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Critical differences between two classical surgical approaches for middle cerebral artery occlusion-induced stroke in mice

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HIGHLIGHTS

- Middle cerebral artery occlusion (experimental stroke) in rodents is achieved by passing a filament up either the external or common carotid artery.
- These entry points, however, result in different outcomes in mortality, neurological scores, inflammation and infarct volume.
- Differences in perfusion of the ipsilateral hemisphere may account for these differences.

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ABSTRACT

Background: Stroke is the third leading cause of death and the leading cause of long-term disability in North America. On average, someone in the US has a stroke every 45 s, and worldwide, stroke claims 15 million lives each year. Therefore, reliable stroke models are vital to the production of effective new therapies for the treatment of this devastating cerebral vascular accident.
New method: Middle cerebral artery occlusion (MCAo) is considered to be the most clinically relevant surgical model of ischemic stroke, in which a variety of methods may be employed to block the MCA (the most common being through insertion of a monofilament). In this study, we have compared two different approaches that are currently used arbitrarily in various laboratories worldwide: one involving insertion of a monofilament via the common carotid artery (Koizumi et al.) and one via the external carotid artery (Longa et al.).
Results and comparisons with existing methods: We assessed various parameters, including: mortality rates, neurological scores, inflammation levels, cellular trafficking (using intravital microscopy) and infarct volumes in mice after using each of the two approaches. We found that the Longa method produced a greater, and robust, inflammatory response, versus the Koizumi method.
Conclusions: In conclusion, we suggest that the Longa method is superior for the study of both short and long-term outcomes of ischemic stroke. These results have considerable implications on stroke model selection for researchers.

1. Introduction

Nearly 800,000 people per year suffer from a stroke in the USA, often with devastating consequences for patients and their families. With no widely effective therapy available, annual expenditure is estimated at $36.5 billion (Go et al., 2014). Reproducible, clinically relevant animal models for the disease are therefore vital to aid the development of new treatments, and optimization of these models is continually sought.

Ischemic strokes in humans (87% of total strokes, others being intracerebral hemorrhage and subarachnoid hemorrhage, 9% versus 3%, respectively) arise most commonly from blockage of the middle cerebral artery (MCA). This is due to disruption of the laminar flow of blood entering the head when it arrives at the pronounced curve at the root of the MCA. MCA occlusion (MCAo) is therefore the "gold standard" in surgical ischemic stroke models. For over 20 years, MCAo has been achieved through passing either a silicon-tipped or flame-blunted monofilament rostrally toward the start of the MCA, via the blood vessels of the neck (Fig. 1). Two approaches to this are typically used: Koizumi's method (Koizumi et al., 1986) and Longa's method (Longa et al., 1989). These have been cited directly 205 and 8472 times, respectively, although owing to varied citation practices among researchers, this is likely to be a vast underestimation of their prevalence.

Abbreviations: MCA/MCAo, middle cerebral artery/occlusion; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery.

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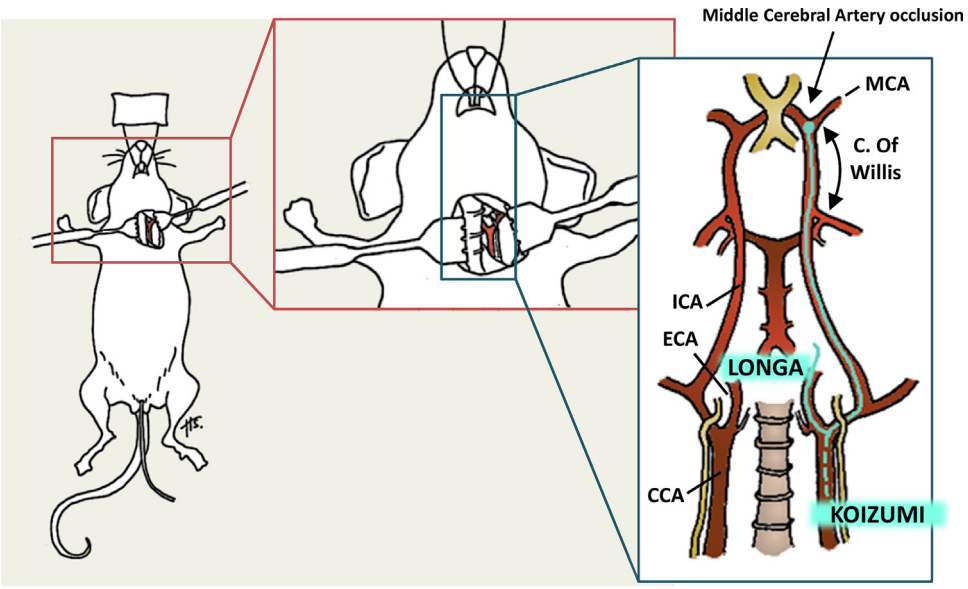

Fig. 1. Schematic showing the location of MCAo surgery and large-vessel vasculature of the cerebral circulation. MCA, middle carotid artery; C. of Willis, Circle of Willis; ICA, interior carotid artery; ECA, external carotid artery; CCA, common carotid artery; L, Longa method of monofilament insertion (via ECA); K, Koizumi method of monofilament insertion (via ICA).

In 1986, Koizumi and colleagues developed a technique in which a silicon-tipped monofilament is passed through an incision in the common carotid artery (CCA), then advanced through the internal carotid artery (ICA) and up to the Circle of Willis, before halting at the entrance of the MCA. Conversely, in 1989, Longa and colleagues used a flame-blunted monofilament, which enters through an incision in the ECA, before being turned around and guided up the ICA (Fig. 1, K = Koizumi filament route, L = Longa filament route). In Koizumi's method, the CCA on the side of the surgery must be permanently tied if the filament is removed to prevent bleeding from the incision in the CCA, while in Longa's method it is the ECA that must be permanently tied. The Circle of Willis is a complete circle connecting vasculature from either side of the brain, and therefore a permanent tie around the CCA should not affect the ipsilateral brain from receiving blood flow from the contralateral carotid arteries.

While use of a silicon-tipped monofilament is widely considered superior in generating reproducible MCAo (due to incomplete occlusion and/or subarachnoid hemorrhage associated with flame-blunted monofilaments) (Tsuchiya et al., 2003), both routes of access (CCA and ECA) are still used regularly and arbitrarily (Bracko et al., 2014; Gavins et al., 2007; Goldmacher et al., 2013). In developing our own parameters for reproducible MCAo in our group, we have discovered profound differences in outcome depending on how the filament is inserted, that is, after using the Koizumi method versus the Longa method. In this study, we discuss considerable differences in mortality and infarct volumes in mice following each of these two methods for filament insertion. In addition, an in-depth stroke scoring system is used to assess behavioral outcome, and varying levels of inflammation in the brain microvasculature are observed in real-time using intravital fluorescence microscopy. Finally, we provide preliminary data that offer an explanation for these significant differences that will be important in all laboratories employing this technique.

2. Materials and methods

2.1. Mice

Male C57BL/6 mice weighing 25–29 g (Jackson Laboratories, Ltd.) were maintained on a standard chow pellet diet with free access to water, under a 12 h light/dark cycle in individually ventilated cages. Animal experimental procedures were approved by the LSUHSC-S Institutional Animal Care and Use Committee and are in compliance with the guidelines of NIH.

2.2. Middle cerebral artery occlusion and reperfusion

Mice underwent 30 min MCAo followed by 24 h or 1 week (wk) reperfusion. Sham-operated animals received all the surgery up until filament insertion. Anesthesia was induced using ketamine/xylazine (150 and 10 mg/kg) and mice were placed in a supine position on a temperature regulated heat mat, with body temperature maintained at 36.5 ± 0.1 °C (dropping 0.5 °C from physiological body temperature enabled temperature to be maintained very tightly versus 37 °C). A midline incision was made at the neck, and the submandibular glands retracted to expose the major arteries on the neck. The CCA and ECA were carefully separated from the surrounding tissue, including the vagus nerve. At this point the surgeries were continued as described by Koizumi (A) or Longa (B).

(A) Koizumi

A permanent tie using 6-0 nylon suture (used throughout for ties) was made around the ECA as well as smaller vessels extending from it. A permanent tie was made around the CCA distal to its bifurcation and a loose tie was made around the CCA proximal to its bifurcation. A microvessel clip was positioned on the ICA and pterygopalatine artery. A small incision was made in the CCA and a 180 μm silicon-tipped monofilament passed into it. The loose tie was secured around the CCA with the filament inserted, the microvessel clip was removed and the filament guided up the ICA until resistance was felt, approximately 9–10 mm beyond the bifurcation of the CCA. After 30 min MCAo-induced ischemia, the filament was removed and the tie around the CCA proximal to the bifurcation secured. Skin wounds were stitched and animals observed throughout recovery from anesthesia in ambient 30 °C.

(B) Longa

A temporary tie was made around the CCA. A permanent tie was made around the ECA and smaller vessels extending from