Endovascular Perforation Murine Model of Subarachnoid Hemorrhage

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Background

Subarachnoid hemorrhage (SAH) is a devastating condition caused by bleeding into the space surrounding the brain. It is a common cerebrovascular disease and one of the subtypes of hemorrhagic stroke. SAH accounts for about 5 % of all strokes, which is reported to be the second leading cause of death in the world. Patients with SAH are left with high rates of disability and mortality with regard to of brain injury [12, 20]. The causes of SAH mainly include ruptured aneurysms and nonaneurysmal perimesencephalic hemorrhage, with ruptured aneurysms accounting for 85 % of patients. The risks of rebleeding and de novo aneurysms contribute to the difficulty in curing the disease. Therefore, a standardized SAH model is needed so that exploration of the treatment of SAH can proceed [2, 13].

To better investigate pathophysiological mechanisms and treatment strategies after SAH, a variety of laboratory techniques have been developed to induce a SAH animal model. Reports in the literature suggest that three surgical procedures are commonly used for inducing SAH animal models: autologous blood or hemolysate injection or infusion, blood clot placement, or endovascular perforation. Injection of autologous fresh blood into the cistern or subarachnoid space is easy to perform and reproducible as the blood volume can be directly determined. In addition, it allows saline injection as a sham control. Unfortunately, there are some aspects of the SAH pathophysiology that cannot be mimicked by this procedure, such as mechanical trauma.

The blood clot placement method mimics some late events, to a certain extent, including delayed vasospasm and ischemia by surgically placed *ex vivo* clot, which is from autologously withdrawn blood on the adventitial surface of arteries. This permits investigation of late pathophysiological mechanisms and pharmacological intervention. The method was determined to have low animal mortality, however, it fails to reproduce early injuries of SAH and mechanical trauma, which is a limitation in these experiments.

Endovascular perforation is demonstrated by puncture of the intracranial artery on the skull base by an endovascular filament. It mimics the pathophysiology in humans most closely. Disadvantages are the inherent technical difficulties in establishing a simple, reliable, and reproducible model in a small rodent and high mortality caused by poor control of bleeding [11, 19].

Establishment of a SAH model in the mouse would be of particularly valuable because the mouse is a common mammalian species available for study; it has a well-characterized genome, making genetic manipulation and generation of genetically modified strains possible; and the species is relatively inexpensive to purchase and house. Thus, increasing efforts have been directed toward establishing a stable and reproducible SAH mouse model with limited surgical procedures and low mortality. Herein, we present an endovascular filament SAH model in C57/BL6 mice, which simulates human cerebral aneurysmal rupture near the bifurcation of the anterior cerebral artery (ACA) and the middle cerebral artery (MCA).

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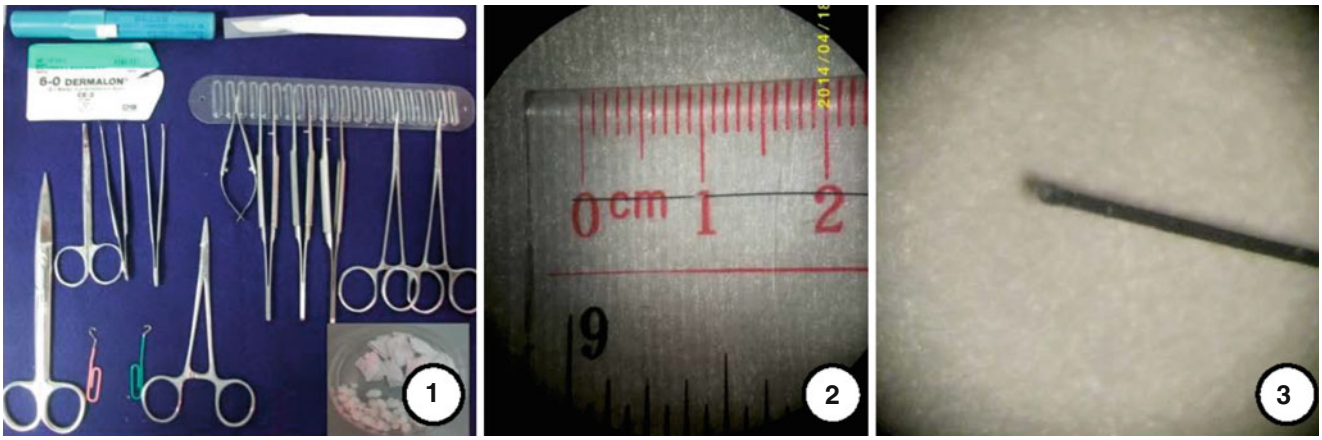

Fig. 1 Microsurgical instruments (①) and the filament (② and ③) used in inducing SAH

Materials and Methods

Animals

Pathogen-free, adult male mice C57BL/6, average weight 23–26 g, were obtained from the Experimental Animal Center of the Lo Kwee Seong Integrated Biomedical Science Building of the Chinese University of Hong Kong. All animals were housed in cages under a temperature-controlled environment at 21 °C with access to food and water. Animals were accepted pretrained by researchers 4 days before any experiment. The protocols for the experimental study received the approval of the Ethics Committee of the Chinese University of Hong Kong.

Experiment Approach

An endovascular perforation procedure was applied to establish the SAH model (n = 15) on the same day and observed for up to 14 days. Ten mice underwent sham surgery. Mice in the model of SAH underwent post-mortem examination to demonstrate evidence of SAH.

SAH Model Establishment

Preoperative Preparation of Instruments (Fig. 1)

A set of micro-neurosurgical apparatus included a microscope, microsurgical scissors, elbow microsurgical forceps, straight microsurgical forceps, bipolar coagulation, and micro-clips. Other equipment included a razor for shaving animal fur, normal forceps, ophthalmological scissors, needle-holder, scalpel, and sutures. Filaments (ShaDong Biological Tech. Co., Ltd., Beijing) marked with a certain scale were smoothed to blunt the top in advance under the microscope. Small cotton balls and gauzes were prepared to stop bleeding during the operation.

Anesthesia

C57BL/6 mice were anesthetized with intraperitoneal administration of 0.2 mL/100 g of a mixed solution consisting of 5 mg/mL ketamine and 2.5 mg/mL xylazine. Under anesthesia, the mice stayed still and unresponsive to any external stimuli, including cutaneous pinching and tail pinching with a sharp forceps, which the mouse would withdraw when pinched. For the righting reflex, mice did not right themselves from dorsal to sternal recumbency and muscles were relaxed when picked up by the tail. Mice had constant breath and heart beat. The absence of the palpebral reflex is considered a respectable depth. Because body temperature is reported to suddenly decrease after induction and remains diminished during anesthesia, hypothermia is considered a potentially fatal injury in small rodents. Therefore, maintenance of body temperature was included in anesthesia management. Mice were placed on a heating pad that was preheated to 37 °C and maintained until termination of surgery [1, 8].

Operation Procedures (Fig. 2)

Mice were fixed in a supine position and shaved of neck fur. A rectal temperature probe was inserted to monitor and maintain the body constantly at 37 °C with a heating pad during surgery. The neck region was disinfected with 70 % ethanol. Under a microscope (Zeiss), a midline neck incision was made and the skin was cut with a pair of scissors from sternum to chin (1 cm). The surrounding tissue was dissected bluntly and the salivary glands pushed aside. The left common carotid artery (CCA) was exposed and gently mobilized . Great care was taken not to harm the vagal nerve, which runs in the same surrounding tissue sheath as the CCA. Using the same technique, the left external carotid artery (ECA) was exposed and mobilized. The first ECA branch, which is the occipital artery, was mobilized and coagulated. The ECA was ligated as far cranially as possible and pulled to the other side of long ligation to the right side and fixed with adhesive plaster. Two 5–0 silk sutures were prearranged (1.5 cm segments)

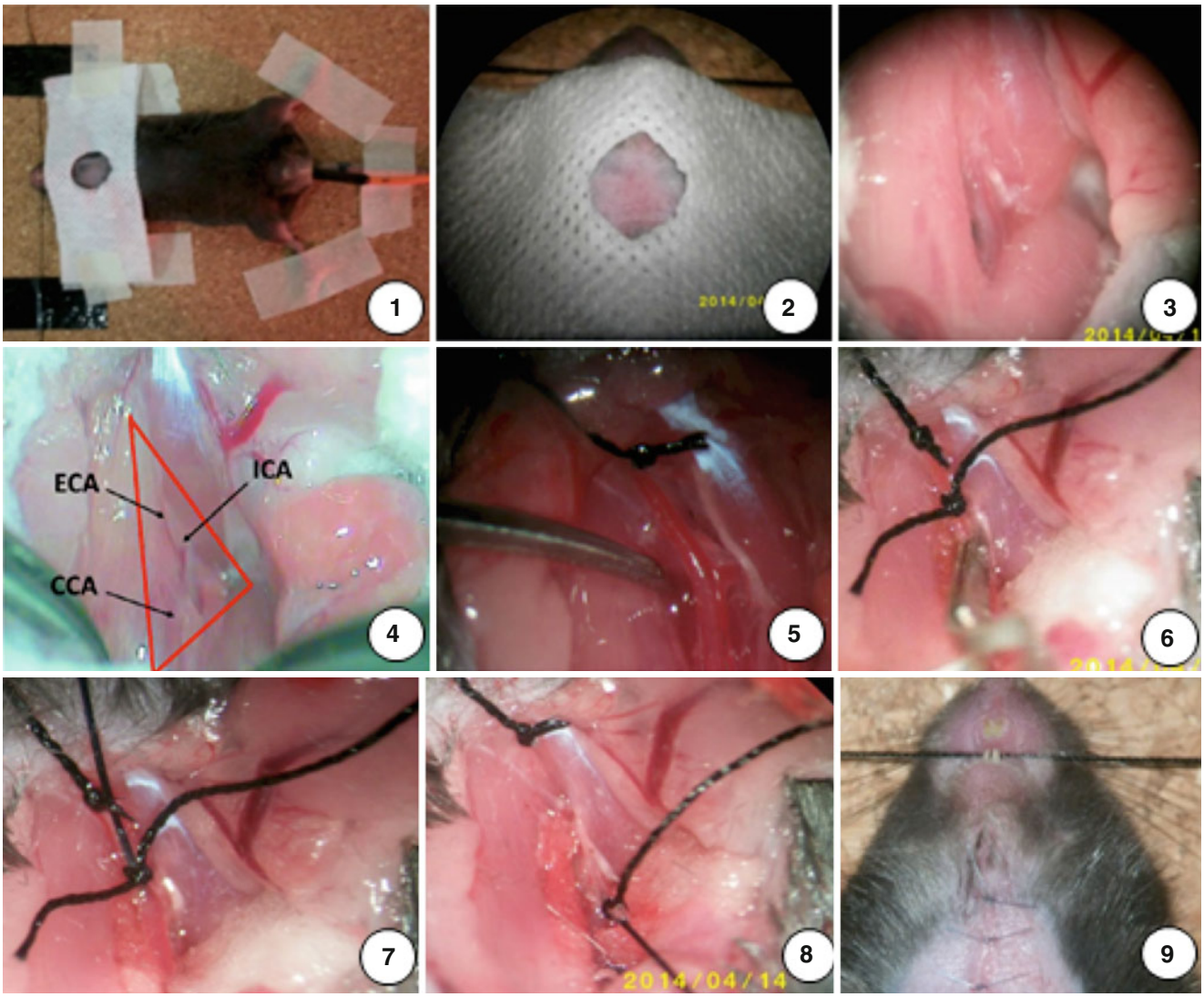

Fig. 2 Surgical steps for inducing SAH model in the mouse (as described in the text)

for the filament around the ECA. The distal of the left internal carotid artery (ICA) was gently exposed. The origin of the ECA was temporarily occluded with one microclip. A hole was cut as near as possible to the previous ligation for the filament insertion with a vessel microscissors. A 20-mm-long blunted 5–0 monofilament nylon suture was inserted into the ECA. Two prearranged sutures were knotted with the appropriate intensity and then the ECA was cut at thr hole point. The micro-clip from the ECA was removed and the filament was advanced with a forceps into the ICA to its bifurcations, where resistance was encountered. The filament was immediately withdrawn after advancing another 2 mm further to perforate the vessel. The total length inserted into the artery was defined to be 10 mm. The ECA was ligated by knotting the prearranged ligations tightly. The skin wound was closed immediately with suture after the removal of the filament. Mice were continuously observed under 23 °C until recovery and were then returned to their cages. Subcutaneous injections of 0.5 ml saline were given twice per day to all mice to standardize hydration. All of SAH mice were analgesized with buprenorphine (0.03 mg/kg body weight intraperitoneally) once per day for at least three days after surgery [3, 14, 17].

Mouse Motor and Sensory Scale (MMSS)

The Mouse Motor and Sensory Scale (MMSS) was used to evaluate the neurologic deficits of SAH mice before operation and at day 3, 7, and 14 after SAH induction [15]. The scale was combined from the prior scales in previously described examinations [4, 7, 9], which were comprised of motor (0–12) (spontaneous activity, symmetry of limb movements, climbing, balance) and sensory (5–15) (proprioception, vibrissae, visual, olfactory, and tactile responses) (Table 1). Neurologic function is graded on a scale of 5–27, with 5 indicating maximum functional deficits and 27 normal neurologic functions; the lower the score, the more severe the brain injury.

Table 1 A Mouse Motor and Sensory Scale (Parra et al. [15], 5–27)

Function		0	1	2	3
Motor	*Activity* (5 min open field)	No movement	Moves, no walls approached	1–2 walls approached	3–4 walls approached
	Limb symmetry (suspended by tail)	Left forelimb, no movement	Minimal movement	Abnormal forelimb walk	Symmetrical extension
	Climbing (on inverted metal mesh)	Fails to hold	Hold < 4 s	Holds, no displacement	Displaces across mesh
	Balance	Falls < 2 s	Falls > 2 s	Holds, no displacement	Displaces across rod
Sensory	*Proprioception* (cotton tip to both sides of neck)		No reaction	Asymmetrical head turning	Symmetric head turning
	Vibrissae (cotton tip to vibrissae)		No reaction	Asymmetrical head turning	Symmetric head turning
	Visual (tip toward each eye)		No reaction	Unilateral blink	Bilateral blink
	Olfactory (lemon juice on tip)		No sniffing	Brief sniff	Sniff >2 s
	Tactile (needle stick to palm)		No reaction	Delayed withdrawal	Immediate withdrawal

Parra scale [15] was combined from two prior scales: Garcia [7] and Crawley [4] comprised of motor (0–12)—spontaneous activity, symmetry of limb movements, climbing, balance—and coordination and sensory (5–15)—proprioception, vibrissae, visual, olfactory, and tactile responses

Results

Mortality

Two mice died in the first 24 h, two mice died with 2 days of SAH induction, and one mouse died within 5 days of SAH induction. In total, five mice died after SAH induction, with most dying in the first 48 h after the surgery, yielding a surgery-associated mortality of 33.3 % in our study.

General Observations and MMSS

Intraoperatively, the mice demonstrated deep breathing and evanescent hyperspasmia when there was a "give" during the puncture of artery. The mice exhibited signs of inspiratory dyspnea with depressed or paradoxical chest and abdomen movement with tachycardia, resulting in high mortality when the vagus nerve was irritated during the operative procedure. Postoperatively, all experimental animals were drowsy in the first 48 h after SAH, with no feeding and drinking behavior. Six mice moaned for about 1 week. Five mice exhibited ischemic symptoms, of which three mice showed ipsilateral drooping eyelids, similar to Horner's syndrome, due to a deficiency of sympathetic activity. Those with severe ischemia symptoms, including hemiplegia and rotation when picked up by the tail, were prone to die. The ischemia symptoms emerged variably from either the left or right side. The body weight of the SAH mice decreased significantly at day 3 after SAH procedure. The average body weight decreased more than 3 g. At day 7, body weight returned to the level of original states in the SAH group.

In the MMSS score analysis, mice showed a significant neurologic dysfunction from day 3 to day 14, with lowest scores on day 3 ($p < 0.05$). However, the sham control group showed an almost normal score of MMSS.

Post-mortem Determination

Immediately after death by sacrifice, isolated brains were examined under magnification. The distribution of hemorrhage was along the Circle of Willis around the cerebral space. The sham-operated mice were sacrificed as controls. There was no visible blood surrounding the cerebral space (Fig. 3).

Discussion

It is important to establish an ideal SAH animal model simulating clinical aneurysm rupture in humans. The SAH murine model depicted in this experiment exhibited the typical signs of SAH with comparable mortality to previous literature

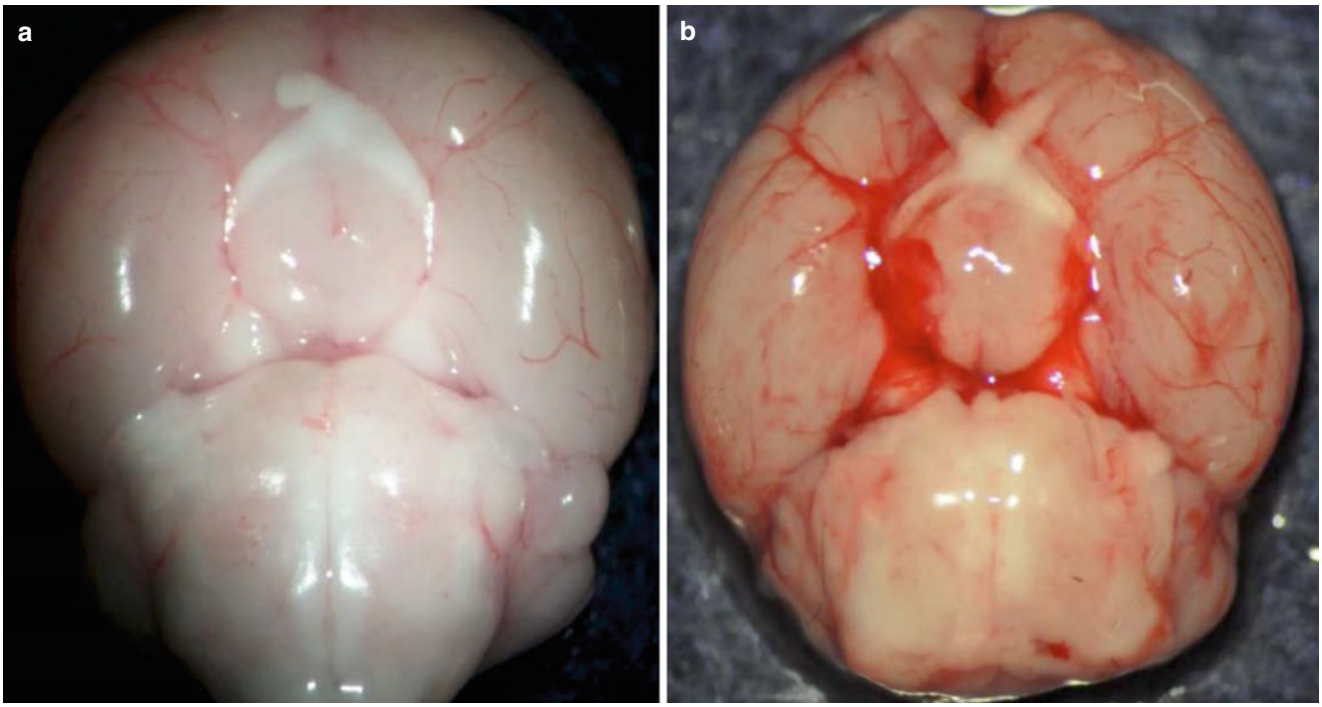

Fig. 3 General appearance of the subarachnoid hemorrhage under microscope. The isolated brains were shown on the ventral surface in sham (**a**) and SAH (**b**) mice

[6, 21]. The evanescent hyperspasmia presented during the operation was probably caused by the sudden increased intracerebral pressure (ICP), which is induced by bleeding into the subarachnoid space. The temporary increased ICP increased signs and symptoms in the animal model similar to the Cushing reflex. This can be considered as a sign by which to judge the condition of vessel perforation. The ischemia symptoms could be the result of hemorrhage-induced delayed cerebral vasospasm [5, 10, 15, 16].

An ideal SAH experimental model exhibits the following important characteristics [18]: (1) consistent and reproducible clot deposition in the space surrounding the brain; (2) uniform and controlled degree of hemorrhage; (3) mechanism of hemorrhage closely mimicking aneurismal rupture and blood distribution connecting with aneurysmal SAH; and (4) performance and reasonable cost. The SAH murine model reported herein satisfied several criteria above. The most frequently mentioned drawback of the filament perforation model in the literature is that bleeding volume cannot be directly controlled.

This problem can be resolved, to some extent, by ensuring the following: (1) suitable marked filament with heparin immersion is used; (2) a minimally invasive procedure; (3) operators capable of skilled microsurgical technique during SAH induction; and (4) good postoperative care. High mortality is a characteristic of SAH in humans. Endovascular puncture in the murine model has a relatively high mortality compared with the blood injection model, which indicates that this model closely mimics aneurismal rupture in humans. The limited attention afforded to mice in this context derives from the inherent technical difficulties in establishing a simple, reliable, and reproducible model in a small rodent. Despite the apparent technical impediments, establishment of a mouse model of SAH would be of considerable value.

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Conflict of Interest Statement All authors read and approved the final manuscript. The authors declare that they have no conflicts of interests.

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