Health Reserch's Journal Volume 02 Nomor 01 January 2025

COMPARATIVE ANALYSIS OF INFARCT VOLUMES IN PERMANENT AND TRANSIENT MCAO MODELS USING TTC STAINING: A REVIEW OF CURRENT RESEARCH AND FUTURE DIRECTIONS

Gherriandi Raja Buana[1], Feda Anisah Makkiyah[2], Dhyani Paramita Wahyudi[3]
Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta[1,2,3]
Corresponding author: fedaanisah@upnvj.ac.id

ABSTRACT

Ischemic stroke research often relies on animal models to understand the pathological processes underlying brain injury. Among the most widely used models are permanent middle cerebral artery occlusion (pMCAO) and transient middle cerebral artery occlusion (tMCAO), both of which simulate ischemic events in the brain. However, while existing studies have compared the effects of pMCAO and tMCAO on infarct volumes, many have been limited by short observation periods (e.g., 4 hours) or have focused only on non-occlusion models. This literature review aims to evaluate the comparative effectiveness of these two models in generating infarct sizes, specifically utilizing triphenyltetrazolium chloride (TTC) staining to measure infarct volumes. Our analysis highlights the variability in infarct volumes between the two models and emphasizes further research incorporating longer observation intervals (e.g., 6, 18, and 24 hours). This gap in research is crucial for refining experimental stroke models and improving the clinical relevance of findings, particularly regarding therapeutic strategies for ischemic stroke. The review also suggests potential research pathways to enhance model standardization and consistency in future studies, aiming for more accurate and reproducible results that will inform the development of effective stroke treatments.

KEYWORDS

Middle Cerebral Artery Occlusion (MCAO); Permanent MCAO; Transient MCAO; TTC Staining; Stroke Research Models

INTRODUCTION

Ischemic stroke remains one of the leading causes of morbidity and mortality worldwide, with profound impacts on both individual and public health. The Global Burden of Disease (GBD) 2021 indicates that ischemic stroke accounts for over 87% of stroke cases, emphasizing the urgent need for a deeper understanding and more effective therapeutic strategies (Feigin *et al.*, 2021). This condition is caused by the obstruction of blood flow to the brain, leading to hypoxia, cellular metabolic failure, and irreversible neuronal injury. Given the devastating effects of ischemic stroke, exploring the mechanisms behind it and finding solutions through clinical and preclinical models is crucial.

One of the most widely used animal models for studying ischemic stroke is the middle cerebral artery occlusion (MCAO) model, which accurately mimics the ischemic events observed in human stroke patients (Liu *et al.*, 2024). The MCAO model targets the middle cerebral artery (MCA), which is involved in approximately 80% of stroke cases and is pivotal in understanding focal cerebral ischemia (Liu *et al.*, 2024; Peng *et al.*, 2022). This model can be categorized into two main variations: permanent MCAO (pMCAO) and transient MCAO (tMCAO). In the pMCAO model, the MCA is permanently occluded, leading to sustained ischemia. In contrast, in the tMCAO model, the occlusion is temporary, and reperfusion follows, allowing for the study of ischemia-reperfusion injury (McBride *et al.*, 2017). Both models play crucial roles in stroke research, but there is still limited data directly comparing the infarct volumes between these two models, especially when measured over extended periods.

Several studies have focused on comparing pMCAO to non-occlusion models or investigating the short-term effects of tMCAO, often with an emphasis on reperfusion damage in the early stages of ischemia (Santo *et al.*, 2023; McBride *et al.*, 2017; Wells *et al.*, 2012). However, many of these studies overlook the timely progression of infarct size. To address this gap, triphenyltetrazolium chloride (TTC)

staining is widely used to quantify infarct volume in stroke research, providing a reliable method to measure infarct size based on mitochondrial dehydrogenase activity (Li *et al*., 2018; Peng *et al.*, 2022). This technique allows for accurate visualization and quantification of infarcted brain tissue, making it a valuable tool in assessing the extent of ischemic damage.

The primary aim of this review is to compare the infarct sizes produced by pMCAO and tMCAO models using TTC staining. Although these models have been extensively studied, the comparative analysis of infarct volume between them remains underexplored, particularly in terms of the long-term ischemic and reperfusion dynamics (McBride *et al*., 2017; Peng *et al.*, 2022). By reviewing and analyzing the findings of prior studies, this review aims to provide a clearer understanding of the infarct progression and size differences between pMCAO and tMCAO, thereby contributing to refining experimental stroke models. Moreover, it highlights the necessity of further research that utilizes standardized methodologies and investigates the long-term effects of ischemia-reperfusion injury, which will ultimately help improve the reproducibility of results in preclinical stroke studies (Biose *et al.*, 2022).

In conclusion, this review aims to enhance the understanding of ischemic stroke by comparing the infarct sizes in pMCAO and tMCAO models, focusing on TTC staining as the gold standard for infarct volume quantification. The insights from this comparison will assist in refining preclinical models and inform the design of more effective therapies for ischemic stroke (Liu *et al.*, 2024; McBride *et al*., 2017). Ultimately, by addressing the gaps in current research, this study will contribute to improving the accuracy and relevance of experimental models, which is crucial for advancing therapeutic interventions in ischemic stroke.

MATERIALS AND METHODS

This literature review examines studies comparing infarct volumes between permanent (pMCAO) and transient (tMCAO) middle cerebral artery occlusion (MCAO) models, using triphenyltetrazolium chloride (TTC) staining for infarct size assessment. A systematic search was conducted across databases, including PubMed, Scopus, and Google Scholar, using the following keywords: "TTC staining" AND (pMCAO OR "Permanent Middle Cerebral Artery Occlusion") AND (tMCAO OR "Transient Middle Cerebral Artery Occlusion"). This search initially yielded 162 papers published between 2015 and 2025. After screening, 11 studies met the inclusion criteria. However, five studies were excluded due to non-compliance with the inclusion criteria as listed in table 1, leaving 6 for final analysis.

Tabel 1. PICO Analysis of studies

PICO Element	Details
Population (P)	Sprague-Dawley rats (adult male, 250–350 g)
Intervention (I)	The permanent MCAO (pMCAO) model, which simulates ischemic stroke without reperfusion, was assessed using TTC staining.
Comparison (C)	Transient MCAO (tMCAO) model, simulating ischemic stroke with reperfusion post-occlusion, assessed similarly for infarct size.
Outcome (O)	Comparison of brain infarct size using TTC staining, MRI imaging, and neurobehavioral/functional tests; effects on cellular damage, edema, and neurological function

RESULTS

Table 2. Comparison of Infarct Size in pMCAO Models Using TTC Staining

No.	Author and Year	Country	MCAO Model	Infarct Size (by TTC Staining)	Occlusion Duration
1	He *et al*., 2022	China	pMCAO	24.21% ± 6.98%	2 hours
2	Liu *et al*., 2024	China	pMCAO	26.72% ± 1.86%	2 hours
3	Kuts *et al*., 2019	Israel	pMCAO	8.27% ± 1.78% (original method) 7.49% ± 0.82% (new method)	24 hours

Table 3. Comparison of Infarct Size in tMCAO Models Using TTC Staining

No.	Author and Year	Country	MCAO Model	Infarct Size (by TTC Staining)	Occlusion Duration	Other
1	Wang *et al*., 2023	China	tMCAO	No specific infarct size reported	90 minutes	Qualitative assessment of infarct region
2	Li *et al*., 2017	China	tMCAO	No specific infarct size reported	2 hours	Immunofluoresc-ence analysis
3	Zhang *et al*., 2020	Israel	tMCAO	No specific infarct size reported	2 hours	Visual identification of infarct areas

DISCUSSION

In this comparative analysis of infarct volumes in pMCAO and tMCAO models using TTC staining, the findings reveal significant insights into the effectiveness of these models for stroke research. This section will critically analyze the results, comparing the infarct volumes measured in both models, and propose future research directions to improve the accuracy and consistency of these experimental models.

Comparison of pMCAO and tMCAO Models

The data in Table 1 indicates the variability in infarct sizes measured by TTC staining between the pMCAO models, with infarct sizes ranging from 8.27% ± 1.78% to 26.72% ± 1.86% across different studies (He *et al.*, 2022; Liu *et al.*, 2024; Kuts *et al.*, 2019). All of these studies used Sprague-Dawley rats, a widely used strain in ischemic stroke research, due to their robust response to ischemic injury and their consistent pathophysiological responses, making them ideal for stroke models. The pMCAO model is characterized by a permanent blockage of the middle cerebral artery, leading to sustained ischemia. This model provides a consistent and reliable way to study brain infarction due to the lack of reperfusion, making it valuable for evaluating stroke therapies aimed at preventing irreversible damage (Zemgylyte *et al*., 2021). In contrast, tMCAO, as shown in Table 2, did not yield consistent infarct size data across studies, with no specific infarct volume or percentage reported. Studies by Wang *et al*. (2023), Li *et al*. (2017), and Zhang *et al*. (2020) instead employed qualitative assessments or visual identification of infarct areas. This discrepancy highlights a significant limitation in current tMCAO-based research, where infarct size quantification remains underreported or inadequately measured.

TTC Staining as an Invaluable Tool

TTC staining has been the gold standard for evaluating infarct size due to its simplicity, cost-effectiveness, and ability to visually distinguish between viable and infarcted tissue. As described by Benedek *et al*. (2006) and Sanchez-Bezanilla *et al*. (2019), TTC is enzymatically reduced by mitochondrial dehydrogenases in viable tissue, which turns the tissue red, while infarcted areas remain white, signifying irreversible damage. This method offers a rapid and reliable means of measuring

infarct volumes and has been widely used in ischemic stroke models (He *et al.*, 2022; Liu *et al.*, 2024). However, the lack of precise data in tMCAO models regarding infarct size poses a challenge to comparing these two models. The absence of standardized methodologies for infarct quantification in tMCAO suggests that future studies should prioritize more quantitative approaches, such as automated image analysis or volumetric assessments, to enhance the reproducibility and clinical relevance of findings.

Limitations of Current Models

A key issue from the reviewed studies is the significant variability in infarct volume measurements and methodologies. For example, while pMCAO models provide measurable infarct volumes, tMCAO models often rely on qualitative assessments, making it difficult to compare results across studies. This inconsistency can lead to challenges in translating preclinical findings into clinical settings, particularly when assessing therapeutic strategies for ischemic stroke. Moreover, many studies, such as those by Liu *et al*. (2024) and Kuts *et al*. (2019), use short observation periods of 2 hours to 24 hours, which may not fully capture the progression of ischemic injury and the potential for therapeutic intervention over longer periods. As Zemgylyte *et al*. (2021) and McBride *et al*. (2017) suggest, incorporating longer observation intervals—ranging from 6 to 24 hours—would provide a more comprehensive understanding of infarct dynamics and the therapeutic window for ischemic stroke treatments.

The Role of Intravascular Filament Occlusion in MCAO

The technique of intravascular filament occlusion of the MCA, used in both pMCAO and tMCAO models, has become the most widely employed method for inducing ischemic stroke in rodents (Zemgulyte *et al.*, 2021). This approach allows for controlled occlusion and reperfusion, mimicking the ischemia-reperfusion injury observed in human stroke patients. However, as indicated by Wang *et al*. (2023) and Si *et al*. (2017), variations in the surgical procedure, such as the depth of filament insertion and the duration of occlusion, can lead to inconsistencies in infarct size measurements. This variability underlines the need for standardized protocols in MCAO surgeries to ensure more reliable and reproducible results.

Recommendations for Future Research

Future studies should focus on a few important areas. First, standardizing experimental protocols for both pMCAO and tMCAO models would help ensure consistency and reliability across studies. This includes using the same methods for occlusion duration, infarct size measurement, and observation times. For tMCAO models, it's crucial to adopt quantitative methods like automated image analysis for more accurate infarct size measurements. Studies should also incorporate longer observation periods, ranging from 6 to 24 hours, with several time intervals in between. This will help better understand how infarct size develops over time and how therapeutic interventions can work at different stages. Lastly, improving the clinical relevance of the models by making them more reflective of real human stroke conditions is essential.

CONCLUSIONS

While pMCAO models provide reliable and measurable infarct volumes, there is a lack of variability in infarct size across different time intervals, as existing studies typically focus on a limited range of observation periods. Moreover, no prior studies have directly compared infarct sizes between pMCAO and tMCAO models across multiple time intervals. The tMCAO model, although widely used, faces challenges due to inconsistent infarct size data, hindering a comprehensive comparison. TTC staining remains a valuable tool for quantifying infarct size, but improvements in methodology especially in tMCAO are necessary for more robust and clinically relevant results. Standardizing experimental protocols and incorporating longer observation periods, including multiple time intervals, along with quantitative methods for infarct size measurement, will enhance the reproducibility and accuracy of preclinical stroke studies. This will ultimately contribute to more informed and effective stroke treatment development.

Acknowledgement

There is no assistance from the government, private companies, or non-profit organizations

Funding Source

No targeted funding source was reported for this study.

Conflict of Interest

The authors declare that no competing interests exist.

REFERENCES

Benedek, A. *et al*. (2006) 'Use of TTC staining for the evaluation of tissue injury in the early phases of reperfusion after focal cerebral ischemia in rats', Brain Research, 1116(1), pp. 159–165. Available at: https://doi.org/10.1016/j.brainres.2006.07.123.

Biose, I.J. *et al*. (2022) 'Optimizing intraluminal monofilament model of ischemic stroke in middle-aged Sprague–Dawley rats', BMC Neuroscience, 23(1), p. 75. Available at: https://doi.org/10.1186/s12868-022-00764-2.

Feigin, V.L. *et al*. (2021) 'Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019', The Lancet Neurology, 20(10), pp. 795–820. Available at: https://doi.org/10.1016/S1474-4422(21)00252-0.

He, Y. *et al*. (2022) 'Evaluating blood-brain barrier disruption and infarction volume concurrently in rats subjected to ischemic stroke using an optical imaging system', Journal of Neuroscience Methods, 378, p. 109630. Available at: https://doi.org/10.1016/j.jneumeth.2022.109630.

Kuts, R. *et al*. (2019) 'A Novel Method for Assessing Cerebral Edema, Infarcted Zone and Blood-Brain Barrier Breakdown in a Single Post-stroke Rodent Brain', Frontiers in Neuroscience, 13, p. 1105. Available at: https://doi.org/10.3389/fnins.2019.01105.

Li, L., Yu, Q. and Liang, W. (2018a) 'Use of 2,3,5-triphenyltetrazolium chloride-stained brain tissues for immunofluorescence analyses after focal cerebral ischemia in rats', Pathology - Research and Practice, 214(1), pp. 174–179. Available at: https://doi.org/10.1016/j.prp.2017.11.016.

Li, L., Yu, Q. and Liang, W. (2018b) 'Use of 2,3,5-triphenyltetrazolium chloride-stained brain tissues for immunofluorescence analyses after focal cerebral ischemia in rats', Pathology - Research and Practice, 214(1), pp. 174–179. Available at: https://doi.org/10.1016/j.prp.2017.11.016.

Liu, P. *et al*. (2024) 'Establishment of a pMCAO model in SD rats and screening for behavioral indicators suitable for long-term monitoring', Brain Injury, 38(9), pp. 716–726. Available at: https://doi.org/10.1080/02699052.2024.2346804.

McBride, D.W. and Zhang, J.H. (2017) 'Precision Stroke Animal Models: the Permanent MCAO Model Should Be the Primary Model, Not Transient MCAO', Translational Stroke Research, 8(5), pp. 397–404. Available at: https://doi.org/10.1007/s12975-017-0554-2.

Peng, J. *et al*. (2022) 'Advancement of epigenetics in stroke', Frontiers in Neuroscience, 16, p. 981726. Available at: https://doi.org/10.3389/fnins.2022.981726.

Sanchez-Bezanilla, S. *et al*. (2019) 'Can We Use 2,3,5-Triphenyltetrazolium Chloride-Stained Brain Slices for Other Purposes? The Application of Western Blotting', Frontiers in Molecular Neuroscience, 12, p. 181. Available at: https://doi.org/10.3389/fnmol.2019.00181.

Santo, B.A. *et al*. (2023) 'Tectonic infarct analysis: A computational tool for automated whole-brain infarct analysis from TTC-stained tissue', Heliyon, 9(4), p. e14837. Available at: https://doi.org/10.1016/j.heliyon.2023.e14837.

Si, Z. *et al*. (2019) 'Effects of thrombolysis within 6 hours on acute cerebral infarction in an improved rat embolic middle cerebral artery occlusion model for ischaemic stroke', Journal of Cellular and Molecular Medicine, 23(4), pp. 2468–2474. Available at: https://doi.org/10.1111/jcmm.14120.

Wang, Y. *et al*. (2023) 'The superiority and feasibility of 2,3,5-triphenyltetrazolium chloride–stained brain tissues for molecular biology experiments based on microglial properties', Animal Models and Experimental Medicine, 6(2), pp. 111–119. Available at: https://doi.org/10.1002/ame2.12312.

Wells, A.J. *et al.* (2012) 'A Surgical Model of Permanent and Transient Middle Cerebral Artery Stroke in the Sheep', *PLoS ONE*. Edited by C. Kleinschnitz, 7(7), p. e42157. Available at: https://doi.org/10.1371/journal.pone.0042157.

Zemgulyte, G. *et al*. (2021) 'Evaluation of the Effectiveness of Post-Stroke Metformin Treatment Using Permanent Middle Cerebral Artery Occlusion in Rats', Pharmaceuticals, 14(4), p. 312. Available at: https://doi.org/10.3390/ph14040312.

Zhang, K.-Y. *et al*. (2020) 'Cathodal tDCS exerts neuroprotective effect in rat brain after acute ischemic stroke', BMC Neuroscience, 21(1), p. 21. Available at: https://doi.org/10.1186/s12868-020-00570-8.