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A rabbit model of aneurysmal subarachnoid hemorrhage by ear central artery-suprasellar cistern shunt

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ABSTRACT

Aneurysmal subarachnoid hemorrhage (aSAH) is a life-threatening hemorrhagic cerebrovascular disease. The concept of early brain injury (EBI), induced by sharply increased intracranial pressure (ICP) and low cerebral perfusion pressure (CPP) with cerebral global ischemia following aneurysm rupture, has been increasingly accepted. However, EBI has not been well studied partly due to lack of an ideal animal model. The purpose of this study was to establish a new aSAH model which can mimic the pathophysiological damage of EBI. Right frontal craniotomy was performed on New Zealand rabbits for placing a PE-50 tube at the suprasella cistern and an ICP probe at the anterior cranial fossa. The central ear artery was punctured and blood was shunted into the suprasellar cistern through the PE-50 tube. ICP, blood pressure, CPP and heart rate peri-aSAH were monitored throughout the experiments. The rabbits were examined for neurological deficits at 24 h post-SAH. Brain coronal sections near the optic chiasma were assessed by HE and Cresyl violet staining. Three minutes after SAH induction, the ICP peaked to 61.7 ± 9.8 mmHg while CPP decreased to nadir 23.5 ± 8.9 mmHg, and both were gradually restored in 15 min. At 24 h post-SAH, significant neurological deficits were found in SAH rabbits as compared to the sham-operated animals. In addition, neuronal degeneration and loss were also detected. Our results indicate that a new rabbit model of aSAH with EBI is successfully established. Moreover, this model is controllable, economical, and no side-injury to the main artery.

1. Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a life-threatening hemorrhagic cerebrovascular disease [1,2]. The intracranial aneurysms that cause aSAH are most frequently located at anterior circulation or at the top of basilar artery and proximal posterior artery, both of which belong to circle of Willis. Therefore, aSAH is distributed around the circle of Willis. Despite major advances in surgical and neurointerventional techniques for intracranial aneurysms in recent years, the mortality and morbidity rate after aSAH have not obviously reduced by solely focusing on cerebral vasospasm (CVS), which was believed to cause delayed ischemic neurological deficits (DIND) [2,3]. Researchers have found that early brain injury (EBI) after aSAH, may be key factor for its poor outcome, especially in patients with higher Hunt-Hess grades [4,5]. EBI was induced by the sharply increased ICP and decreased CPP with cerebral global ischemia following rupture of aneurysm [4,6]. However, the literature concerning EBI are still in initial stages, and more extensive and profound animal studies are necessary to fully understand the pathophysiology of EBI.

To closely mimic aSAH, several animal models have been proposed and applied, of which only two models (cisterna magna blood injection and endovascular puncture) are frequently used [7]. The cisterna magna blood injection aSAH model cannot mimic the sharp increase of ICP followed by global ischemia after rupture of aneurysm. Besides, blood injected into the cisterna magna sometimes accumulated on the surface of medulla oblongata that may suppress the respiratory and cardiovascular center functions, and cause death of animals. Moreover, distribution of bleeding does not correspond to aSAH in patients [7,8].

The endovascular puncture rat and mouse aSAH models are increasingly applied at present. With filament perforating the circle of Willis in the bifurcation of anterior and middle cerebral artery, they can mimic the pathophysiology of EBI after aSAH, resulting in sharp increase of ICP followed by global ischemia [7,9]. However, there are several limitations as follows: (1) Cerebral vascular is pushed and damaged by the filament, and the extent of bleeding

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is hard to control with high mortality of about 50%, which does not correspond to typical aSAH patients; (2) Operation itself can cause additional injury such as ligation of the external carotid artery and disruption of internal carotid artery endothelium, which may cause thrombosis. Patients suffering from SAH may not have these pathological changes. These two characteristics demonstrate that this model is only suitable for study focusing on acute or super-acute phase of aSAH [9]; (3) Larger animals such as rabbit, which is more suitable for examination with MRI, CTA, or DSA, cannot be used to establish a model by this technique [10].

Therefore, a better animal model of aSAH should mimic the location, clinical, neurological and pathophysiological characteristics, and can be examined by conventional imaging such as MRA, CTA and DSA. It should also be reasonable in price and easy to establish. In this study, we aimed to establish an ear central artery-suprasellar cistern shunt rabbit model of aSAH, which conformed with above requirements.

2. Materials and methods

2.1. Animals

The research protocol was approved by the Animal Care and Use Committee of Fujian Medical University and in accordance with the guidelines of National Institutes of Health. Fifteen male New Zealand rabbits (3–3.5 kg body weight) were used in this study. Three animals served as sham-operated controls. Experimental aSAH was performed in remaining 12 animals as described below.

2.2. Operation procedure for aSAH induction

General anesthesia was given by intramuscular injection of ketamine (50 mg/kg) and chlorpromazine (7.5 mg/kg). Blood pressure and heart rate were monitored through a 20 G vascular detaining needle inserted into the left ear central artery and connected via a pressure transducer to a multi-parameter monitor (Codman corporation, USA). The rabbit was kept in prone position and its head was fixed on a stereotactic frame. A midline scalp incision about 3.5 cm in length was made and the right cranial vault was exposed. With an operating microscope, a bone flap of about 3.5 mm × 5 mm was removed from the right supraorbital frontal-parietal bone with a micro drill. Then, a dura incision was made near the orbital margin. With protection provided by a small gasket made from rubber gloves, a PE-50 tube filled with heparin solution was inserted into the suprasellar cistern near the circle of Willis to a depth of 12 mm from the orbital margin. Then an ICP monitor (Codman corporation, USA) catheter tip was inserted under the frontal brain tissue. The dura was tightly sutured, and sealed with a gelatin sponge. The bone flap was placed back into the bone window and sealed with a strong adhesive to maintain the closed cranium, and the scalp was sutured. The stereotactic frame was removed and rabbit was kept freely prone. A 20 G vascular detaining needle was inserted into the right ear central artery and connected to the PE-50 tube with a three-way cock (Fig. 1).

SAH was initiated by opening the shunt to let the blood stream into the suprasellar cistern under arterial pressure. ICP and blood pressure were recorded from 1 min before to 15 min after the aSAH. Sham-operated animals underwent the frontal osteotomy with PE-50 tube and ICP monitoring placement as well as puncture of the left ear central artery but without opening the shunt.

2.3. Neurological evaluation

For neurological status evaluation, the animals were observed by an investigator blinded to the surgical group. Neurological status was graded at 24 h post-aSAH according to the four-point grading system by S. Endo et al. [11].

2.4. Euthanasia and brain tissue processing

After excessive anesthesia, intracardiac perfusion-fixation was performed at 24 h after aSAH with 1000 ml 0.01 M phosphate-buffered solution (PBS), followed by 500 ml 4% paraformaldehyde in PBS, under a perfusion pressure of 110 cm H_2O. The brain was cautiously removed from the skull, and the basal and hemispheric surfaces were observed and photographed to identify the distribution of subarachnoidal blood. Then, the brain was cut into post-optic chiasma coronal block, immersed in 4% paraformaldehyde for 24 h, embedded in paraffin, and cut into 6 μm consecutive sections.

2.5. Histology staining

Sections were stained with Hematoxylin-eosin and Cresyl violet (Beyotime, China) according to the manufacturer's protocols for paraffin-embedded tissues.

2.6. Statistical analysis

Values were expressed as mean ± SD. Statistical analyses were performed using SPSS 17.0. Neurological scores were analyzed using Kruskal-Wallis test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. General condition of the rabbits

There were no significant differences between the aSAH group and the sham-operated group in body weight, arterial blood pressure and heart rate before and after the operation (data not shown). After opening the shunt to induce aSAH, the ICP of the third experimental rabbit peaked to 95 mmHg after three minutes, followed by increase in blood pressure to 113/83 mmHg. The rabbit died after four minutes, and could not be revived by cardiopulmonary resuscitation for 10 min. In the other experimental rabbits, consciousness changed from shallow anesthesia in which their head and limbs had slight activity to coma, accompanied with whole body loss of tension, and tachypnea or bradypnea. Four out of ten rabbits had gatism. The neurological status gradually improved. The sham-operation group survived with no neurological status changes.

3.2. Neurological status at 24 h after SAH

The experimental aSAH rabbits showed worse neurological status at 24 h and significant neurological deficit of grade 3 or 4 with mean ± SD of 3.40 ± 0.51, while in the sham-operated group the mean was 1 (Fig. 2, $P < 0.01$).

3.3. ICP, CPP, MABP, and HR changed at peri-aSAH induction

After opening the shunt to induce aSAH, ICP increased sharply from the baseline of 5.6 ± 3.6 mmHg to peak at 61.7 ± 9.8 mmHg in three minutes, then slowly decreased to 16.7 ± 8.0 mmHg after 15 min. Correspondingly, CPP decreased sharply from the baseline of 68.3 ± 7.0 mmHg to nadir at 23.5 ± 8.9 mmHg, then slowly returned to 56.9 ± 14.0 mmHg. Changes of MABP and HR were slow, rarely added up in three minutes, and then dropped below the baseline (Fig. 3).