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Neurological deficits and hematological changes in a rat model of ischemic stroke induced by middle cerebral artery occlusion

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ABSTRACT: Stroke is a neurological disorder caused by impaired blood flow to specific regions of the brain, often resulting in paralysis and functional deficits in the affected area. Among its subtypes, ischemic stroke is the most prevalent in the general population. This study aimed to establish a rat model of ischemic stroke induced by middle cerebral artery occlusion (MCAO). Eight male Sprague-Dawley rats aged 8–10 weeks were randomly assigned to two groups: control and treatment group. In the treatment group, the middle cerebral artery was occluded for 45 min. Neurological assessment using the Bederson scale revealed a neurological deficit score (NDS) of 1, characterized by contralateral flexion after left-brain injury. Hematological analysis demonstrated significantly higher white blood cell (WBC) counts ($p < 0.05$), whereas red blood cell (RBC) counts, hemoglobin concentration, and hematocrit values were significantly lower ($p < 0.05$) than those in the control group. These findings indicate that the MCAO method with a 45-minute occlusion successfully induced a mild neurological deficit accompanied by distinct hematological alterations, thereby providing a reproducible animal model for ischemic stroke research.

Keywords:
hematology, ischemic stroke, *middle cerebral artery occlusion*, neurological deficit score, rats

■ INTRODUCTION

Stroke is a leading cause of death and disability worldwide. Each year, more than 12.2 million individuals suffer strokes (Feigin *et al*. 2022). It is a neurological disorder caused by impaired blood flow to the brain, leading to paralysis and other functional deficits in the body. Stroke can be classified into two main types: hemorrhagic stroke, which results from the rupture of cerebral blood vessels, and ischemic stroke, which occurs due to reduced blood and oxygen supply to the brain. Among these, ischemic stroke is the most common form in the general population (Hui *et al*. 2024).

Rodents, particularly rats, are widely used in stroke research because their cerebrovascular anatomy and physiological characteristics closely resemble those of human beings (Fluri *et al*. 2015). Experimental stroke can be induced using several approaches, including middle cerebral artery occlusion (MCAO), photothrombosis, and intracerebral hemorrhage models (Li & Zhang 2021). Among these, the MCAO method has become one of the most frequently applied techniques because of its reproducibility and relevance to human ischemic stroke pathophysiology.

The present study aimed to establish an ischemic stroke model in rats using the MCAO method and evaluate post-induction hematological changes.

■ MATERIALS AND METHODS

Eight male Sprague-Dawley rats (8–10 weeks old) were acclimatized for two weeks. The rats were randomly assigned to the control and treatment groups, with the latter group subjected to stroke induction. All rats were provided with commercial feed and water ad libitum.

Ischemic stroke was induced using the middle cerebral artery occlusion (MCAO) model. Rats were anesthetized with ketamine (70 mg/kg) and xylazine (4 mg/kg) administered intraperitoneally. A midline incision was made in the left anterior cervical region. Using a 26G needle, an opening was created in the internal carotid artery, and a 4.0 nylon monofilament was inserted until it reached the middle cerebral artery bifurcation to occlude blood flow. After 45 min of occlusion, the incision was sutured with 4.0 vicryl, and the rats were returned to their cages.

Neurological and hematological assessments were performed 24 h postoperatively. Neurological deficits were evaluated using the neurological deficit score (NDS) based on the Bederson test (Desland *et al*. 2014), where a score of

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0 indicated normal function, 1 indicated contralateral flexion after left-brain injury, 2 indicated contralateral circling, and 3 indicated immobility. Rats were considered to have had a stroke when the score was between 1 and 3. Hematological parameters were analyzed according to the protocol described by Mahantayya *et al*. (2016).

■ RESULTS AND DISCUSSION

The MCAO method induced ischemic stroke, as confirmed by histopathological findings (Figure 1) and neurological deficit scores (NDS) (Table 1), consistent with previous studies (Shao *et al*. 2021). A 45-minute occlusion produced mild neurological deficits, although longer times may be needed for severe impairment. Occlusion of the middle cerebral artery disrupts the central nervous system, causing neurological and motor function deficits (Bouët *et al*. 2007).

Hematological analysis showed increased white blood cell (WBC) counts, whereas red blood cell (RBC) counts, hemoglobin, and hematocrit were significantly reduced compared to the control group (Table 2). Erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), showed no significant intergroup differences and remained within the reference range (He *et al*. 2017).

Tissue injury after ischemic stroke activates the immune response through elevated WBC counts, counteracting neuronal damage and inflammation. This process increases the levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 (Doll *et al*. 2014). Cerebral occlusion causes hypoxia and the generation of reactive oxygen species (ROS). ROS induce lipid peroxidation, compromising erythrocyte membrane integrity and increasing hemolysis susceptibility (Rubattu *et al*. 2019).

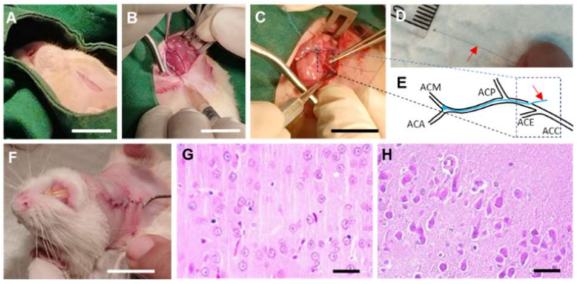

Figure 1 Middle cerebral artery occlusion (MCAO) procedure and histopathology. (A) Orientation; (B) Opening in the internal carotid artery (ICA); (C) Filament insertion for MCA occlusion; (D) Nylon filament with bulbous tip; (E) Schematic of MCAO; (F) Skin suturing; (G) Control cortex; (H) MCAO cortex. ACA: anterior cerebral artery; PCA: posterior cerebral artery; ECA: external carotid artery; CCA: common carotid artery. Scale bars: A–F = 2 cm, G–H = 50 µm.

Table 1 Neurological deficit score (NDS) of rats before (K) and after middle cerebral artery occlusion (MCAO)

Score	Category	K	MCAO
0	Normal	4/4	-
1	Contralateral flexion of the left-brain injury	-	4/4
2	Contralateral rotation	-	-
3	Immobility	-	-

Table 2 Hematological values of rat before (K) and after induction of middle cerebral artery occlusion (MCAO)

Parameter	K (n=4)	MCAO (n=4)
White blood cell (10^3/µL)	5.3 ± 2.5^a	9.1 ± 2.4^b
Red blood cell (10^6/µL)	7.7 ± 0.4^a	4.8 ± 1.0^b
Hemoglobin (g/dL)	14.2 ± 0.3^a	8.7 ± 3.0^b
Hematocrit (%)	42.8 ± 3.0^a	26.3 ± 8.2^b
Mean corpuscular volume (fL)	55.3 ± 1.1^a	54.1 ± 4.9^a
Mean corpuscular hemoglobin (pg)	18.4 ± 0.9^a	17.9 ± 2.0^a
MCHC (g/dL)	33.3 ± 2.4^a	33.1 ± 0.9^a

Note: MCHC=mean corpuscular hemoglobin concentration

■ CONCLUSION

The MCAO was used to establish an ischemic stroke model in rats. A 45-minute occlusion caused mild neurological deficits, increased WBCs, and reduced RBCs, hemoglobin, and hematocrit.

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