Supplemental Digital Content 1

Supplemental Materials and Methods

Dose Determination and Preparation of Drugs

For pharmacological interventions, the following two inhibitors were used: 1) a potent and specific SphK inhibitor DMS (Enzo Life Sciences Inc., Plymouth Meeting, PA) dissolved in dimethyl sulfoxide (DMSO; final concentration, 0.17µg/0.5µL); and 2) a S1P1- and S1P3- receptor antagonist VPC23019 (Avanti Polar Lipids Inc., Alabaster, Alabama) dissolved in DMSO/1N hydrogen chloride (95:5; final concentration, 0.26µg/0.5µL). These drugs were further diluted in phosphate-buffered saline (PBS) to a total volume of 0.5µL, and administered intracerebroventricularly 1 hour before surgery. The vehicle groups were given the same volume (0.5µL) of DMSO (1.1g/mL/kg) diluted in PBS.

The dose for intracerebroventricular injections was calculated by determining the conversion coefficient by taking the ratio between blood volume (84.7±1.2mL/kg; 2.3mL per mouse) (1) and cerebrospinal fluid (CSF) volume (0.04mL per mouse) (2). The dose for systemic administration (DMS, 0.33mg/kg=0.01mg/mouse; VPC23019, 0.5mg/kg=0.015 mg/mouse) that was used in previous studies (3, 4) was divided by the conversion coefficient (0.01mg/57.5=0.17µg for DMS; 0.015mg/57.5=0.26µg for VPC23019) to achieve an equivalent CSF concentration of the inhibitors.

Severity of SAH

The severity of SAH was blindly evaluated using the SAH grading scale with high-resolution photographs at the time of sacrifice (5). 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.

Neurological Scores

The neurological score was blindly evaluated at 24 and 72 hours after SAH, based on the scoring system of Garcia et al. (6) with modifications (Table 1). The evaluation consists of six tests that can be scored 0 to 3 or 1 to 3. These six tests include: spontaneous activity; symmetry in the movement of all four limbs; forelimbs outstretching; climbing; side stroking; and response to vibrissae (whisker stimulation). Animals were given a score of 3 to 18 in 1-number steps (higher scores indicate greater function).

Table 1. Modified Garcia's score

	0	1	2	3
Spontaneous activity (position changes in a cage for 5min)	No movement	Barely move	Touch 1-2 sides of cage	Touch 3-4 sides of cage
Spontaneous movement of all four limbs	No movement	Limited movement of limbs	Move fully but slowly	Move the same as pre-SAH
Forepaw outstretching	No outstretching	Slight outstretching	Limited outstretching	Outstretch the same as pre-SAH
Climbing (1min)	…….	Failed climbing	Weak climbing	Normal climbing
Body proprioception	…….	No response	Weak response	Normal response
Response to vibrissae touch	…….	No response	Weak response	Normal response

References

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