Journal of Neuroscience Methods 213 (2013) 63–69

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

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

Basic Neuroscience

Concurrent middle cerebral artery occlusion and intra-arterial drug infusion via ipsilateral common carotid artery catheter in the rat

Jessica A. Van Winkle*, Bo Chen, I.-Farn Lei, Benedict Pereira, Padmesh S. Rajput, Patrick D. Lyden

Department of Neurology, Cedars-Sinai Medical Center, 8730 Alden Drive, Los Angeles, CA 90048, United States

HIGHLIGHTS

- We describe a novel method to deliver test substances directly into the ischemic area of the brain during stroke in rats.
- This method provides a powerful tool in the pre-clinical testing of ischemic stroke therapies.
- We validate our method by comparing the amount of dye present in the ischemic brain after intra-arterial verses intravenous administration routes.
- We have successfully used this method in over 100 rats.

ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised form
17 November 2012
Accepted 10 December 2012

Keywords:
Cerebral ischemia
Stroke
Middle cerebral artery occlusion
Intraluminal filament
Drug delivery

ABSTRACT

Pre-clinical development of therapy for acute ischemic stroke requires robust animal models; the rodent middle cerebral artery occlusion (MCAo) model using a nylon filament inserted into the internal carotid artery is the most popular. Drug screening requires targeted delivery of test substance in a controlled manner. To address these needs, we developed a novel method for delivering substances directly into the ischemic brain during MCAo in the awake rat. An indwelling catheter is placed in the common carotid artery ipsilateral to the occlusion at the time of the surgical placement of the occluding filament. The internal and common carotid arteries are left patent to allow superfusion anterograde. The surgeries can be completed quickly to allow rapid recovery from anesthesia; tests substances can be infused at any given time for any given duration. To simulate clinical scenarios, the occluding filament can be removed minutes or hours later (reperfusion) followed by therapeutic infusions. By delivering drug intra-arterially to the target tissue, "first pass" loss in the liver is reduced and drug effects are concentrated in the ischemic zone. To validate our method, rats were infused with Evans blue dye either intra-arterially or intravenously during a 4 h MCAo. After a 30 min reperfusion period, the dye was extracted from each hemisphere and quantitated with a spectrophotometer. Significantly more dye was measured in the ischemic hemispheres that received the dye intra-arterially.

1. Introduction

As more treatments for human stroke fail in clinical trials (Tymianski, 2010), indictments of pre-clinical stroke models have emerged. For example, putative stroke treatments are often given to test animals immediately after ischemia onset, an impossible clinical scenario (Fisher and Tatlisumak, 2005). Patients are not anesthetized during stroke, and thus pre-clinical models must be developed to allow drug infusions in awake animals because anesthetic have neuroprotective effects (Kawaguchi et al., 2005). Study sample sizes may be constrained because test substances might be in short supply; therefore, to allow larger group sizes, intra-arterial drug delivery could enable testing with much lower quantities in each subject.

Conventional methods of drug screening in an animal model of stroke are often conducted by occluding the middle cerebral artery (Macrae, 2011) and delivering the test substance by intravenous or intraperitoneal injection. These methods allow high-throughput modeling, as they are easy to perform, but the obvious problem is that the distribution of the test substances are unpredictable and often dependent on peripheral drug metabolism (Alavijeh et al., 2005). A large quantity of test substance will be metabolized or excreted or both by the liver or kidney before reaching the brain. As a result, a larger dose of test substance is needed to achieve an effective level in the brain. Such larger doses might be accompanied by side effects and complications in other tissues. To solve these issues, there is a need to develop a simple model that allows target delivery of test substances to the brain in a controllable manner. Here we establish a new method for implanting a catheter in the

* Corresponding author. Tel.: +1 310 423 7612.
E-mail address: jessica.vanwinkle@cshs.org (J.A. Van Winkle).

0165-0270/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneumeth.2012.12.004

carotid artery that will deliver test substances intra-arterially to ischemic brain.

2. Materials and methods

2.1. Animals and Evans blue preparation

Adult male Sprague Dawley rats weighing 290–310 g (obtained from Harlan Laboratories, San Diego, CA) were used in this experiment. Animals were housed under standard conditions (21–23 °C, 12 h light–dark cycle) with unlimited access to standard food and water. All procedures were carried out in accordance with the Institutional Animal Care and Use Committee guidelines. A solution of 4% Evans blue (40 mg/mL) was prepared in sterile 0.9% sodium chloride and was protected from light.

2.2. Pre-surgical preparation

2.2.1. Suture and catheter preparation

4-0 Ethilon monofilament suture was heat blunted by waving each filament briefly through a flame; the tip diameter was measured under a microscope (Fig. 1A) and recorded for later use. To prepare the infusion catheter, 60 cm of 2 French silicone tubing (0.3 mm ID × 0.6 mm OD; Access Technologies; Cat. No. BC-2S) was attached to a 27 guage female luer stub adaptor (Access Technologies Cat. No. LSA-27). The adaptor was then attached to a 1 mL syringe filled with saline. Two 3 French retention beads (Access Technologies; Cat. No. RB-3S) were sleeved over the distal end of the catheter. The plunger was depressed to expel all of the air and load the catheter line with saline. A bevel was cut on the distal end of the catheter to aid in insertion into the vessel (Fig. 1B).

2.2.2. Animal preparation

Twenty-one animals were weighed and randomly assigned to one of four groups: intra-arterial (IA) infusion during MCAo ($n = 8$), intravenous (IV) infusion during MCAo ($n = 8$), IA infusion without MCAo (IA sham group, $n = 2$), and IV infusion without MCAo (IV sham group, $n = 3$). To increase the likelihood of successful MCA occlusion (a known issue with this method), for each animal a heat blunted filament was selected based on the previously measured tip diameter, ranging between 290 and 310 μm. Through extensive trial-and-error, we found a 1:1 ratio to be the most reliable method to select suture size, i.e., a 300 μm suture tip would be used with a 300 g rat. The suture was marked 1.7 cm from tip using a wax pencil. This mark served as a visual aid for placement depth when advancing the suture up the internal carotid artery (ICA) later. Anesthesia was induced with 4% isoflurane, in 70% N_2O and 30% O_2, in a closed plastic induction box in a chemical fume hood. Once induced, the animal was then transferred to the surgery table and fitted into a nose cone with a bite bar for stabilization throughout surgery. The vacuum line for gas scavenging was set to 5 L/min. Isoflurane was then reduced to 2–2.5% for maintenance. An empty 10 cc syringe was placed under the neck to keep the airway open and to aid in the exposure of the carotids. The anesthetized animal then received intraperitoneal (IP) injection of 0.05 mg/kg atropine and 0.4 mg/kg carprofen subcutaneously (SQ) and the eyes were lubricated with a petrolatum ophthalmic ointment. A lubricated thermometer was then inserted into the rectum for the servo-controlled warming blanket to regulate core body temperature to 37 °C. The fur on the ventral cervical area from the mandibles to the sternum was shaved. Another area dorsally between the shoulder blades also was shaved (catheter exit site). From both areas, loose hair was removed with a lint roller, and the skin swabbed with betadine, followed by 70% alcohol. Sterile instruments were organized so that they were within easy reach. All materials and the surgical procedures were performed under sterile conditions.

2.3. Reversible middle cerebral artery occlusion surgery

2.3.1. Anesthetic depth assessment

Before starting surgery, the depth of anesthesia was assessed by pinching the interdigital skin. If no response was elicited, the surgery was started. If the animal responded, the isoflurane was slightly increased (0.5%), a minute would elapse and the interdigital skin was pinched again.

2.3.2. Approach

Under the operating microscope with 7× magnification, a midline skin incision was made cranial to the sternum. Blunt dissection was used to expose the intersection of the digastric, sternohyoid, and sternomastoid muscles (Fig. 2). This triangular intersection reliably marks the underlying carotid bifurcation. Cotton tip applicators were used to blot any blood from small vessels. A retractor was positioned so that the sternomastoid and mandibular glands were gently retracted laterally. Blunt dissection was used in the center of the intersection to expose the pulsating common carotid artery (CCA) directly underneath. 4-0 silk was then looped around the belly of the omohyoid muscle that lies directly over and diagonal to the CCA to retract the muscle medially (Supplementary Fig. S1). The weight of hemostats clamped on the ends of the suture kept the muscle retracted medially to improve the exposure of the CCA. The external carotid (ECA), the ICA, and the CCA were bluntly dissected and the surrounding fascia was carefully separated.

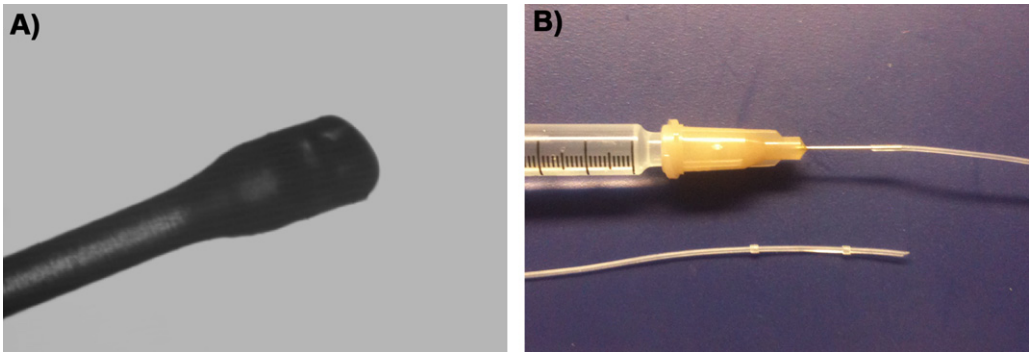

Fig. 1. (A) 4-0 Ethilon monofilament with tip heat blunted. The widest part of this particular occlusion monofilament measures 308 μm. (B) Catheter set-up. Top: Saline filled 1 cc syringe with a 27 gauge luer stub adaptor and catheter attached. Bottom: 2 French silicone catheter (0.3 mm ID × 0.6 mm OD) with 3 French retention beads sleeved over the distal beveled end.