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A new percutaneous model of Subarachnoid Haemorrhage in rats

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HIGHLIGHTS

- We described a new rat model of SAH by percutaneous injection of autologous, non-heparinised blood into the intracisternal space.
- The model is reproducible, easy-to-perform and quick.
- The model presents low mortality rates and subsequent ischemic–haemorrhagic lesions.

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ABSTRACT

Objective: Describe the results obtained with a new percutaneous, intracisternal model of Subarachnoid Haemorrhage (SAH) in Wistar rats by a single injection of non-heparinised, autologous blood.
Methods: Once anaesthetized the rat was fixed prone in a stereotaxic frame. After identifying the projection of the occipital bone, the needle of the stereotaxic frame aspirated towards the foramen magnum until it punctured through the atlanto-occipital membrane and obtained cerebrospinal fluid. Autologous blood (100 μl) was withdrawn from the tail and injected intracisternally. This procedure was repeated in the sham group, injecting 100 μl of isotonic saline. On the fifth day post-intervention, the rats were anaesthetized and the brain was exposed. After a lethal injection of ketamine the brain was explanted and fixed in paraformaldehyde. Gross and microscopic inspection of the slices revealed the existence or non-existence of pathological findings.
Results: A total of 26 rats were operated on (13 in the SAH group/13 in the sham group). The average time between obtaining the blood and the start of the intracisternal injection was 10 (±1.2) s. The mortality rate was 16.12%. Intra- and extraparenchymal ischemic–haemorrhagic lesions were found in three animals (23.07%) – all from the SAH group – with ischemic neuronal cell injury detected in two of the three.
Conclusions: The new murine model of SAH is easy to perform, with low mortality, minimally invasive, which makes it interesting for future studies on vasospasm-related delayed SAH complications.

1. Introduction

Patients with Spontaneous Subarachnoid Haemorrhage (SAH) have a high mortality rate within thirty days. This elevated level shows a slight tendency to descend, which is possibly related to the new diagnostic techniques and treatment (Sandvei et al., 2011). Although some clinical factors exist which improve prognosis by means of suitable intervention, a high percentage of the variables are non-modifiable (Andaluz and Zuccarello, 2008; Goldacre et al., 2008; Muñoz-Sánchez et al., 2009). Vasospasm secondary to SAH is the leading cause of sequelae following a ruptured aneurysm in the cerebral arteries (Dorsch, 2002). After day four, 30% of patients go on to develop symptomatic vasospasm (SV) (Vergouwen, 2011). At present our understanding of the pathophysiological events that occur after SAH is insufficient and this leads to two crucial facts: firstly, we do not have reliable tools to unmistakeably select the patients who are at risk for SV, and secondly, with the exception of nimodipine, there is no existing therapy to avoid or solve this complication. This leads to variability in the clinical management of patients worldwide (Stevens et al., 2009). For these reasons we must promote new lines of research that facilitate pathophysiological understanding of the phenomena associated with vasospasm after SAH, and test treatments that may induce changes in the mortality rates

Abbreviations: SAH, Subarachnoid Haemorrhage; SV, symptomatic vasospasm; CSF, cerebrospinal fluid.

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and in the number of disabling sequelae (Laskowitz and Kolls, 2010).

Despite the large number of species (rats, rabbits, dogs, etc.) used to conduct studies about vasospasms after SAH, rat models of SAH are quite widespread. This is due to the ease in handling, housing and cost (Megyesi et al., 2000). Since the studies by Barry et al. in 1979, multiple invasive models have been generated with large differences between them, without achieving the optimal model (Barry et al., 1979). We have aimed to produce a rat model of SAH that meets all the characteristics of an ideal experimental model: simple to perform, minimally invasive, efficient and that accurately reproduces the natural development of the disease.

2. Methods

The procedures were performed in the experimental operating room of the Seville Biomedicine Institute (Instituto de Biomedicina de Seville [IBiS]), Virgen del Rocío University Hospital, Seville, Spain. This research project was overseen and approved by our hospital's Animal Experimentation Committee. It met all ethical standards for research and legal requirements as established by the corresponding legislation (Directive 2010/63/EU).

Wistar rats (300–350 g) were used as the model. The procedure was performed in males, to eliminate the hormonal influence of the oestrus cycle in the cardiovascular system. After the procedure, the animals were housed in independent cages, in a dry place away from infectious sources and surgical areas. A stable temperature between 23 °C and 27 °C was maintained and a 12 h light/dark cycle. Food and water was provided without restrictions, both before and after surgery.

2.1. Study groups

There were two study groups:

1. The SAH group comprising of 13 rats with SAH caused by percutaneous injection of autologous, non-heparinised, fresh blood.
2. The sham group which included 13 rats using the same method but with saline serum injected into the subarachnoid space.

2.2. Procedures: SAH group

The animals were anaesthetized with an intraperitoneal injection of a preparation consisting of: ketamine hydrochloride 50 mg (Ketolar©, Pfizer, 50 mg/cc), Xylazine 2 cc (Rompun© 2%, Bayer; 20 mg/cc) and 1 mg atropine (Atropine Bayer©, 1 mg/cc). The optimal dose to achieve a good degree of analgesia and sedation was established in 0.025 cc of solution per gram of body weight. Anaesthetic depth was considered appropriate at the absence of corneal reflex and the absence of withdrawal reflex after pressure on the hind legs. The cardiac and respiratory rate was measured during the procedure. During the surgical procedure the animal was spontaneously breathing.

2.2.1. Positioning

First, the posterior cephalic cervical surface was shaved, as well as the base of the tail to allow visibility of the vessels. Then the rat was fixed prone in a stereotaxic frame (Stoelting©). The animals are positioned with the nose on the same axis as the body, using the fastening system of the frame and being careful not to pinch the tongue. The head was immobilized by ear bars, which were adjusted to recognize the tympanic-ocular reflex. A 24-gauge needle with a syringe (1 cc BD Plastipak©) was mounted on the arm of the stereotaxic frame. The body was rotated 45° to allow free access to the margins of the tail. The direction of rotation, whether left or right, is chosen according to the macroscopic accessibility of the vessels under a cold light source. Then a tourniquet was applied to the proximal part of the tail, and gauze soaked in 37 °C water was applied.

2.2.2. Location of the cisterna magna

Once the previous position was reached, and by palpating the occipital region, the occipital protuberance at the start of the cervical spine was identified. This point was marked by blunt forceps, positioning the arm of the stereotaxic frame by combining the joints, allowing the needle to stay in the same direction as the tip of the forceps. In this way the needle is directed towards the foramen magnum. Previously we have carried out this procedure by opening the skin and dissecting by planes until the membrane is visually located, followed by methylene blue injection. These experiences have shown us the reliability of positioning by palpation (see Fig. 1). The needle was carefully inserted through the skin and progressively aspirated the embolus until it was felt that the atlanto-occipital membrane was punctured. Here the advance of the needle was detained, and it was verified that the syringe filled with cerebrospinal fluid (CSF). An estimated 50 μl was aspirated, and the arm and the syringe were kept in position. If during the manoeuvres to locate the cisterna magna, blood was obtained instead of CSF, or the needle progressed further than expected, the experiment was automatically stopped and the animal was sacrificed.

2.2.3. Obtaining autologous blood and intracisternal injection

To obtain autologous blood an incision was performed in the lateral margin of the tail, and a total amount of 100 μl of fresh blood was collected. This blood was collected using a non-heparinised syringe (1 cc BD Plastipak©). A rapid second aspiration of CSF reconfirmed that the needle mounted on the arm of the stereotaxic frame remained in the intracisternal position. After aspirating a total volume of CSF equal to 100 μl, the syringe on the needle was replaced by mounting the new syringe with fresh blood. At that very moment the blood injection was started. Infusion time took 30 s. Once the blood was injected, the animal's tail was sutured with braided silk. As a final step of the procedure the needle was removed from the intracisternal space and the animal was kept upside down for 10 min at 45°, to facilitate the spread of blood in the cisterns. Later the animal was returned to its cage. During the awakening from anaesthesia stage, the cages were maintained on a heating stand (KDH Hot Plate, Kedee, China JinhuaKedi Co. Ltd.) thus ensuring a 37 °C temperature. Fig. 1 illustrates all the steps described above, to be able to perform the percutaneous intracisternal injection model. The image shows a rat of the SAH group.

2.3. Procedures: Sham group

The SAH procedure and all the steps were repeated identically for the creation of a sham group, which was injected with only 100 μl isotonic saline instead of blood.

2.4. Euthanasia and collection of the brain

On day five post-intervention the rats were again anaesthetized in the same manner as described on the first day. Once the degree of sedation-analgesia was ensured, a craniectomy was performed. On exposing the brain, a lethal dose of ketamine (0.01 cc/kg) was injected into the intraperitoneal cavity. When respiratory arrest was detected the brain was rapidly removed. The brain was fixed for a minimum of 18 h in 4% paraformaldehyde, on a tilting system at a temperature of +4 °C. Then, with the brain resting in a methacrylate mould, it was coronally sectioned with razor blades into 1 mm slices. A macroscopic examination was performed on all the brains and the sections to show the presence or absence of pathological findings. Finally, the slices were cut by microtome to