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Simple and efficient rat model for studying delayed cerebral ischemia after subarachnoid hemorrhage

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HIGHLIGHTS

- Rat models for SAH-induced DCI studies are lacking.
- Complete exposure of the atlanto-occipital membrane or burr hole is avoided in our SAH model.
- Our model with many merits including lower mortality, reduced operative trauma, shorter procedure time and minimized learning curve.
- Pearl-string-like microvascular spasm was visualized in vivo in our SAH model.
- Reduced cerebral blood flow was observed in our model by MR PWI.

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ABSTRACT

Background: Delayed cerebral ischemia (DCI) is a late phase of consequences of subarachnoid hemorrhage (SAH) that causes poor outcome and has become the focus of current research. The aim of this study was to characterize an experimental SAH technique for studying DCI after SAH.
New method: A double injection SAH rat model with a tiny incision was introduced. At 7 days post-SAH induction, the diameter and luminal cross-sectional area (CSA) of the basilar artery (BA) were measured. In vivo fluorescence microscopy and magnetic resonance perfusion-weighted imaging (MRPWI) were used to evaluate the occurrence of DCI. Normal and sham-operated groups served as References
Results: Compared to the sham group, in SAH group, the diameter and CSA of the BA were decreased, and the CBF in the SAH group was also reduced to barely half of the level in the sham group. Moreover, both the proportion and severity of microarterial constrictions were increased significantly in the SAH group when compared to those in the sham group.
Comparison with existing methods: Complete exposure of the atlanto-occipital membrane is avoided, only a tiny region is exposed to identify the puncture spot. Lower mortality, reduced operative trauma and shorter procedure time are advantages to existing models. Multiple techniques for DCI assessment were used including in vivo microscopy and MRPWI.
Conclusions: The current study demonstrates that our SAH model was successfully established and may serve to help identify a novel target for the treatment of DCI after SAH.
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1. Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is a stroke with a case fatality rate of nearly 25–50% (Hop et al., 1997; Kolias et al., 2009; Lovelock et al., 2010; Steiger et al., 2015). As mentioned in prior studies(Lang et al., 2001), the pathophysiological changes that occur after SAH are divided into two distinct phases: initial pathophysiological changes known as "early brain injury (EBI)" and a sequence of events occurring thereafter that is summarized by the term "delayed cerebral ischemia (DCI)", which causes poor outcome or death in up to 30% of patients with SAH (Macdonald, 2014). Due to the limited possibilities of research in humans, a good animal model is a prerequisite to better understand the pathophysiology of DCI after SAH.

For studying and developing therapies for DCI, various SAH models were established in animals such as rabbits, dogs, pigs, primates, rats and mice (Hashi et al., 1972; Prunell et al., 2002; Marbacher et al., 2008; Nikitina et al., 2010; Wang et al., 2013).

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Among them, rats were the most widely used. Since the induction of SAH was first described in a rat model in 1979 (Barry et al., 1979), it has been further divided into an endovascular arterial perforation model (Park et al., 2008; Feiler et al., 2010) and a blood injection model (Meguro et al., 2001; Gules et al., 2002; Sugawara et al., 2008; Güresir et al., 2015a,b). As mentioned in the existing meta-analysis of mortality in different DCI models(Kamp et al., 2017), the mortality of the cisterna magna double injection model was much lower than that of the endovascular perforation model. Within all the double injection models that have been established so far, the two most commonly used surgical approaches to the cisterna magna were either though a burr hole (Prunell and Diemer, 2003; Cai et al., 2012; Güresir et al., 2015a,b) or by fully exposing the atlanto-occipital membrane (Güresir et al., 2015a,b) for to identify the puncture spot. However, these methods have a number of well-known limitations, including heavy bleeding, severe operative trauma, limited blood injection volume, cerebrospinal fluid (CSF) leakage, a longer recovery course and a relatively high mortality.

In the present study, we primarily describe how to perform a simple and efficient double injection SAH model with less invasion and a lower mortality rate. To better mimic the pathogenesis of DCI, including angiographic vasospasm, microcirculation constriction and cerebral blood flow (CBF) reduction (Rosengart et al., 2007a,b; Macdonald, 2014), all the animals were examined using magnetic resonance perfusion-weighted imaging (MRPWI), intravital fluorescence microscopy and hematoxylin-eosin staining on day 7 after the induction of SAH.

2. Materials and methods

All animal procedures conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 86-23, revised 1985); animal use and welfare followed a protocol that was reviewed and approved by the First Affiliated Hospital of Nanchang University.

2.1. Study design

Forty-five healthy adult male Sprague-Dawley rats weighing 250–450 g were purchased from the laboratory animal science center of Nanchang University for the experiments. The animals were raised in an animal facility at 25 °C with a 12-h light/dark cycle; the animals were supplemented with food and water ad libitum. They were randomized into three groups, including the normal group (n = 15), the sham-operated group (n = 15) and the SAH group (n = 15). All surgical procedures were performed under sterile conditions at the Experimental Surgical Institute in the Department of Neurosurgery at the First Affiliated Hospital of Nanchang University.

2.2. Anesthesia, monitoring, perioperative care and sacrifice

The rats were anesthetized by intraperitoneal injection of 100 mg/kg ketamine (Sigma-Aldrich Co., St. Louis, MO, USA) and 10 mg/kg xylazine (Sigma-Aldrich Co., St. Louis, MO, USA), which appears to be practicable and safe (Meguro et al., 2001; Longo et al., 2002; Satoh et al., 2002; Cambj-Sapunar et al., 2003; Ono et al., 2003; Lee et al., 2009; Güresir et al., 2013), and the likelihood for no significant changes in circulation parameters or in CBF has been shown with this medication (Rousselle et al., 1998; Lei et al., 2001; Dittmar et al., 2004). During the procedure, the animal was placed in a prone position on a heating blanket (Harvard Apparatus Ltd., UK) coupled to a rectal probe to maintain body temperature (37.5 ± 0.5 °C). In addition, a Desktop Digital Stereotaxic Instrument (RWD Life Science Co., Shenzhen, China) was used for rigid cranial fixation (Prunell et al., 2002). A polyethylene tube (Portex, luminal diameter: 0.96 mm) was inserted into the femoral artery for blood pressure monitoring, arterial gas sampling and autologous blood withdrawal. Euthanasia was performed 7 days post-SAH induction by overdose with carbon dioxide (CO_2) gas.

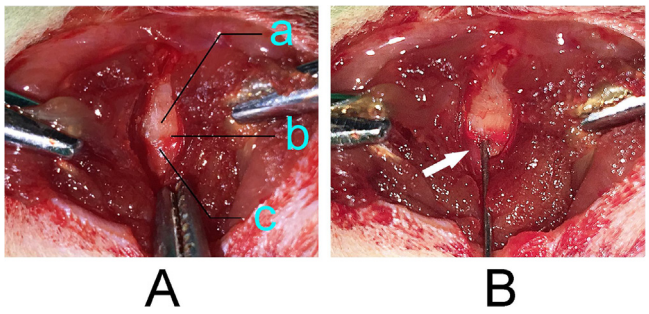

Fig. 1. Exposure of atlanto-occipital and the puncture spot. (A) Only a tiny region is exposed by merely identified the boundary of the occipital bone and the atlanto-occipital membrane. (a) occipital bone; (b) the boundary between the occipital bone and the atlanto-occipital membrane; (c) atlanto-occipital membrane. (B) After insertion of a sterile syringe into the cisterna magna (white arrow), 0.15 ml CSF is gently aspirated followed by injection of 0.15 ml non-heparinizated autologous arterial blood to induce SAH.

2.3. Induction of SAH

After anesthesia, the area of the scruff was shaved. A skin incision was made to span the suboccipital region and the arch of C1. The splenius muscle layer was strictly divided along the midline to reduce bleeding and to make the operation field clearer, which is crucial throughout the procedure. After retracting the muscle layers laterally and holding them with hemostatic forceps, the suboccipital region and the atlanto-occipital membrane were exposed (Fig. 1A). With the aim of increasing the survival rate by minimizing trauma and avoiding unnecessary tissue destruction, it is important to clearly identify the boundary of the occipital bone and the atlanto-occipital membrane but not dissect further. The atlanto-occipital membrane was visualized as a shiny white membrane then a 26 gauge needle was inserted into the membrane (Fig. 1B). With the needle inserted 1–2 mm and withdrawn, it was considered placed into the cisterna magna when transparent CSF back-flowed into the syringe. Under manual manipulation, 0.15 ml CSF was gently aspirated. Thereafter, 0.15 ml of non-heparinized autologous arterial blood from the femoral artery was withdrawn into a sterile syringe. Once extracted from the artery, the blood was immediately injected into the cisterna magna.

Given that the cisterna magna is in close proximity to the brainstem, any stimulus over the medulla oblongata cardiovascular center or respiratory center on the brainstem may induce cardiac or respiratory arrest(Fàbregas et al., 2000). For this reason, blood injection is the most important step during the surgery. The needle was introduced into the cisterna magna at an angle of approximately 60° to the occipital bone with the bevel facing the ventral side. The blood was injected slowly at a rate of 0.05 ml every 30 s. When the injection was over, the needle was left in place for 30 s and then carefully withdrawn. In the “sham” group, the atlanto-occipital membrane was exposed, but an equivalent volume of normal saline was injected to determine if the surgery significantly altered the outcome of the current study. After covering the injection point with a gelatin sponge, the skin was sutured. The head of the animal was then placed in a downward position for 20 min on the heating blanket to ensure the distribution of the administered blood through the subarachnoid space. After suffering from the first injection, due to the surgical strikes and the compression of clots to the brainstem, breathing problems and limited mobility may occurred to a few animals even died before the second injec-

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