glycation end-products (RAGE), a multiligand inflammatory receptor, is involved in the damaging effects of various cellular processes, contributing to neuroinflammation and neurodegeneration. However, the potential role of RAGE in the acute period of POCD has not been fully investigated. C57BL/6 male mice undergoing surgery of the tibia under isoflurane anesthesia were treated with the RAGE antagonist FPS-ZM1 or vehicle control intraperitoneally for a period of 7 days. The cognitive function of the animals was tested using trace fear conditioning on the third postoperative day. To determine astrocytic activation, microgliosis, p65 expression, inflammatory factor levels and postsynaptic density protein-95 (PSD-95) expression in the hippocampus, the animals were euthanized on either the first, third or seventh postoperative day. Compared with the control group, the cognitive function of the surgical animals was impaired on the third postoperative day. Astrocytic activation, microgliosis and the expression levels of p65, interleukin (IL)-1 β , IL-6, and PSD-95 were significantly increased on the first, and third postoperative days. However, tumor necrosis factor- α expression was significantly increased only on postoperative day 1. All of the surgical effects observed were partially inhibited by treatment with FPS-ZM1. In summary, the results of the present study suggest that RAGE serves an important role in the acute inflammatory process of POCD, and blocking RAGE

can inhibit neuroinflammation and attenuate POCD. Thus, the RAGE signaling pathway may be a novel target in the prevention, and treatment of POCD.

Introduction

Postoperative cognitive dysfunction (POCD), which is commonly experienced in elderly patients following surgery, is mainly characterized by impaired memory, decreased information processing capability, and executive dysfunction (1,2). POCD may occur after cardiac surgery (3), non-cardiac surgery (4) and even after non-invasive surgeries under sedation (5). It tends to affect 16-21% of elderly patients (4,6), and is associated with long-term hospitalization and decreased quality of life, along with an increase in mortality and morbidity (1). Thus, it is vital to identify the pathophysiological mechanisms involved and develop effective strategies for the prevention or treatment of POCD, especially in older patients.

Although POCD may be regarded as a multifactorial process, there is evidence that suggests that neuroinflammation resulting from surgery significantly contributes to the development of POCD (7-10). Surgery activates an immune response and induces expression of peripheral pro-inflammatory cytokines (11). Pro-inflammatory cytokines originating from the periphery can affect the central nervous system (CNS) directly and indirectly (12). Within the CNS, microglia and astrocytes, which are activated by peripheral pro-inflammatory cytokines, can release additional pro-inflammatory cytokines. Continued activation of microglia and astrocytes together with pro-inflammatory cytokine release may ultimately lead to POCD via different downstream signaling pathways (13).

Receptor for advanced glycation end-products (RAGE) is a pattern-recognition receptor that is part of the immunoglobulin superfamily, and can bind a variety of ligands, including advanced glycation end-products (AGEs), high mobility group box 1 (HMGB1), S100 and amyloid- β (14). Recently, RAGE has increasingly been discussed in the context of neuroinflammation; it is reported that RAGE can promote neuroinflammation through the NF- κ B signaling pathway, which is critical for transducing a variety of inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β and IL-6 (15,16). In our previous study,

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we reported that RAGE and its ligands are highly expressed in mice with POCD (17). Thus, we hypothesized that RAGE may be involved in the development of POCD by promoting neuroinflammation. The main aim of the present study was to elucidate whether RAGE signaling may be involved in POCD and whether a RAGE antagonist can attenuate POCD in older mice.

Materials and methods

Animals. A total of 120 male C57BL/6 mice (age, 14 months; weight, 26–38 g) were acquired from the Guangdong Medical Laboratory Animal Centre (Guangdong, China). The mice were housed in a temperature-controlled ($25\pm 2^\circ\text{C}$) and humidity-controlled ($55\pm 5\%$) room wherein a 12-h light and dark cycle was maintained for ~ 2 weeks prior to surgery. All mice had free access to food and water. The experimental procedures were carried out in accordance with the National Institutes of Health 'Guidelines for the Care and Use of Laboratory Animals', and were approved by the Animal Ethics Committee of the Capital Medical University.

Study design. The 14-month-old mice ($n=36$) were randomly divided into three different groups: Control group ($n=12$; mice were not subjected to any treatment); surgical group ($n=12$; mice were subjected to open tibia fracture surgery 30 min after trace fear conditioning training); and the drug group ($n=12$; mice were subjected to open tibia fracture surgery with RAGE antagonist intervention). On the third postoperative day, cognitive function was assessed in all mice using fear conditioning tests.

The remaining 14-month-old mice ($n=84$) were randomly divided into seven experimental groups ($n=12$ per group). In one group, the mice were treated in the same way as the control group (C group); the other six groups of mice underwent open tibia fracture surgery with or without the RAGE antagonist intervention and were sacrificed on postoperative days 1, 3 and 7 (S1, S3, S7, D1, D3 and D7 group, respectively). Half of all mice ($n=6$ /measurement point/group) were utilized for the western blot analysis and RT-qPCR experiments, while the other half were utilized for immunohistochemical analyses.

In the D1, D3 and D7 groups, the RAGE antagonist FPS-ZM1 (EMD Millipore, Billerica, MA, USA) was administered at a dose of 10 mg/kg via intraperitoneal injection (IP) 30 min prior to surgery, and 1.5 mg/kg once per day postoperatively prior to sacrifice (18). The remaining surgical animal groups (S1, S3 and S7) were given normal saline of a similar quantity at the same time as the RAGE antagonist groups in order to control for the effects of injection-related stress.

Anesthesia and surgery. As previously described (19), the surgical mice were subjected to open tibia fracture surgery under general anesthesia (2.1% isoflurane in 30% FiO_2). The left hind limb of each mouse was shaved, and sterilized using povidone-iodine. Following a central skin cut, a 22 G pin was inserted into the intramedullary canal. Subsequently, the periosteum was exposed and osteotomy was conducted. Finally, the lesion was dampened and the skin was sewn together with 8-0 sutures. The mice were then allowed to recover consciousness following anesthesia spontaneously

in a separate polypropylene cage. By use of warming pads, the temperature was checked and sustained at $36.5\text{--}37.5^\circ\text{C}$ throughout the process.

Trace fear conditioning. Trace fear conditioning was utilized to evaluate the hippocampus-dependent memory of the mice, as described previously (20). The behavioral investigations were performed by use of a dedicated trace fear conditioning chamber. The chamber comprised a grid floor composed of stainless steel bars attached to a shock release system. During training, the mice were allowed to explore this environment for 3 min. Subsequently, three tone-foot shock pairings (tone: 2,000 Hz, 85 dB, 30 sec; foot shock: 0.6 mA, 2 sec) were delivered with an intertribal interval of 60 sec. Animals were returned to their home cage after an additional 30 sec. Three days after training, animals were returned to the same chamber for five min without tone or shock. Freezing behavior was described as a deficiency of any noticeable movement with the exception of respiration. The freezing behavior related to context was expressed as a percentage of the observation period, and was automatically documented using the software (Xeye Fcs; Beijing MacroAmbition S&T Development Co., Ltd., Beijing, China).

Immunohistochemical staining. The animals were transcardially perfused with saline followed by 4% paraformaldehyde (PFA) under anesthesia. The brains were harvested, and post-fixed in 4% PFA for 2 h, then dried in 30% sucrose under 4°C overnight, after which they were embedded in paraffin. The paraffin-embedded hippocampal tissues were cut into $5\text{-}\mu\text{m}$ sections, which were subsequently deparaffinized and rehydrated prior to antigen retrieval. After blocking with 3% H_2O_2 in phosphate-buffered saline for 10 min, the sections were incubated with a rabbit polyclonal antibody against ionized calcium-binding adaptor molecule 1 (IBA-1; 1:500; 019-19741; Wako Chemicals Inc., Richmond, VA, USA) or a rabbit anti-glial fibrillary acidic protein (GFAP) antibody (1:500; Dako, Copenhagen, Denmark) at 4°C overnight. Subsequent to washing, the sections were incubated with secondary antibody for 2 h at room temperature in the dark. Finally, DAB was added to visualize the microglia and astrocyte proportions of the hippocampus. Quantification was conducted through the use of ImageJ 1.47n software. Positive immunoreactivity levels were quantified as the proportion of positively stained divided by the total image area. Quantitative analyses were conducted in a blinded manner.

RT-qPCR. On day 1, 3 and 7 postoperatively, mice ($n=6$) were sacrificed via cervical dislocation under anesthesia. The total RNA was extracted and purified from homogenized hippocampal tissues using TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the guidelines of the manufacturer. RNA was reverse transcribed into a cDNA template by use of a PrimeScript™ Reverse Transcription Reagent kit (Takara Bio Inc., Shiga, Japan) in a $20\text{-}\mu\text{l}$ reaction system. Following this, cDNA was amplified and analyzed by qPCR using SYBR-Green qPCR Master mix (Invitrogen Life Technologies) and an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The specific primers utilized were as follows: TNF- α forward, 5'-CATGAT

CCGAGATGTGGAAGTGGC-3' and reverse, 5'-CTGGCT CAGCCACTCCAGC-3'; IL-1 β forward, 5'-CATGATCCG AGATGTGGAAGTGGC-3' and reverse, 5'-CTGGCTCAG CCACTCCAGC-3'; IL-6 forward, 5'-ACTCACCTTTCAGA ACGAATTG-3' and reverse, 5'-CCATCTTTGGAAGGTTCA GGTTG-3'; and GAPDH forward, 5'-GGAGCGAGATCCCTC CAAAAT-3' and reverse, 5'-GGCTGTTGTCATACTTCT CATGG-3'. The PCR conditions were set at 95°C for 3 min (initial denaturation), followed by 40 cycles of 95°C for 30 sec and 60°C for 5 sec (amplification). Relative mRNA levels were then normalized with respect to the GAPDH control values, and quantified with the $2^{-\Delta\Delta Ct}$ technique.

Western blot analysis. A total of 6 hippocampal tissue samples were homogenized on ice and lysed in cold radioimmunoprecipitation assay buffer with a protease-inhibitor cocktail. The homogenates were then centrifuged at 12,000 x g at a temperature of 4°C for ~30 min. Supernatant was then collected, and the proportion of protein in the supernatants was quantified using a BCA Protein Assay kit, according to the protocol of the manufacturer. After denaturing with SDS sample buffer, proteins (20 μ g) were separated via SDS-polyacrylamide gel electrophoresis, and transferred to a polyvinylidene difluoride membrane. The membranes were blocked in 5% non-fat dried milk dissolved in Tris-buffered saline with Tween-20 (TBST) at room temperature for 1 h, then incubated with rabbit anti-p65 (1:1,000) and anti-GAPDH (1:2,000; both from Cell Signaling Technology, Inc., Danvers, MA, USA) primary antibodies overnight at 4°C. After thorough washing, the membranes underwent incubation with a secondary goat anti-rabbit antibody conjugated to horseradish peroxidase (1:5,000; Cell Signaling Technology, Inc.) for 1 h at room temperature. Thereafter, the membranes were treated with an enhanced chemiluminescence detection kit (Pierce; Thermo Scientific, Inc., Shanghai, China), and the concentration of each band was measured by densitometry using Bio-Rad Quantity One software. The relative expression levels were normalized to GAPDH.

Statistical analyses. All statistical analyses were carried out with SPSS (version 14; SPSS, Inc., Chicago, IL, USA) and Prism software. Data are presented as the mean \pm standard deviation. The variations between the means were assessed by one-way ANOVA. Additionally, Bonferroni post hoc tests were used when the ANOVA test indicated significance. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

RAGE antagonist ameliorates POCD in 14-month-old mice. In order to investigate whether inhibition of RAGE could improve POCD following surgery, the effect of FPS-ZM1 on learning and memory impairment was evaluated using the conditioned fear test. In this study, surgery considerably reduced the freezing time percentage compared with the control group (40.51 \pm 9.63 vs. 59.28 \pm 11.37%; $n=12$; $P < 0.001$). Exposure to the RAGE inhibitor FPS-ZM1 attenuated the surgery-induced reduction in freezing behavior; these mice showed no significant difference in freezing time compared with the non-surgical control group (54.38 \pm 10.32 vs. 59.28 \pm 11.37%; $n=12$; $P > 0.05$) (Fig. 1).

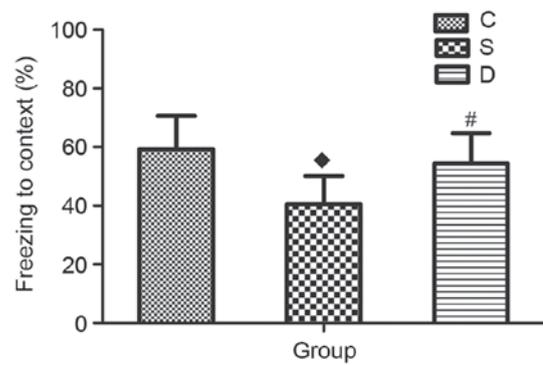


Figure 1. Effects of FPS-ZM1 on postsurgical memory impairment among 14-month-old mice. Contextual fear response shows hippocampal-dependent memory damage on the third postoperative day. Bars represent the mean \pm standard deviation ($n=12$). * $P < 0.01$ vs. the normal control group; # $P < 0.05$ vs. the corresponding surgical group. C, control group; S, surgical group; D, surgery + FPS-ZM1 group.

FPS-ZM1 inhibits the post-surgical upregulation of GFAP and IBA-1 expression in the hippocampus. To examine the surgery-associated activation of inflammatory cells and the role of RAGE antagonism, IBA-1 and GFAP expression levels in the mouse hippocampi were investigated by immunohistochemical staining. Microglial activation was characterized by increased numbers of microglia and a trend towards increased microglial cell body-to-cell size ratio. Astrocyte activation was characterized by an increased astrocyte cell soma size and greater width and length of the stained processes. IBA-1 and GFAP immunoreactivity were enhanced in the mice of the surgical groups on the first and third postoperative days relative to the control mice (those which did not undergo open tibia fracture surgery). These effects were attenuated by treatment with the RAGE antagonist (Figs. 2 and 3). The findings suggest that the RAGE antagonist may inhibit surgery-induced activation of microglia activation and astrocytes.

FPS-ZM1 inhibits the elevation of NF- κ B p65 expression in hippocampal tissues following open tibia fracture surgery. Compared with the control group, NF- κ B p65 protein levels were significantly increased on postoperative day 3 ($P < 0.01$) and decreased to baseline by the seventh postoperative day in the surgical group. This increase was inhibited by FPS-ZM1 treatment (Fig. 4).

FPS-ZM1 inhibits pro-inflammatory cytokine elevation in hippocampal tissues. The TNF- α , IL-1 β and IL-6 mRNA expression levels in hippocampal tissues of 14-month-old mice were determined using RT-qPCR on the first, third and seventh postoperative days, with or without RAGE antagonist administration. As illustrated in Fig. 5, the expression levels of TNF- α , IL-1 β and IL-6 were considerably upregulated on the first postoperative day. Upregulation of IL-1 β and IL-6, relative to the control group, remained until the third postoperative day. However, IP administration of the RAGE antagonist considerably attenuated the surgery-induced upregulation of these pro-inflammatory cytokines.

FPS-ZM1 inhibits the elevation of PSD-95 at postoperative days 1 and 3, but increases its expression on the seventh day.

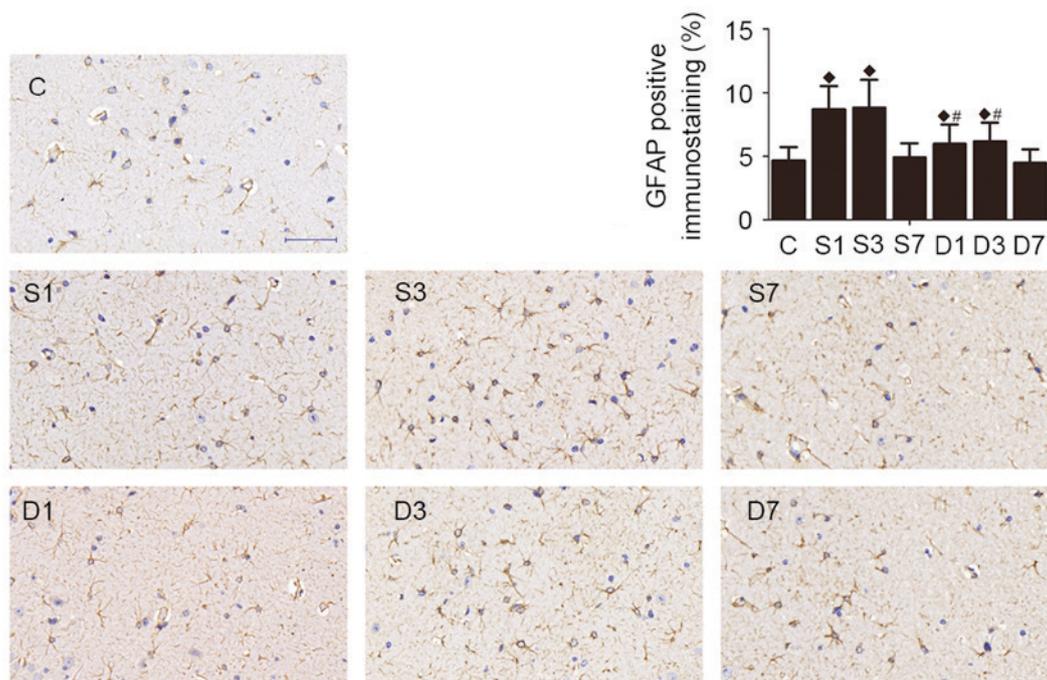


Figure 2. FPS-ZM1 inhibited the upregulation of GFAP expression in the hippocampus following surgery. Histogram depicts the area of HMGB1-positive cells in the CA1 region of the hippocampus. Data are presented as the mean \pm standard deviation ($n=6$). * $P<0.01$ vs. the normal control group; # $P<0.05$ vs. the corresponding surgical group. C: control group; S1, S3 and S7: surgical groups on postoperative days 1, 3 and 7, respectively; D1, D3 and D7: surgical group with FPS-ZM1 on postoperative days 1, 3 and 7, respectively. Scale bar, 50 μ m. GFAP, glial fibrillary acidic protein; HMGB1, high mobility group box 1.

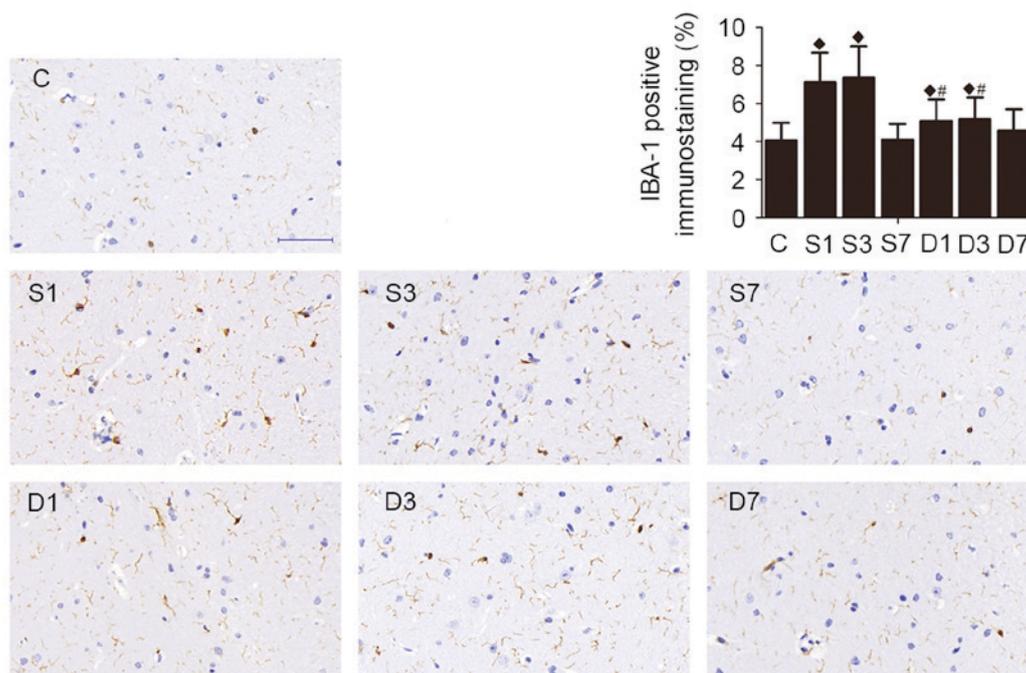


Figure 3. FPS-ZM1 inhibited the upregulation of IBA-1 expression in the hippocampus following surgery. Histogram depicts the area of IBA-1-positive cells in the CA1 region of the hippocampus. Data are presented as the mean \pm standard deviation ($n=6$). * $P<0.01$ vs. the normal control group; # $P<0.05$ vs. the corresponding surgical group. C: control group; S1, S3 and S7: surgical groups on postoperative days 1, 3 and 7, respectively; D1, D3 and D7: surgical group with FPS-ZM1 on postoperative days 1, 3 and 7, respectively. Scale bar, 50 μ m. IBA-1, ionized calcium-binding adaptor molecule 1.

To investigate the results of surgery and RAGE antagonism on the expression of synaptic proteins, we measured the levels of postsynaptic density protein-95 (PSD-95, also known as

DLG4). PSD-95 was increased markedly on the first postoperative day in the surgical group, reaching a peak, decreased slowly on the third day and then returned to baseline on the

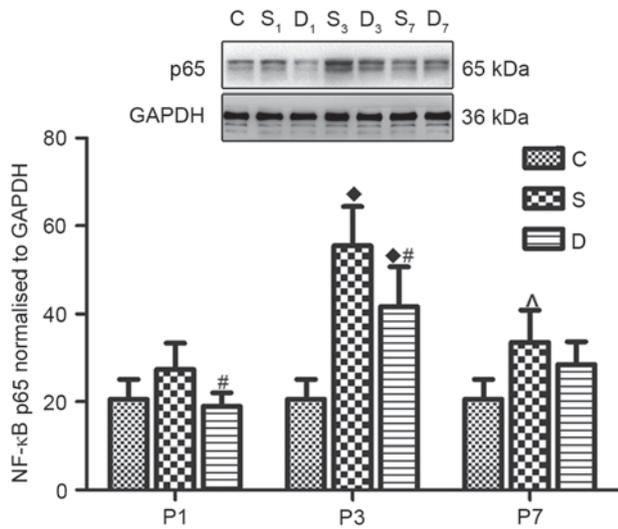


Figure 4. Western blot analysis revealing the expression of NF-κB p65 in the hippocampi of 14-month-old mice following tibia fracture surgery. Data are presented as the mean ± standard deviation (n=6). [^]P<0.05 and ^{*}P<0.01 vs. the normal control group; [#]P<0.05 vs. the corresponding surgical group. S1, S3 and S7: surgical groups on postoperative days 1, 3 and 7, respectively; D1, D3 and D7: surgical group with FPS-ZM1 on postoperative days 1, 3 and 7, respectively; P1, P3 and P7: postoperative days 1, 3 and 7, respectively.

seventh day. The RAGE antagonist inhibited the elevation of PSD-95 on the first and third postoperative days, but increased the overexpression of PSD-95 on the seventh day after surgery (Fig. 6).

Discussion

The present study demonstrated that a RAGE antagonist inhibited the activation of inflammatory cells and release of inflammatory factors, partly by inhibiting the NF-κB pathway, and this could lead to an improvement of postoperative cognitive function in mice. In addition, the results indicated that PSD-95 may be an antagonist of POCD in the early postoperative period.

Microglia are specialized macrophages of the CNS, constituting 5-20% of overall glial cells in mice. Risk signals, such as the peripheral pro-inflammatory factors TNF-α, IL-1β and IL-6, can trigger latent microglia and lead to their activation (8). High levels of pro-inflammatory mediators, which are released by activated microglia, are potentially neurotoxic, and thus may lead to acute and reversible behavioral effects such as delirium. Severe and permanent behavioral effects through bystander damage to neighboring neurons may also occur (21-23). Terrando *et al* (8) reported that peripheral macrophages can infiltrate the parenchyma and periventricular areas of the hippocampus prior to the activation of resident microglia. Thus, peripheral macrophages may play crucial roles in early hippocampal inflammation. Consistently with these results, the present study demonstrated that IBA-1 immunoreactivity was enhanced in the hippocampi of animals that had undergone surgery, suggesting the activation of macrophages or microglia, and these enhancements were attenuated by treatment with a RAGE antagonist.

Astrocytes are widespread in the brain. They directly communicate with neurons and microglia. By regulating the

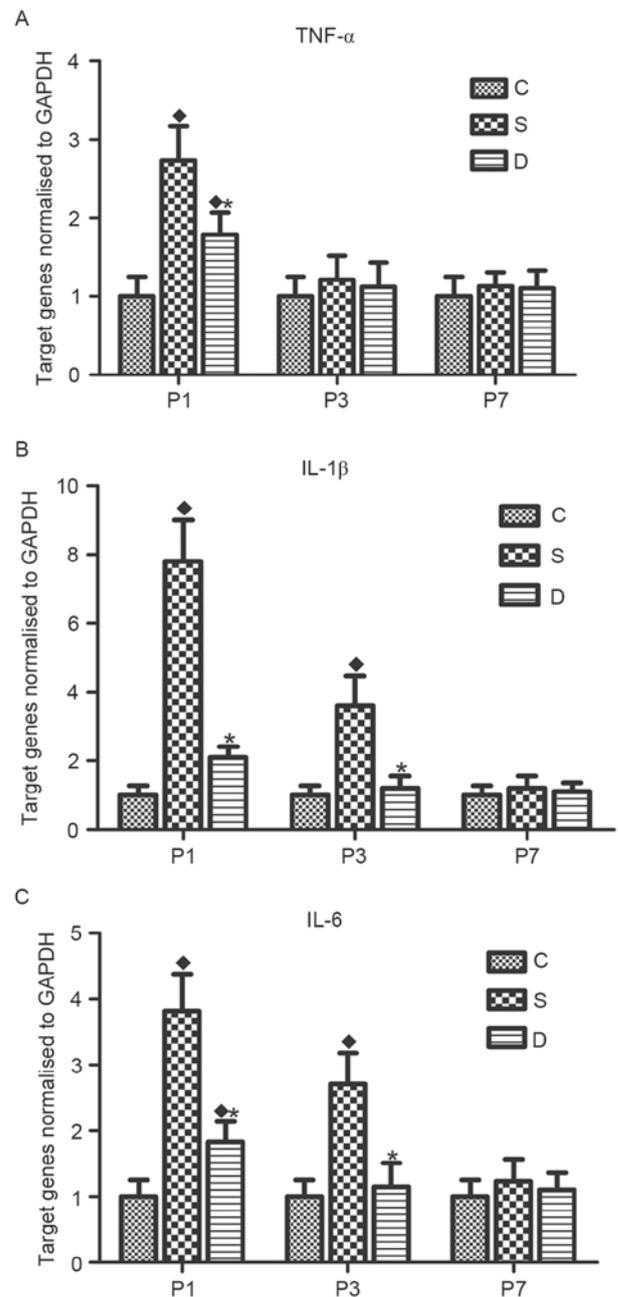


Figure 5. FPS-ZM1 attenuated the increase of pro-inflammatory cytokines in the hippocampal tissues following surgery. Hippocampal (A) TNF-α, (B) IL-1β, and (C) IL-6 mRNA levels in 14-month-old mice were quantified on the first, third and seventh postoperative days. Bars represent the mean ± standard deviation (n=6). ^{*}P<0.01 vs. the normal control group; [#]P<0.01 vs. the corresponding surgical group. P1, P3, and P7: postoperative days 1, 3 and 7, respectively. C, control group; S, surgical group; D, surgery + FPS-ZM1 group; TNF-α, tumor necrosis factor-α; IL, interleukin.

permeability of the blood-brain barrier (BBB) and microgliosis, astrocytes are able to coordinate immune responses caused by pro-inflammatory cytokines that enter the brain from the periphery. Firstly, pro-inflammatory cytokines enhance the permeability of the BBB. Using transmission electron microscopy, enlarged astrocyte end-feet as well as detachment of end-feet plasma membranes from the basal lamina can be observed, which shows activation of astrocytes and BBB damage (24). Meanwhile, IL-1β and TNF-α can increase the expression of pro-inflammatory genes in the

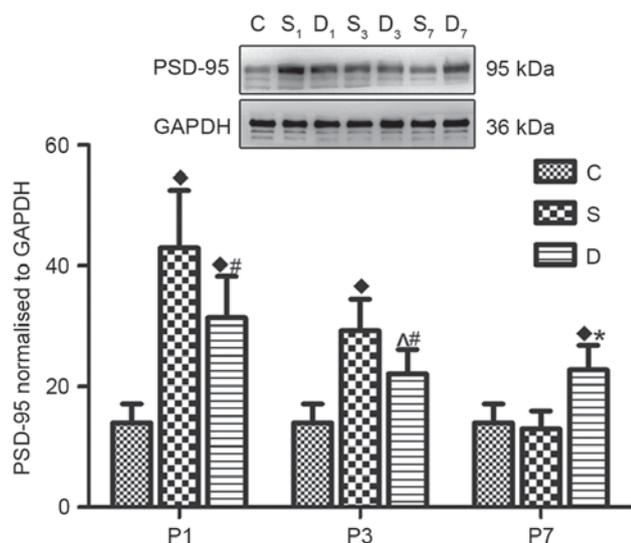


Figure 6. Treatment with the RAGE antagonist inhibited the elevation of PSD-95 expression on days 1, 3 and 7, but promoted its expression on day 7 after surgery. Data are presented as the mean \pm standard deviation ($n=6$). $^{\wedge}P<0.05$ and $^*P<0.01$ vs. the normal control group; $^{\#}P<0.05$ and $^*P<0.01$ vs. the corresponding surgical group. S1, S3 and S7: surgical groups on postoperative days 1, 3 and 7, respectively; D1, D3 and D7: surgical group with FPS-ZM1 on postoperative days 1, 3 and 7, respectively; P1, P3 and P7: postoperative days 1, 3 and 7, respectively. RAGE, receptor for advanced glycation end-products; PSD-95, postsynaptic density protein-95.

astrocytes. Pro-inflammatory factors that are derived from astrocytes may also be capable of activating the microglia, potentially resulting in microgliosis (23). Finally, crosstalk between classically stimulated microglia and astrocytes may cause amplification of inflammatory reactions. In agreement with previous studies, the present study showed that surgery enabled the activation of astrocytes in the hippocampus on the first and third days after surgery. Additionally, the activation of astrocytes could be significantly inhibited by pre-operative application of FPS-ZM1.

In mammals, p65 is an important member of the NF- κ B family and is a component of the classical NF- κ B pathway. It forms heterodimers with p50 to promote the transcription of NF- κ B target genes in the nucleus (25). Evidence indicates that multiple RAGE ligands can activate neuroinflammation via the RAGE/NF- κ B pathway (16,26). The present study demonstrated that surgery can upregulate NF- κ B expression, and this effect was significantly inhibited by FPS-ZM1. Interestingly, on the first day after surgery, although the expression of NF- κ B was inhibited, inflammatory cell activation and release of pro-inflammatory cytokines were still apparent. One possible explanation for this observation could be that there are other forms of NF- κ B, which do not include p65, leading to inflammatory cell activation and pro-inflammatory cytokine release. Another possible explanation could be that other inflammatory signaling pathways are triggered (27).

In the present study, TNF- α , IL-1 β and IL-6 were strongly upregulated after surgery, illustrating the activation of pro-inflammatory cytokines. Pro-inflammatory cytokines are involved in specific memory processes, such as acquisition, consolidation and retrieval. Under physiological conditions, IL-1 β largely contributes to hippocampus-dependent memory formation (28). Nevertheless, IL-1 β application also inhibits

the initiation and preservation of long-term potentiation (LTP), resulting in impaired consolidation (29) and memory reconsolidation (30). On the contrary, TNF- α inhibition (31) or IL-6 genetic deletion (32) have been shown not to interrupt memory or learning, whereas TNF- α overexpression (33) or IL-6 application (34) can lead to widespread memory damage and decreased LTP, respectively. In the present study, the FPS-ZM1-treated mice in the surgical group had decreased mRNA expression of TNF- α , IL-1 β and IL-6 relative to the control surgical mice. This suggests that RAGE may be involved in the inflammatory process stimulated by surgery, and that treatment with a RAGE antagonist may reduce the production and release of inflammatory cytokines by inhibiting the activation of the inflammatory cells mentioned above.

PSD-95 is a neuronal scaffolding protein that is involved in anchoring membrane proteins and regulating protein trafficking (35). By regulating the interaction between proteins, PSD-95 contributes to synaptic plasticity as well as LTP (36). Decreased levels of PSD-95 have been reported in Alzheimer's disease, and are considered to be associated with learning and memory deficits (37). Nonetheless, an increase in PSD-95 has also been reported in cases of Alzheimer's disease, which was considered to be a compensatory mechanism at the postsynaptic terminal (38). In agreement with the previous study by Sultana *et al* (38), the presented study showed that PSD-95 markedly increased after surgery; this may be a compensatory measure for postoperative cognitive impairment. Since FPS-ZM1 inhibited surgery-induced neuroinflammation and cognitive impairment, the compensation was equally weakened. Although such a compensatory response has been reported in Alzheimer's disease, to the best of our knowledge, the present study is the first to describe this compensatory phenomenon in association with POCD. As shown in Fig. 6, we found that the levels of PSD-95 in the surgical groups were higher than those in the corresponding drug treatment groups on postoperative days 1 and 3; however, the level of PSD-95 in the drug treatment group was higher than that in the surgical group on the seventh day after surgery. The reasons for this might be that FPS-ZM1 can slow down and extend the compensatory response of PSD-95, and that the compensation can be exhausted.

In previous studies, it was noted that different timings of inflammatory stimuli could lead to different effects on learning and memory. Pre-existing inflammation impaired the learning process although it did not affect retrieval (7). After learning, early inflammatory stimuli damaged memory consolidation. Conversely, late stimulation did not affect memory consolidation or retrieval, but tended to shorten the duration of memory (29). In agreement with the study by Vacas *et al* (39), our results confirmed that after learning, early inflammatory stimuli caused by surgical injury interrupted the memory consolidation process. To avoid cognitive disturbance from postoperative depression caused by neuroinflammation, we carried out the fear conditioning test on the third day after surgery.

The current study had a number of limitations. First, the study failed to observe the long-term effects of surgery and medication on cognition. Therefore, it mainly reflected on the acute phase of POCD. Additionally, to reduce the potential impacts of estrogen and progesterone on memory and

learning, this study utilized only male mice. Therefore, the effects of FPS-ZMI on surgery-stimulated cognitive damage in females are unknown. Furthermore, animal models may not entirely replicate the clinical state observed in humans or its complexity. Thus, it is necessary to assess whether comparable modifications occur in human patients following surgery.

In summary, POCD is associated with the RAGE signaling pathway. RAGE antagonism has a neuroprotective role in the mouse hippocampus by inhibiting neuroinflammation. The results suggest that the RAGE signaling pathway may be a novel target for the prevention and treatment of POCD.

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