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Selective intra-arterial drug administration in a model of large vessel ischemia

Michael Maniskas[a,c], Gregory Bix[a,c,d,*], Justin Fraser[a,b,d,e,**]

[a] Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY, United States
[b] Department of Neurosurgery, University of Kentucky, Lexington, KY, United States
[c] Sanders Brown Center on Aging, University of Kentucky, Lexington, KY, United States
[d] Department of Neurology, University of Kentucky, Lexington, KY, United States
[e] Department of Radiology, University of Kentucky, Lexington, KY, United States

HIGHLIGHTS

- Demonstrate reliable and reproducible mouse stroke model similar to human condition.
- Retro-engineered intra-arterial drug delivery model for mice from human condition.
- Optimized drug injection volume and flow rate for intra-arterial delivery model.

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ABSTRACT

With continuing disconnect between laboratory stroke treatment models and clinical stroke therapy, we propose a novel experimental model to study stroke and vessel recanalization that mirrors acute management of large vessel stroke, with concomitant directed pharmacotherapy. Using the tandem transient ipsilateral common carotid/middle cerebral artery occlusion (MCAO) model to induce stroke in mice we then added selective intra-arterial (IA) drug administration for directed pharmacotherapy. The IA model uses micro-angio tubing placed at the bifurcation of the CCA to selectively administer the drug to the internal carotid distribution. We have shown that delivery of pharmacotherapy agents selectively through an IA injection is feasible in a mouse model, which will permit studies involving pharmacotherapy, transgenic modification, and/or a combination. Our IA model has similarities to previously published models of IA injection but differs in that we do not leave an indwelling micro-port or catheter in our animals, which is not clinically relevant as it does not reflect the human condition or current clinical management. Furthermore, we optimized our model to selectively direct therapy to the ipsilateral, stroke affected hemisphere. By developing an IA drug delivery model that mirrors clinical conditions, we are bridging the gap between basic stroke research and what is standard practice in acute ischemic stroke intervention. The IA model of drug delivery can target agents directly to the site of injury while blunting systemic effects, dose penetration issues, and administration delay that have plagued the intraperitoneal and oral drug administration models.

1. Introduction

Large vessel ischemic stroke, which affects the vital arteries of the brain, is a leading cause of morbidity and mortality in the United States. At present, the only FDA-approved pharmacotherapy for thrombolysis in acute ischemic stroke is administration of tissue plasminogen activator (tPA), which acts to dissolve the clot, allowing blood flow to resume. The therapeutic window for intravenous administration of tPA is 4.5 h after onset, and when combined with additional exclusion criteria, a large number of individuals are eliminated from receiving treatment. Results from a multicenter study

* Corresponding author at: Associate Professor of Anatomy and Neurobiology, and Neurology, Paul G. Blazer, Jr. Professor of Stroke Research, University of Kentucky, Sanders Brown Center on Aging, Room 430, 800 South Limestone Street, Lexington, KY 40536, United States. Tel.: +1 859 218 3859; fax: +1 859 218 2866.
** Corresponding author at: Assistant Professor of Cerebrovascular, Endovascular, and Skull Base Surgery, Director, Cerebrovascular Surgery, Department of Neurological Surgery, University of Kentucky, 800 Rose Street Room MS108A, Lexington, KY 40536, United States. Tel.: +1 859 323 0616; fax: +1 859 257 8902.
E-mail addresses: gregorybix@uky.edu (G. Bix), jfr235@uky.edu (J. Fraser).

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between 2001 and 2004 evaluating the rate of tPA administration demonstrated an increase in tPA use from 14.0% to 37.5% (Lichtman et al., 2009). While these results are positive it shows a need for alternative therapies that can be administered when an individual is not eligible for tPA. One such alternative is intra-arterial (IA) endovascular thrombectomy or mechanical removal of a clot using a retrieval device threaded through the patient's vasculature (Investigators, 2007; Saver et al., 2012). Both forms of thrombolysis have the potential to restore blood flow to the affected area but show poor correlations with clinical outcomes (Investigators, 2007; Broderick et al., 2013; Fargen et al., 2013). Currently there is no therapy, thrombolysis included, that provides direct neuroprotective or neuroreparative effects.

The practice of endovascular thrombectomy utilized by a neurointerventionalist to remove a clot begins with the advancing of a catheter within the femoral artery in the leg. From there the catheter is threaded through the arterial vasculature of the body until it reaches the common carotid artery (CCA) which leads to the occluded middle cerebral artery (MCA) via the internal carotid artery (ICA). Navigating the catheter into the occluded internal carotid, basilar, or middle cerebral arteries provides access to not only remove the clot but to also deliver potential neuroprotective/neuroreparative compounds. Such a stroke treatment paradigm has many potential advantages over systemic routes of administration including better affected brain penetration/targeting and less systemic side-effects.

There has been a failure to bring neuroprotective agents successfully to the bedside in the treatment of acute ischemic stroke. The cause is multifactorial, but is best summarized as a failure of translation from the bench to clinic. Our approach to this topic was to retro-engineer a mouse model to mirror the clinical condition of intra-arterial (IA) thrombectomy with the opportunity for direct selective IA pharmacotherapy immediately following recanalization. In our method, a catheter is advanced through the vasculature of the mouse neck into the ICA so the potential drug delivery can be selective. Our model is different in that we initiate a stroke, recanalize and then inject IA therapeutics without leaving an indwelling microport or catheter (Chen et al., 2009; Van Winkle et al., 2013). Direct IA administration of neuroprotective agents represents a novel method of drug delivery for acute stroke. Coupled with recanalization and restoration of blood flow, this method mimics clinical practice.

Furthermore, because our mouse model is based on the clinical treatment of stroke, it was also essential to verify that our model could be effectively utilized to deliver compounds directly and specifically to the site of ischemia. The efficacy of selective delivery of potential pharmacotherapies is dependent upon optimized flow rate and injection volume. The study of flow rate and injection volume through carbon black injection allowed us to demonstrate that a compound administered in such a fashion could reach the affected area and its injection rate and volume could be optimized for our model. Through this retro-engineered model, flow rate and injection volume study we hope to overcome many of the hurdles that have long plagued potential pharmacologic agents in acute ischemic stroke.

2. Materials and methods

2.1. Nylon suture, metal wire, micro-angio tubing and syringe preparation

Two different sized sutures were used: one 2-0 nylon monofilament suture cut 2 cm in length for occlusion of the CCA and three 6-0 nylon braided silk sutures cut 1 cm in length for permanent occlusion of the external carotid artery (ECA) and securing of the micro-angio tubing. Metal wire (Small Parts, Logansport, IN) 0.0127 cm in diameter were cut to a length of 0.1–0.15 cm. Using micro forceps under a dissecting microscope, a 34 gauge needle (Hamilton Syringe Co., Reno, NV) was fitted with a 10 cm length of micro-angio tubing (MRE 010-Braintree Scientific, Braintree, MA), the combined needle and tubing were then attached to a 100 μl Hamilton Gas Tight syringe (Fig. 1C).

2.2. Animal preparation for surgery

In accordance with University of Kentucky guidelines, 3 month old C57/Bl6 (Jackson Laboratory) mice were anesthetized via intraperitoneal injection with a combination Ketamine/Xylazine mixture (1:1.33) in a saline solution using a weight based dosing scale. The mice were shaved on the left temporal region of the head from the lateral corner of the left eye to the medial region of the left ear with the resultant shaved region being 0.75 cm by 0.75 cm (Fig. 1A). The shaved area of the cervical and thoracic region started at the angle of the mandible and extended to the apex of the ribcage with a width spanning from forelimb axillary region to the opposite forelimb axillary region (Fig. 1B). The shaved areas were cleaned 3 times with alcohol prep pads followed by a cotton tipped applicator moistened with betadine.

2.3. Surgical preparation

Once the mouse was fully anesthetized it was placed on a heated (to control body temperature) elevated platform. With the mouse in the supine position, the head was secured using teeth restraints and the forelimbs and tail were secured to the platform using surgical tape (Fig. 1A and B).

2.4. MCAO stroke surgery

To induce focal cerebral ischemia, we used the previously described transient tandem ipsilateral CCA/MCA occlusion stroke model (MCAO) (Aronowski et al., 1999; Lee et al., 2011). With the mouse in the supine position a midline incision was made allowing us to isolate and elevate the CCA to place a 2-0 suture underneath. It is important to note that the vagus nerve runs parallel to the CCA and should be separated so that it is not clamped with the CCA. We then removed the surgical tape and rotated the mouse so that it was in a pronated position and re-secured. Another incision was made in the left temporal region for the lateral corner of the left eye to the medial region of the left ear exposing the temporal region. The temporalis muscle was reflected, the MCA verified and a small burr hole drilled to allow the metal wire to be placed under the MCA. The mouse was repositioned to the supine position for temporary clamping of the CCA, (the occlusion time of the CCA/MCA can be varied; our lab occludes for 60 min). Before the head was sutured, blood flow measurements using the Laser Doppler and Speckle were taken using the process detailed in the following section. All surgical sites were temporarily sutured with 4-0 pre-needled nylon suture.

2.5. Laser Doppler and Laser Speckle for blood flow measurement

To measure blood flow in the ipsilateral MCA, a Perimed Laser Doppler flow meter (Periflux System 5000, Perimed) probe was placed directly over the MCA touching the skull, a reading was taken pre-occlusion and the probe was placed in the same spot to take the post-occlusion measurements after insertion of the metal wire under the MCA to confirm a decrease in blood flow. The ipsilateral hemisphere total blood flow was measured using a Laser Speckle (Pericam PSI HR, Perimed) which was positioned 15 cm above the exposed skull of the mouse. The laser sighting system