ACTA VETERINARIA INDONESIANA
P-ISSN 2337-3202, E-ISSN 2337-4373

Research Vol. 13, No. 1: 67–76, Maret 2025

Method Modification of Developing Ischemic Stroke Animal Models Using Intravenous Catheters in Rats

Ika Satya Perdhana[1,2], Ekowati Handharyani[2], Huda Shalahuddin Darusman[2], Setyo Widi Nugroho[3], Hera Maheshwari[2*], Arni Diana Fitri[4]

[1]Faculty of Medicine, Gunadarma University, Undergraduate Program in Medicine, Gunadarma University
[2]School of Veterinary Medicine and Biomedical Sciences, Study Program in Veterinary Biomedical Sciences, Bogor Agricultural University
[3]Faculty of Medicine, University of Indonesia
[4]Teaching Animal Hospital, Bogor Agricultural University
*Correspondence author: hera_maheshwari@apps.ipb.ac.id
Received: 11 October 2024, Approved: 13 January 2025

ABSTRACT

In various parts of the world, including Indonesia, the incidence of ischemic stroke remains high, and its impact poses a significant burden. This condition is influenced by numerous limitations in ischemic stroke therapy. Research on ischemic stroke is therefore crucial to develop, making the creation of appropriate animal models a vital necessity. One of the most commonly used methods by researchers to create animal models is the Middle Cerebral Artery Occlusion (MCAO) method. However, in Indonesia, its application remains limited due to the lack of available facilities. Method modifications can be implemented to enable the development of animal models in Indonesia. This study employed modifications to the MCAO method on eight Sprague Dawley rats. The modification involved using an intravenous catheter to facilitate the insertion of the filament as an occluder in the middle cerebral artery of the rats. Based on clinical and pathological observations, it can be concluded that the modified MCAO method used in this study is suitable for creating ischemic stroke animal models. With this modification, the creation of ischemic stroke animal models can be carried out in a simpler and more cost-effective manner.

Keywords: ischemic, model, modification, intravenous catheter, rats

ABSTRAK

Di berbagai belahan dunia termasuk Indonesia, angka kejadian strok iskemik masih tinggi dan dampak yang ditimbulkannya menjadi beban yang cukup besar. Keadaan ini dipengaruhi oleh banyaknya keterbatasan dalam terapi strok iskemik. Penelitian mengenai strok iskemik ini sangat penting untuk dikembangkan sehingga pembuatan hewan model yang sesuai menjadi kebutuhan yang sangat penting. Salah satu metode pembuatan hewan model yang paling sering digunakan oleh para peneliti adalah Middle Cerebral Artery Occlusion (MCAO) namun di Indonesia belum banyak dilakukan karena kendala ketersediaan fasilitas. Modifikasi metode dapat dilakukan agar dapat mengembangkan pembuatan hewan model di Indonesia. Penelitian ini menggunakan modifikasi dalam pelaksanaan metode MCAO pada delapan ekor tikus Sprague Dawley. Modifikasi ini dilakukan dengan menggunakan kateter intravena untuk memudahkan masuknya filamen sebagai okluder pada arteri serebri media tikus. Pengamatan klinis menggunakan skala Bederson menunjukkan adanya penurunan fungsi motorik tikus kelompok induksi sedangkan pengamatan patologis menunjukkan peningkatan jumlah sel otak yang mati pada kelompok tikus induksi. Berdasarkan pengamatan klinis maupun patologis tersebut dapat disimpulkan bahwa modifikasi metode MCAO yang dilakukan pada penelitian ini dapat digunakan untuk membuat hewan model strok iskemik. Dengan modifikasi ini pembuatan hewan model strok iskemik dapat dilakukan dengan sederhana dan ekonomis.

Kata kunci: iskemik, kateter intravena, model, modifikasi, tikus

INTRODUCTION

Stroke remains a major global health issue, ranking as the third leading cause of death worldwide (Jayaraj et al., 2019). The mortality rate due to stroke is significantly high, and it also results in a considerable number of permanent disabilities (Jia et al., 2022). In fact, stroke is the leading cause of death across all age groups (Kristianto Wijaya, 2013). The long-term effects of stroke impose a substantial burden on society (Soliman et al., 2020).

This condition requires appropriate medical intervention. However, the success rate of stroke therapy remains relatively low (Kuriakose & Xiao, 2020). Effective stroke management necessitates a comprehensive understanding of its causes and pathophysiological mechanisms at the molecular level. It is well established that the most common cause of stroke is cerebral ischemia (Halliday, 2021), which necessitates the use of animal models for further study (Tekam et al., 2021).

The use of experimental animal models is crucial for advancing research in both the pathogenesis and drug development of ischemic stroke (Handayani et al., 2019). The necessity of using animal models in neurological research, particularly in ischemic stroke studies, is driven by several factors. Firstly, ischemic stroke presents diverse clinical manifestations, etiologies, and anatomical locations. Secondly, experimental studies must be reproducible and standardized. These two factors highlight the importance of in vivo ischemic stroke research using animal models, as they can accurately simulate the pathogenesis, anatomical location, and clinical manifestations observed in human ischemic stroke (Li & Zhang, 2021).

Furthermore, animal models play a vital role in understanding the molecular, biochemical, and physiological mechanisms of ischemic stroke. In many cases, invasive procedures are required to access brain tissue directly, which is not feasible in human studies. Another critical reason for using animal models is that the pathophysiological dynamics of ischemic stroke often occur within the first few minutes, making them difficult to study in humans. These early-stage events can only be thoroughly examined through animal models (Mentari et al., 2018).

Given the importance of animal models in ischemic stroke research, numerous researchers have attempted to establish the most effective model. Various methods have been developed and evaluated, each with its own advantages and limitations (Li & Zhang, 2021). Among these, the Middle Cerebral Artery Occlusion (MCAO) method is the most widely used technique for inducing ischemic stroke in animal models (Rousselet et al., 2012). This method closely mimics human ischemic stroke, which frequently results from occlusion of the middle cerebral artery (Fluri et al., 2015). While researchers worldwide extensively employ the MCAO method, its implementation in Indonesia remains limited. This is concerning, as stroke incidence is higher in developing countries, including Indonesia (Hidayat et al., 2023).

The limited adoption of the MCAO method in Indonesia is primarily due to the lack of necessary facilities and equipment (Makkiyah & Sadewo, 2019). Essential tools and materials, such as rounded-tip nylon filaments, surgical microscopes with 40x magnification, microvascular clips, and vascular Doppler devices, are difficult to obtain in Indonesia (Dirnagl, 2010). These challenges have prompted Indonesian researchers to modify the MCAO method using simpler equipment (Makkiyah & Sadewo, 2019; Ramli et al., 2017). However, modifying the MCAO technique is not an easy task.

Overcoming resource limitations requires creative solutions to achieve results comparable to those obtained using standard facilities. In their technical report, Makkiyah and Sadewo (2019) described modifications involving the use of temporary knots as substitutes for vascular clips and surgical loupes instead of high-magnification surgical microscopes. These adaptations are cost-effective and more feasible for Indonesian researchers, who often lack access to advanced laboratory equipment. The modifications provide an accessible alternative to the standard MCAO method (Makkiyah & Sadewo, 2019). Similarly, Ramli et al. (2017) modified the occluder used in MCAO by heating the tip of a monofilament to create a rounded end, replacing the commercially available blunted monofilament, which is relatively expensive. This modification allows researchers in Indonesia to conduct ischemic stroke studies without relying on costly imported materials.

This technical report presents a new modification of the MCAO method, utilizing an intravenous catheter to facilitate the insertion of the rounded monofilament into the blood vessel, thereby occluding the middle cerebral artery's perfusion area. The monofilament used in this study was modified based on Ramli's technique. The adaptation of MCAO using an intravenous catheter in this study makes the procedure more accessible, cost-effective, and time-efficient while still yielding results comparable to previous research findings. It is hoped that this modified MCAO technique can be implemented throughout Indonesia and in other countries facing similar resource constraints.

MATERIALS AND METHODS

Materials and Equipment

This study utilized male Sprague Dawley rats aged 6–11 months with body weights of 300–400 grams. The anesthetic agents used included ketamine and xylazine for general anesthesia, as well as isoflurane for inhalational anesthesia when necessary during surgery. A 1 cc disposable syringe was used to administer anesthesia. The occlusion was induced using 6.0 nylon filament, while Vicryl sutures were used for wound closure. A 26G intravenous catheter was employed to facilitate the insertion of the filament into the blood vessel. The primary surgical instruments included minor surgical tools and a 2.5x magnification loupe.

Experimental Animals

The male Sprague Dawley rats used in this study were obtained from Dramaga Agri Satwa. A total of eight rats, aged 4–5 months and weighing 300–400 grams, underwent a two-week acclimatization period at the Veterinary Teaching Hospital, Bogor Agricultural Institute (RSHP IPB). The rats were housed individually in cages under controlled temperature conditions of 22–24°C. They were provided with T21 feed (PT Indofeed) and ad libitum access to water. The rats were divided into two groups:

1. Sham group – underwent only a skin incision.
2. Induction group – underwent middle cerebral artery occlusion (MCAO) using a 6.0 nylon filament.

Filament Preparation

In this study, 0.6 mm monofilament nylon surgical suture (Arthylon) was used as the occluder. Before insertion, the filament tip was rounded using heat from a lighter at a distance of approximately 5 cm.

Surgical Procedure

The rats were anesthetized using intraperitoneal ketamine (70 mg/kg BW) and xylazine (4 mg/kg BW). Additional anesthesia with 1% isoflurane was provided via inhalation during surgery. This study was approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, Bogor Agricultural Institute (Approval No. 140/KEH/SKE/XII/2023). The MCAO procedure was performed by a veterinarian following the Koizumi and Longa MCAO method, with modifications to accommodate available resources. The modifications included using an intravenous catheter and a rounded-tip nylon filament as the occluder for the middle cerebral artery (MCA).

The surgery began with a 2 cm midline cervical incision. The subcutaneous tissue and platysma muscle were dissected to expose the submandibular gland, which was then retracted to reveal the jugular vein. Medial to the jugular vein, the common carotid artery (CCA) was identified, which bifurcates into the external carotid artery (ECA) laterally and the internal carotid artery (ICA) medially. The ICA was carefully isolated from surrounding tissues.

Once the ICA was exposed, an intravenous catheter was inserted into the ICA. Proper placement was confirmed by observing blood flow into the catheter. After confirming the catheter's position, the trocar (needle) was removed, and to minimize excessive blood loss, the vessel was clamped using anatomical forceps. The catheter hub was then cut, and the rounded-tip nylon filament was inserted through the catheter. The filament was advanced gently until a resistance point was felt, indicating that it had reached the ICA bifurcation and occluded the MCA. This occlusion led to ischemia in the MCA-perfused area.

The next step involved removing the catheter while ensuring the filament remained in place. The ICA was clamped distal to the catheter to prevent filament displacement. After catheter removal, the filament position was confirmed, and 2–3 cm of the filament was left protruding outside the insertion site for later removal. The occlusion was maintained for 60 minutes. The muscles and tissues were repositioned, and the incision was sutured using 5.0 Vicryl. The externalized 2–3 cm filament was left accessible for easy removal after the occlusion period. Following 60 minutes of occlusion, the filament was removed without reopening the incision. The wound was cleaned, and antibiotic ointment was applied as infection prophylaxis before returning the rats to their cages.

Clinical and Histopathological Observations

Following stroke induction, clinical and histopathological assessments were conducted. Clinical observations were recorded at 1 hour, 24 hours, and 48 hours post-induction. At 48 hours post-induction, the rats were euthanized using exsanguination under general anesthesia, a method widely used in research due to its efficiency and minimal contamination of the surgical field (Hickman & Johnson, 2011). Ketamine and xylazine anesthesia were administered before cardiac blood collection

using a syringe. Once exsanguination was complete, decapitation was performed (Hidayat & Wulandari, 2021). Following euthanasia confirmation, the brain tissue was collected. The excised brain samples were processed and stained using Hematoxylin-Eosin for histopathological examination (Digambiro & Parwanto, 2023).

RESULTS

Clinical Observations

Prior to induction, the rats' body weight, general health, and motor function were assessed. All rats weighed 300–400 grams, appeared healthy, and exhibited no motor disabilities. Post-induction observations were conducted at 1 hour, 24 hours, and 48 hours after ischemic induction, evaluating the same parameters. No significant changes in body weight were observed at any time point.

At 1 hour post-induction, there were no observable differences between Group 1 (sham) and Group 2 (induced stroke). All rats remained under the effects of anesthesia, preventing motor function evaluation.

At 24 hours post-induction, rats in Group 1 showed no noticeable changes, while rats in Group 2 exhibited reduced general health and impaired motor function. Group 2 rats appeared less active and displayed unsteady gait. Motor behavior was assessed using the Bederson Score (Ruan & Yao, 2020). All Group 1 rats maintained a score of 0, while Group 2 rats, which had a score of 0 before induction, scored 3 at 24 hours post-induction (Table 1).

At 48 hours post-induction, Group 1 remained unchanged, whereas Group 2 continued to show motor function decline, including limb flexion and reduced resistance to lateral push. However, Group 2 rats no longer exhibited consistent circling behavior observed at 24 hours post-induction (Table 2).

Histopathological Observations

At 48 hours post-induction, histopathological assessments were conducted following euthanasia, examining brain tissue both macroscopically and microscopically. Microscopic observations using histological slides (Figure 1) revealed distinct differences between the two groups.

In Group 1, normal brain cells were observed, with minimal cell death in both the cortex and hippocampus. In contrast, Group 2 displayed a higher number of dead neurons, indicating ischemic damage.

DISCUSSION

Research on ischemic stroke and its therapeutic development remains a highly relevant topic due to the high incidence and low treatment success rate (Gibson & Trotman-Lucas, 2021). Unfortunately, in Indonesia, ischemic stroke research is hindered by limited facilities, particularly in terms of equipment and materials, which are essential for developing animal models to study pathophysiological mechanisms and explore natural compounds as potential treatments (Ramli et al., 2017). The lack of research infrastructure has led Indonesian scientists to modify existing methods and develop creative solutions to conduct studies using available resources (Rasjad Indra & Parindra Gasmara, 2016).

Similar to other ischemic stroke studies in Indonesia, this study modified the MCAO (Middle Cerebral Artery

Table 1. 24h post induction Bederson Score

Bederson Scale	Rat							
Observed motoric	1.1	1.2	1.3	1.4	2.1	2.2	2.3	2.4
Flexion • Any degree of forelimb flexion (score=l) • Both forelimbs extended toward floor (score=0)	0	0	0	0	1	1	1	1
Lateral push • Consistent reduction in resistance to lateral push toward paretic side (score=l) • No reduction in resistance to lateral push toward paretic side (score=0)	0	0	0	0	1	1	1	1
Circling • Consistent circling (score=l) • No circling (score=0)	0	0	0	0	1	1	1	1

Rats number 1.1, 1.2, 1.3 and 1.4 are group 1 (*sham*), number 2.1, 2.1, 2.3 dan 2.4 are group 2 (inducted with MCAO)

Tabel 2. Skor Bederson 48 jam pasca induksi

Skala Bederson	Tikus							
Observed motoric	1.1	1.2	1.3	1.4	2.1	2.2	2.3	2.4
Flexion								
• Any degree of forelimb flexion (score=l)	0	0	0	0	1	1	1	1
• Both forelimbs extended toward floor (score=0)								
Lateral push	0	0	0	0	1	1	1	1
• Consistent reduction in resistance to lateral push toward paretic side (score=l)								
• No reduction in resistance to lateral push toward paretic side (score=0)								
Circling	0	0	0	0	0	0	0	0
• Consistent circling (score=l)								
• No circling (score=0)								

Rats number 1.1, 1.2, 1.3 and 1.4 are group 1 (sham), number 2.1, 2.1, 2.3 dan 2.4 are group 2 (inducted with MCAO)

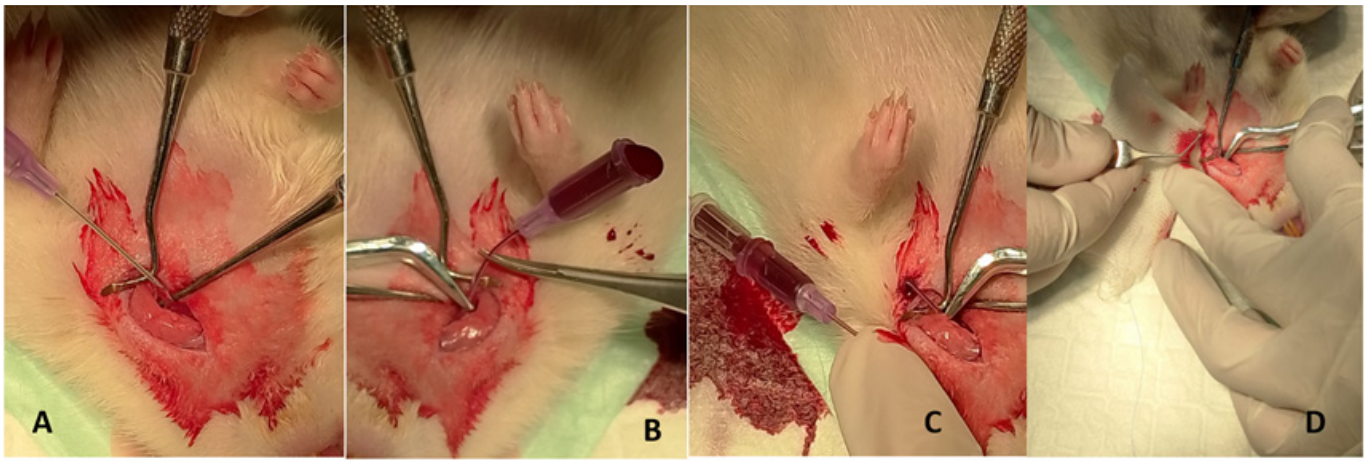

Figure 1. Surgical procedures. Puncture the IV catheter (A). Cut the IV hub (B). Clamping blood vessels to prevent blood from flowing after the hub was cut (C). Insert filamen through the IV catheter (D).

Occlusion) method, originally introduced by Koizumi and Longa, which involves filament insertion into a blood vessel to induce MCA occlusion. The Koizumi-Longa MCAO method is considered one of the most accurate models for replicating ischemic stroke in humans (Morris et al., 2016).

Methodological Modifications

The modifications in this study were driven by challenges in fully replicating previous research methods, particularly due to limited access to necessary equipment and materials in Indonesia. Compared to developed countries, research facilities in Indonesia are still underdeveloped, and obtaining standardized research tools is often difficult and expensive. One example is the laser Doppler flowmetry, which is typically used to monitor cerebral blood flow during induction surgery (Biose et al., 2022). However, this equipment is not available in Indonesia and was therefore not used in this study.

Previous studies using MCAO employed specialized rounded-tip filaments to prevent endothelial injury and ensure complete occlusion (Biose et al., 2022). Since these filaments are unavailable in Indonesia and costly, this study adopted Ramli's modification, in which nylon sutures were rounded using heat (Ramli et al., 2017). Additionally, past studies used microsurgical loupes with up to 8x magnification, which were also unavailable in this study due to facility limitations.

Besides modifying the filament, this study also adjusted the surgical procedure. Earlier studies applied temporary ligation to the proximal and distal carotid artery instead of using aneurysm clips (Makkiyah & Sadewo, 2019). In contrast, our study did not perform arterial ligation or arteriotomy but instead used a 26G intravenous catheter. This modification simplified filament insertion into the blood vessel.

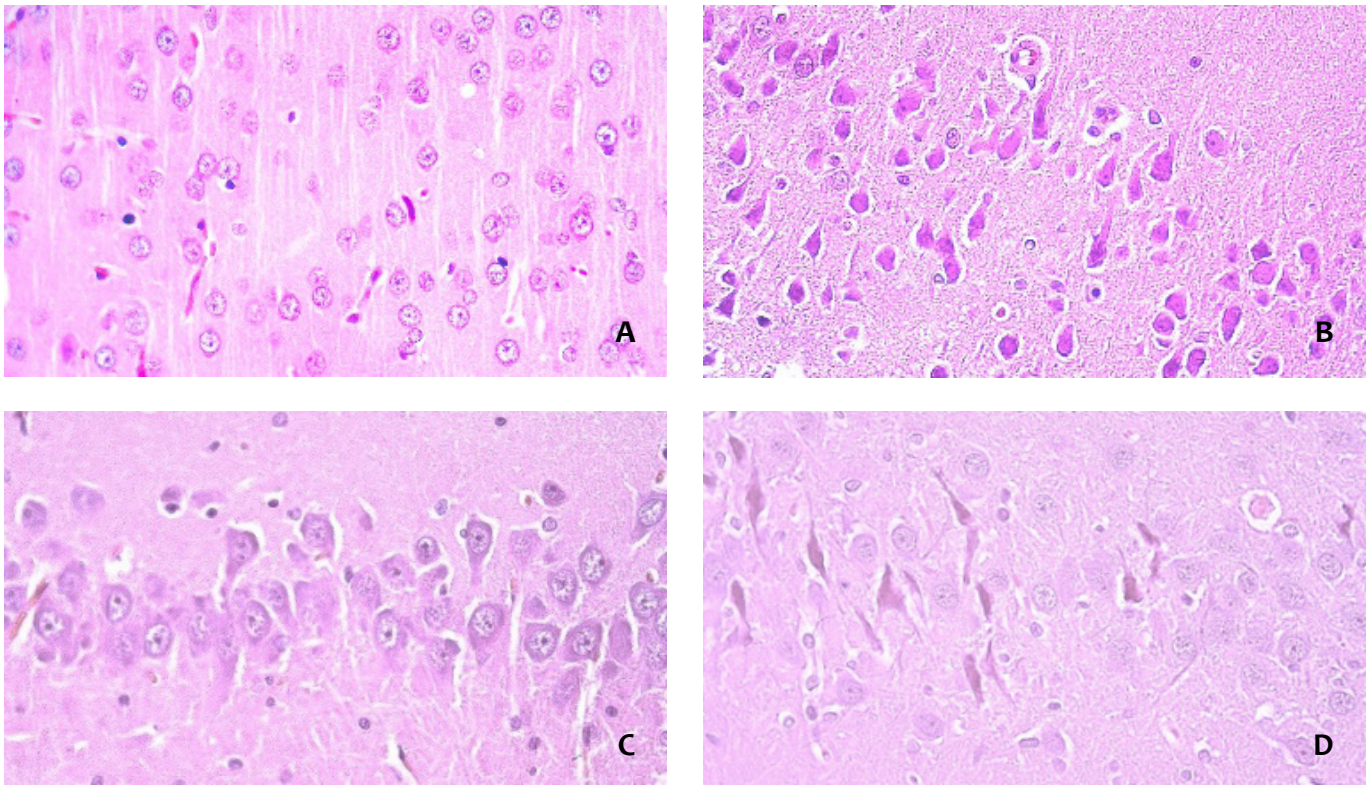

Figure 2. Histopathological observation. Group 1 cerebral cortex (A). Group 2 cerebral cortex (B). Group 1 cerebral hipocampal (C). Group 2 cerebral hipocampal (D). Bar : 50um

Previous researchers performed arteriotomy using a 1 cc syringe under an 8x magnification loupe. Without sufficient magnification, identifying the arteriotomy site for filament insertion becomes challenging. By employing an intravenous catheter, the filament could be inserted more easily through the catheter's lumen.

Effectiveness of the Modified MCAO Method

This study demonstrated that ischemic stroke animal models could be successfully developed using basic surgical tools and readily available nylon sutures in Indonesia. The use of an intravenous catheter eliminated the need for distal or proximal vessel ligation before filament insertion.

The modified MCAO procedure successfully induced ischemic stroke, as shown by differences between the sham and ischemic induction groups. The right MCA was occluded using a rounded nylon filament inserted through an intravenous catheter. Ischemic stroke was confirmed via clinical observations using the Bederson scale, which assesses motor function and body symmetry (Ruan & Yao, 2020).

Clinical observations revealed that ischemic stroke induction led to motor impairment in the contralateral forelimb and hindlimb (Table 2). This confirms that MCA occlusion caused brain damage, manifesting in contralateral motor deficits. The sham group did not exhibit such changes. These findings align with previous studies using advanced MCAO techniques with microcatheters and zirconia spheres under fluoroscopic guidance (Komatsu et al., 2021).

Furthermore, our clinical findings at 24 hours post-induction were consistent with previous studies that modified MCAO using temporary ligatures instead of arterial clips (Makkiyah & Sadewo, 2019). The Bederson score in this study at 24 hours post-induction was 3 for all ischemic stroke-induced rats, matching previous research (Makkiyah & Sadewo, 2019). However, this study extended observations to 48 hours, whereas previous studies only observed rats up to 24 hours post-induction.

Histopathological Analysis

The 48-hour observation period was selected based on studies showing that cerebral infarction peaks at 24 hours and begins to decline at 48 hours (Jiang et al., 2017). The clinical observations in this study aligned with Jiang et al.'s findings, as Bederson scores showed improvement at 48 hours compared to 24 hours, specifically in reduced circling behavior.

Histological analysis further supported the clinical findings. Brain tissue samples (Table 3) showed a higher number of dead neurons in the cortex and hippocampus in stroke-induced rats compared to the

sham group. The average number of dead neurons in the ischemic group was 59.5 (SE = 4.09), whereas the sham group had only 17.2 (SE = 3.56). These findings confirm that the rounded nylon filament successfully induced ischemia, leading to neural damage and cell death, consistent with previous studies (Makkiyah & Sadewo, 2019; Biose et al., 2022).

Table 3. Rat clinical observation 24h post induction

Rats	Group 1		Group 2	
	Pre-induction	Post-induction	Pre-induction	Post-induction
Activity	active	less active	active	less active
Apetite	normal	less	normal	less
Bederson score	0	0	0	3

Table 4. Number of cerebral dead cells

Group	Dead cells number ± SE
1	17,2 ± 3,56
2	59,5 ± 4,09

Makkiyah's study used double ligation on the external carotid artery (ECA), common carotid artery (CCA), and internal carotid artery (ICA) as a substitute for microvascular clips, along with ligating the pterygopalatine artery (PPA) to facilitate filament insertion under an 8x magnification loupe (Makkiyah & Sadewo, 2019). On the other hand, Biose's study used Doccol monofilaments with rounded silicone tips for MCA occlusion, guided by laser Doppler flowmetry. Both studies reported clinical and histopathological evidence of brain damage, similar to the results of this study (Biose et al., 2022).

Safety and Limitations

MCAO models are categorized into permanent and temporary occlusion models. This study adopted the temporary MCAO method, which involves removing the filament after a set period. This reperfusion process closely mimics clinical ischemic stroke in humans (McBride & Zhang, 2019). Although temporary MCAO has high reproducibility, it carries risks such as subarachnoid hemorrhage (Singh et al., 2021).

Since MCAO is an invasive procedure affecting a vital organ (brain), it carries a mortality risk. The reported surgical mortality rate is around 5%, while postoperative mortality (within 7 days) is 1–2%. Deaths are primarily caused by subarachnoid hemorrhage or anesthesia complications (Llovera et al., 2014). However, no deaths occurred in this study, indicating that the modified MCAO method using an intravenous catheter was safe and did not increase mortality risks. Nonetheless, future studies should further validate this method by assessing long-term survival rates.

This study has certain limitations. One key limitation is the lack of infarct volume measurement. Instead, this study quantified dead neurons in Hematoxylin-Eosin-stained histological samples. This staining method was chosen due to its simplicity and widespread use among researchers (Digambiro & Parwanto, 2023). While infarct volume measurement could provide more precise data, the selected analysis method was cost-effective and practical, aligning with the study's goal of developing an affordable ischemic stroke model in Indonesia. Despite these limitations, the results were consistent with previous studies using more advanced techniques (Halliday, 2021).

CONCLUSION

Ischemic stroke remains a global health concern, with a particularly severe impact on developing countries like Indonesia. Given the high burden of disease, extensive research is needed to develop effective therapeutic options and reduce its impact on society. However, ischemic stroke research in Indonesia, particularly animal model studies, is often hindered by limited facilities.

This study successfully developed an ischemic stroke animal model using a modified MCAO (Middle Cerebral Artery Occlusion) method, making the procedure simpler and more efficient. The modifications, which included the use of an intravenous catheter and a heat-rounded nylon filament, provide a viable alternative for researchers in resource-limited settings like Indonesia. Clinical observations, motor function scoring using the Bederson scale, and histopathological analysis confirmed that the modified MCAO method effectively induced ischemic stroke, producing results comparable to those obtained with standard laboratory facilities.

Given the urgency of stroke treatment, research into ischemic stroke therapies plays a critical role in medicine, particularly in Indonesia, where abundant natural resources may offer therapeutic potential. The high prevalence of stroke in Indonesia and its limited research infrastructure necessitate methodological adaptations to facilitate further therapeutic developments.

The simplicity of this modified MCAO method, along with its accessibility and consistency with previous research, provides a promising approach for future ischemic stroke studies. This study encourages further research on ischemic stroke using MCAO animal models with a more efficient

approach, allowing researchers in Indonesia and other developing countries to actively contribute to stroke-related studies. These efforts will ultimately advance global ischemic stroke management.

ACKNOWLEDGMENTS

This study was funded by BIMA Kemendikbudristekdikti under contract number 102/E5/PG.02.00.PL/2023.

The authors declare no conflict of interest regarding this study.

REFFERENCES

Biose, I. J., Chastain, W. H., Wang, H., Ouvrier, B., & Bix, G. J. (2022). Optimizing intraluminal monofilament model of ischemic stroke in middle-aged Sprague–Dawley rats. BMC Neuroscience, 23(1). https://doi.org/10.1186/s12868-022-00764-2

Digambiro, R. A., & Parwanto, E. (2023). *Panduan Prosesing Dan Pewarnaan Jaringan Dalam Histopatologi*. Penerbit Lakeisha. Hal 59-60

Fluri F, Schuhmann MK, Kleinschnitz C. 2015. Animal models of ischemic stroke and their application in clinical research. Drug Des Devel Ther. 9:3445–3454. doi:10.2147/DDDT.S56071.

Gibson, C. L., & Trotman-Lucas, M. (2021). A review of experimental models of focal cerebral ischemia focusing on the middle cerebral artery occlusion model. F1000Research, 10. https://doi.org/10.12688/f1000research.51752.1

Halliday, S. (2021). Ischemic stroke. *Preoperative Assessment: A Case-Based Approach*, 221–226. https://doi.org/10.1007/978-3-030-58842-7_33

Handayani ES, Susilowati R, Setyopranoto I, Partadiredja G. 2019. Transient bilateral common carotid artery occlusion (Tbccao) of rats as a model of global cerebral ischemia. Bangladesh J Med Sci. 18(3):491–500. doi:10.3329/bjms.v18i3.41616.

Hickman, D. L., & Johnson, S. W. (2011). Evaluation of the aesthetics of physical methods of euthanasia of anesthetized rats. Journal of the American Association for Laboratory Animal Science, 50(5), 695–701.

Hidayat, R., & Patricia Wulandari. (2021). Euthanasia Procedure of Animal Model in Biomedical Research. Bioscientia Medicina : Journal of Biomedicine and Translational Research, 5(6), 540–544. https://doi.org/10.32539/bsm.v5i6.310

Hidayat R, Rima SPP, Pangeran D, Mesiano T, Kurniawan M, Rasyid A, Harris S. 2023. Optimizing Stroke Care in Indonesia: A Policy Brief on Expanding Access to Thrombolysis for Improved Outcomes. Acta Neurol Indones. 1(01):1–5. doi:10.69868/ani.v1i01.12.

Jayaraj, R. L., Azimullah, S., Beiram, R., Jalal, F. Y., & Rosenberg, G. A. (2019). Neuroinflammation: Friend and foe for ischemic stroke. Journal of Neuroinflammation, 16(1), 1–24. https://doi.org/10.1186/s12974-019-1516-2

Jia J, Yang L, Chen Yan, Zheng L, Chen Yanting, Xu Y, Zhang M. 2022. The Role of Microglial Phagocytosis in Ischemic Stroke. Front Immunol. 12. doi:10.3389/fimmu.2021.790201.

Jiang, L. J., Zhang, S. M., Li, C. W., Tang, J. Y., Che, F. Y., & Lu, Y. C. (2017). Roles of the Nrf2/HO-1 Pathway In The Anti-Oxidative Stress Response To Ischemia-Reperfusion Brain Injury In Rats. European Review For Medical And Pharmacological Sciences, 21(7), 1532–1540.

Komatsu, T., Ohta, H., Motegi, H., Hata, J., Terawaki, K., Koizumi, M., Muta, K., Okano, H. J., & Iguchi, Y. (2021). A novel model of ischemia in rats with middle cerebral artery occlusion using a microcatheter and zirconia ball under fluoroscopy. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-92321-w

Kristianto Wijaya A. 2013. Patofisiologi Stroke Non-Hemoragik Akibat Trombus. https://ojs.unud.ac.id/index.php/eum/issue/view/927.

Kuriakose D, Xiao Z. 2020. Pathophysiology and treatment of stroke: Present status and future perspectives. Int J Mol Sci. 21(20):1–24. doi:10.3390/ijms21207609.

Li Y, Zhang J. 2021. Animal models of stroke. Anim Model Exp Med. 4(3):204–219. doi:10.1002/ame2.12179.

Llovera, G., Roth, S., Plesnila, N., Veltkamp, R., & Liesz, A. (2014). Modeling stroke in mice: Permanent coagulation of the distal middle cerebral artery. *Journal of Visualized Experiments*, *89*, 1–8. https://doi.org/10.3791/51729

Makkiyah, F. A., & Sadewo, W. (2019). Technical report: Simple method of animal stroke model of luminal occlusion of middle cerebral artery in Indonesia. Surgical Neurology International, 10(143). https://doi.org/10.25259/SNI_62_2019

McBride, D. W., & Zhang, J. H. (2019). HHS Public Access Author manuscript Transl Stroke Res. Author manuscript; available in PMC 2019 January 17. Precision Stroke Animal Models: The Permanent MCAO Model Should be the Primary Model, Not Transient MCAO. Transl Stroke Res., 176(1), 100–106. https://doi.org/10.1177/0022146515594631.

Mentari IA, Naufalina R, Rahmadi M, Khotib J. 2018. Development Ischemic Stroke Model by Right Unilateral Common Carotid Artery Occlusion

(RUCCAO) Method. Folia Medica Indones. 54(3):200. doi:10.20473/fmi.v54i3.10015.

Morris, G. P., Wright, A. L., Tan, R. P., Gladbach, A., Ittner, L. M., & Vissel, B. (2016). A comparative study of variables influencing ischemic injury in the longa and koizumi methods of intraluminal filament middle cerebral artery occlusion in mice. *PLoS ONE*, *11*(2). https://doi.org/10.1371/journal.pone.0148503

Ramli, Y., Alwahdy, A. S., Kurniawan, M., Juliandi, B., Wuyung, P. E., & Susanto, Y. D. B. (2017). Permanent flame-blunted monofilament of middle cerebral artery occlusion technique for ischemia stroke induction in animal models. Medical Journal of Indonesia, 26(3), 183–189. https://doi.org/10.13181/mji.v26i3.1645

Rasjad Indra, M., & Parindra Gasmara, C. (2016). UCAO (Unilateral Cerebral Artery Occlussion) Method Increases The Level Of Mmp- 9 Brain Tissue In Rats Model Of Ischemic Stroke. MNJ (Malang Neurology Journal), 2(2), 46–50. https://doi.org/10.21776/ub.mnj.2016.002.02.1

Rousselet E, Kriz J, Seidah NG. 2012. Mouse model of intraluminal MCAO: cerebral infarct evaluation by cresyl violet staining. J Vis Exp.(69). doi:10.3791/4038.

Ruan J, Yao Y. 2020. Behavioral tests in rodent models of stroke. Brain Hemorrhages. 1(4):171–184. doi:10.1016/j.hest.2020.09.001.

Biose, I. J., Chastain, W. H., Wang, H., Ouvrier, B., & Bix, G. J. (2022). Optimizing intraluminal monofilament model of ischemic stroke in middle-aged Sprague–Dawley rats. BMC Neuroscience, 23(1). https://doi.org/10.1186/s12868-022-00764-2

Digambiro, R. A., & Parwanto, E. (2023). Panduan Prosesing Dan Pewarnaan Jaringan Dalam Histopatologi.

Gibson, C. L., & Trotman-Lucas, M. (2021). A review of experimental models of focal cerebral ischemia focusing on the middle cerebral artery occlusion model. F1000Research, 10. https://doi.org/10.12688/f1000research.51752.1

Halliday, S. (2021). Ischemic stroke. Preoperative Assessment: A Case-Based Approach, 221–226. https://doi.org/10.1007/978-3-030-58842-7_33

Hickman, D. L., & Johnson, S. W. (2011). Evaluation of the aesthetics of physical methods of euthanasia of anesthetized rats. Journal of the American Association for Laboratory Animal Science, 50(5), 695–701.

Hidayat, R., & Patricia Wulandari. (2021). Euthanasia Procedure of Animal Model in Biomedical Research. Bioscientia Medicina : Journal of Biomedicine and Translational Research, 5(6), 540–544. https://doi.org/10.32539/bsm.v5i6.310

Jayaraj, R. L., Azimullah, S., Beiram, R., Jalal, F. Y., & Rosenberg, G. A. (2019). Neuroinflammation: Friend and foe for ischemic stroke. Journal of Neuroinflammation, 16(1), 1–24. https://doi.org/10.1186/s12974-019-1516-2

Jiang, L. J., Zhang, S. M., Li, C. W., Tang, J. Y., Che, F. Y., & Lu, Y. C. (2017). Roles of the Nrf2/HO-1 pathway in the anti-oxidative stress response to ischemia-reperfusion brain injury in rats. European Review for Medical and Pharmacological Sciences, 21(7), 1532–1540.

Komatsu, T., Ohta, H., Motegi, H., Hata, J., Terawaki, K., Koizumi, M., Muta, K., Okano, H. J., & Iguchi, Y. (2021). A novel model of ischemia in rats with middle cerebral artery occlusion using a microcatheter and zirconia ball under fluoroscopy. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-92321-w

Llovera, G., Roth, S., Plesnila, N., Veltkamp, R., & Liesz, A. (2014). Modeling stroke in mice: Permanent coagulation of the distal middle cerebral artery. Journal of Visualized Experiments, 89, 1–8. https://doi.org/10.3791/51729

Makkiyah, F. A., & Sadewo, W. (2019). Technical report: Simple method of animal stroke model of luminal occlusion of middle cerebral artery in Indonesia. Surgical Neurology International, 10(143). https://doi.org/10.25259/SNI_62_2019

McBride, D. W., & Zhang, J. H. (2019). HHS Public Access Author manuscript Transl Stroke Res. Author manuscript; available in PMC 2019 January 17. Precision Stroke Animal Models: The Permanent MCAO Model Should be the Primary Model, Not Transient MCAO. Transl Stroke Res.,176(1),100–106. https://doi.org/10.1177/0022146515594631.

Morris, G. P., Wright, A. L., Tan, R. P., Gladbach, A., Ittner, L. M., & Vissel, B. (2016). A comparative study of variables influencing ischemic injury in the longa and koizumi methods of intraluminal filament middle cerebral artery occlusion in mice. PLoS ONE, 11(2). https://doi.org/10.1371/journal.pone.0148503

Ramli, Y., Alwahdy, A. S., Kurniawan, M., Juliandi, B., Wuyung, P. E., & Susanto, Y. D. B. (2017). Permanent flame-blunted monofilament of middle cerebral artery occlusion technique for ischemia stroke induction in animal models. Medical Journal of Indonesia, 26(3), 183–189. https://doi.org/10.13181/mji.v26i3.1645

Rasjad Indra, M., & Parindra Gasmara, C. (2016). UCAO (Unilateral Cerebral Artery Occlussion) Method Increases The Level Of Mmp- 9 Brain Tissue In Rats Model Of Ischemic Stroke. MNJ (Malang Neurology Journal), 2(2), 46–50. https://doi.org/10.21776/ub.mnj.2016.002.02.1

Ruan, J., & Yao, Y. (2020). Behavioral tests in rodent models of stroke. Brain Hemorrhages, 1(4), 171–184. https://doi.org/10.1016/j.hest.2020.09.001

Singh, A. A., Kharwar, A., & Dandekar, M. P. (2021). A Review on Preclinical Models of Ischemic Stroke: Insights Into the Pathomechanisms and New Treatment Strategies. Current Neuropharmacology, 20(9), 1667–1686. https://doi.org/10.2174/1570159x19666210907092928

Soliman SM, Sheta NM, Ibrahim BMM, El-Shawwa MM, Abd El-Halim SM. 2020. Novel intranasal drug delivery: Geraniol charged polymeric mixed micelles for targeting cerebral insult as a result of Ischaemia/Reperfusion. Pharmaceutics. 12(1). doi:10.3390/pharmaceutics12010076.

Tekam CS, Ranjana P, Shinde S, Mahto SK. 2021. Bilateral Common Carotid Artery Occlusion: Stroke Model. Model Tech Stroke Biol. October:1–115. doi:10.1007/978-981-33-6679-4.