Journal of Neuroscience Methods 209 (2012) 90–96

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Clinical Neuroscience

A novel embolic stroke model resembling lacunar infarction following proximal middle cerebral artery occlusion in beagle dogs

Sheng Liu[a], Wei-Xing Hu[b], Qing-Quan Zu[a], Shan-Shan Lu[a], Xiao-Quan Xu[a], Lei Sun[c], Wei-Zhong Zhou[a], Hai-Bin Shi[a,*]

[a] Department of Radiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029, China
[b] Department of Neurosurgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029, China
[c] Department of Pathology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029, China

HIGHLIGHTS

- The white thrombus of 1.7 mm in diameter is found to be optimal for producing segmental occlusions in the proximal MCA trunk through ipsilateral ICA injection.
- We generate an ischemic model which can recapitulate the radiologic and histologic changes in lacunar infarcts in beagle dogs.
- We are the first group to study the time course of lacunar infarction in acute phase using diffusion weighted imaging in beagle dogs.

ARTICLE INFO

Article history:
Received 27 February 2012
Received in revised form 6 June 2012
Accepted 10 June 2012

Keywords:
Lacunar infarction
Thrombus
Middle cerebral artery occlusion
Diffusion weighted imaging
Beagle dogs

ABSTRACT

It is estimated that lacunar infarcts account for 25% of all ischemic strokes, but its exact etiology is still on debating. The existing controversies include whether the embolisms can indeed cause lacunar stroke in humans or animal models. We hypothesized that lacunar infarction can be induced by the proximal middle cerebral artery (MCA) segmental occlusion involving the orifices of lenticulostriate arteries in animal models, which have abundant distal cerebral collateral anastomosis. Our work here establishes a proximal MCA occlusion model using thrombi (autologous blood clots about 1.7 mm in diameter and 5 mm in length) in 8 beagle dogs, evaluates the progression of ischemic lesions at 30 min interval within 6 h after embolization using the diffusion weighted imaging (DWI), and discusses the potential mechanisms of lacunar infarction. Our results indicate that the left proximal MCAs can be successfully occluded in all dogs using interventional single-thrombus method. The small solitary or multiple ischemic lesions shown in DWI were observed in the deep brain area, with the mean detecting time of 1.21 ± 0.45 h using DWI and diameter of 6.62 ± 0.60 mm in 6h-DWI after procedure. In conclusion, our method established an ischemic model which can recapitulate the radiologic and histologic changes in lacunar infarcts, suggesting that emboli can cause lacunar infarcts in animal model.

1. Introduction

Lacunar infarction accounts for about 25% of all ischemic strokes, but the exact etiology is still on dabating (Bamford et al., 1987). The intrinsic disease theory of the deep perforating lenticulostriate arteries, including lipohyalinosis, arteriosclerosis, has been emphasized as the most predominant mechanism of lacunar infarction (Jackson and Sudlow, 2005). However, no clinical stroke syndrome is absolutely specific with respect to the pathophysiology, and the clinical value of the lacunar hypothesis has been questioned (Potter et al., 2011). Several investigators observed that intracranial large arteries embolism, or any other form of the endovascular damage involving the orifices of deep penetrating arteries, can cause small deep infarcts (Fisher, 1982; Kloska et al., 2010; Lee et al., 2005, 2006; Min et al., 2000). The controversies exist that whether the embolism has indeed been proven to cause lacunar stroke in humans or animal models (Futrell, 2004).

Reproducible animal models of focal ischemic infarction are crucial in cerebral ischemia study to replicate ischemic damage encountered in human. In contrast to large artery occlusive ischemic stroke, the lack of clarity regarding the pathophysiological events in lacunar stroke makes this subtype of stroke particularly difficult to model (Bailey et al., 2009), which may be contributing to the current debate on the causes of lacunar stroke. Additionally,

* Corresponding author at: Department of Radiology, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Gulou district, Nanjing, Jiangsu 210029, China. Tel.: +86 25 68136299/13601463365; fax: +86 25 83724440.
E-mail address: shihb@vip.sina.com (H.-B. Shi).

0165-0270/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneumeth.2012.06.009

with the advent of diffusion weighted imaging (DWI) and perfusion weighted imaging (PWI), MRI has made the most exciting advances in stroke imaging within the last decade (Lee et al., 2006; Liu et al., 2003; Muir et al., 2006). To date, unfortunately, monitoring the progression of lacunar stroke using MRI has not been broadly studied.

Stroke in humans is highly heterogeneous both clinically and radiographically. The major factor in determining whether an infraction will follow the occlusion of a vessel is the degree of collateral circulation that can develop distally to the point of occlusion before an irreversible ischemic insult occurs (Gerraty et al., 2002; Lee et al., 2009; Lyrer et al., 1997; Min et al., 2000). Here, we hypothesized that lacunar infarction can be induced by the proximal middle cerebral artery (MCA) segmental occlusion using thrombi if the orifices of perforating lenticulostriate arteries are involved in animal models, which have well formed distal cerebral collaterals. This paper will (1) describe generating an embolic lacunar stroke model in beagle dogs using autologous clots by segmental occlusion of the proximal MCA trunk; (2) identify and follow the fate of induced lacunar ischemic lesions after proximal MCA occlusion by using DWI-MR.

2. Methods

2.1. Animal preparation

A total of 8 adult beagle dogs of either sex weighing 12–15 kg were anesthetized with intravenous injection 3 mg/kg of pentobarbital (Pentobarbital Sodium Salt, Chemical Reagent Company, Shanghai, China). The airway was secured using an oral endotracheal tube with spontaneous respiration. Bilateral femoral arterial and left femoral venous accesses were obtained using 5 French sheaths for catheterization, physiological monitoring, and drug administration. Body temperature was maintained at 37–39 °C by using a heating blanket, and eye ointments were applied to animal eyes to prevent cornea dryness during anesthesia, procedure and recovery. Strict sterile techniques were utilized in all cases, and intramuscular ampicillin (20 mg/kg) was given to experimental animals daily for up to 7 days. These protocols in accordance with European Communities Council Directive were reviewed and approved by our institutional Animal Care and Use Committee (Nanjing Medical University, Nanjing, China).

2.2. Embolus preparation

After placement of the sheaths, 10 ml venous blood sample was taken and centrifugated at 4000 rpm for 10 min. Then the upper layer serum (about 4 ml) was taken out and mixed with 1.0 ml (250 IU/ml) of bovine thrombin (Yige Pharmaceutical Co Ltd, Hunan, China). This mixture was aspirated in liquid phase and immediately injected into a sectioned 8F guiding catheter (20 cm long and approximate 2.5 mm inner diameter). After maintaining at room temperature for 2 h, the threadlike clot was removed from the catheter and washed for three times with normal saline solution. The coagulated and dehydrated column clots measuring about 1.7 mm in diameter then were cut into pieces with 5 mm in length.

2.3. Procedure

A standard 5F vertebral catheter (Terumo Medical Corporation, Somerset, NJ, USA) was introduced through the right femoral arterial sheath. Catheter manipulations were monitored using X-ray (Axiom Artis; Siemens AG, Muenchen, Germany) to the bilateral vertebral arteries, external carotid arteries (ECAs), and internal carotid arteries (ICAs). A 4–6 mL radiographic contrast medium (Omnipaque 300; GE Healthcare, Shanghai, China) was injected for angiography. DSA was performed to visualize the intracranial vasculature and obtain baseline angiograms. After a full cerebral arteries angiogram, catheter was inserted into the left ICA about 2 cm in the ascending part. Then one piece of premade whitish thrombus with high fibrin content was transferred into the hub of a 10 ml syringe filled with normal saline, and attached directly to the hub of the catheter placed in the left ICA. When the clot was injected into the catheter, a 2 ml syringe with contrast medium was then exchanged and injected slowly using intermittent pressure on the syringe. After the clot went into the left proximal MCA, additional two to three milliliters of saline were injected to douche the thrombus. Confirmatory DSA was performed to document the presence and site of the occlusion 0 h, 24 h, and 7 days after the procedure, respectively. It usually took about 20 min to finish the embolization procedure. Then the animals were transported to the MR scanner in the same suite as the procedure room.

2.4. Evaluation of embolization

2.4.1. MR imaging studies

MRI examinations were started 30 min after embolization in a 3.0-T whole-body MR scanner (Magnetom Trio, Siemens Medical Solutions, Erlangen, Germany), using a transmit-receive extremity coil with a diameter of 15 cm. The imaging protocols consisted of an axial and coronal turbo spin echo T1-weighted (TR/TE 1390/11, matrix 320 × 320), T2-weighted (TR/TE 5000/66, matrix 320 × 320), and diffusion-weighted (TR/TE 5500/96, 192 × 192 matrix, *b* values of 50 and 800 s/mm^2) sequences with 22 sections and no skip 2-mm section thickness. These above serial scannings were repeated at 30-min intervals to track the evolution of diffusion changes in the stroke region until 6 h after procedure. Then the MR images were obtained at 24 h and 7 days after embolization using the same parameters. Image J (National Institutes of Health, Bethesda, MD) was used for infarct volume calculation from coronal DWI (6 h) and T2-weighted MR images (24 h and 7 days) by two independent observers (Lu and Zhou).

The recent lacunar infarction in beagle dogs was defined as round or ovoid lesion (single or multiple) of increased signal on DWI, T2, or of white area on gross pathological specimens view of 2,3,5-triphenyltetrazolium chloride (TTC) staining, measuring < 10 mm in maximal diameter located in the cerebral hemispheric basal ganglia or white matter. This definition was described by Potter et al., with some modifications according to the relatively smaller volume of dog's brain compared with humans in our study.

2.4.2. Neurobehavioral scoring

Neurobehavioral scoring was performed using a standardized categorical rating scale as described previously (Kang et al., 2007; Purdy et al., 1989). This scoring system was used to evaluate motor function (no deficit = 1, hemiparetic but able to walk = 2, stands only with assistance = 3, hemiplegic and unable to stand = 4, comatose or dead, not testable = 4), consciousness (normal = 1, mildly reduced = 2, severely reduced = 3, comatose or dead = 4), head turning (absent = 0, posturing and turns toward side of infarct = 1, unable to lift head, comatose, or dead = 1), circling (absent = 0, present = 1, does not walk or dead = 1), and hemianopsia (absent = 0, present = 1, unable to test because of reduced consciousness or death = 1). Each dog was assessed by 2 independent observers (Zu and Xu) prior to anesthesia, 24 h after embolization, and then daily until euthanasia at 7 days.

2.4.3. Pathological examinations

Seven days after inducing of focal cerebral ischemia, the animals were euthanatized by intravenous overdose pentobarbital sodium (80 mg/kg). The entire brain was taken and soaked in ice-cold saline for 10 min, and cut into 2-mm thick serial coronal sections in a