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Effects of Isoflurane on Brain Inflammation After Subarachnoid Hemorrhage in Mice

Supplementary Material and Methods

Neurological Scores

Neurological score was blindly evaluated at 24 hours after subarachnoid hemorrhage (SAH) as previously described (Altay et al., 2012a, 2012b). 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).

Brain Water Content (BWC)

Brains were quickly removed and separated into the left and right cerebral hemispheres, cerebellum, and brain stem, and weighed (wet weight) at 24 hours (n=7 per group) after surgery. Next, brain specimens were dried in an oven at 105°C for 72 hours and weighed again (dry weight). BWC was calculated as ([wet weight-dry weight]/wet weight) X100% (Altay et al., 2012a, 2012b).

BBB Disruption

At 24 hours after operation, we injected a 2% solution of Evans blue dye (4mL/kg of body weight) intraperitoneally and allowed to circulate for 3 hours. Under deep anesthesia, mice

(n=6 per group) were sacrificed by intracardial perfusion with phosphate-buffered saline (PBS), and brains were removed and divided into the same regions as the BWC study. Brain specimens were weighed, homogenized in PBS, and centrifuged at 15,000*g* for 30 minutes. Then, 0.5mL of the resultant supernatant was added to an equal volume of 50% trichloroacetic acid. After overnight incubation and centrifugation at 15,000*g* for 30 minutes at 4°C, the supernatant was taken for spectrophotometric quantification of extravasated Evans blue dye at 610 nm as described previously (Manaenko et al., 2011).

Supplementary References

Altay, O., Hasegawa, Y., Sherchan, P., Suzuki, H., Khatibi, N.H., Tang, J., et al., 2012a. Isoflurane delays the development of early brain injury after subarachnoid hemorrhage through sphingosine-related pathway activation in mice. Crit. Care Med. 40, 1908-1913.

Altay, O., Suzuki, H., Hasegawa, Y., Caner, B., Krafft, P.R., Fujii, M., et al., 2012b. Isoflurane attenuates blood-brain barrier disruption in ipsilateral hemisphere after subarachnoid hemorrhage in mice. Stroke 43, 2513-2516.

Manaenko, A., Chen, H., Kammer, J., Zhang, J.H., Tang, J., 2011. Comparison Evans Blue injection routes: intravenous versus intraperitoneal, for measurement of blood-brain barrier in a mice hemorrhage model. J. Neurosci. Methods 195, 206-210.

Supplementary Figures and Figure Captions

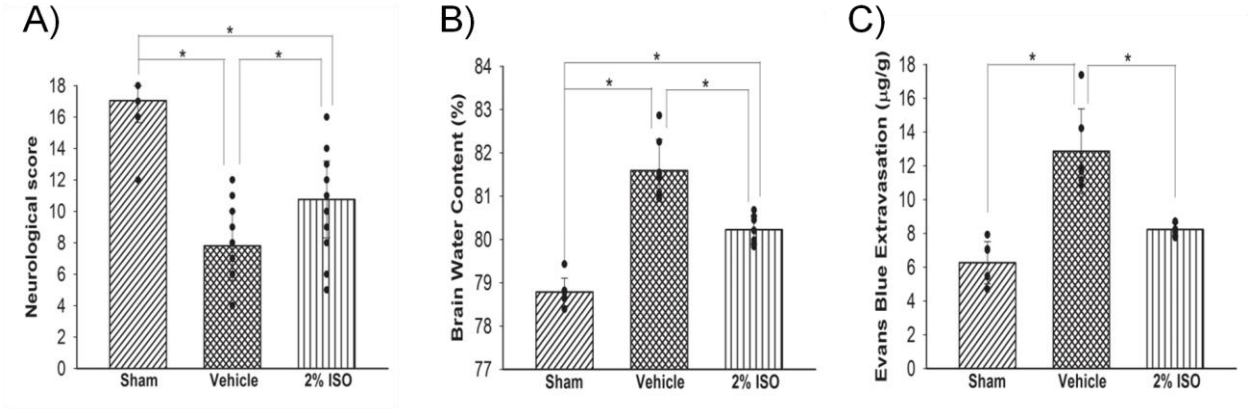

Figure S1. Neurological score (n=21 per group) (**A**), brain water content (n=7 per group) (**B**), and Evans blue dye extravasation (n=6 per group) (**C**) in the left cerebral hemisphere at 24 hours after SAH. Vehicle, SAH+vehicle-air group; 2% ISO, SAH+2% isoflurane group; values, mean±SD; **P*<0.05, ANOVA.

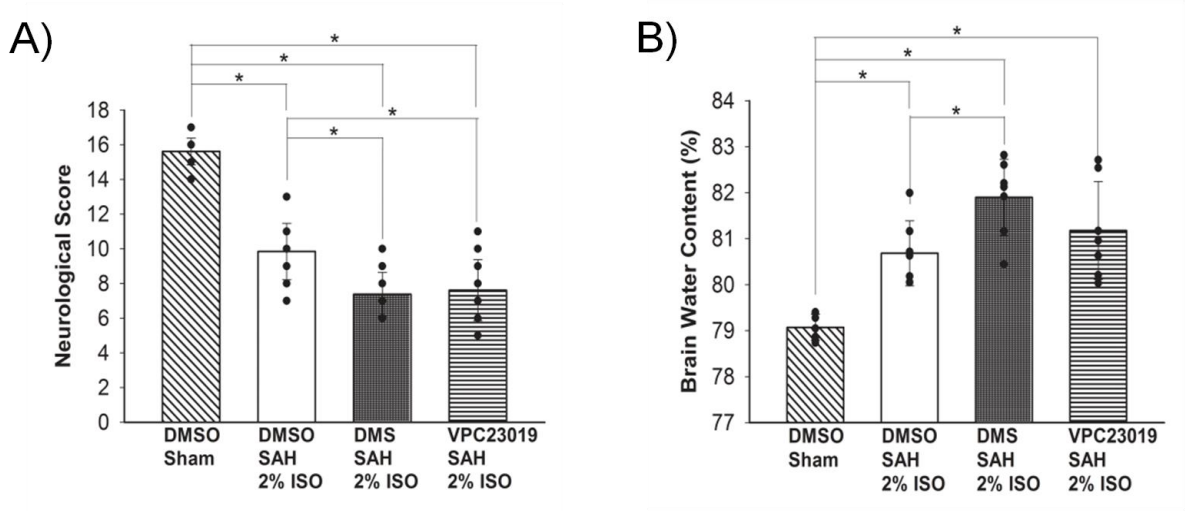

Figure S2. Neurological score (n=13 per group) (**A**), and brain water content (n=7 per group) in the left cerebral hemisphere (**B**) at 24 hours after SAH. 2% ISO, 2% isoflurane group; values, mean±SD; **P*<0.05, ANOVA.