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Study Protocol

A Video-Documented Mouse Model of Internal Capsule Stroke Induced by Photothrombotic Method Using Fiber Optic Technology: A Novel Approach

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Abstract
The internal capsule is a critical structure that is highly susceptible to ischemic injury, yet traditional stroke models often fail to isolate and precisely target this region. Laser-based methods offer a novel approach for inducing controlled, localized ischemia with high spatial accuracy. This study aimed to develop a precise mouse model of internal capsule stroke induced by the photothrombotic method using fiber optic technology, providing a reproducible platform for studying ischemic injury and recovery mechanisms in this vital brain region. To this end, an adult male BALB/c mouse was anesthetized, and 150 µg Rose Bengal/g dissolved in normal saline was intraperitoneally injected for photothrombotic ischemia induction. The optic fiber was positioned into the brain's internal capsule using a stereotaxic frame. The laser was activated for 10 minutes to induce ischemia, and stroke was validated three days post-procedure using 2,3,5-triphenyl-tetrazolium chloride staining to confirm lesion location and extent. This novel photothrombotic stroke model provides a powerful and reliable tool for investigating ischemic injury and recovery in the internal capsule. The precision and reproducibility of this method make it a significant advancement over traditional stroke models, with potential applications in understanding stroke pathophysiology and evaluating therapeutic interventions.
Keywords: Ischemic stroke, Internal capsule, Photothrombotic ischemia, Mouse

Introduction

Stroke, an acute neurological event caused by the interruption of blood flow to the brain, is the third leading cause of mortality and the fourth leading cause of disability-adjusted life-year worldwide.[1,2] It has devastating effects on brain function and is a major contributor to long-term disability.[3,4] The internal capsule, a critical area housing sensory and motor networks, is a significant risk site in brain ischemia. Injury to this area can significantly hinder motor coordination, sensory processing, and other essential brain activities. This vulnerability makes the internal capsule a focal point for stroke research, as understanding its response to ischemic damage can offer insights into stroke pathophysiology and recovery.[5-8]

The investigation of stroke predominantly relies on various animal models, such as the middle cerebral artery occlusion and mechanical injury techniques.[9,10] Although these methodologies have yielded significant discoveries, they include inherent limits. They often struggle with precision in targeting specific brain regions, leading to variability in the size and location of induced infarcts.[9-11] These challenges hinder reproducibility and limit the scope of targeted investigations into stroke mechanisms and recovery pathways. The necessity for enhanced models facilitating accurate, localized brain damage assessment has become more evident. By simulating the intricate and regional consequences of stroke, such models would enable researchers to gain a deeper understanding of its effects and possible treatment strategies.

Recent advancements in laser-based technologies have provided new tools for investigating brain function and pathology. The photothrombotic method using fiber optics has emerged as a powerful technique in neuroscience, offering unparalleled spatial and temporal precision.[12,13] This method enables researchers to target specific brain regions with minimal collateral damage, making them ideal for studies that require controlled, reproducible conditions.[14] Photothrombotic models in stroke research signify a substantial advancement, enabling the generation of targeted damage with enhanced consistency and less variability relative to conventional models.[14-16] This accuracy is especially beneficial for examining areas

such as the internal capsule, where little ischemic injury can lead to significant neurological impairments.[5,10]

The present study introduces a novel method for inducing stroke in the internal capsule of the mouse brain using the photothrombotic method via fiber optic technology. This study represents a step forward in stroke research, providing a robust and innovative model that addresses the limitations of existing methods (Supplementary file 1, Chapter 1).

Methods

Preparation and Anesthesia

An adult male BALB/c mouse (8–12 weeks) weighing 20–22 g was maintained in the Neuroscience Research Center of Tabriz University of Medical Sciences for one week for acclimatization. Before and after the surgical treatment, the mouse was housed in a standard cage under a 12-hour light/dark cycle at 23 ± 1 ˚C, with unrestricted access to food and water.

To begin the procedure, the animal was anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (9 mg/kg). The depth of anesthesia was monitored by the absence of pedal reflex, and supplemental doses were administered as necessary.

Following shaving skull hair, a longitudinal incision (1.0–1.5 cm) was generated from surface to depth using a surgical blade to expose coronal and sagittal sutures. The interest site (antero-posterior of -1.58 mm, medio-lateral of + 2 mm, and dorso-ventral of -4.5) was targeted using the stereotaxic atlas of Paxinos and Watson.[17] Before photothrombotic stroke induction, 150 µg sterile Rose Bengal/g dissolved in normal saline was administered per g/body weight and allowed to disseminate into the blood circulation.[18]

Photothrombotic Laser Setup and Ischemia Induction

An optical fiber (200 µm core diameter) coupled with a 523 nm laser diode was used to induce ischemia.[19] The optic fiber was stereotaxically positioned at the target site, and the device was activated for 10 minutes. This photochemical process involving Rose Bengal resulted in localized thrombosis and ischemia confined to the internal capsule.

Validation of Infarction

Three days post-induction, the mouse was euthanized, and its brain was harvested according to the method suggested by Norouzi-Bonab et al.[20] Serial brain sections were prepared and stained with 2,3,5-triphenyl-tetrazolium chloride to visualize and confirm the ischemic lesion (Figure 1) (Supplementary file 1, Chapter 2).[18]

Conclusion

This study has presented an innovative and accurate photothrombotic technique using fiber optic technology for a stroke model that targets the internal capsule in the mouse brain, overcoming the notable limitations of conventional stroke models. Techniques such as middle cerebral artery occlusion or mechanical injury frequently result in inconsistent infarct sizes, non-specific damage, and difficulties in separating effects on tiny, deep brain areas (e.g., the internal capsule). The photothrombotic method utilizing fiber optic technology presents significant advantages, including the capacity to precisely target specific areas, such as the internal capsule, thereby reducing inadvertent harm to adjacent structures and facilitating concentrated investigations of internal capsule ischemia. This approach offers fine control over the time and degree of ischemia damage, enhancing repeatability and dependability across trials. This model enhances the representation of subcortical ischemic strokes, thus bridging the divide between preclinical studies and clinical situations and improving the relevance of findings to human stroke research (Supplementary file 1, Chapter 3).

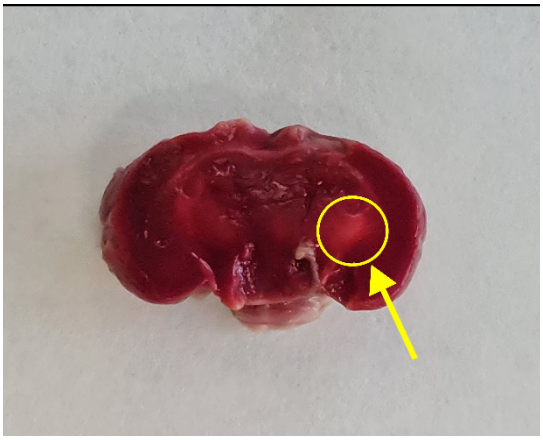

Figure 1. The Ischemic Lesion Side in the Left Internal Capsule of the Mouse*. *Note*. *It is shown with a yellow arrow in the encircled area

Authors' Contribution

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Competing Interests

None.

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Supplementary Files

Supplementary file 1. Video-Documented Mouse Model of Internal Capsule Stroke Induced by the Photothrombotic Method Using Fiber Optic Technology: A Novel Approach (Movie).

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