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# PHOTOTHROMBOSIS COMBINED WITH THROMBIN INJECTION ESTABLISHES A RAT MODEL OF CEREBRAL VENOUS SINUS THROMBOSIS

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**Abstract—*Objective:* Cerebral venous sinus thrombosis (CVST) is a rare but life-threatening disease and an animal model for in-depth study of CVST is needed. This study aimed to develop a rat model suitable for studying clinically relevant aspects of CVST and investigating its dynamic pathophysiological changes during a 7-day period. *Method:* A photothrombosis method was used to create a rat sinus-vein thrombosis model. A spot size-adjustable Diode Pumped Solid State laser (DPSS) combined with thrombin injection occluded the rostral and caudal superior sagittal sinus (SSS). The model was used to evaluate pathophysiological changes at different time points over 7 days. Evans Blue dye injection was used to detect alterations in blood–brain barrier (BBB) permeability. Brain water content was also measured. Moreover, we examined changes in brain infarct volume, neurological function, as well as histology after induction of CVST. *Result:* CVST in rats significantly altered BBB permeability, consistent with the development of brain edema. It was accompanied by an increase in brain infarct volume and deficits in neurological function that began on day 1, peaked on day 2, and typically improved by day 7 due to the neuroprotective effects of angiogenesis and gliocyte proliferation. *Conclusion:* In this study, we describe a rat model that produces clinically relevant pathophysiology and pathology that will facilitate evaluation of therapeutic regimens for CVST. Furthermore, our results indicate a period of optimal clinical intervention for patients with CVST, which may reduce the probability of dependency and death.** 

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*Abbreviations:* 2-VO, 2-vein occlusion; ANOVA, analysis of variance; BBB, blood–brain barrier; CVST, cerebral venous sinus thrombosis; DPSS, Diode Pumped Solid State laser; HE, hematoxylin-eosin; MCAO, middle cerebral artery occlusion; PFA, paraformaldehyde; SEM, standard error of the mean; SSS, superior sagittal sinus; TCA, trichloroacetic acid; TTC, 2,3,5-triphenyltetrazolium chloride; VEGF, vascular endothelial growth factor.

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**Key words: cerebral venous sinus thrombosis, model, blood–brain barrier, edema, photochemical reaction.**

## INTRODUCTION

Cerebral venous sinus thrombosis (CVST) is a rare but life-threatening disease with highly variable symptoms and clinical features accounting for 1% of all strokes (Saadatnia et al., 2009). Due to thrombosis of the cortical veins and major sinuses, brain edema and venous infarction as well as intracranial hypertension frequently occur in patients with CVST, resulting in deep coma, deficits in neurological function, and even death (Stam, 2005).

Recently, animal models have elucidated pathophysiologies such as edema formation, disruption of the blood–brain barrier (BBB) and hemodynamical alterations which allow evaluation of new therapies for CVST (Haggendal and Johansson, 1971; Liszczak et al., 1984; Mayhan and Heistad, 1986; Alexander et al., 1990; Fries et al., 1992; Frerichs et al., 1994; Nakase et al., 1995, 1997, 1998; Hayashi et al., 1997; Forbes et al., 2001; Miyamoto et al., 2001; Röttger et al., 2005b; Siddiqui and Kamal, 2006; Srivastava et al., 2007, 2009a,b; Lo and Rosenberg, 2009; Saadatnia et al., 2009; Krysl et al., 2012; Sawada et al., 2014). However, an in-depth understanding of CVST requires an animal model that has the following three critical features: (i) reproduces the pathophysiology observed in humans with CSVT; (ii) follows the pattern of thrombosis and pathology observed in humans; and (iii) provides a platform for the assessment and development of novel therapeutic approaches illustrated by Rahal et al. (2013).

Previous studies have utilized various techniques to establish animal models, including superior sagittal sinus (SSS) ligation (Schaller et al., 2003), injection of thrombogenic substances (Gotoh et al., 1993; Ungersböck et al., 1993; Frerichs et al., 1994; Kanaiwa et al., 1995; Miyamoto et al., 2001; Wang et al., 2007, 2013; Li et al., 2012), application of $FeCl_3$ (Kim and Schellingerhout, 2005; Röttger et al., 2005a; Srivastava et al., 2007), vascular interventional embolization (Fries et al., 1992; Stracke et al., 2006), as well as photochemical thrombosis (Nakase et al., 1995). Unfortunately, it is difficult to find an animal model that involves thrombosis of both sinuses and veins without permanent ligation of the SSS.

We utilized a method based on existing experimental models, in which photochemical thrombosis combined with thrombin injection created a clinically relevant cerebral sinus-vein model in the rat. This model may provide a better platform for in-depth research on the pathology of CVST and assessment of possible treatments.

The mechanism underlying disruption of BBB permeability following CVST has been investigated in recent studies (Kim and Schellingerhout, 2005; Nagai et al., 2010). Time-dependent, dynamic changes in BBB permeability play an important role in infarct progression and prognosis in patients with venous stroke (Stam, 2005). However, little is known about alterations in BBB permeability over several days. In this experiment, we employed a sinus-vein thrombosis model in the rat to evaluate the effect of CVST on BBB permeability, brain edema, brain tissue infarction, as well as neurological function over 7 days. Our results suggest an optimal period of clinical intervention for patients afflicted with CVST.

## EXPERIMENTAL PROCEDURES

### Animal preparation

Experiments were performed using male Wistar rats (260–310 g, $N = 143$, Animal Experimental Center of Southern Medical University, Guangzhou, China). Procedures that used animals were reviewed and approved by the Southern Medical University Ethics Committee and were in accordance with the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Research Council. Washington, DC: National Academy Press, 1996). In all, 143 rats were randomly divided into three groups: a model group ($n = 66$), a sham-operated group ($n = 66$), and a normal control group ($n = 6$ for brain water content, $n = 5$ for the Evans Blue quantitative test). Rats in the experimental and sham-operated groups were allocated to time point subgroups: day 1, day 2, and day 7 ($n = 22$ per time point in the model group, $n = 22$ per time point in the sham-operated group). In each subgroup, we conducted: (1) measurement of brain water content ($n = 6$); (2) the Evans Blue dye extravasation test ($n = 5$ for quantitative and $n = 3$ for qualitative investigations, respectively); (3) 2,3,5-triphenyltetrazolium chloride (TTC) staining ($n = 4$); histologic examination and immunochemistry ($n = 4$); (4) the rotarod test ($n = 11$, randomly selected from the day 7 group). The animals were maintained on an alternating 12-h light/dark cycle with free access to food and water.

### Induction of CVST

Each rat was anesthetized by intraperitoneal injection of chloral hydrate (36 mg/100 g body weight). The animal was then placed in the sphinx position and fixed within a stereotaxic frame (Stoelting, Wood Dale, U.S.A.). The skull was exposed by performing a 1.5-cm midline skin incision. A high-speed dental drill (Strong-90, Korea) monitored with a surgical microscope (OPTON-Microscope, Zeiss, Wetzlar, Germany) created a cranial window (4 mm × 7 mm) between the lambda and bregma. We avoided thermal injury to the cortex by continuously cooling the drill tip with cold saline. The SSS and bridge veins were gently exposed and care was taken to keep the dura intact. A diagrammatic sketch of the operation is shown in Fig. 1.

We then turned on a spot size-adjustable, stabilized green light Diode Pumped Solid State laser (DPSS) laser system (LE-LS-532-15TAF, LEO Photonics, Shenzhen, China) that was affixed to the gripper of the stereotaxic frame. Green light at 532 nm was directed vertically from the laser device onto the SSS. Irradiation intensity was calculated from the formula: irradiation

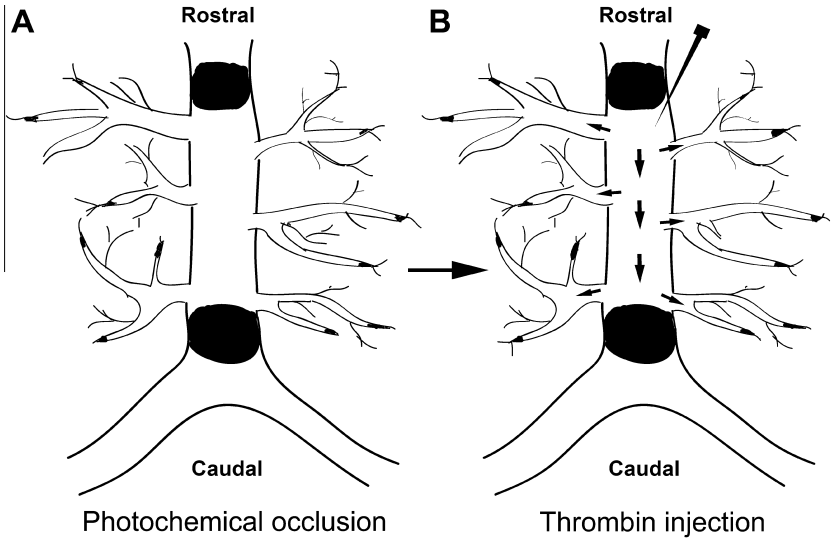


**Fig. 1.** Schematic diagram of the operation procedure. (A) The superior sagittal sinus (SSS) was occluded 0.5 mm rostral to lambda and 0.5 mm caudal to bregma using a Diode Pumped Solid State laser (DPSS) combined with occlusive photothrombosis. (B) Thrombin was injected into the SSS with an injection unit.