Specific antagonist of receptor for advanced glycation end-products attenuates delirium-like behaviours induced by sevoflurane anaesthesia with surgery in aged mice partially by improving damage to the blood-brain barrier

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Received July 15, 2022; Accepted February 16, 2023

DOI: 10.3892/etm.2023.12016

Abstract. Postoperative delirium (POD), which occurs in hospital up to 1-week post-procedure or until discharge, is a common complication, especially in older adult patients. However, the pathogenesis of POD remains unclear. Although damage to blood-brain barrier (BBB) integrity is involved in the neuropathogenesis of POD, the specific role of the BBB in POD requires further elucidation. Anaesthesia using 2% isoflurane for 4 h results in the upregulation of hippocampal receptor for advanced glycation end-products (RAGE) expression and β-amyloid accumulation in aged rats. The present study investigated the role of RAGE in BBB integrity and its mechanisms in POD-like behaviours. The buried food, open field and Y maze tests were used to evaluate neurobehavioural changes in aged mice following 2.5% sevoflurane anaesthesia administration with exploratory laparotomy. Levels of tight junction proteins were assessed by western blotting. Multiphoton *in vivo* microscopy was used to observe the ultrastructural changes in the BBB in the hippocampal CA1 region. Anaesthesia with surgery decreased the levels of tight junction proteins occludin and claudin 5, increased matrix metalloproteinases (MMPs) 2 and 9, damaged the ultrastructure of the BBB and induced POD-like behaviour. FPS-ZM1, a specific RAGE antagonist, ameliorated POD-like behaviour induced by anaesthesia and surgery in aged mice. Furthermore, FPS-ZM1 also restored decreased levels of occludin and claudin 5 as well as increased levels of MMP2 and MMP9. The present findings suggested that RAGE signalling was involved in BBB damage following anaesthesia with surgery. Thus, RAGE has potential as a novel therapeutic intervention for the prevention of POD.

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Key words: postoperative delirium, blood-brain barrier, advanced glycation end-product receptor, inhalation anaesthetic, surgery, ageing, mice

Introduction

Postoperative delirium (POD) refers to delirium that occurs within 7 days of surgery or before discharge from the hospital (1) and is characterized by acute onset, fluctuating course, inattention, disorganized thinking and altered levels of consciousness (2,3). POD is a common postoperative complication frequently observed in older adult surgical patients, with incidence of 5.1-52.2% (4,5). POD is associated with longer hospital stay, increased hospital and societal costs, poorer recovery and increased morbidity and mortality by a factor of 2-20 (6,7), resulting in increased personal medical burden and a series of medical, social and economical problems (8). Furthermore, POD has the potential to lead to short-term or long-term postoperative complications, such as delayed neurocognitive recovery, postoperative cognitive dysfunction and dementia (9,10). However, to the best of our knowledge, the neuropathogenesis of POD remains unclear.

The blood-brain barrier (BBB) is involved in maintaining the microenvironment homeostasis of the central nervous system by blocking toxic substances from circulation (11).

BBB disorders are involved in multiple neurological diseases, such as Alzheimer's disease (AD) (12,13), cerebral ischemia, stroke (14,15) and traumatic brain injury (16,17). Several studies have shown that volatile anaesthetic, such as isoflurane and sevoflurane, exposure alone or with surgery, disrupts the ultrastructural and functional integrity of the hippocampal BBB, which leads to postoperative cognitive impairment (18-21). A recent clinical study found that perioperative increases in cerebrospinal fluid/plasma albumin ratio and plasma S100β are associated with delirium severity, indicating that POD is associated with a breakdown of the BBB (22). These findings suggest that the BBB may be a novel therapeutic target for treatment of POD.

BBB disruption is linked to proinflammatory cytokine transport and β-amyloid (Aβ) deposition (23,24), which serve a critical role in the progression of postoperative cognitive impairment (25). Therefore, it is key to search for specific and effective targets against BBB disruption for the treatment of POD. The receptor for advanced glycation end-products (RAGE), a member of the immunoglobulin superfamily, mediates Aβ-peptide transport across the BBB and accumulation in the brain (26,27). Specifically, RAGEs are enriched at the lumina of microvascular endothelial cells and mediate circulating Aβ influx across the BBB, leading to Aβ deposition in brain parenchyma. RAGE activation is also involved in BBB damage in a variety of human brain disorders, including AD, Parkinson's disease (PD) and amyotrophic lateral sclerosis (13,28). However, to the best of our knowledge, the role of RAGE activation in BBB disruption under anaesthesia and surgery remains unclear. Our previous study showed that RAGE activation is involved in BBB damage following 4 h isoflurane exposure and induces spatial learning and memory impairment in aged rats (29). However, the effect of RAGE on the BBB under anaesthesia with surgery remains unclear.

Thus, the present study investigated if RAGE-mediated BBB modulation participates in anaesthesia with surgery-induced delirium-like behaviour in aged mice. In addition, it also examined the neuroprotective role of the RAGE-specific inhibitor FPS-ZM1 on surgery-induced destruction of the BBB and delirium-like behaviour.

Materials and methods

Study design. The present study was a prospective, randomized, controlled animal study, which started in January 2020 and ended in January 2021. The duration of the animal experiment was 5 months. The present study was approved by the Peking University Biomedical Ethics Committee Experimental Animal Ethics Branch (approval no. LA2019318) and experiments on animals were performed at the Peking University Health Science Center (Beijing, China).

Experimental animals. A total of 40 aged male C57BL/6J mice (age, 20 months; body weight, 30-40 g) were purchased from Dongchuang Laboratory Animal Center (Changsha, Hunan, China) and housed (n=4/cage) under standard environmental conditions (25˚C, 60% humidity and 12/12-h light/dark cycle). The animals were given 2 weeks to adapt to the environment before the experiments and food and water were freely accessible. Normal heart rate, core body temperature, locomotor activity, body weight and food and water consumption were recorded for each animal. Every effort was made to minimize the number of animals used and their discomfort. Unrelievable severe pain and inability to move freely or eat on their own were humane endpoints used to determine when the animals should be sacrificed to minimize suffering. There was no death in each group before decapitation.

Anaesthesia and surgery. The anaesthesia and surgical procedures were based on previous reports (30,31). Each animal was initially anaesthetized with 2.5% sevoflurane (in 100% oxygen) in a transparent chamber (RWD Life Science Co., Ltd.) for 5 min and a face mask equipped with a 16-gauge sensor monitor (S/5 Compact Anesthesia Monitor, GE health care, United States) was used to detect the sevoflurane concentration and maintain anaesthesia. The mice were then placed on a heating pad to maintain body temperature at 37˚C. A longitudinal midline incision was made from the xiphoid to the pubic symphysis, cutting through the skin, abdominal muscle and peritoneum. A segment of the intestine ~1.0 cm long was exteriorized and vigorously rubbed between the surgeon's thumb and index finger for 30 sec. The intestine was placed back into the peritoneal cavity. Subsequently, the incision was sutured layer by layer using 5-0 suture sterile silk sutures and cleaned with iodophor (LIRCON, Shandong, China) three times followed by 0.25% bupivacaine infiltration (Aladdin, Shanghai, China). The mice recovered on a thermal blanket in a chamber containing 100% oxygen. The anaesthesia and surgery lasted 25 min. The mucus colour was monitored every 5 min until the mice could maintain an upright posture and walk normally. To relieve pain and stress from the surgery, 1 mg (0.33 ml) oxybuprocaine hydrochloride gel (Shenyang Oasis Pharmaceutical Manufacturing Co., Ltd., China) was aseptically applied to the wound every 8 h during the first 24 h after the operation. The normal heart rate, core body temperature, locomotor activity, body weight, and food and water consumption were recorded for each animal every 8 h during the first 24 h after the operation. The sham operation group was treated in the same way as the surgery group, except no exploratory laparotomy was performed.

Group assignment. The mice were divided into the following groups using computer-based randomization (n=10/group): Vehicle + sham operation (SHAM); sham operation + FPS-ZM1 (SHAM + FPS-ZM1); vehicle + anaesthesia + surgery (A + S) and the anaesthesia + surgery + FPS-ZM1 (A + S + FPS-ZM1). The SHAM group received 0.1 ml FPS-ZM1 vehicle intraperitoneal (i.p.) injection [0.1% dimethyl sulfoxide (DMSO)] once daily for 7 consecutive days; on the seventh day, the vehicle was i.p. injected 1 h before the sham operation. For the SHAM + FPS-ZM1 group, 1.0 mg/kg FPS-ZM1 (purity, 99.56%; RennoTech Co., Ltd.) was i.p. injected (32) once daily for 7 consecutive days. On the seventh day, FPS-ZM1 was i.p. injected (1.0 mg/kg) 1 h before the sham operation. The A + S group received 0.1 ml i.p. injection of vehicle (0.1% DMSO) once daily for 7 consecutive days; on the seventh day the vehicle was i.p. injected 1 h before sevoflurane anaesthesia and exploratory laparotomy. In the A + S + FPS-ZM1 group, 1.0 mg/kg FPS-ZM1 was i.p. injected once daily for

7 consecutive days. On the seventh day, FPS-ZM1 was i.p. injected (1.0 mg/kg) 1 h before anaesthesia and surgery.

Behavioural tests. The buried food test (BFT), open field test (OFT) and Y maze test (YMT) were performed to measure impairments in neurobehavioural performance. All animals underwent neurobehavioural tests 24 h before and after the surgery or sham operation (Fig. 1). The protocols of these tests were based on previous studies with slight modifications (3,33,34). All mice were moved to the behavioural testing room 1 h before the start of the tests. A total of four mice from each group were tested and all tests were completed within 1 h to minimize the impact of circadian rhythm. To avoid odours that could influence the results, all test equipment was cleaned with 70% ethanol after every trial. All behavioural data were analysed with an animal tracking system (Smart 3.0; RWD Life Science Co., Ltd.). The interval between each neurobehavioural test was 2 h. All behaviours were evaluated by experienced technicians who were blinded to the treatments.

BFT. Each mouse was given two pieces of sweetened cereal every day for 2 days before the test. An opaque plastic test box (50x30x20 cm) with clean bedding (3-cm thick) was used as the test device. A sweetened cereal pellet was buried 0.5 cm under the bedding so that it was not visible and the location of the cereal pellet was changed to a random location for each trial to avoid the potential confounding impact of memory. The mouse was placed in the centre of the test box and the time until the time the mouse uncovered the food pellet and grasped it in the forepaws and/or teeth was defined as the latency to eat the food. Each mouse was allowed to find the pellet for 5 min and then return to the home cage. If the mouse did not find the food pellet within 5 min, the latency time was recorded as 300 sec. The bedding was emptied and the test box was cleaned with a 70% ethanol solution to prevent olfactory transmission. The experimenter also changed gloves after each test.

OFT. A 40x40x40 cm grey polyvinylidene open-field chamber was used for the open-field test. The mouse was placed in the centre of the chamber under dim light and was allowed to move freely for 5 min. The total travelled distance (m) and the time spent in the centre of the open field (sec) were recorded and analysed.

YMT. The Y maze apparatus, made of grey polyvinylidene, consisted of three identical arms (30x8x15 cm) separated by 120˚. The three arms were as follows: Start arm, in which the mouse began to explore (always open); novel arm, which was blocked during the first trial but open during the second trial and the other arm (always open). The apparatus was placed in a quiet and illuminated room. In the first trial, the mouse was allowed to explore the start and the other arm for 10 min (the novel arm was blocked) and then returned to its home cage. In the second trial, which was performed 2 h after the first trial, the mouse was returned to the start arm with free access to all three arms for 5 min. The duration and entries into the novel arm were monitored and analysed using a video camera installed 60 cm above the apparatus, which was linked to the aforementioned animal tracking system software.

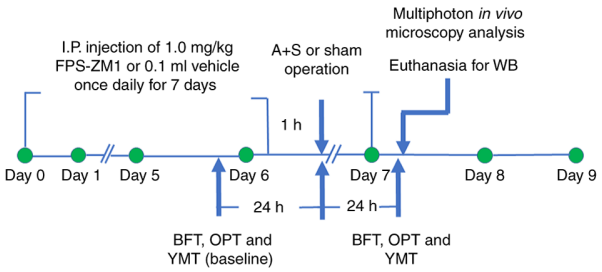

Figure 1. Experimental schematic diagram. Mice received 1.0 mg/kg FPS-ZM1 or 0.1 ml vehicle (0.1% DMSO) i.p. injected once daily for 7 consecutive days. On the seventh day, vehicle was i.p. injected 1 h before anaesthesia and surgery or sham operation. Baseline neurobehavioural measurements of mice were obtained 24 h before and after the surgery or sham operation to evaluate the delirium-like behaviour. Rodents in each group were euthanized and the hippocampus was extracted immediately after neurobehavioural tests. Western blotting was performed. *In vivo* multiphoton laser confocal fluorescence microscopy analysis was performed 24 h post-S. A, anaesthesia; S, surgery; BFT, buried food test; OPT, open field test; YMT, Y maze test; WB, western blotting; i.p., intraperitoneal.

Western blotting. The animals in each group (n=6) were euthanized by decapitation. Respiratory and cardiac arrest, loss of nerve reflex and muscle relaxation were used to confirm death. The hippocampus was extracted immediately after completion of the neurobehavioural tests (Fig. 1). In brief, hippocampus tissue was homogenized in cold RIPA Assay buffer (Applygen Technologies, Inc.) and the quantity of protein in the supernatant was determined using BCA protein assay kit (Applygen Technologies. Inc.). Protein samples (40 μg/lane) were separated by 8 or 10% SDS-PAGE and used the maker (WB1902S, Biotides) as loading control. After electrophoresis, the proteins were transferred onto a polyvinylidene fluoride membrane. The membrane was blocked with 10% skimmed milk in Tris-buffered saline (TBS) at room temperature for 30 min and incubated with primary antibodies (18-21,29). Membranes were incubated overnight with the following primary antibodies at 4˚C (all Abcam; diluted with 1% skimmed milk in TBS): Anti-RAGE (ab216329, 1:1,000); anti-MMP-2 (cat. no. ab92536, 1:1,000); anti-MMP-9 (ab283575, 1:1,000); anti-occludin (ab216327, 1:1,000) anti-claudin 5 (ab131259, 1:1,000) and used β-actin as the reference antibody (Santa Cruz Biotechnology, Inc.; cat. no. sc-47778, 1:1,000). The membranes were incubated with horseradish enzyme-labelled goat anti-rabbit IgG or anti-mouse IgG (cat. nos. ZSGB-BIO, ZB-2301, ZB-2305, 1:10,000) at 37˚C for 1 h to detect primary antibody binding. Immunoreactivity was visualized by pro-light HRP chemiluminescent kit (TIANGEN, PA112) and scanned the membrane using C-DiGit® (LI-COR Biosciences). For densitometry analysis, the results were normalized to the mean values of the corresponding sham animals.

Multiphoton in vivo microscopy analysis. *In vivo* multiphoton imaging was performed 24 h postoperatively as described previously (35-38). Following i.p. injection of pentobarbital sodium (40 mg/kg), the craniums of the mice were firmly fixed onto a stereotaxic frame. A square cranial window was opened using a high-speed drill. Tetramethyl rhodamine (TMR)-conjugated dextran [40 Kda; 0.1 ml (10 mg/ml); Invitrogen; Thermo Fisher Scientific, Inc.] was injected via

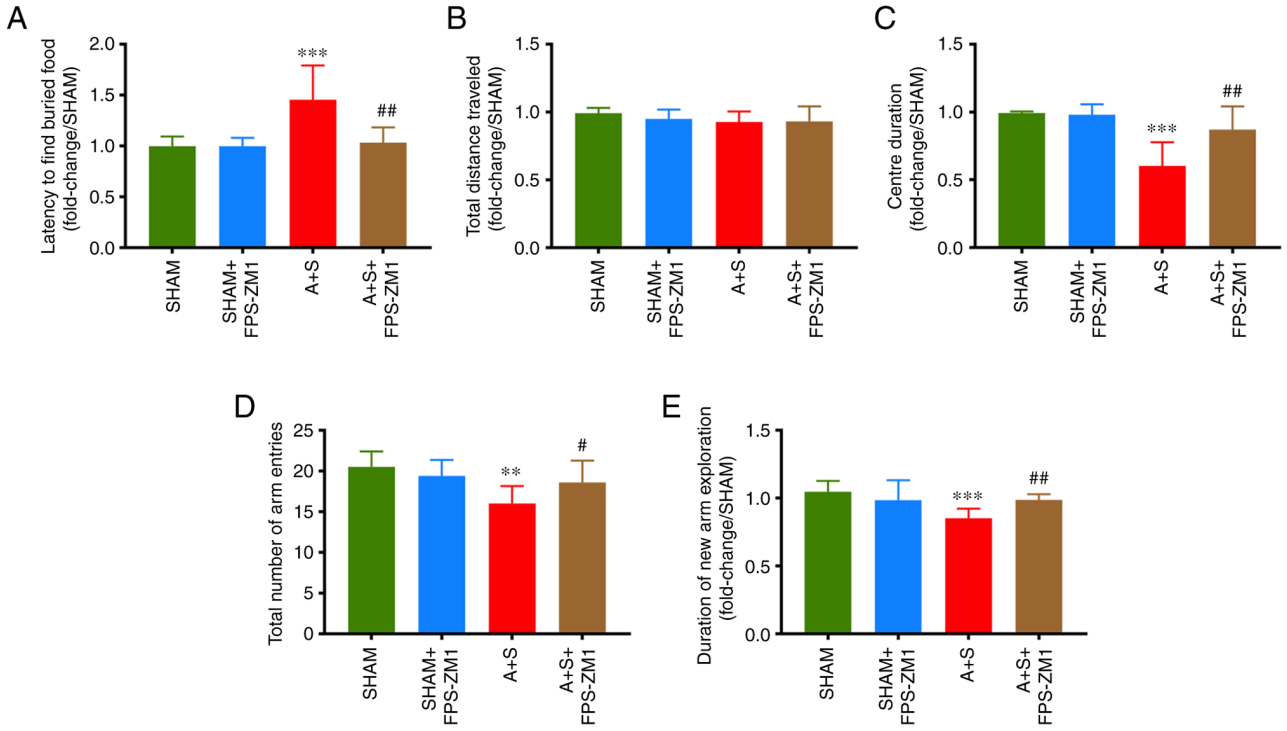

Figure 2. Receptor for advanced glycation end-products-specific antagonist FPS-ZM1 administration alleviates POD-like behaviour in aged mice. (A) Buried food test. A + S increased the latency to eat the food of aged mice 24 h after surgery, whereas FPS-ZM1 administration decreased the latency. Open field test. A + S did not affect the (B) total travelled distance but decreased the (C) time spent in the centre. The shortened centre duration was also prolonged by FPS-ZM1 pre-administration. Y maze test. A + S decreased (D) number and (E) duration of novel arm visits in mice with POD-like behaviour, whereas FPS-ZM1 increased these. - (n=10/group). **P<0.01 and ***P<0.001 vs. SHAM. $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. A + S. A, anaesthesia; S, surgery; POD, postoperative delirium.

the tail vein. *In vivo* images were acquired using a two-photon microscope (Leica TCS SP5 MP; Leica Microsystems, Inc.) at 850 nm wavelength excitation, 20.0/1.0 water immersion objective and a 2-mm working distance. Once the area of interest was defined, 200 μm hick stacks in the z-axis (5-μm steps) were obtained using TCS-SP8 DIVE (Leica Microsystems, Inc.). Specifically, all images were subjected to threshold processing and extravascular fluorescent intensity was measured using the integrated density measurement function. The *in vivo* BBB permeability for TMR-dextran was estimated as the permeability surface-area product (PS) using the following formula: $PS=(1-H_{ct})\ 1/I_v \times V \times dI_t/dt$, where H_{ct} is the haematocrit (45%), I_v is the initial fluorescence intensity of the region of interest (ROI) within the vessel, I_t is the intensity of the ROI in the brain at time t and V is the vessel volume assuming 1 g brain is equivalent to 50 cm^2. The fluorescence intensity was normalised to the SHAM group. The relative fluorescence intensity across the cross-section of vessels was analysed with Image J1 software. (National Institutes of Health).

Data analysis. Statistical analysis of experimental data and images was performed using SPSS 26.0 for Windows (IBM Corp.) and GraphPad Prism 8.0 (GraphPad Software, Inc.; Dotmatics). Data from neurobehavioural tests (n=10), biochemistry (n=6) and multiphoton *in vivo* microscopy results (n=4) are expressed as the mean ± standard error of the mean. Data were analysed using one-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

RAGE-specific antagonist administration alleviates anaesthesia/surgery-induced POD-like behaviour in aged mice. The BFT was used to assess natural behaviours of mice; latency to eat food reflected attention, organized thinking and level of consciousness of mice (39). A + S group significantly increased the latency to eat food 24 h after surgery compared with the SHAM group (Fig. 2A), which suggested that surgery/anaesthesia impaired the ability to find and eat food. However, the prolonged latency to eat food was significantly shortened by FPS-ZM1 pretreatment (Fig. 2A).

OFT was used to evaluate autonomous behaviours and the exploratory ability of mice in a novel environment. The total travelled distance was not affected by anaesthesia/surgery (Fig. 2B), which suggested that anaesthesia/surgery did not cause motor dysfunction. However, anaesthesia/surgery decreased the time spent in the center 24 h after surgery (Fig. 2C), and less time spent in the centre represented more anxiety. Moreover, 1.0 mg/kg FPS-ZM1 treatment reversed the decrease in time spent in the centre caused by anaesthesia and surgery (Fig. 2C) but had no impact on the motor function of mice (Fig. 2B). The OFT data indicated that RAGE-specific antagonist administration prevented neurobehavioural disorder following anaesthesia/surgery in aged mice.

YMT was used to evaluate spatial recognition memory, which requires attention and organized thinking (40). Anaesthesia/surgery significantly decreased entries and time spent in the novel arm at 24 h post-surgery compared with the

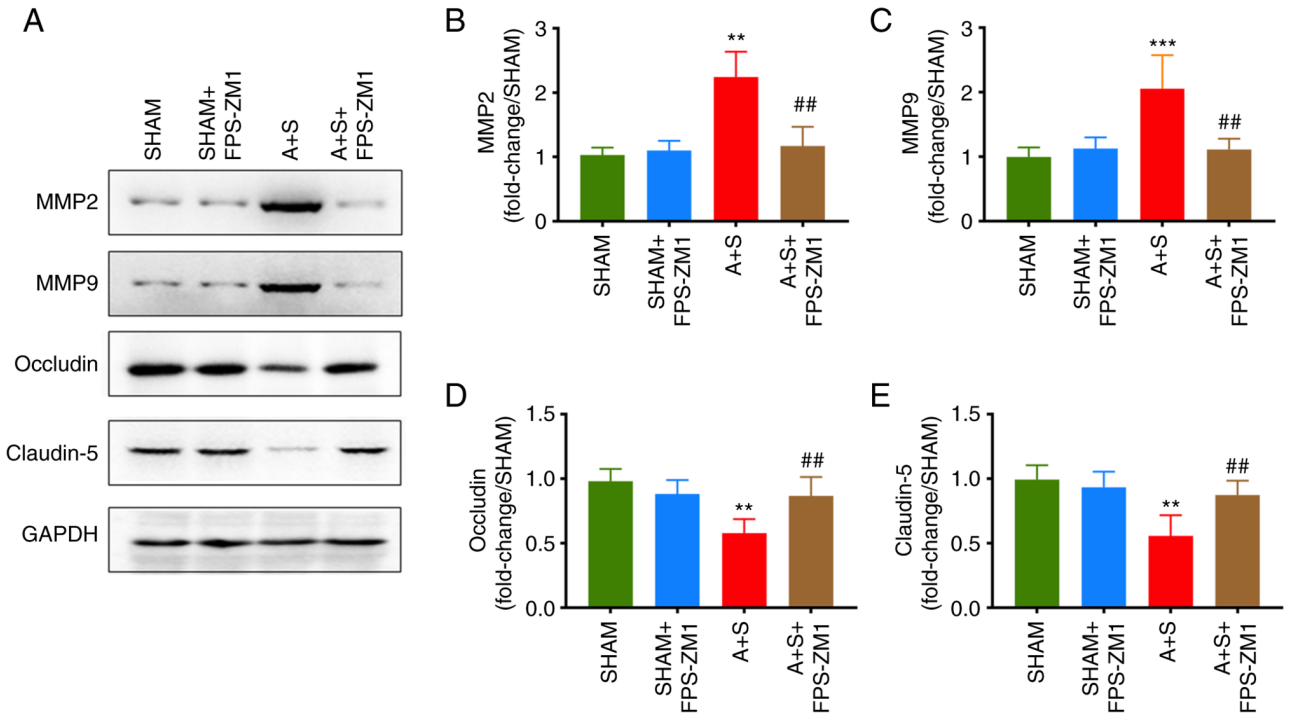

Figure 3. Pre-administration of FPS-ZM1 enhances hippocampal BBB integrity. (A) Representative immunoblots illustrating MMP2, MMP9, occludin and claudin 5 protein expression levels in the hippocampus. FPS-ZM1 significantly reduced the increase in (B) MMP2 and (C) MMP9 expression induced by A + S. FPS-ZM1 increased the reduction in hippocampal (D) occludin and (E) claudin 5 expression induced by A + S. Data are expressed as mean ± standard error of the mean (one-way ANOVA followed by Bonferroni's correction post hoc test; n=6 per group). **P<0.01 and ***P<0.001 vs. SHAM. $^{\#\#}$P<0.01 vs. A + S. SHAM, vehicle + sham operation; A, anaesthesia; S, surgery.

SHAM group (Fig. 2D and E). These results demonstrated that anaesthesia/surgery impaired spatial working and reference memory in aged mice. Moreover, FPS-ZM1 pretreatment also significantly increased entries and duration spent in the novel arm in the A + S + FPS-ZM1 group compared with the A + S group (Fig. 2D and E).

RAGE-specific antagonist pretreatment improves BBB damage following anaesthesia and surgery. MMPs, especially MMP2 and MMP9, are involved in maintaining BBB integrity (41,42). MMP2 and MMP9 expression significantly increased following anaesthesia and surgery, both of which were decreased by FPS-ZM1 pretreatment in the hippocampus (Fig. 3A-C). Occludin and claudin 5 are important tight-junction proteins for maintaining BBB integrity (43-45). Semi-quantitative analysis by western blotting showed that anaesthesia and surgery induced decreases in occludin and claudin 5 (Fig. 3A, D and E). This decrease was significantly inhibited by FPS-ZM1 pretreatment (Fig. 3A, D and E). Additionally, no significant difference in MMP2, MMP9, occludin or claudin 5 expression levels was measured between the SHAM and SHAM + FPS-ZM1 groups.

Pre-administration of FPS-ZM1 inhibits upregulation of hippocampal RAGE levels induced by anaesthesia and surgery. To determine the anaesthesia and surgery-mediated RAGE upregulation and the inhibitory effects of FPS-ZM1, RAGE protein expression was evaluated immediately after neurobehavioural tests. The data indicated that RAGE

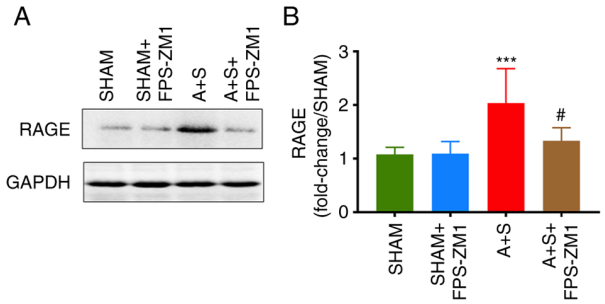

Figure 4. Effect of FPS-ZM1 on RAGE protein levels in the hippocampus. (A) Representative western blot bands. (B) Ratios of the target protein to the internal standard β-actin (n=6/group). ***P<0.001 vs. SHAM. $^{\#}$P<0.05 vs. A + S. SHAM, vehicle + sham operation; FPS-ZM1, A, anaesthesia; S, surgery; RAGE, receptor for advanced glycation end-products.

expression was significantly increased in the A + S group compared with SHAM group and decreased significantly by the RAGE-specific antagonist (Fig. 4A and B).

FPS-ZM1 pretreatment ameliorates brain microvascular leakage in aged mice with delirium-like behaviour. BBB disruption and the effect of FPS-ZM1 were evaluated by TMR-dextran leakage using live two-photon microscopy. Results revealed intact micro-vessel networks in the SHAM group (Fig. 5A). The microvessels (arrows) in the A + S group (Fig. 5A and B) were significantly leakier than those in the SHAM group. Administration of FPS-ZM1 significantly attenuated anaesthesia and surgery-induced microvascular leakage (Fig. 5B) and the relative fluorescence intensity also

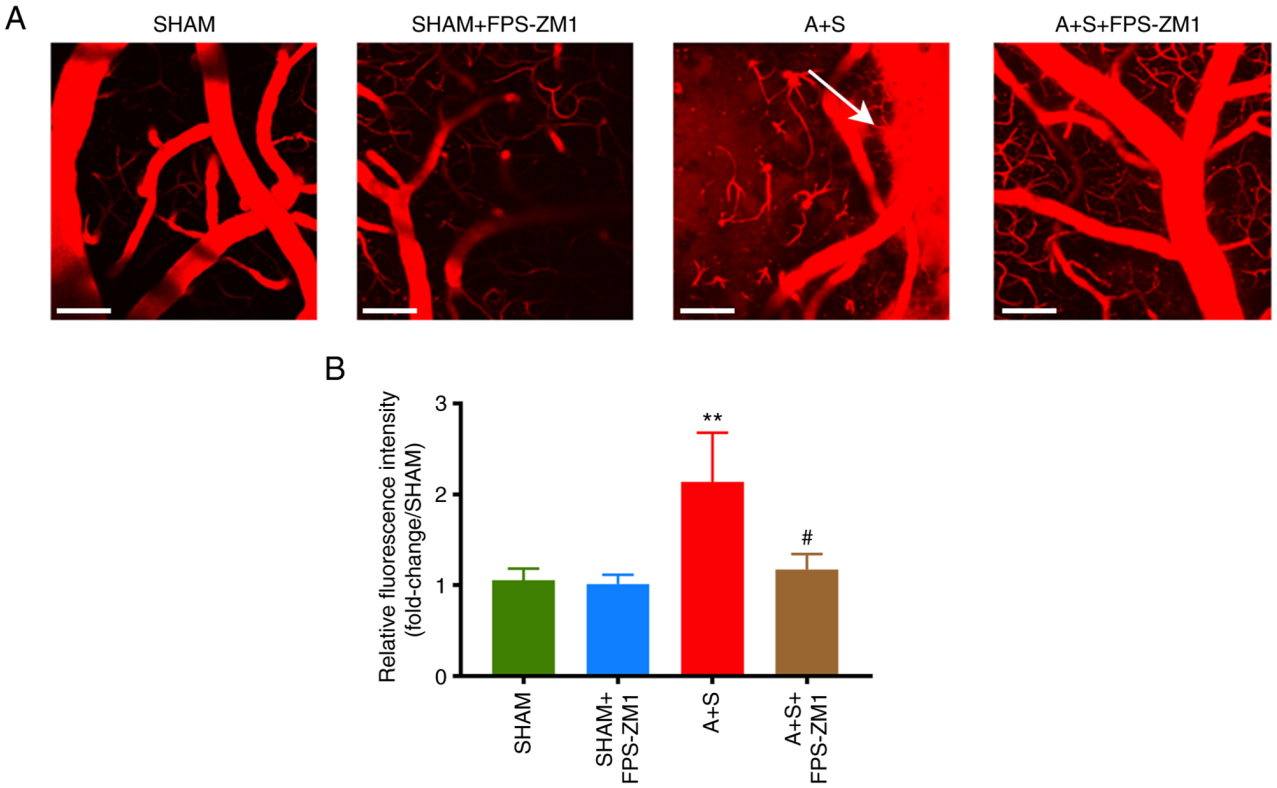

Figure 5. Blood-brain barrier leakage induced by anaesthesia and surgery is attenuated by FPS-ZM1. (A) At 24 h post operation, live optical multiphoton laser confocal microscopy of tetramethyl rhodamine-dextran (red) was used to observe brain microvascular integrity. The arrow indicated that microvascular with leakage showed patchy lumen and extravasation of red tracer. (B) Quantification of relative fluorescence intensity across the cross-section of vessels (n=4/group). Scale bar, 100 μm. **P<0.01 vs. SHAM. $^{\#}$P<0.05 vs. A + S. SHAM, vehicle + sham operation; A, anaesthesia; S, surgery.

decreased. There was no significant difference in microvascular leakage between the SHAM and SHAM + FPS-ZM1 groups.

Discussion

The present study demonstrated that exploratory laparotomy under sevoflurane exposure induced hippocampal RAGE upregulation and BBB damage, resulting in delirium-like behaviour in aged mice (Fig. 6). Moreover, treatment with the RAGE-specific antagonist FPS-ZM1 for 7 consecutive days suppressed hippocampal RAGE expression and improved BBB damage. Furthermore, pre-administration of the RAGE-specific antagonist significantly mitigated the neurobehavioural impairment resulting from the stress of sevoflurane anaesthesia with laparotomy.

POD is a common postoperative complication characterized by acute and fluctuating disorder of attention, consciousness and cognition within 7 days of surgery or before discharge from the hospital (1). Based on previous studies (3,33,34), the present study established a POD mouse model using exploratory laparotomy under sevoflurane anaesthesia inhalation. Neurobehavioural tests (BFT, OFT and YMT) were used to evaluate postoperative behavioural changes in mice. BFT and YMT assess the capabilities of rodents with intact attention, organized thinking and OFT evaluate the ability of rodents with exploration and autonomous behaviours (3). However, anaesthesia with surgery increased the latency of mice to find and eat food, indicating that anaesthesia/surgery

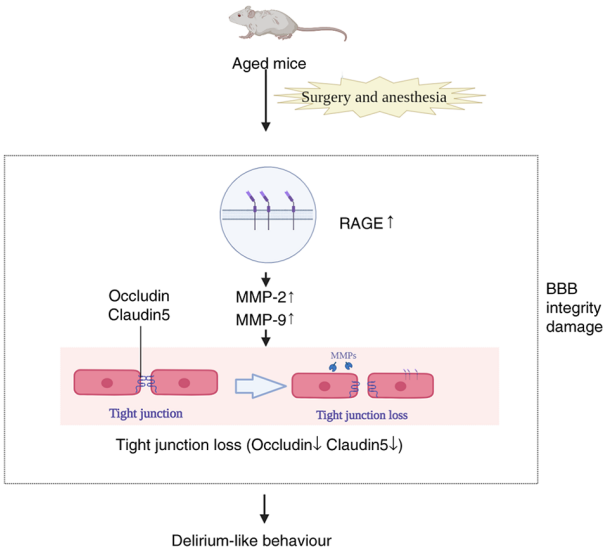

Figure 6. Schematic diagram of protective effects on postoperative delirium-like behaviour by FPS-ZM1 administration after exploratory laparotomy under sevoflurane anaesthesia in aged mice, which may be mediated by the BBB/RAGE signalling pathway. BBB, blood-brain barrier; RAGE, receptor for advanced glycation end-products.

induced delirium-like behaviour (inattention, disorganized thinking and altered levels of consciousness (46,47) in mice. OFT is typically used to study the emotional state of animals and the time spent in the centre reflects levels of anxiety (48). A previous study in mice demonstrated that anxiety decreases

the time spent in the centre (49). In the present study, anaesthesia with surgery decreased the time spent in the centre of the OFT, which suggested that anaesthesia/surgery induced anxiety, and OFT also requires attention, consciousness and organized thinking (48). Finally, surgery/anaesthesia decreased the number and duration of novel arm visits, which suggested that surgery/anaesthesia disrupted the spatial memory of mice. Collectively, these behavioural tests revealed that sevoflurane anaesthesia and exploratory laparotomy induced POD-like behaviours in aged mice.

In our previous study, 4 h 2% isoflurane exposure resulted in the upregulation of hippocampal RAGE expression, disruption of BBB integrity, neuroinflammation and Aβ accumulation in aged rats (29). In the present study, exploratory laparotomy under 2.5% sevoflurane exposure for 25 min induced hippocampal RAGE upregulation and BBB disruption, resulting in delirium-like behaviour in aged mice. Sevoflurane was used because our previous studies (30,50) found that surgery plus sevoflurane could induce postoperative cognitive impairment in aged rodents. Qu *et al* (43) found that 2.5% sevoflurane for 4 h altered expression profiles including gene expression and transcription factor activity of long non-coding RNAs in aged rats, which are associated with mitochondrial dysfunction, oxidative stress, ageing-associated metabolism alteration, DNA damage and apoptosis, as well as neurodegenerative features in the hippocampus. He *et al* (31) found that 2.5% sevoflurane + exploratory laparotomy for 25 min induces postoperative cognitive impairment. Li *et al* (30) also demonstrated that 2.5% sevoflurane with exploratory laparotomy for 25 min leads to deteriorated postoperative learning and memory. Therefore, sevoflurane was adopted in the present study.

In our previous study, anaesthesia and exploratory laparotomy impaired hippocampus-dependent spatial cognition in aged mice, whereas 2.5% sevoflurane anaesthesia for 25 min was not effective (30,31). However, 2.5% sevoflurane anaesthesia alone for 25 min + exploratory laparotomy could result in delayed neurocognitive recovery (30,31). It was hypothesised that delirium-like behaviours are more affected by surgical invasion than by anaesthesia in the present study. Moreover, previous studies have demonstrated that sevoflurane inhalation for 4 h + bilateral intrahippocampal injections of Aβ, but not normal saline, deteriorate cognition in adult rats by inducing neurotoxicity, neuroinflammation and neuronal apoptosis in the hippocampus (51-53). Nonetheless, another study demonstrated that 2.5% sevoflurane exposure alone for 5 h could induce neuroinflammation and cognitive dysfunction in aged rats (54). Therefore, preoperative states (such as low cognitive reserve), surgical stress as well as prolonged exposure to anaesthesia may promote postoperative cognitive impairment. However, short-term anaesthesia may have no effect on neurocognitive function.

Both male and female POD animal models are used. Lu *et al* (34) used 18-month-old female C57BL/6 mice that underwent a 10-min simple laparotomy under 1.4% isoflurane anaesthesia lasting 2 h; Liu *et al* (33) studied 16-month-old female C57BL/6 mice that underwent exploratory laparotomy that took ~20 min with 1.4-2.0% isoflurane anaesthesia lasting up to 2 h; Chen *et al* (3) used 3-month-old female Sprague-Dawley rats that underwent ~10 min surgery with 1.4% isoflurane anaesthesia lasting up to 2 h; Illendula *et al* (46) had 18-20-month-old male C57BL/6J mice undergo laparotomy under 3 h 3.0-3.5% sevoflurane anaesthesia, followed by 2 h sedation with propofol and 12 h intensive care unit conditions with exposure to intermittent lights, sounds and cage shaking. The present study was performed on 20-month-old male mice because a previous study reported that both older age and male sex are predisposing factors for delirium (8). Moreover, an exploratory laparotomy that lasted 25 min was performed under 2.5% sevoflurane.

A well-functioning BBB is key for maintaining brain homeostasis. BBB damage leading to efflux and influx transporter dysfunction results in influx of neurotoxic blood-derived debris, cells and microbial pathogens into the brain, which contributes to the pathogenesis of neurodegenerative disease, such as AD and PD (12,55). Given that such neurotoxic influxes are associated with inflammatory and immune responses, they may also underlie the mechanisms of postoperative impairment. Previous studies have demonstrated that inhalational anaesthesia with or without surgery can induce postoperative cognitive impairment via an increase in neuroinflammation, Aβ accumulation and BBB disruption (18,21,29,56). Of these, BBB disruption plays a key role because inflammatory responses increase BBB permeability (19), while abnormal Aβ accumulation causes neuroinflammation, which leads to further BBB dysfunction (29). Because RAGE mediates Aβ peptide transport across the BBB and accumulation in the brain (13), upregulation of RAGE expression leads to abnormal Aβ levels in the postoperative ageing brain, as demonstrated in our previous study (29). The present study found that laparotomy under sevoflurane anaesthesia induced hippocampal RAGE upregulation and BBB damage, as well as abnormal neurobehavioural performance, which may have been due to hippocampal Aβ accumulation, resulting in neuroinflammation; this needs further investigation.

The MMPs studied for BBB disruption were MMP2 and MMP9, which can degrade the extracellular matrix of the basal membrane and tight junction proteins (57,58). In the present study, both MMP2 and MMP9 expression increased significantly, whereas tight junction protein claudin 5 and occludin expression decreased significantly 24 h after anaesthesia/surgery, which indicated that anaesthesia/surgery significantly disrupted the BBB ultrastructure. FPS-ZM1 is a BBB-permeable, non-toxic, tertiary amide compound that serves as a high affinity, potent, multimodal blocker of RAGE to inhibit RAGE-mediated Aβ influx into the brain (58). A previous study reported that FPS-ZM1 (1.0 mg/kg i.p.) inhibits hippocampal NF-κB signalling, decreases neuronal apoptosis, enhances hippocampal plasticity and improves neurobehavioural performance in db/db mice (32). The focus of future research should be the specific mechanisms of the effect of the RAGE-specific inhibitor FPS-ZM1 on surgery-induced increased permeability of the BBB and POD.

The present study had limitations. It is well established that RAGE is a significant signal transduction receptor that evokes an array of signal transduction cascades and is directly involved in the development of multiple neurodegenerative diseases, such as AD, PD and amyotrophic lateral sclerosis (32,59,60). Specifically, RAGE participates in inflammation, immunity, apoptosis and cellular senescence in response to Aβ binding (32,61-63). However, the present study

did not determine by which specific cell type and RAGE signalling pathway the structure of the BBB is affected by anaesthesia and surgery. Second, 1.0 mg/kg FPS-ZM1 i.p. once daily for 7 days before surgery was adopted without determining the optimal drug dosage or time window. Third, since delirium is characterized by an acute onset and a transient fluctuating disturbance of consciousness, attention, cognition, and perception, the data from postoperative behaviour tests should be presented continuously. However, the present study examined the behavioural changes only once at 24 h (3,33,34). Fourth, the severity of BBB disruption, which was not elucidated by the present study, should be examined using electron microscopy to investigate structural changes and by analysing molecular tracers, such as NaF and IgG, to determine leakage of blood to the brain to evaluate the functional changes (20).

In conclusion, 1.0 mg/kg RAGE-specific inhibitor FPS-ZM1 ameliorated delirium-like cognitive deficit following anaesthesia and surgery in aged mice. These beneficial effects were likely due to restoration of the structure of the BBB. The present results suggested that the RAGE transduction pathway was involved in BBB damage after anaesthesia and surgery in the ageing brain; therefore, targeting RAGE signalling of the BBB may be a novel option for the prevention and treatment of POD.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant nos. 81801070 and 81901095) and Shanghai Pudong New District Science and Technology Development Fund Medical and Health Project of China (grant no. PKJ2018-Y56).

Availability of data and materials

All data generated and analysed during this study are included in this published article.

Authors' contributions

CMS, NY, XYG and DYH conceived and designed the study. YD and JSH wrote and edited the manuscript. YYC edited the manuscript. YD, JSH, NK, YTL, LC and RZ performed the experiments. DYH constructed the figures and revised the manuscript. ZQL and YYC conducted the statistical analyses. XYG and NY confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Peking University Biomedical Ethics Committee Experimental Animal Ethics Branch (approval no. LA2019318) and experiments on animals were performed at the Peking University Health Science Center (Beijing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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