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[^{18}F]FDG/PET: a non-invasive technique for the study of stroke in murine and rat models

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ABSTRACT

The use of mouse and rat models in conjunction with anatomic functional imaging techniques has directly contributed to expanding knowledge about the complex pathophysiology of stroke. Therefore, this study aims to identify the most relevant mouse and rat models of stroke and how [^{18}F]FDG/PET can contribute to this pathology study. A narrative review of the literature was performed to describe applications of positron emission tomography in conjunction with the radiopharmaceutical [^{18}F]FDG in stroke models. PubMed, Scopus, and Web of Science were searched for relevant articles published between 2015 and 2022. In this study, we describe applications of positron emission tomography in combination with the radiopharmaceutical [^{18}F]FDG in mouse and rat stroke models. The most commonly used model was middle cerebral artery occlusion (MCAO) in rats. This study demonstrates that using murine and rat models in conjunction with anatomic functional imaging techniques has directly contributed to expanding knowledge about the complex pathophysiology of stroke. In addition, they have been essential for studies aimed at discovering and developing therapeutic and prophylactic strategies for the disease.

Keywords: **Positron Emission Tomography, [^{18}F]FDG, Murine and Rat Stroke Models, Stroke.**

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1. INTRODUCTION

Vascular disease is the leading cause of death. Stroke is the second leading cause of death worldwide after ischemic heart disease and the third leading cause of disability-adjusted life years (DALYs) [1, 2]. Stroke can be ischemic (IS) or hemorrhagic (HS). Ischemic stroke is responsible for most disease liabilities and affects a greater number of people; however, the hemorrhagic form has caused a more significant number of deaths. It is responsible for 80% of the mortality rate in low- and middle-income countries [2].

The most important primary health goal for stroke is to reduce stroke incidence. In high-income countries, stroke risk factors such as hypertension, smoking, obesity, diabetes mellitus, atrial fibrillation, dyslipidemia, and physical inactivity are the targets for stroke prevention. This strategy has reduced stroke incidence and increased DALYs in these countries [2]. However, the need for effective therapies such as thrombolytics and reperfusion procedures, as well as rehabilitation and long-term follow-up to prevent stroke recurrence and improve functional outcomes, should be recognized as important measures to reduce stroke incidence [1].

To optimize these therapies, it is necessary to understand the pathophysiology of stroke, which is highly complex and involves processes such as inflammation, excitotoxicity, and oxidative stress. The neuronal damage that occurs after stroke extends beyond the acute phase and needs to be better understood to develop effective therapeutic and preventive strategies [1]. The use of animal models, including rats and mice, that mimic human stroke is a very useful resource in the quest to understand these processes.

Most studies using animal models obtain results through histological and biochemical techniques. However, these techniques are performed after the animal's death, requiring a large sample size and preventing longitudinal studies [3]. An interesting alternative is functional neuroimaging, such as [^{18}F]fluoro-2-deoxyglucose/positron emission tomography ([^{18}F]FDG/PET). This technique allows the evaluation of changes in glycolytic metabolism as a predictor of brain damage in neurological disease. Furthermore, because [^{18}F]FDG/PET is a non-invasive technique that can be performed in vivo, it allows monitoring stroke disease progression and its manifestations in the acute and chronic phases [3]. Thus, the aim of this review was to collect important information regarding: [1] the pathophysiology of stroke, [2] the applicability of the [^{18}F]FDG/PET

technique in the study of ischemic brain function, and [3] the main mouse models used in pre-clinical studies of stroke.

2. MATERIALS AND METHODS

2.1. Search strategy and selection

Multiple databases were searched to identify relevant papers for the review. First, targeted searches were performed to find pre-clinical studies associated with [^{18}F]FDG/PET technique published from 2015 to 2022. For this purpose, it was used PubMed, Scopus, Web of Science, and Google Scholar using appropriate keywords; 'Positron Emission Tomography', 'Fluorodeoxyglucose' and acronyms; 'rat', 'mouse' and the same plural words; 'ischemic stroke' and related terms/synonyms. In addition, to obtain recent information regarding the epidemiology and pathophysiology of the stroke disease, other keywords were also used, such as: "incidence of ischemic stroke" and "epidemiology of ischemic stroke" and "clinical features of stroke".

2.2. Inclusion/Exclusion criteria

To address and meet about the recent applications of the [^{18}F]FDG/PET technique for the pre-clinical study of stroke, were evaluated papers published from 2015 to 2022. The search was also limited to those papers published in peer-reviewed scientific journals. After reading the abstract of the papers and removal of duplicate articles, some exclusion criteria were adopted, such as: studies that used pre-clinical models that were not cerebral ischemia, articles that did not present the appropriate description of the animal model, articles that used another methodology to study the stroke, the use of [^{18}F]FDG/PET was not to evaluate or study stroke, studies that used another radiopharmaceutical that was not [^{18}F]FDG, studies regarding another pathology and reviews article.

3. RESULTS AND DISCUSSION

3.1 Main features of stroke

Acute cerebral stroke is mainly characterized by occlusion of a supplying arterial vessel, which occurs IS. On the other hand, HS is characterized by vascular rupture followed by bleeding. The HS occurs less frequently, about 20% of strokes [4]. Ischemic stroke is caused by three major events: rupture of an atherosclerotic plaque in a large vessel, cardioembolism, and probable occlusion of

deep arteries [4]. Hemorrhagic stroke can occur in two different ways, one is intracranial hemorrhage, and the other is subarachnoid hemorrhage. The most common risk factor for HS is hypertension, myocardial infarction, and the use of thrombolytics [5].

In these cases, the lack of blood flow during a stroke leads to a complicated pathophysiological response that results in neuronal damage. This neuronal cell loss may result from various mechanisms such as excitotoxicity, mitochondrial response, free radical release, protein misfolding, and inflammatory changes [6].

Ischemia of the brain leads to neuronal deprivation of oxygen and glucose, making neurons unable to maintain a normal ion gradient. When depolarization of these affected neurons occurs, excessive glutamate release leads to an intracellular calcium influx. This event initiates cell death pathways, such as apoptosis, autophagocytosis, and necrotic pathways. This process is called excitotoxicity and promotes brain edema, which is clinically significant in the first days after stroke [6].

In addition to neuronal damage from excitotoxicity, brain ischemia also affects mitochondrial homeostasis and disrupted energy balance, resulting in altered ATP synthesis. The intracellular calcium influx resulting from excitotoxicity leads to excessive accumulation in mitochondria, which is responsible for the opening of the mitochondrial permeability transition pore and release of cytochrome c. This event culminates in the collapse of the mitochondrial membrane, initiating the apoptosis cascade. In addition, reactive oxygen species (ROS) generated by mitochondria also play a role in reperfusion injury leading to neuronal damage and cell death [7]. Calcium influx also triggers the production of nitric oxide (NO) by nitric oxide synthase (NOS), which leads to injury through the generation of oxygen free radicals and the production of peroxynitrite ($ONOO^-$). Free radicals in ischemic tissue contribute to oxidative stress and neuronal death [6, 7].

The intracellular calcium influx after cerebral ischemia also affects the endoplasmic reticulum (ER) functions. This organelle regulates protein synthesis, and the ER stress induced by ischemic injury leads to protein misfolding. One protein involved in protein misfolding in this context is inositol-requiring enzyme 1 (IRE1), which is responsible for initiating apoptotic processes in times of ER stress [6].

Inflammatory processes in response to brain ischemia play a vital role in the pathophysiology of the disease. They are involved in blood-brain barrier breakdown and poststroke tissue remodeling and also provide a margin of neuroprotection from the harsh excitotoxic poststroke environment in which increased free radicals and enzymes are generated [6]. Moreover, inflammatory markers are

associated with poor prognosis in stroke patients and brain inflammation mediated by microglia, the major innate immune cells of the brain parenchyma, which is a key component in triggering injury [8]. In addition to releasing pro-inflammatory mediators, microglia also increase their capacity for phagocytosis in acute ischemic stroke and increase the number of phagocytosing microglia after 24 hours, which helps remove damaged tissue and enables synaptic remodeling [8, 9].

Many inflammatory cells are involved and respond to an ischemic insult. Initially, microglia are activated, followed by an increase in dendritic cells, macrophages, and lymphocytes, and after the breakdown of the blood-brain barrier, an influx of neutrophilic cells invades the infarct and peri-infarct region [6]. This process is upregulated by pro-inflammatory cytokines such as tumor necrosis factor-a (TNF-a), interleukin-1b (IL -1b), and free radicals released by inflammatory cells. Immune cells also release NO synthase, which contributes to the deleterious effects of NO in brain ischemia. In addition, the immune response increases the production of matrix metalloproteins (MMPs) and myeloperoxidase (MPO), both of which lead to blood-brain barrier breakdown [10].

On the other hand, immune cells such as eosinophils may significantly impact postinfarction plasticity through the production of trophic factors such as nerve growth factor (NGF) and neurotrophin-3, which promote neuronal growth. Microglia also play an important role in promoting neuronal growth and healing through the production of glial cell-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF). Cytokines such as transforming growth factor-b (TGF-b) and interleukin-10 (IL -10) often have dual roles: driving the inflammatory response but also promoting tissue repair and resolution of inflammation, depending on the timing and environment [6].

The presence of inflammatory processes plays a fundamental role in the pathophysiology of stroke, having both beneficial and detrimental effects on surviving tissue [6]. Therefore, a balance of the inflammatory response after stroke is critical for patient recovery, and accurate knowledge of these inflammatory components and disease characteristics could lead to improved therapeutic approaches. In addition, further research and translational investigations are needed to understand how stroke recovery and plasticity occur and to improve the diagnosis and treatment of worse ischemic damage.

3.2 Applicability of ^{18}F-fluoro-2-deoxyglucose/ PET on the investigation of ischemic brain function

Neuroimaging can be performed by structural or molecular imaging techniques such as magnetic resonance imaging (MRI) or positron emission tomography (PET). However, molecular imaging techniques play an important role in neuroimaging by allowing the assessment of brain function in vivo. In this context, PET is an invaluable non-invasive technique that provides information about metabolism by imaging the distribution of biologically active molecules labeled with positron-emitting radioisotopes [11, 12]. One of the radiopharmaceuticals most commonly used in clinical practice for PET imaging is ^{18}F-fluoro-2-deoxyglucose ([^{18}F]FDG), a tracer that can detect changes in glucose metabolism in various tissues, including the brain [13, 14].

Glucose is the main energy source for the brain cells, and [^{18}F]FDG uptake is related to glucose consumption during neuronal function. Thus, the evaluation of [^{18}F]FDG uptake is one way to measure brain activity [15]. Indeed, in recent years, has been observed an increasing use of [^{18}F]FDG/PET imaging, in basic and clinical studies of brain function in various physiological and pathological processes, including metabolic brain networks, epilepsy, dementia, Alzheimer's disease, brain tumor, and ischemic brain injury [16-23]. In addition, [^{18}F]FDG/PET has also become a useful tool in clinical practice mainly when used in conjunction with MRI to diagnose various pathological brain conditions [24].

Scientific studies have provided evidence that [^{18}F]FDG/PET is a potential tool to improve stroke diagnosis and subsequent damage assessment in medical practice. Liang et al. [25] demonstrated that [^{18}F]FDG/PET can detect abnormal metabolic connectivity of the brain in rats in the acute stage of middle cerebral artery occlusion (MCAO). Zhang et al. [23] obtained similar results after inducing focal ischemia at the bilateral motor cortex of rats and measuring glucose metabolism in different brain regions. [^{18}F]FDG/PET imaging showed a significant change in glucose uptake in brain regions associated with the sensorimotor domain [23].

In ischemic stroke, the ischemic and irreversibly damaged region is called the core, and the area surrounding the core is called the ischemic penumbra. In the ischemic penumbra, metabolism is impaired, but the tissue remains structurally intact [26]. Preventing the breakdown of the ischemic penumbra appears to improve neurological recovery after stroke, making identification of the penumbra region essential for stroke outcome. Sobrado et al. [27] investigated the efficacy of [^{18}F]FDG/PET imaging, perfusion-weighted imaging (PWI), and diffusion-weighted imaging

(DWI) MRI techniques to identify the ischemic penumbra in two models of ischemic stroke in rats, the permanent model and the mild transient cerebral ischemia model. Their results showed that combining the three imaging techniques could identify two distinct penumbra regions, one that could develop into infarcted tissue even after reperfusion and a recoverable region that could be salvaged by early reperfusion. Remarkably, [^{18}F]FDG/PET proved to be the most sensitive imaging technique for detecting the recoverable region early after stroke.

Interestingly, studies have demonstrated the efficiency of the [^{18}F]FDG/PET technique in evaluating the therapeutic potential of various drugs and therapeutic procedures in cerebral ischemia. The [^{18}F]FDG/PET was able to demonstrate the beneficial effect of electro-acupuncture on glucose metabolism in the brain of rats subjected to MCAO [28]. Similarly, a higher accumulation of the tracer was observed in the ipsilateral brain infarct area of rats subjected to MCAO followed by stem cell transplantation, and this finding was correlated with the best neurofunctional recovery [29, 30]. With [^{18}F]FDG/PET, facilitated glucose utilization was also demonstrated in rat brains ipsilateral ischemic cortex and the perinfarct region after treatment with various potential angiogenic drugs [31-33].

Despite the invaluable potential of the [^{18}F]FDG/PET imaging technique for the evaluation of ischemic brain function, some features should be considered for its use in basic and clinical research as well as in medical practice. Previous studies found some changes in [^{18}F]FDG uptake in the brain due to sex and normal aging [34-36], and recent results suggest higher [^{18}F]FDG uptake in certain brain regions in young and adult women compared with men of the same age. The differences were found in brain regions such as the frontal, parietooccipital, and temporal lobes [35]. Meanwhile, Bentourkia et al. [34] observed a significant decrease in cerebral blood flow and glucose metabolism due to the natural aging process. Shamchi et al. [35] observed similar results and, interestingly, the effect of aging on [^{18}F]FDG uptake was found to be more pronounced in males. Based on these results, it is clear that it is of utmost importance to take into account the normal sex- and age-related changes in [^{18}F]FDG uptake to correctly evaluate the data obtained for both scientific and diagnostic purposes. However, these facts do not diminish the importance and applicability of the technique.

3.3 [^{18}F]FDG/PET and murine models of ischemic stroke

Several pre-clinical studies using mouse models of ischemic stroke have used [^{18}F]FDG/PET imaging as the primary technique to evaluate ischemic brain function and treatment efficacy. All research cited in this review on mouse models of ischemic stroke published between 2015 and 2022 found that the most commonly used mouse model was MCAO performed by intraluminal suturing [22, 25, 28, 30-33, 37-46]. In the context of the goal of the research, variations of this technique are used, such as. MCAO by microwire, embolic MCAO, or distal MCAO [47-50]. The second most common model for pre-clinical stroke research is common carotid artery occlusion (CCAO), which can be performed by unilateral or bilateral carotid artery occlusion [51-54], followed by the photothrombotic model [23, 55]. A summary of these representative studies using [^{18}F]FDG/PET is provided in Table 1.

Table 1: Main experimental stroke models – studies performed by [^{18}F]FDG/PET.

Stroke Model	Procedures	Post stroke timing	Relevant findings with [^{18}F]FDG/PET
Middle cerebral artery occlusion (MCAO) in male Sprague-Dawley rats. Yu et al. [23]; Liang et al. [25]; Wu et al. [28]; Zhang et al. [30]; Han et al. [31] ; Hui et al. [32]; Hwang et al. [33]; Choi et al. [38]; Deng et al. [39]; Dong et al. [40]; Joya et al. [41]; Li et al. [42] ; Svoboda et al. [44] Xu et al. [45]; Yu et al. [46]; He et al. [67]; Yu et al. [50]	[^{18}F]FDG was allowed to circulate in the blood for 30 min [28], [33], [41], He et al. [67], 40 min [25], [40], [42], 45 min [23] or 60 min [31], [32], [38], [39], [44], [45], [46], [50], and then the animals were fixed on the special scanning bed for a 10 min [30], [31], [32], [39], [40], 15 min [28], [42], He et al. [67] 20 min [33], [38] or 30 min [23], [25], [41], [44], [50] static acquisition.	45 min after surgery [67], 12 h after surgery [25], at 24 h [38], [40], [41], [44], [45], [46], 3 days [41], [45], [46], 7days [23], [28], [33], [38], [39], [41], [42], [45], [46], [50] 14 days [41], 21 days [41], and 28 days [41] after MCAO.	- Treatment with SalA-4g, an analog of salidroside, resulted in a significant improvement in the recovery of cerebral glucose metabolism [23]. - Infarct regions were observed by decreased striatum, auditory cortex, and somatosensory metabolic activity. [^{18}F]FDG/PET showed hypermetabolism in the contralesional regions [25]. - Treatment with EA at the LI11 and ST36 acupoints enhanced glucose metabolism of the CPu, MCTX and SCTX regions [28]. - Dynamic metabolic and functional recovery after treatment (iPSCs or NSCs combined with QKL) [30]. - HUK (exogenous human tissue kallikrein) treatment enhanced cerebral perfusion in the penumbra region [31]. - The treatment with PTS (Panaxatriol saponins) significantly improved rat neurological deficit scores, reduced infarct volumes, enhanced [^{18}F]FDG uptake in ischemic brain tissue, and increased cerebral perfusion after surgery [32]. - In response to liposomal delivery of angiogenic peptides, cortical [^{18}F]FDG uptake was increased, which was correlated with the extent of improvement in cerebral perfusion [33]. - Decreased metabolic activity in the affected (left) hemisphere by more than 50% than the contralateral hemisphere after surgery. However, the SUVs in the cortex and striatum of the affected hemisphere were significantly higher in the treated group at 7 days [38]. - It was observed higher [^{18}F]FDG accumulation in the right cerebral infarction among all of the drug treatment groups Hydroxysafflor yellow A (HSYA) and acetylglutamine (NAG) [39]. - Treatment with liraglutide increased glucose metabolism [40]. - Decreased [^{18}F]FDG uptake in the infarction region followed by a progressive recovery during the first week after ischemia [41]. - [^{18}F]FDG uptake was decreased in brain regions ipsilateral to the MCAO

			surgery. [^{18}F]FDG uptake was increased subregions of the midbrain, such as the superior and inferior colliculus, the VTA, as well as the entorhinal cortex, posterior hippocampus, pons, and medulla of the contralesional hemisphere at day 7 [42]. - Reduction in focal brain metabolism and outline ischemic brain region. Ischemic region has been detected as region with decreased [^{18}F]FDG activity in comparison to remaining brain tissue [44]. - Decrease in metabolic activity in the affected hemisphere [45]. - Treatment with SalA-4g led to a significant increase in the recovery of cerebral glucose metabolism, in association with a neurological functional recovery [46]. - Automatic assessment of the quality of the MCAO procedure [67]. - Combined treatment with tPA and SalA-4g led to a significant increase in the recovery of cerebral glucose metabolism, in association with a reduction of neurological deficits and infarct volume [50] .
MCAO in male Wistar rats. Suh et al. [43]; Backes et al. [48]	[^{18}F]FDG was allowed to circulate in the blood for 60 min [43], [48], and then the animals were fixed on the special scanning bed for a 10 min [43] static acquisition.	At 75 min [48], At 24h [43], 2 days [48], 7 days [48], 14 days [48], 21 days [48], and 42 days [48] after procedure.	- Significant decrease in [^{18}F]FDG uptake in the ischemic brain [43]. - PET scanning with double tracer, [^{18}F]FDG and [^{11}C]PK11195, showed that glucose metabolism of inflammatory cells can mask metabolic deficits of neuronal damages [48].
MCAO in C57BL/6J mice. Cao et al. [37]; Ni et al. [66]	[^{18}F]FDG was allowed to circulate in the blood for 60 min [37]; [66] static acquisition.	At 24h [37]; [66] after procedure.	- Improved brain glucose metabolism after treatment (YQFM), which was consistent with the results of TTC staining [37]. - Reduced [^{18}F]FDG uptake was observed in the ischemic striatum of tMCAO mouse brain at 24 h after reperfusion [66].
Bilateral common carotid artery occlusion (BCCAO) in male Sprague-Dawley rats. Bai et al. [51]	[^{18}F]FDG was allowed to circulate in the blood for 45 min [51], and then the animals were fixed on the special scanning bed for a 15 min [51] static acquisition.		-Treatment with PYTN capsule led to a significant increase in the cerebral glucose metabolism, in association with an improvement of performance [51].

Photothrombotic ischemia model in male Sprague-Dawley rats. Zhang et al. [16]; Liu et al. [55]	[^{18}F]FDG was allowed to circulate in the blood for 40 min [16] or 60 min [55] and then the animals were fixed on the special scanning bed for a 20 min [16] or 30 min [55] static acquisition.	24 after ischemia [16]; [55]; 3 days [55], 7 days [55] and 14 days [55] after ischemia.	- Construction of metabolic brain network and glucose metabolic changes of all brain regions after ischemia [16]. - [^{18}F]FDG/PET showed that glucose metabolism of the infarct and peri-infarct region gradually recovered [55].
Photothrombotic stroke (PTS) indution in adults male Wistar rats. Krämer et al. [71]; Krämer et al. [72]	PET acquisition was started between 3 and 6 min after [^{18}F]FDG injection for a 60 min acquisition. Krämer et al. [71, 72]	2, 3 and 4 days after ischemia. Krämer et al. [71, 72]	- Reduced glucose metabolism in the right cortico-striatal thalamic loop after PTS compared to the state before intervention. Krämer et al. [71] - High-frequency stimulation of the subthalamic nucleus might retune neuronal networks involved in upper limb motor function and corroborate recent studies that impairments after ischemic stroke are due to distributed brain circuit disruption. Krämer et al. [72]
Unilateral common carotid artery occlusion (UCCAO) followed by exposure to hypoxic air, hypoxia-ischemia in female and male Lewis rats. Chevin et al. [52]	[^{18}F]FDG was allowed to circulate in the blood for 45 min [52], and then the animals were fixed on the special scanning bed for a 30 min [52] static acquisition.	1 day after MRI scans [52].	- As compared to untreated rats, rats treated with hypothermia had a higher uptake of [^{18}F]FDG in the motor cortex, the hippocampus, and the caudate-putamen [52].
UCCAO followed by exposure to hypoxic air, hypoxia-ischemia in female and male Wistar rats. Odorcyk et al. [53]	[^{18}F]FDG was allowed to circulate in the blood for 40 min [53], and then the animals were fixed on the special scanning bed for a 10 min [53] static acquisition.	24 and 72 h after HI [53].	- [^{18}F]FDG hypometabolism in the lesion area of group HIP11[53].
UCCAO followed by exposure to hypoxic air, hypoxia-ischemia in mice. Ouyang et al. [54]	Micro-PET scanning was conducted for 60 min acquisition. [54]	10-15 min before exposure, during and after HI [54].	- The PET/MRI method presented was able to detect physiological changes generated by the model [54].

3.4 Middle Cerebral Artery Occlusion

MCAO by intraluminal suture is the most commonly used model for pre-clinical stroke studies because it does not require craniotomy and allows reversible and focal ischemia [56, 57]. In general, after anesthetizing the animals, a midline cervical incision is made to expose the common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA). An appropriate monofilament is inserted into the lumen of the ECA or CCA and carefully advanced into the lumen of the ICA to occlude the middle cerebral artery (MCA) origin. In some models, permanent occlusion is performed, whereas in others, reperfusion occurs when the monofilament is removed. In these ischemia/reperfusion studies, the occlusion time ranged from 40 to 120 minutes [22, 25, 28, 30-33, 37-46].

Evidence suggests that this method results in variable infarcts that may include areas of the thalamus, hypothalamus, hippocampus, and substantia nigra in addition to the MCA territory. This variability occurs when the inserted monofilament may affect the blood flow of the ICA to its branches [58, 59]. In addition, it has been observed that the size of the infarct may reach up to 50% of the cerebral hemisphere and, therefore, does not correspond to the features seen in the most common cases of stroke in humans, which vary from 4.5 to 14% of the ipsilateral hemisphere [56]. Because of these and other potential limitations, some studies opt for alternative techniques of MCAO.

In the microcatheter triggered model of MCAO, a midline incision is made on the ventral side of the tail. A microcatheter connected to a microwire is inserted into the ventral artery and advanced into the aorta [60]. Fluoroscopy is used to guide the microwire to the MCA to selectively occlude branches of the artery. Arnberg et al. [47], for example, occluded the M2 branch. After 90 minutes, the microcatheter is removed to allow reperfusion. Like the suture model, this technique allows reversible and focal ischemia. However, it results in infarcts limited to the somatosensory cortex or striatum, depending on which segment of the MCA is occluded. This selective occlusion allows studies of neuronal repair after stroke [60].

Among the studies that have used the MCAO model, two have chosen embolic models. The MCAO embolic model consists of macrosphere injection or clot injection [48, 50]. In the macrosphere injection method, a modification of the technique of Gerriets et al. [61], a PE -50 catheter filled with saline and two TiO2 macrosphere is inserted into the CCA and advanced to the origin of the pterygopalatine artery, where it is fixed. The macrospheres are then injected to perform MCAO [48]. This method results in similar infarcts to permanent suture closure of the MCA, but

with a more significant standard deviation in size. The advantage is that the likelihood of hypothalamic infarcts is low [56, 61].

In the clot injection method, a PE -50 catheter with an outer diameter of 0.3 mm filled with an autologous clot of 25 mm is inserted into the ECA and advanced to the ICA [50, 62, 63]. When the tip of the catheter is found to be at the origin of the MCA, the clot is injected to occlude the artery. This technique allows selective occlusion of the MCA, resulting in infarcts confined to the area perfused by the MCA [62]. Therefore, this method is useful for evaluating treatments with thrombolytic drugs, alone or in synergy with neuroprotective agents [57, 62, 63].

Another variant of the MCAO model is performed through an incision between the lateral part of the orbit and the external auditory canal. After partial resection, the parotid gland and temporal muscle are moved aside. Next, a small craniotomy is performed over the distal part of the MCA and the dura mater is removed. Then, the MCA is coagulated [64, 65]. This method has high reproducibility and produces infarcts limited to the MCA area [56-58]. However, this technique requires a craniotomy, which requires good surgical skills to avoid damage to the underlying tissue. Another limitation is the impossibility of controlled reperfusion, which may be caused by local collateral blood flow [56, 57].

In most of these studies, which used the MCAO model, the micro-PET imaging by [^{18}F]FDG administration was used to attend to important goals, such as evaluating the therapeutic efficacy of potential new drugs or understanding the mechanism of action for the potential treatments of ischemic stroke [22, 23, 28-34, 37-43, 45-47, 50, 51]. For instance, Hwang et al. [33] investigated the therapeutic efficacy of liposomal angiogenic peptides. It was observed that the peptides increased the [^{18}F]FDG uptake in the ipsilateral ischemic cortex, which correlated with the increase in cerebral perfusion [33]. Therefore, the [^{18}F]FDG/PET technique is promising in the pre-clinical studies of new treatment evaluation for stroke. Looking at non-pharmacological therapy, a study performed by Li et al. [42] aimed to understand the therapeutic effects of constraint-induced movement therapy (CIMT). It was observed that, in animals that suffered cerebral ischemia, CIMT promoted both an increase in [^{18}F]FDG uptake in the contralateral hemisphere and a reduction in the ipsilateral hemisphere, in parallel with an improvement in behavioral performance. A possible explanation for this phenomenon observed, reduction of [^{18}F]FDG uptake, is the reduction of glucose metabolism in the injured hemisphere, which leads to an optimization of the compensatory

role of the contralateral hemisphere [42]. So, the [^{18}F]FDG/PET technique can help to elucidate the therapeutic effects of stroke treatments.

Other studies aim to understand glucose metabolism in this model, for instance, Ni et al., 2022 [66] show a reduced [^{18}F]FDG in the ischemic striatum of tMCAO mouse brain at 24 h after reperfusion. Besides being used to study brain metabolism under cerebral ischemia and infarction, PET imaging with FDG can automatically assess the quality of the MCAO procedure [67]. A new protocol using [^{18}F]FDG/PET was developed that allows users to evaluate their own image data with only one scan after the MCAO [67].

3.5 Common Carotid Artery Occlusion

Besides the MCAO, the second stroke model most used was the CCAO [51-54]. For the unilateral stroke model to be made, it is necessary to add hypoxic stress, as collateral blood flow compensates for the difference in flow between the hemispheres [68]. Therefore, a permanent ligation is performed on the CCA with a suture. After occlusion, the animals are exposed to hypoxic air (8% oxygen). The exposure time ranged from 15 to 90 minutes [52-54]. This method is widely used in neonatal stroke studies [42, 53, 68, 69]. However, it does not ideally model the human condition [68, 69]. Thus, this methodology has variations, such as the model that adds LPS injection to induce inflammation [52, 68]. As for the unilateral model in general, there is a generation of very variable results, because of the difference in the collateral flow between animals [54].

In the bilateral model, the arteries are permanently or temporarily occluded to induce global cerebral ischemia. The death of neurons in certain areas and the degeneration of the retina are characteristic consequences of this ischemia in murine models. These areas can encompass hippocampal subregions, the cerebral cortex, and the striatum. Some limitations of this model are high mortality and the possibility of seizures. Furthermore, it has high variability due to the same reasons for the unilateral model [70].

Half of the studies that used the CCAO model used the [^{18}F]FDG/PET technique to assess the therapeutic efficacy of stroke treatments [51, 52]. The other half of these studies analyzed the diagnostic potential of PET in this pathology [53, 54]. For example, a study performed by Chevin et al [52] investigated the therapeutic efficacy of hypothermia in treating neonatal stroke. Hypothermia resulted in a higher cerebral uptake of [^{18}F]FDG in parallel with a lower volume of infarcted area in the treated animals [52]. Thus, the potential of [^{18}F]FDG/PET in evaluating stroke

treatments was confirmed [4]. Odorcyk et al [53] evaluated the [^{18}F]FDG/PET predictive potential of neonatal hypoxia-ischemia (HI) consequences. Interestingly, the data obtained by [^{18}F]FDG/PET images, 72 hours after IH, had significant correlations with behavioral and histological data in animals in adulthood [53]. Therefore, the [^{18}F]FDG/PET technique is promising for the diagnosis of stroke in this model [4, 5].

3.6 Photothrombotic model

In the photothrombotic stroke (PTS) model, the photosensitive dye Rose Bengal is injected intravenously 10 minutes before an animal's skull is exposed to a laser or during the first 2 minutes of irradiation. Stereotactic coordinates determine the irradiated region and the procedure takes 20 minutes [23, 55]. This technique leads to dye oxidation, generating singlet oxygen, endothelial damage, platelet activation, and, consequently, ischemia throughout the irradiated region [56, 57]. It is important to note that it is performed by a minor invasive surgery, has high reproducibility, can be used for neural repair studies, and has a small and well-defined infarctions area [23, 56, 69]. However, it results in a little penumbra, and there is no possibility of reperfusion by collateral blood flow [56, 69].

Studies performed by Liu et al. [55] applied the [^{18}F]FDG/PET imaging to assess cerebral glucose metabolism concomitantly with the evolution of the lesion induced by the photothrombotic model. This methodology was efficient for the characterization of this model, which can be widely used in studies evaluating treatments for ischemic stroke [55]. Zhang et al. [23] used [^{18}F]FDG/PET to investigate whether the cerebral metabolic network has a modular architecture and to analyze a disturbance's effect on this network. This disturbance was induced by focal ischemia in the photothrombotic model. With the aid of [^{18}F]FDG/PET, it was possible to verify the existence of a modular architecture. Furthermore, evidence was obtained that this modularity limits the propagation of the disturbance effect to the affected module [23].

A recent study, performed by Kramer et al. [71, 72] demonstrated a reduced glucose metabolism in the right cortico-striatal thalamic loop after PTS model compared to the state before intervention, by [^{18}F]FDG-PET imaging. These results could demonstrate the changes in cerebral network activity after invasive stimulation of the mesencephalic locomotor region in a rat stroke model.

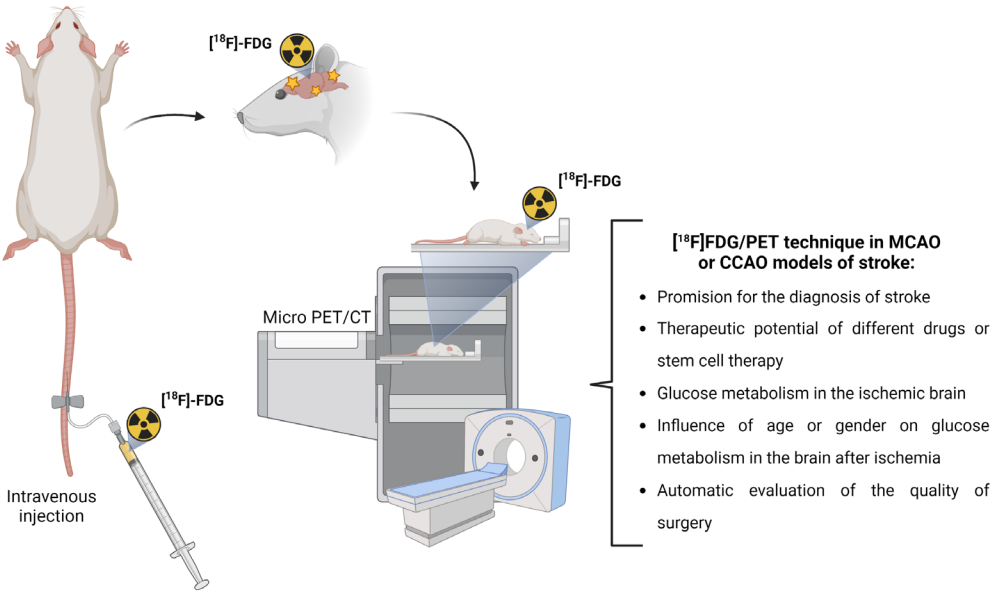
4. CONCLUSIONS

As previously mentioned, there is still no effective cure or treatment for ischemic injury and there is still much to be understood regarding its pathophysiology. The use of animal models of stroke, associated with the study of new therapies, is the main source of information currently available to fill this gap [70]. Although no pre-clinical model perfectly reproduces human stroke, these have been very useful for obtaining information associated with the pathogenesis of the disease and adding basic support for developing new therapeutic approaches.

The [^{18}F]FDG/PET technique has proven valuable for clinical diagnosis and determination of the patient's prognosis, providing relevant functional information about the penumbra region. It has been useful in pre-clinical studies of new therapeutic approaches against stroke, mainly in focal cerebral ischemia models using rats. [^{18}F]FDG/PET studies using mice are not frequent. The reason for this is likely limitations related to the size of the animal's brain, small blood volume for sampling, and cerebral vascular variability between individuals of the same species and lineage [73].

Although PET equipment generally has high sensitivity, spatial resolution, especially concerning studies of small animals, is still limited. Values range from 5 to10 mm in equipment for clinical use, and 0.9 mm for devices dedicated to pre-clinical research using fluorine-18 labeled radiopharmaceuticals. Although the absolute values are small, the ratio between resolution and volumes of interest is worse in practice and distortions such as those related to the positron reach before annihilation become more pronounced [73]. Therefore, choosing the most appropriate model for the proposed research and using strategies to homogenize the results are important.

Figure 1: [^{18}F]FDG/PET is an important tool for stroke study. A review of the literature shows several experimental models, but the most commonly used are MCAO and CCAO in rats. The use of MicroPET provides a non-invasive approach to monitoring stroke in an experimental model. With this tool, it is possible to study the characteristics of stroke and, for example, to investigate the therapeutic potential of some drugs.



In conclusion, although there is no ideal model for studying stroke since all have associated limitations, the [^{18}F]FDG/PET technique is a non-invasive tool with great potential for the investigation of ischemic brain metabolism and new potential therapeutic approaches using the available stroke models. Besides, [^{18}F]FDG/PET technique seems to be a promising tool in diagnosing and staging stroke in humans since it allows the identification of the affected regions and those with potential for recovery, which can be potential targets of early therapeutic intervention.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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