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Research article

Establishment and characterization of porcine focal cerebral ischemic model induced by endothelin-1

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HIGHLIGHTS

- The endothelin-1 induced porcine cerebral ischemia model was less technically challenging and has high animal survival rate, compared to the traditional porcine stroke model.
- Our research provided a more detailed characterization of the endothelin-1 induced porcine model of focal cerebral ischemia.
- We kept the pigs alive for 72 h and evaluated the change of neurological behavior and the brain lesion monitored by MRI.
- We concluded that it could be a promising candidate for further cerebral stroke research.

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ABSTRACT

Background and purpose: Due to well-developed Circle of Willis in pigs, it is technically challenging to make persistent focal ischemic stroke based on occlusion of cerebral arteries. Endothelin-1 could cause a focal lesion by forcing transient but strong vasoconstriction in the circumscribed injected area. Its use in porcine stroke model has drawn attention lately. However, all the porcine endothelin-1 induced models were euthanized soon after surgery. Whether the brain lesion is persistent, and whether they could cause neurological deficit are not known. This research aims to provide a more detailed characterization of endothelin-1 induced porcine cerebral ischemic model by evaluating the change of neurological function and the brain lesion monitored by MRI of the pigs.

Methods: Danish Domestic pigs were randomly divided into two groups: a group receiving endothelin-1 (ET-1 group, n = 6) and a sham group (n = 6). After the fronto-temporal craniotomy, pigs in the ET-1 group received 200 μl endothelin-1 injected within a cortical area of one cm^2; pigs in the sham group received only saline injections. Neurological deficit evaluation and MRI scanning were done 24 h and 72 h after operation. Afterwards, hematoxylin and eosin staining was conducted to detect the morphological characteristics of the damaged brain tissue.

Results: The average performance score in the pigs of the ET-1 group was 9.67 ± 1.03 and 9.00 ± 1.26 respectively, at 24 h and 72 h after surgery, which was significantly higher than that of the pigs in sham group. The brain lesion percentage detected by MRI was $12.26 \pm 0.60\%$, and $10.33 \pm 0.51\%$ respectively, at 24 h and 72 h after surgery in the ET-1 group. Microscopy showed extended pyknotic neuronal perikarya in neurons located in the ischemic area.

Conclusions: The endothelin-1 induced porcine cerebral ischemic model is technically easier, and able to create cerebral ischemia severe enough to cause a functional neurological deficit as well as observable lesions on MRI. It is a suitable model for long-term cerebral ischemia research.

1. Introduction

Cerebral ischemic stroke is one of the worldwide leading causes of death and permanent disabilities in patients, leading to reduced

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life quality and an increased burden on the healthcare system [1]. Thus, exploring new therapeutic agents for stroke prevention or for reduction of sequela has been the purpose of a large number of studies [2–4]. Most experimental studies on ischemic stroke have focused on the triggering factor, subsequent cytotoxic reactions and re-vitalization of brain cells in the penumbra area. These studies were performed on rodents with permanent or temporary occlusion of one of the cerebral arteries followed by an ischemic stroke of various sizes [5]. Up to 80% of ischemic strokes in humans are due to occlusion of part of the cerebral arterial branches. These experimental stroke models seem attractive for studying mechanisms of stroke and the possible effects of new drugs on stroke prevention. However, compared to the human brain, the rodent brain presents differences with respect to size, gray and white matter composition and other key elements of brain architecture. These differences limit transfer of results from studies of neuro-protective drugs conducted on rodents to man.

Because there is higher cerebral anatomical similarity between man and larger animals, such as the pig, more sophisticated brain ischemic models for pigs have been developed [6]. The size of the pig's brain is large enough to allow neurosurgery and in vivo conventional multi-modality imaging using MRI, CT or PET scans [7]. The cerebral circumvolutions of the pig brain are relatively well defined. Additionally, the porcine cerebrovascular system is in general more similar to that of humans. It has a Circle of Willis, but with even more extended branching. The diameter of the posterior communicating artery is comparable to that of the internal carotid artery. One difference however, is that unlike the human cerebrovascular system, the pig's cerebrovascular system has two middle cerebral arteries originating from the internal carotid artery in each hemisphere, one coursing laterally and another rostrally over the olfactory tract [8].

Ligation of the common carotid artery and distal middle cerebral artery occlusion is the most commonly used methods for introducing cerebral ischemia on rodents [9]. In the pig, however, due to the well-developed system of arterial shunts, it is technically challenging to make consistent focal ischemic stroke by direct arterial ligation. Moreover, the operation has a high mortality rate in the following hours or days. Therefore, many methods have been conducted to find a simpler and less traumatic method to induce a focal ischemic lesion. One of these methods is direct intra-cortical injection of a potent vasoconstrictor endothelin-1 (ET-1) through a craniotomy. ET-1 is known to cause a focal lesion by forcing strong vasoconstriction, thus acutely reducing blood flow in the circumscribed injected area [10]. ET-1 is also produced endogenously during ischemic stroke, and known to accelerate overall loss of neurons due to vasoconstriction followed by ischemia. This means that direct intra-cortical injections of ET-1 have the potential of introducing stroke and cell death by sustained vasoconstriction. But as the constriction is not permanent, a reperfusion will follow hours later [11]. In practice, ET-1 is most often injected in the cortical area covered by the middle cerebral artery in microliter volumes to induce very focal brain ischemia [12]. One advantage of this practice to introduce stroke is that it causes highly reproducible infarcts with very low mortality rates. The practice has been tried in both rat and mice experimental models, but the results showed there was a higher efficiency in rats when compared to mice [13]. Recently, the ET-1 induced porcine cerebral ischemia model was used in two studies[14,15]. However, the animals were all euthanized 3 h later. The functional outcome was not evaluated, and the long time prognosis of the pigs was not known.

The objective of the present study was to provide a more detailed characterization of the ET-1 induced porcine model of focal cerebral ischemia, and evaluate the change of neurological behavior and the brain lesion monitored by MRI scans.

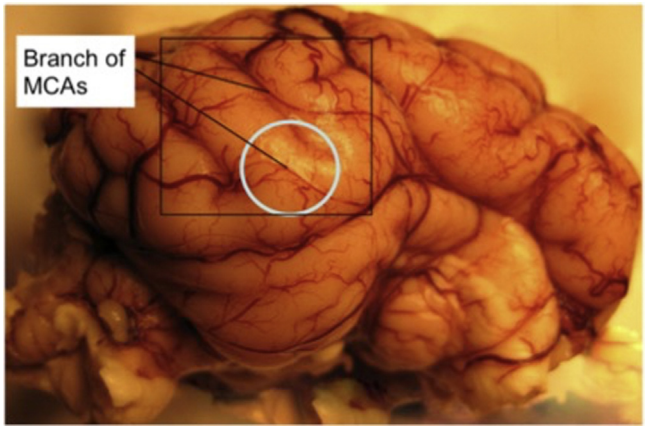

Fig. 1. Following the opening of dura-mater, two branches of middle cerebral arteries were explored. This was taken as the landmark of the injecting area and the specific injection points were around the lower branch of middle cerebral artery, which were more thoroughly explored (shown in white circle). The operation field was shown in the black square.

2. Materials and methods

All procedures were approved by Danish Animal Experiments Inspectorate (no. 2012-15-2934-00132).

2.1. Subjects

12 male Danish Domestic pigs (30–35 kg) were randomly divided into an ET-1 group (n = 6) receiving endothelin-1 injection and a sham group (n = 6) receiving only saline but subjected to same surgical procedure.

2.2. Surgical procedures

The pigs were sedated with an intramuscular injection of 60 mg/kg ketamine hydrochloride, intubated and artificially ventilated with 1.0–1.9% isoflurane in a 2:1 nitrous oxide/oxygen mixture. An arterial catheter was inserted through the right femoral artery. The mean arterial blood pressure (MABP) and respiratory status in each animal were monitored. The pigs were maintained normo-tensive and normo-capnic, and adequately oxygenated. Rectal temperature was maintained at 38 °C using a heating blanket. Haldid was injected intravenously at the rate of 8 mg/min to ameliorate the pain during operation.

Curved skin incision started at the zygoma of the right side 10 mm anterior to the auricle and was extended to above the right orbit. The temporal fascia and muscle were incised and reflected together with the skin flap. Craniotomy (diameter 15 mm) was applied at the anterior aspect of the superior temporal line. The orbital contents were protected with a spatula. The dura mater was opened with a five mm incision and retracted as a semicircular flap, thus allowing direct inspection of the arterial branches on the cortical surface.

A total amount of 200 μl endothelin-1 solution was injected 1–2 mm deep into the brain parenchyma not more than 1.5 mm away from a visible arterial branch. Within that distance the injection was divided into four (50 μl) close locations with two on each side of the artery. The injecting area is shown in Fig. 1. The dura was sutured with 5-0 vicryl to avoid further CSF leakage. The muscle and fascia were reattached and the skin was closed; Buprenorphine (Temgesic, 0.01 mg/kg) was administered for postoperative pain relief.