Anterior Circulation Model of Subarachnoid Hemorrhage in Mice

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Abstract Subarachnoid hemorrhage (SAH) remains one of the most morbid subtypes of stroke around the world and has been the focus of hemorrhagic stroke research for longer than five decades. Animal models have been instrumental in shaping the progress and advancement of SAH research, particularly models that allow for transgenic manipulation. The anterior circulation mouse model provides the research community with a rodent model that depicts very similar clinical findings of SAH; from the location of the hemorrhages to the secondary complications that arise after the hemorrhagic insult. The model allows for the recreation of clinically relevant findings such as large vessel vasospasm, oxidative stress, microcirculatory spasm and microthrombosis, and delayed neuronal injury – all of which appear in human cases of SAH. The model is also not technically demanding, is highly reproducible, and allows for an array of transgenic manipulation, which is essential for mechanistic investigations of the pathogenesis of SAH. The anterior circulation mouse model of SAH is one of a few models that are currently used in mice, and provides the research community with a relatively easy, reliable, and clinically relevant model of SAH – one that could be effectively be used to test for early brain injury (EBI) and delayed neurological injury after SAH.

Keywords Anterior circulation • Subarachnoid hemorrhage • Vasospasm • Mouse • Perichiasmatic • Early brain injury • Delayed brain injury • Animal models • SAH

Introduction of Model

The prechiasmatic animal model was first introduced by the group of Prunell et al. in 2002 when it was first constructed and used on rats for the induction of experimental subarachnoid hemorrhage (SAH) [2]. The prechiasmatic model focuses on using the anterior circulation and allows for the injection of autologous or nonautologous blood into the prechiasmatic cistern using an anterior approach. The model was first constructed as an experimental SAH model to approximate the clinical picture of SAH. Clinically speaking, approximately 80 % of aneurysms found in aSAH patients were often located in the anterior circulation and anterior fossa [1]. As a result, constructing a model with an anterior distribution of hemorrhage was thought to be beneficial and more translatable to the clinical picture. The model was then adapted to be used in mice, and some technical modifications were made to maximize the proximity of

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recreating a hemorrhagic event in mice [3]. Adapting the anterior model in mice was a big objective because it allows for the use of transgenic technology to further dissect the pathogenesis of SAH.

Model Techniques and Methodology

For our model and our studies, all experiments and protocols are approved by the Animal Care Committee associated with our hospital base and comply with all regulations of the Canadian council of animal care. Mice used in our experiments are randomized by gender and weigh between 17 and 25 g. Experimental model characteristics have been published by our laboratory in great detail [3]. In brief, animals can be anesthetized with either injectable anesthetics (ketamine 120 mg/kg and xylazine 30 mg/kg) or spontaneous inhalation of isofluorane. Body temperature is maintained at 37.0 ± 0.5 °C with a homeothermic heating pad and rectal temperature probe (Harvard apparatus, Holliston, MA, USA). The head is fixed in a stereotactic frame equipped with a mouse adaptor (Harvard apparatus) and stereotactic manipulators to hold the laser Doppler flow probe (BLT21, Transonics Systems, New York, NY, USA) and a spinal 27-gauge needle (BD Biosciences, San Jose, CA, USA). A simple incision is made midline of the anterior scalp to expose the skull and to visualize the sagittal sinus. A burr hole is drilled 4.5 mm anterior to the bregma and slightly lateral to the midline using a 0.9 mm STARRET drill (TRANSCAT, New York, NY, USA). The needle is angled ventrally at 35–40° (depending on the breed of mice), which allows the needle to advance between both hemispheres without penetrating any parenchyma or causing any brain damage (Fig. 1). Cerebral blood flow (CBF) is monitored on the contralateral aspect of the skull to monitor changes; recording times vary depending on experimental purposes. Once a stable recording is established, nonautologous blood from a donor animal or autologous blood is extracted from the mouse's tail artery and injected through the spinal needle. The spinal needle is advanced to the base of the skull until a fine resistance is detected; the needle is then pulled back (0.4–0.5 mm) to position the needle in the subarachnoid space in the prechiasmatic cistern. Either blood (experimental) or normal sterile saline (control) is injected at a steady speed, ranging from 5 to 15 s (depending on the model severity desired). The volume injected also depends on model severity desired and mouse breed, and usually varies from 50 to 100 µl. The needle is kept in this position for ~ 2 min to prevent backflow or CSF leakage.

Advantages and Disadvantages

Constructing the perfect model of any pathology requires a number of essential criteria to be considered efficient and useful. Schwartz et al. suggested a few criteria that may assist in the creation of an ideal model of SAH [4]. The model should be reproducible with very little variation, inexpensive, produce a documented and controlled volume of hemorrhage in the correct location, and reproduce the clinical complications and secondary sequelae of SAH. The anterior approach model of SAH was constructed and adapted in mice keeping all of these criteria in mind. The model provides the research community with a number of advantages and with ease of reproducibility, some of which are listed in Table 1.

The prechiasmatic anterior model has demonstrated to be reproducible, reliable, and have very little intergroup variability. This model would be classed under injectable SAH models, and it allows for controlling the volume and source of blood injected – allowing the severity of the hemorrhage to be controlled and the use of either autologous or nonautologous blood (donor animals of the same genetic background). The model also reliably recreates the Cushings reflex, with an increased intracranial pressure (ICP) and reduced CBF with the induction of SAH. The model has been used in our laboratory extensively, and reliably reproduced a number of essential secondary complications such as microthrombosis, microcirculatory spasm, neuronal degeneration and apoptosis, and large vessel vasospasm. This makes the anterior model one of the most flexible and reliable models available to the SAH community, and certainly one that could be used for research involving early brain injury (EBI) after SAH. Other models that exist in mice provide a number of advantages and disadvantages that are listed in Table 2.

Conclusions and Future Directions

The prechiasmatic anterior model adapted in mice provides the SAH community with a flexible model that is technically not challenging and that has been demonstrated to be increasingly reliable. Despite extensive research in our laboratory with this model, it is still in its early stages and requires further research and time-series analysis to truly understand whether this model is best used for EBI research, delayed-type complications research, or can effectively model both modalities of pathogenesis. Additionally, further work and research is needed to construct a model that truly encapsulates the pathogenesis of SAH and one that could take into account the multifactorial nature of this pathology.

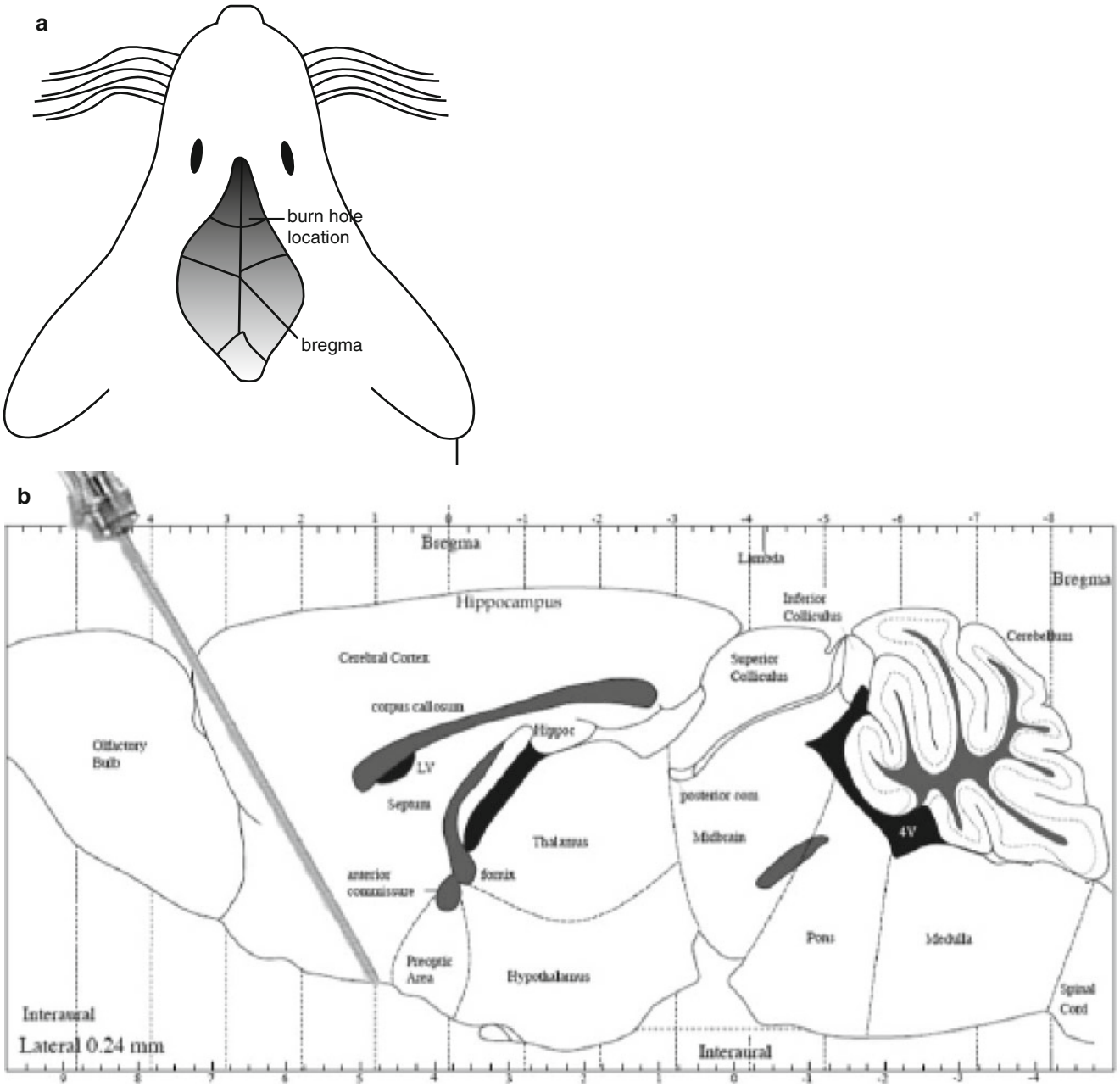

Fig. 1 (**a**) Location of the burr hole, at 4.5 mm from the bregma, slightly off midline. (**b**) Spinal needle is advanced at 40° to the base of the brain into the prechiasmatic cistern

Table 1 Summary of the advantages and disadvantages of the anterior circulation SAH model

Advantages	Disadvantages
Controlled volume of injected blood and reproducible	Does not recreate the natural aneurysmal rupture
Not challenging technically, with a low mortality	Difficult to monitor blood pressure (mouse tail cannulation)
Nonautologous or autologous sources of blood can be used	
Recreates an ICP spike	
Recreates primary complications of SAH	
Recreates secondary complications of SAH	

Table 2 Summary table of experimental SAH models and the advantages and disadvantages associated with each

Model	Advantages	Disadvantages
Endovascular perforation		Uncontrolled blood volume
		Variable severity
		High mortality/ morbidity
	Mimics spontaneous rupture of aneurysm	No experimental control
Blood injection models (basal cistern injection)	Severity controlled, volume of blood controlled	Location and distribution of blood not clinically similar
	Experimental controls exist	Does not mimic spontaneous rupture
Vein puncture	Mimics spontaneous rupture of aneurysm	Uncontrolled blood volume
		Variable severity
		Venous blood
		No experimental control
Hypertension and vascular fragility model	Mimics spontaneous rupture of aneurysm	SAH data does not exist yet
		Variable blood volume and severity
		No proper experimental control

Conflict of Interest Statement We declare that we have no conflict of interest.

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