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Isoflurane on brain inflammation ☆

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ABSTRACT

Brain inflammation may play an important role in the pathophysiology of early brain injury after subarachnoid hemorrhage (SAH). Our aim was to demonstrate brain inflammation development and to determine whether isoflurane, a clinically available volatile anesthetic agent, prevents brain inflammation after SAH. This study used 162 8-week-old male CD-1 mice. We induced SAH with endovascular perforation in mice and randomly assigned animals to sham-operated (n = 21), SAH + vehicle-air (n = 35) and SAH + 2% isoflurane (n = 31). In addition to the evaluation of brain injury (neurological scores, brain edema and Evans blue dye extravasation), brain inflammation was evaluated by means of expression changes in markers of inflammatory cells (ionized calcium binding adaptor molecule-1, myeloperoxidase), cytokines (tumor necrosis factor [TNF]-α, interleukin-1β), adhesion molecules (intercellular adhesion molecule [ICAM]-1, P-selectin), inducers of inflammation (cyclooxygenase-2, phosphorylated c-Jun N-terminal kinase [p-JNK]) and endothelial cell activation (von Willebrand factor) at 24 h post-SAH. Sphingosine kinase inhibitor (N, N-dimethylsphingosine [DMS]) and sphingosine-1-phosphate receptor-1/3 antagonist (VPC23019) were used to block isoflurane's effects (n = 22, each). SAH caused early brain injury, which was associated with inflammation so that all evaluated markers of inflammation were increased. Isoflurane significantly inhibited both brain injury ($P < 0.001$, respectively) and inflammation (myeloperoxidase, $P = 0.022$; interleukin-1β, $P = 0.002$; TNF-α, $P = 0.015$; P-selectin, $P = 0.010$; ICAM-1, $P = 0.016$; p-JNK, $P < 0.001$; cyclooxygenase-2, $P = 0.003$, respectively). This beneficial effect of isoflurane was abolished with DMS and VPC23019. Isoflurane may suppress post-SAH brain inflammation possibly via the sphingosine-related pathway.

Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is a life-threatening disease carrying the risk of sudden death of 12.4% before receiving medical intervention (Huang and van Gelder, 2002) while a majority of deaths occur within the first 48 h post ictus owing to the impact of the initial bleeding (Broderick et al., 1994). Subarachnoid blood, elevation of intracranial pressure, and reduced cerebral perfusion initiate an acute injury cascade such as microvascular disturbance and inflammatory reaction, leading to early brain injury (EBI), one of the important causes of unfavorable outcomes after SAH (Fujii et al., 2013; Sehba and Bederson, 2006).

Isoflurane is a volatile anesthetic and a lipophilic molecule (Antkowiak, 2001). We reported that 2% isoflurane prevented post-SAH neuronal apoptosis and blood–brain barrier (BBB) disruption through sphingosine-related pathway activation (Altay et al., 2012a, b). Preconditioning with isoflurane was also reported to reduce lipopolysaccharide-induced inflammation in vivo (Plachinta et al., 2003). However, it remains undetermined whether isoflurane post-treatment prevents brain inflammation in an acute stage of SAH. In this study, thus, we examined effects of post-treatment isoflurane on brain inflammatory markers after SAH in mice and whether the treatment mechanism involved the sphingosine-related pathway, which was verified by using a sphingosine kinase (SphK) inhibitor and a sphingosine-1-phosphate (S1P) receptor-1/3 (S1P1/3) antagonist (VPC23019).

Abbreviations: SAH, Subarachnoid hemorrhage; EBI, Early brain injury; SphK, Sphingosine kinase; S1P, Sphingosine 1-phosphate; S1P1/3, Sphingosine-1-phosphate receptor-1/3; BWC, Brain water content; iba-1, Ionized calcium binding adaptor molecule-1; MPO, Myeloperoxidase; TNF-α, Tumor necrosis factor-alpha; IL-1β, Interleukin-1beta; ICAM-1, Intercellular adhesion molecule-1; COX-2, Cyclooxygenase-2; p-JNK, Phosphorylated c-Jun N-terminal kinase; vWF, von Willebrand factor; DMS, N, N-dimethylsphingosine.

☆ The authors report no conflicts of interest.

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Material and methods

Experimental design and animal groups

The animal and ethics review committee at Loma Linda University approved all protocols. One hundred sixty two 8-week-old male CD-1 mice (30–38 g; Charles River, Wilmington, MA) were used.

To examine whether isoflurane attenuated EBI and brain inflammation after SAH, animals were randomly divided into 3 groups, and evaluated at 24 h: sham-operated + 30% O_2 + 70% medical air (O_2-medical air; sham group, n = 21), SAH + O_2-medical air (vehicle group, n = 35) and SAH + 2% isoflurane (isoflurane group, n = 31).

To confirm that isoflurane had anti-inflammatory effects on brain after SAH, we used a potent and specific SphK inhibitor, N, N-dimethylsphingosine (DMS) and a S1P1- and S1P3-receptor antagonist, VPC23019, both of which were reported to block isoflurane's action on post-SAH brain. Animals were randomly divided into 4 groups, and evaluated at 24 h post-SAH: dimethyl sulfoxide (DMSO; a vehicle) + sham-operated + O_2-medical air (n = 13), DMSO + SAH + 2% isoflurane (n = 18), DMS + SAH + 2% isoflurane (n = 22), and VPC23019 + SAH + 2% isoflurane (n = 22).

Mouse SAH model

SAH endovascular monofilament model was produced as described previously (Altay et al., 2012a). Briefly, animals were anesthetized with an intraperitoneal injection of ketamine/xylazine (100/10 mg/kg). A sharpened 4–0 monofilament nylon suture was advanced through the internal carotid artery (ICA) to perforate the anterior cerebral artery. In the sham surgery, the filament was advanced 5 mm through the ICA without perforating the artery. Body temperature was kept constant (37.5 ± 0.5 °C) during the operation.

Drug administration

One hour after SAH induction, 2% isoflurane (Baxter, Deerfield, IL) was continuously administered for 1 h with O_2-medical air.

DMS (Enzo, Plymouth Meeting, PA; final concentration, 0.17 μg/0.5 μL) and VPC23019 (Avanti Polar Lipids Inc., Alabaster, Alabama; final concentration, 0.26 μg/0.5 μL) were automatically infused at a rate of 0.1 μL/min intracerebroventricularly, 60 min before the SAH induction, because there was little information if both drugs can pass the BBB. The dose for intracerebroventricular injections was determined based on our previous studies (Altay et al., 2012a,b). The vehicle groups were given the same volume (0.5 μL) of DMSO (1.1 g/mL/kg) diluted in phosphate-buffered saline (PBS). Mice were placed in a head holder (Stoelting Stereotactic Instrument, Wood Dale, IL) and a 26 s-gauge needle of a 10 μL Hamilton syringe (Microliter #701; Hamilton, Reno, NV) was inserted through a burr hole perforated on the skull into the right lateral ventricle using the following coordinates relative to bregma: 0.1 mm posterior; 0.9 mm lateral; and 3.1 mm below the horizontal plane of bregma (Hirt et al., 2004). The needle was removed 10 min after completion of the infusion, and the burr hole was quickly plugged with bone wax.

Severity of SAH

The severity of SAH was blindly evaluated using the SAH grading scale at sacrifice (Altay et al., 2012a,b). The SAH grading system was as follows: the basal cistern was divided into six segments, and each segment was allotted a grade from 0 to 3 depending on the amount of subarachnoid blood clot in the segment; grade 0, no subarachnoid blood; grade 1, minimal subarachnoid blood; grade 2, moderate blood clot with recognizable arteries; and grade 3, blood clot obliterating all arteries within the segment. The animals received a total score ranging from 0 to 18 after adding the scores from all six segments. Thirteen mice with SAH grading scores ≤7, which had no significant brain injury (Altay et al., 2012a,b) were excluded.

Mortality and neurological scores

We calculated mortality at 24 h after SAH. Neurological score was blindly evaluated at 24 h after SAH as previously described (Altay et al., 2012a,b: Supplementary Material and Methods).

Brain water content and BBB disruption

Brain water content (n = 7 per group) (Altay et al., 2012a,b) and Evans blue dye extravasation (n = 6 per group) (Manaenko et al., 2011) were measured as previously described (Supplementary Material and Methods).

Western blotting

We isolated and collected the left cerebral hemisphere (perforation side) at 24 h after SAH (n = 5 per group in the first study; n = 6 per group in the second study). Western blotting was performed as previously described (Altay et al., 2012a,b) using the following primary antibodies: anti-phospho-c-Jun N-terminal kinase (JNK), anti-myeloperoxidase (MPO), anti-P-selectin, anti-interleukin (IL)-1β, anti-tumor necrosis factor (TNF)-α (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), anti-intercellular adhesion molecule (ICAM)-1 (1:1000, Millipore, Temecula, CA) and anti-cyclooxygenase (COX)-2 (1:1000, Abcam, Cambridge, MA) antibodies.

Immunofluorescence

Animals were euthanized 24 h after surgery and brains were processed (n = 3 per group) as previously described (Altay et al., 2012a). Ten-micron-thick coronal sections at the level of bregma 1 mm (caudally) were cut on a cryostat (LM3050S; Leica Microsystems, Bannockburn, Ill). Double-fluorescence labeling was performed using the following primary antibodies: anti-von Willebrand factor (vWF; 1:400, Abcam, Cambridge, MA) and anti-P-selectin (1:200, Santa Cruz Biotechnology, Santa Cruz, CA) antibodies. Besides, anti-ionized calcium binding adaptor molecule (iba)-1 antibody (1:50, Abcam, Cambridge, MA) was applied and stained alone.

Statistics

Neurological scores were expressed as median ± 25th to 75th percentiles and other data were expressed as mean ± SD. After confirming that each population being compared followed a normal distribution using Barlett's tests, neurological scores were analyzed using Kruskal–Wallis test, followed by Tukey's multiple comparisons. Other statistical differences were analyzed using unpaired *t* tests and one-way analysis of variance (ANOVA) with Tukey–Kramer post hoc tests. Differences in mortality were tested using Fisher's exact tests or chi-square tests as appropriate. $P < 0.05$ was considered statistically significant.

Results

Isoflurane prevents post-SAH brain injury and inflammation

Mortality rate was not significantly different between the SAH groups (vehicle, 32.3% [10 of 31 mice]; and isoflurane, 22.2% [6 of 27]). No sham-operated mice died. SAH grade was equivalent between the groups ($P = 0.657$). SAH significantly aggravated neurological scores, brain edema and Evans blue dye extravasation in the left cerebral hemisphere compared with the sham group ($P < 0.05$, respectively), which were all significantly ameliorated by the isoflurane