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A novel mouse model of thromboembolic stroke

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HIGHLIGHTS

- We developed a new mouse model of thromboembolic stroke.
- Stroke induction does not require craniotomy.
- The model requires short surgery time and a single anesthesia exposure.
- Model produces consistent infarction, with low variability and mortality.
- We validated the model using tissue plasminogen activator in two mouse strains.

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ABSTRACT

Background: We previously demonstrated that tissue plasminogen activator (tPA) reduces infarct size after mechanical middle cerebral artery occlusion (MCAO) in wild-type (WT) mice and transgenic mice expressing human leukocyte antigen DR2 (DR2-Tg). Clinically, tPA limits ischemic damage by dissolving the clot blocking blood flow through a cerebral artery. To mimic the clinical situation, we developed a new mouse model of thromboembolic stroke, and tested the efficacy of tPA in WT and DR2-Tg mice.

New Method Autologous blood is withdrawn into a PE-8 catheter filled with 2 IU α-thrombin. After exposing the catheter briefly to air, the catheter is reintroduced into the external (ECA) and advanced into the internal carotid artery (ICA) to allow for intravascular injection of thrombin at the MCA bifurcation. To validate the model, we tested the effect of tPA on laser-Doppler perfusion (LDP) over the MCA territory and infarct size in WT and DR2-Tg mice.

Results: The procedure results in a consistent drop in LDP, and leads to a highly reproducible ischemic lesion. When administered at 15 min after thrombosis, tPA restored LDP and resulted in a significant reduction in infarct size at 24 h after thrombosis in both WT and DR2-Tg.

Comparison with Existing Methods: Our model significantly reduces surgery time, requires a single anesthesia exposure, and produces a consistent and predictable infarction, with low variability and mortality.

Conclusion: We validated the efficacy of tPA in restoring blood flow and reducing infarct in a new model of endovascular thromboembolic stroke in the mouse.

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1. Introduction

Stroke is the fourth leading cause of death and a leading cause of serious, long-term disability in the United States (Towfighi and Saver, 2011). Approximately 87% of acute strokes are ischemic (Go et al., 2013), in which the middle cerebral artery (MCA) is most frequently affected (Gillum, 2002). Ischemic stroke occurs as a result of cerebral thrombosis or embolism. Cerebral thrombosis is the most common type of ischemic stroke (Zhang et al., 2013; Casals et al., 2011), which refers to a thrombus that develops at the clogged part of cerebral vessel due to atherosclerosis of brain arteries, caused by a build-up of fatty deposits inside the blood vessels. An embolus is most often a piece of a thrombus that has broken free and travels to brain usually from an atherosclerotic plaque or heart.

Several stroke models have been developed in rodents to mimic ischemic stroke in humans, including the mechanical (intraluminal filament) occlusion model (Longa et al., 1989; Belayev et al., 1996; Kitagawa et al., 1998; Hata et al., 2000), the embolic (fibrin-rich clot) occlusion model (Overgaard et al., 1992; Busch et al., 1997; Zhang et al., 1997a, b, 2005; Ren et al., 2012) and the photochemical in-situ thrombosis occlusion model (Yao et al., 1996; Cai et al., 1998; Watson et al., 2002; Yao et al., 2003). These models were useful in understanding the consequences of cerebral ischemia, although none reflected the natural course and pathophysiology of cerebral thrombosis (Hossmann, 2012). Zhang et al. (1997c) described a rat thrombotic stroke model, whereby an autologous clot is formed as a result of direct injection of thrombin endovascularly. A mouse model of in situ thromboembolic stroke has also been described, in which an autologous thrombus is directly induced inside the MCA by local microinjection of purified thrombin, resulting in a stable and reproducible infarct volume with low mortality rate (Orset et al., 2007, p. 23; Ansar et al., 2014). However, this model requires craniotomy, penetration of dura mater and may alter intracranial pressure (ICP). Finally, and more recently, an atherothrombotic model of stroke in rats and mice was developed by collagen injection directly into the cerebral circulation (Schunke et al., 2015). Authors note, however, that in the mouse, the model produces multifocal strokes with variable size infarcts.

In the present study, we developed a reproducible thromboembolic mouse model with low mortality and variability, which requires short surgery time and a single anesthesia exposure. The model, which is based on the endovascular introduction of autologous blood and thrombin, was used in wild-type (WT) mice and mice expressing human leukocyte antigen DR2 transgene (DR2-Tg), to test the effect of thrombolysis by intravenous administration of recombinant tissue plasminogen activator (tPA) on infarct size and cerebral blood flow (CBF) recovery. We have previously demonstrated that Recombinant T Cell Receptor Ligands (RTL), partial MHC class II (pMHC) molecules covalently bound to myelin peptides, reverse brain tissue infiltration by leukocytes, which contributes to increased infarct size and worse outcome after middle cerebral artery occlusion (MCAO) (Subramanian et al., 2009). However, the potential application of RTL therapy to human stroke is limited by the need to match recipient MHC class II with the pMHC construct. In order to test the efficacy of human pMHC in experimental stroke, we used humanized transgenic mice, which express the human MHC class II region carrying the *HLA-DR2* haplotype (DR2-Tg mice). For RTL therapy to be used in humans, it has to be combined with tPA, the standard stroke therapy. Therefore, in addition to using wild-type (WT) in the current study, we also validated tPA therapy in DR2-Tg mice, in anticipation of combining tPA with pMHC therapy in future studies.

2. Materials and methods

2.1. Experimental animals

All animal procedures were conducted in accordance with the National Institutes of Health guidelines for the use of animals in research, and protocols were approved by the Animal Care and Use Committees at Oregon Health & Science University and the Portland Veteran Affairs Medical Center.

Male C57BL/6 mice (WT, 10–14 weeks of age, 23–28 g body weight) were purchased form Jackson Laboratory (Sacramento, CA, USA) and male HLA-DRB1*1502 transgenic mice (DR2-Tg, 10–14 weeks of age and weighing 23–28 g), which are also on a C57BL/6 background, were produced at the Portland VA Medical Center using foundation breeders provided by Dr. Chella David (Zhu et al., 2014). The mice were housed in temperature-controlled rooms on a 12-hour light and 12-hour dark cycle with water and food ad libitum. Mice were randomized to one of the following experimental groups: sham surgery, control (experimentally naïve) group and a treatment group (vehicle or tPA). Surgeons were blinded to treatment groups.

2.2. Pre-surgery catheter preparation

The tip of a PE-8 tubing (inner diameter (I.D.): 0.20 mm, outer diameter (O.D.): 0.35 mm, Strategic Applications Inc., Lake Villa, IL, USA) was stretched under a heating lamp to further reduce its O.D. to 0.23–0.25 mm. This will also smooth the catheter's tip and make it more flexible, decreasing the risk of damaging vessel wall by the rigid tubing and sharp tip. A 31-gage small hub removable needle (Hamilton Co., Reno, NV, USA) was inserted into a 15-cm long modified PE-8 catheter and attached to a 50 μL Hamilton syringe (Model 1705 RN SYR, Reno, NV, USA). Bovine α-thrombin (10 μL, 0.2 NIHU/μL; T7513, Sigma-Aldrich, Co. LLC, St. Louis, MO, USA) was filled into catheter and syringe and subsequently stored at 4 °C until needed for infusion.

2.3. Animal model

Mice were anesthetized with 5% isoflurane for induction, and anesthesia was maintained with 1.0–1.5% isoflurane in