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Phosphodiesterase type 5 Inhibitors (PDE-5I) as a potential therapeutic drug in stroke: A systematic narrative review

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ABSTRACT

Objectives. Stroke is an acute cerebrovascular disease with high morbidity and mortality rate. Many stroke management strategies can improve prognosis and quality of life, but only to a certain extent. Phosphodiesterase inhibitor-5 (PDE-5i) has shown benefits in numerous preclinical studies, but human studies have been inconclusive.

Material and Methods. For this narrative review, we conducted a systematic search following the PRISMA statement guideline from inception until November 2022. The search was conducted in PubMed, ScienceDirect, EBSCOhost, and ProQuest. We included all human and animal studies on this topic, including preclinical studies, randomized clinical trials, and case studies. All the excluded studies are reviews or non-English studies.

Review. Many PDE-5i have shown benefits in anatomical and functional outcomes in preclinical acute experimental stroke models. PF-03049423 was safe and well tolerated in humans but had no significant impact on neurological or functional outcomes. Sildenafil on acute/subacute was deemed safe to use and demonstrated improvement over baseline, but power remains unknown due to the lack of a control group. Tadalafil use resulted in a reduction in regional cerebral blood flow in subjects with a history of stroke.

Conclusions. Despite promising results in preclinical studies, current evidence shows that PDE-5i does not affect clinical or functional recovery in people with acute stroke. The disparity in the intricacy of stroke pathophysiology between human and animal models was crucial. Further research in a larger population with more consistent stroke onset, medicines, and doses is required for a more conclusive result.

Keywords: Phosphodiesterase 5 Inhibitor, PDE 5 inhibitor, stroke, cerebral stroke, CVA, brain vascular accident

BACKGROUND

Stroke is still considered a disease with a high morbidity and mortality rate. It was estimated that 1 in 6 cardiovascular-related deaths was due to stroke, and three-fourths of survivors are disabled, with 15 to 30% having severe disability [1,2]. According to available data, more than a million new cases of ischemic and hemorrhagic stroke occur each year worldwide. These rapid new cases significantly burden families and society due to significant expenditure on complication treatment in healthcare systems. Concerningly, global population aging and dietary changes are increasing the incidence and burden of a stroke every year [3].

Stroke has a complex pathophysiology that includes an inflammatory response, neuronal apoptosis, ischemia-reperfusion injury, blood-brain barrier damage, neurotoxic substance release, free radical generation, oxidative stress, and brain edema. At present,

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stroke management/therapies mainly concentrate on thrombolytic agents, neuroprotective drugs, mechanical thrombectomy, stent, angioplasty, surgical treatment (decompressive craniectomy and carotid endarterectomy), and rehabilitation training [4]. Comprehensive therapy and early thrombolysis/mechanical thrombectomy have been shown to improve prognosis and quality of life but only to a certain extent.

PDE-5Is, such as Sildenafil (Viagra®), are widely available and primarily used to treat erectile dysfunction and pulmonary hypertension (PH). Its use in stroke patients was still debatable [5]. Many preclinical studies, mostly in animals, have suggested that PDE-5I may improve functional outcomes after an ischemic stroke. These studies have shown the potential use of PDE-5I, which exerted neuronal and synapse protection, improved angiogenesis, cerebral blood flow, and overall brain function, and reduced apoptosis, oxidative stress, and neurodegeneration [6-9].

The study in humans yielded varying results, whereas many animals benefited. Due to the ambiguity of the evidence, the current study aims to compile and compare all relevant studies related to the therapeutic potency of PDE-5i in stroke subjects, both in animals and humans, to reach a more definitive conclusion.

METHODS

Search strategy and selection criteria

For this narrative review, a structured literature search was conducted from inception until November 2022 to identify published articles on the potential use of PDE-5i in the recovery of stroke patients. The search was conducted in PubMed, ScienceDirect, EBSCOhost, and ProQuest, by using the following MeSH term keywords "Phosphodiesterase 5 Inhibitor", "PDE 5 inhibitor", "Stroke," "Cerebral stroke," "CVA," and "Brain vascular accident." The search terms, especially for PDE 5 inhibitors, were broad to encompass every drug with a PDE 5 inhibitor mechanism. We did a systematic searching method for this narrative review following the PRISMA statement guideline [10], with a predetermined search strategy in which we identified potential studies, screened titles and abstracts, assessed full-text articles, and determined relevant studies.

All studies related to this topic, both in humans and animals, including preclinical studies, randomized clinical trials, and case studies, were included in the present study. We also reviewed each article's references to find other relevant studies or reports not identified by the search. After eliminating duplicates, the authors reviewed all titles and abstracts and excluded those articles with full text that failed to be retrieved. The exclusion criteria included review studies and non-English studies.

TABLE 1. Search terms strategy

Source	Search Term	Number of Studies
PubMed	((Phosphodiesterase 5 inhibitor) OR (PDE 5 inhibitor)) AND ((stroke) OR (cerebral stroke) OR (CVA) OR (Brain vascular accident))	236
ScienceDirect	((Phosphodiesterase 5 inhibitor) OR (PDE 5 inhibitor)) AND ((stroke) OR (cerebral stroke) OR (CVA) OR (Brain vascular accident))	4642
ProQuest	(title(Phosphodiesterase 5 inhibitor) OR title(PDE 5 inhibitor) OR title(PDE-5i) OR abstract(Phosphodiesterase 5 inhibitor) OR abstract(PDE 5 inhibitor) OR abstract(PDE-5i)) AND (title(stroke) OR title(brain vascular accident) OR title(cerebral stroke) OR title(CVA) OR abstract(stroke) OR abstract(brain vascular accident) OR abstract(CVA) OR abstract(stroke))	58
EBSCOhost	(AB phosphodiesterase inhibitor OR AB phosphodiesterase-5 inhibitors OR AB phosphodiesterase type 5 inhibitors OR AB PDE5 inhibitors) AND (AB stroke OR AB cerebral stroke OR AB CVA OR AB Brain vascular accident)	286

Data extraction

The following data were extracted from the studies selected for inclusion: (1) first author; (2) publication year; (3) region; (4) study design; (5) sample size; (6) age; (7) gender; (8) intervention; (9) results. To ensure the accuracy of all the data, the information was extracted by four independent authors, and conflicting data were resolved with consensus among all the authors.

The data retrieved in animal and human studies differed since many animal trials involved brain removal, whereas only clinical assessments were performed in humans. In animals, we obtained anatomical (infarct size, edema size, penumbra enlargement, angiogenesis, cerebral blood flow, vascular density, neuronal density) and functional (modified six-point scoring, beam walking test, rotarod test, adhesive removal test) outcomes. In humans, we retrieved clinical (mRS day 90, BI score, NIHSS score, cerebral perfusion)

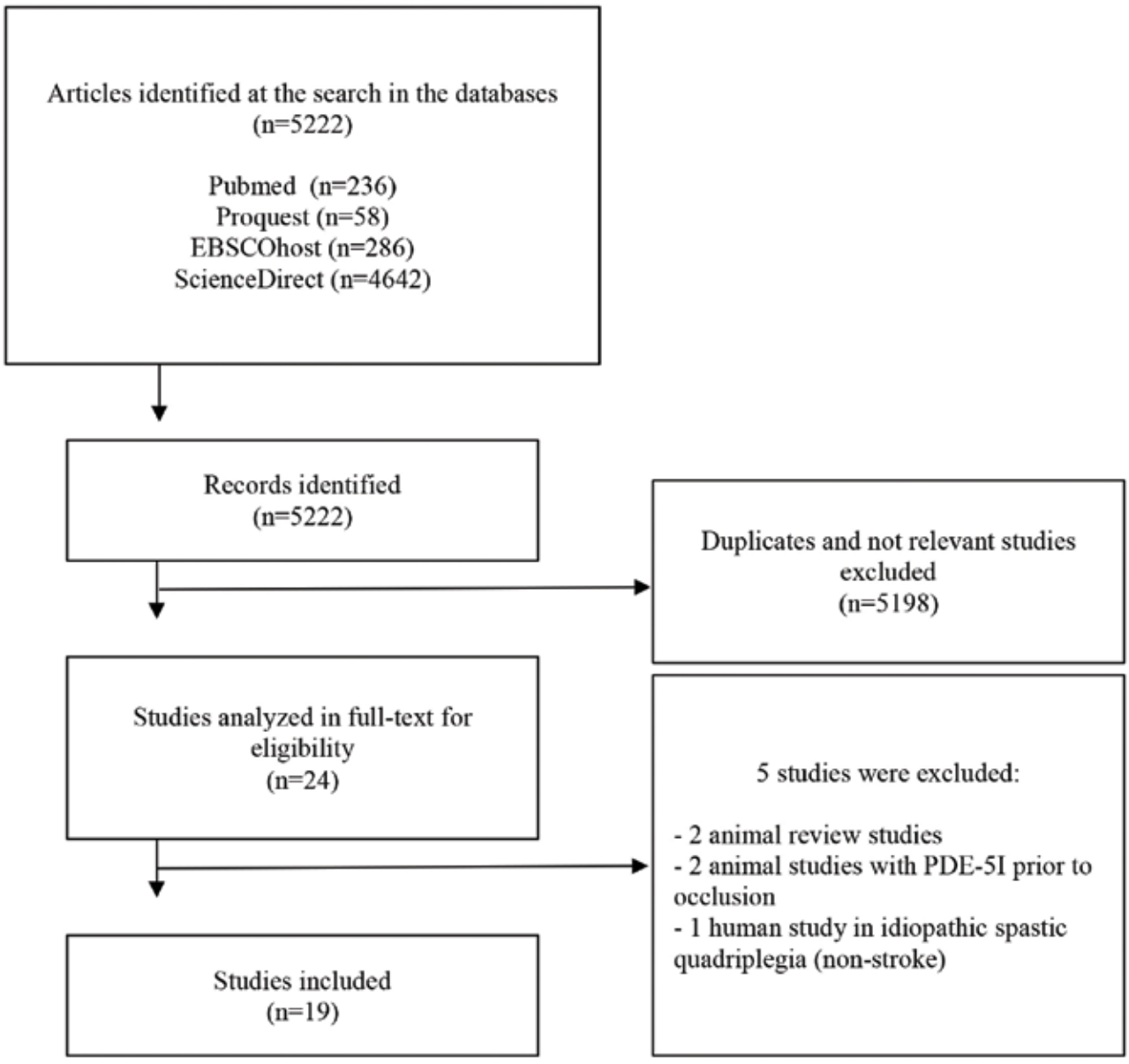

FIGURE 1. Study selection process following PRISMA guidelines

and functional (sensorimotor, box and blocks test, hand-grip strength test, 10-meter walk test, repeatable battery assessment of neuro-psychological status naming and codings subtests, line cancellation test, and recognition memory test) outcomes for analysis.

Cerebral vascular dysregulation in cerebral ischemia

The advancement in technology and techniques enable us to measure regional cerebral blood flow (CBF), providing us with new insights into the alteration of CBF distribution following focal or global cerebral ischemia. Occlusion of a major cerebral artery severely impaired CBF to the area of the brain supplied by this artery. This impairment can be observed more clearly in the center of the ischemic territory, where the CBF reduction is at its greatest. Surrounding the 'core' area of cerebral ischemia is an area with less severe CBF reduction termed ischemic penumbra [11]. The fate of the penumbra then depends on the residual CBF and the duration of the flow reduction. Studies found that CBF in the penumbra ranged between 12 to 22 cc/100 g per minute. A further drop in the CBF below this critical point for a suffi-cient period resulted in tissue damage [12].

Re-establishment of the CBF in ischemic areas following perma-nent/transient arterial occlusion is beneficial to be done as early as possible. On the other hand, reperfusion may also exacerbate brain injury/reperfusion injury through the development of hemorrhagic transformations or cerebral edema [13]. After reopening the vessel occlusion, reperfusion in the ischemic core typically shows a biphasic pattern in

which CBF increases transiently (post-ischemic hyperperfu-sion/luxury perfusion) followed by more sustained reduction (hypoperfusion), confirmed by laser Doppler flowmetry studies. Post-ischemic hyperperfusion has been demonstrated in several animal and human stroke models [14-18]. What is interesting is that this hyperemic phase is not mediated by an increase in oxygen or glucose utilization and can be attributed to abnormal vasodilatation in the ischemic territory. Such abnormal vasodilatation has been observed to cause multiple negative effects, including lactic acidosis secondary to ischemia-induced anaerobic glycolysis [19] and or release of vasoactive mediators from the ischemic brain, including ions, metabolites, and reactive oxygen species [20]. The mechanisms leading to post-ischemic hypoperfusion are still unclear, but the hypothesis circle around the increase in cerebrovascular resistance due to microvascular compression and vasospasm, as suggested by a scanning electron microscopy study in animal models. Microvessels appeared constricted, compressed, and narrowed, presumably by vasoconstrictors released by the ischemic brain [21].

Vasodilatation in stroke

Nitric oxide (NO) was first named endothelium-derived relaxing factor (EDRF) in the late 1970s, a colorless, odorless gas that transmits biological information to relax smooth muscle cells and cause vasodilatation. This signaling molecule's primary functions include maintaining vascular tone, reducing inflammation response, balancing thrombotic-thrombolytic homeostasis, and regulating cell growth [22]. In the acute stage of cerebral ischemia, one of our physiological self-defense mechanisms is to increase focal NO production in the ischemic area, sustained for at least one hour [23,24].

Guanosine 3',5'-cyclic monophosphate (cGMP) is another critical regulatory factor mediating the vasodilatory effect on the cerebral vessels. cGMP is regulated through synthesis by guanylyl cyclase and degradation by phosphodiesterases, especially the phosphodiesterase type 5 (PDE5) enzyme, as it is a particular enzyme for hydrolysis cGMP. This molecule is closely related to the expression of NO, which activates soluble guanylate cyclase. cGMP is formed in response to nitric oxide (NO) by NO-sensitive guanylyl cyclases in two isoforms (NO-GC1 and NO-GC2). The other way to increase the cGMP level without raising the NO level was to block its degradation [25].

Drug profile and mechanism of action

Several mechanisms of action from PDE-5Is had been proposed: (1) PDE-5i increase expression of nitric oxide (NO) synthases. The NO increase will then activate soluble guanylate cyclase in myocytes, which converts guanosine triphosphate to cGMP. The increase in cGMP concentrations then reduced smooth muscle tone and caused vasodilations, meaning there was an increase in cerebral blood flow. (2) PDE-5i prevented the cGMP conversion to GMP. Thus, the cGMP accumu-lates and increases phosphokinase G (PKG), improving cerebrovascular perfusion and producing neurogenesis, angiogenesis, and synaptogenesis [26]. (3) PDE-5i prevented the activation of calpain and CKD5, and increased the p25/p35 ratio. The formation of p25/CKD5 complexes had been associated with the aggregate for-mation in stroke [27]. (4) PDE-5i increased the expression of the antiapoptotic proteins Bcl-2 and Bcl-xL and reduced cell death. (5) PDE-5i reduced proapoptotic pro-teins Bax and caspase-3 expression [28]. (6) PDE-5i increased postsynaptic density protein-95 (PSD-95), an important membrane-bound protein for synaptic plasticity.

PDE-5i, such as Tadalafil and Sildenafil, showed neurogenesis enhancement and increased proliferating neural progenitor cells in the penumbra [29]. Several animal studies have demonstrated the protective effects on neuronal networks and synaptogenesis by reducing neuron loss, enhancing axonal remodeling, and modulating microglial function [6,7]. Further, Sildenafil reduced the capillary density loss, induced angiogenesis, and cerebral blood flow in the ischemic penumbra shown by MRI. These findings were accompanied by reduced neuronal apoptosis by the expression of apoptotic factors [8,9].

Current evidence of PDE-5i in acute stroke

A total of 16 preclinical studies in animal models and three studies in hu-mans were included in the present study. Table 2 summarizes the studies in animals, and Table 3 summarizes the studies in humans.

Infarct size and edema size after PDE-5i intake was improved in 7 studies, with no changes in 5 studies. Among the 7 studies, improvement was observed with Yonkenafil [6], Sildenafil [7,29,33], Zaprinast [23], Bevacizumab [34], and Verdenafil [35]. All three Sildenafil studies used single intravenous/intraperitoneal doses rang-ing from 12 to 32 mg/kg. A dose-dependent effect was observed in many studies [6,7,33]. No significant improvement was observed in studies with Tadalafil [39] and Sildenafil [31,36-38]. Almost all [36-39] non-significant results were observed in oral administration at doses of 0.3 to 5 mg/kg. One study with subcutaneous administration found a smaller mean infarct area when compared to placebo, but it was not statistically significant [31]. This reduction in infarct size was a result of PDE-5i's ability to increase cerebral blood flow [23] (CBF), enhance angiogenesis [23], antioxidant effect [39], and provide neuroprotection (reactive astrogliosis suppression [33], S100B and AQP4 co-expression suppression [29], synaptophysin activation [7], and cGP-PKG activation [29]).

TABLE 2. Animal studies of PDE-5i in acute stroke rat models

First Author, Year	Region	Study Design	Population	Groups (intervention, size/n)	Interventions	Outcomes
Chen et al., 2014 [6]	China	Blinded Experimental study	Male Sprague-Dawley rats, eight weeks, subjected to MCA occlusion	Experimental group (MCAO+PDE-5I, n=10-13[a])	Yonkenafil (4/8/16/32) mg/kg IV/IP, single dose, given (2/4/6) hours post-reperfusion	**Anatomical** Significant infarct size and edema reduction in the Yonkenafil group (dose-dependent). Significant penumbra enlargement in the Yonkenafil group Considerable reduction of ischemic cell apoptosis and neuronal loss in the Yonkenafil group **Functional** Significant neurological function (modified six-point scoring) and behavioral function improvement (the beam walking test and rotarod test) in the Yonkenafil group.
				Experimental group (MCAO+ Inhibitor)	Inhibitors were administered immediately after MCAO	
				Experimental group (MCAO+None)	No interventions were given post-reperfusion	
				Control group (Saline, n=10-13[a])	Normal saline 10 mg/kg IP/IV, once daily for 7 days, given (2/4/6) hours post-reperfusion	
Chen et al., 2014 [7]	China	Blinded Experimental study	Male Sprague-Dawley rats, 8 weeks, subjected to MCA occlusion	Experimental group (MCAO+PDE-5I, n=10–12[a])	Sildenafil (4/8/16/32) mg/kg IV/IP, single dose, given (2/4/6) hours post-reperfusion	**Anatomical** Significant infarct size and edema reduction in the Sildenafil group (dose-dependent). Significantly reduced degenerated neurons, neuronal loss, and increased surviving neurons in the Sildenafil group. **Functional** Significant neurological function (modified six-point scoring) and behavioral function improvement (the beam walking test and rotarod test) in the Yonkenafil group.
				Experimental group (MCAO +Inhibitor)	Inhibitors were administered immediately after MCAO	
				Experimental group (MCAO+None)	No interventions were given post-reperfusion.	
				Control group (Saline, n=10–12[a])	Normal saline 10 mg/kg IP/IV, once daily for 7 days, given (2/4/6) hours post-reperfusion	
Ding et al., 2011 [8]	China	Blinded Experimental study	Male Wistar rats, 18 months, subjected to MCA occlusion	Experimental group (MCAO+PDE-5I, n=10)	Sildenafil 10 mg/kg SC, once daily for 7 days, given 24 hours post-reperfusion	**Anatomical** Significant increase in angiogenesis, axonal remodelling, cerebral blood flow, and ventricle expansion inhibition in the Sildenafil group. **Functional** Significant improvement in mNSS score in the Sildenafil group
				Control group (MCAO+Saline, n=10)	Normal saline 10 mg/kg IV, once daily for 7 days, given 24 hours post-reperfusion.	
Ding, et al., 2008 [9	China	Experimental study	Male Wistar rats, 8 to 12 weeks, subjected to MCA occlusion	Experimental group (MCAO+PDE-5I, n=11)	Sildenafil 10 mg/kg SC, once daily for 7 days, given 24 hours post-reperfusion	**Anatomical** Significant acceleration in angiogenesis detected by T2*WI and SWI in the Sildenafil group. Significant enhancement in cerebral angiogenesis measured histologically in the Sildenafil group
				Control group (MCAO+Saline, n=10)	Normal saline 10 mg/kg SC, once daily for 7 days, given 24 hours post-reperfusion	
Gao et al., 2005 [23]	China	Blinded Experimental study	Male Wistar rats, subjected to MCA occlusion	Experimental group (MCAO+PDE-5i, n=8)	Zaprinast 10 mg/kg IV, single dose, given 10 minutes post-reperfusion	**Anatomical** Significant infarct size reduction in the Zaprinast group. Significant increase of regional cerebral flow for 10-20 minutes after infusion in the Zaprinast group
				Experimental group (MCAO+SNAP, n=7)	SNAP 100 mcg/kg/min IV, single dose, given 10 minutes post-reperfusion	
				Control group (MCAO+Saline, n=8)	Phosphate-buffered saline IV, single dose, given 10 minutes post-reperfusion.	

First Author, Year	Region	Study Design	Population	Groups (intervention, size/n)	Interventions	Outcomes
Gao et al., 2020 [30]	China	Blinded Experimental study	Male Sprague-Dawley rats subjected to MCA occlusion	Experimental group (MCAO+PDE-5I, n=10*)	Icariside II (4/8/16) mg/kg IV, twice daily for 7 days, given 2 hours post-reperfusion	**Anatomical** Significant neuronal and organelles damage reduction in the Icariside group. **Functional** Significant improvement in mNSS score in the Icariside group post ischemia (dose dependent) Significant neurological function (discrimination index test) in the Icariside group. Significant behavioral function improvement (adhesive removal test and rotarod test) in the Icariside group
				Control group (MCAO+Saline, n=10*)	Saline (4/8/16) mg/kg IV, twice daily for 7 days, given 2 hours post-reperfusion	
Li et al., 2007 [31]	China	Experimental study	Male Wistar rats, 12 to 16 weeks, subjected to MCA occlusion	Experimental group (MCAO+PDE-5I, n=11)	Sildenafil 10 mg/kg SC, once daily for 7 days, given 24 hours post-reperfusion	**Anatomical** No significant difference in ischemic lesion size, but mean values is smaller in Sildenafil group. Significant increase of cerebral vessel number in the lesion boundary area in the Sildenafil group. **Functional** Significant improvement in neurological (NSS score) and behavioral (foot-fault test) function in the Sildenafil group
				Control group (MCAO+Saline, n=10)	Saline 10 mg/kg IV, once daily for 7 days	
Menniti et al., 2009 [32]	USA	Experimental study	Male Sprague-Dawley rats, subjected to MCA occlusion	Experimental group (MCAO+PDE-5I, n=10)	Sildenafil 10 mg/kg SC, twice daily for 7 days, given 24 hours post-reperfusion	**Functional** Significant improvement in AUEC scores of neurological/ sensorimotor function (fore-/ hind-limb placement task and ipsilateral body-swing test) in Sildenafil and PF-5 group. (dose-dependent)
				Experimental group (MCAO+PF-5, n=10*)	PF-5 (0.1/1/10) mg/kg SC, twice daily for 7 days, given 24 hours post-reperfusion.	
				Control group (MCAO+Vehicle, n=10)	Saline 10 mg/kg SC, twice daily for 7 days, given 24 hours post-reperfusion	
Moretti et al., 2016 [33]	USA	Experimental study	C57B1/6 rats, 9 days, subjected to MCA occlusion	Experimental group (MCAO+PDE-5i, n=8*)	Sildenafil (0.5/2.5/10/15) mg/kg IP, single dose, given 5 minutes post-reperfusion	**Anatomical** Significant mean lesion reduction in the Sildenafil group. (dose-dependent) Significant decrease of microglial density in the Sildenafil group. Significant decrease of COX2+ microglial/macrophage in the Sildenafil group. Modulation of M1- (ptgs2, CD32 and CD86) and M2-like (CD206, Arg-1 and Lgals3) microglia/ macrophages in the late phase in the Sildenafil group
				Control group (Saline, n=8)	Saline 10 mg/kg IV, single dose, given 5 minutes post-reperfusion	
Novitzky et al., 2016 [34]	Israel	Experimental study	C57Bl/6 rats, subjected to MCA occlusion	Experimental group (MCAO+PDE-5i, n=16)	Sildenafil 24 mcg/mcL IP, single dose, given 1 hour post-reperfusion	**Anatomical** Largest penumbra area in the early Bevacizumab group. Significant smaller total stroke area in both the early and delayed Bevacizumab group. Significant lowest injured brain hemispheric percentage in the delayed Bevacizumab group.
				Experimental group (MCAO+early VEGF inhibitor, n=19)	Bevacizumab 75 mcg/mcL IP, single dose, given 1 hour post-reperfusion	
				Experimental group (MCAO+delayed VEGF in-hibitor,n=7)	Bevacizumab 75 mcg/mcL IP, single dose in 6 hours after induction	
				Control group (MCAO+Saline, n=16)	Saline 0.3 mL IP, single dose, given 1 hour post-reperfusion	

First Author, Year	Region	Study Design	Population	Groups (intervention, size/n)	Interventions	Outcomes
Royl, et al., 2009 [35]	Germany	Experimental study	Male C57Bl/6 rats, 7 weeks, subjected to MCA occlusion	Experimental group (MCAO+PDE-5i, n=20)	Verdenafil 10 mg/kg PO, twice daily for 14 days, given 45 minutes post-reperfusion.	**Anatomical** Significant smaller lesion volume in the Verdenafil group) Significant lower relative atrophy of ischemic hemi-sphere in the Verdenafil group. No effect of Verdenafil on the cerebral blood flow in the is-chemic and non-ischemic hemisphere **Functional** No significant improvement in functional recovery (pole test) in the Verdenafil alone. No difference in functional recovery (wire hanging test) between groups
				Experimental group (MCAO+Surgery + PDE-5i, n=20)	Surgery followed by Verdenafil 10 mg/kg PO, twice daily for 14 days, given 3h post-surgery,	
				Experimental group (MCAO+Surgery + Vehicle, n=20)	Surgery followed by Solutol HS15 2500 mcL PO, twice daily for 14 days, given 3h post-surgery	
				Control group (MCAO+Vehicle, n=20)	Solutol HS15 2500 mcL, twice daily for 14 days, given 45 minutes post-reperfusion	
Wang, et al., 2013 [36]	China	Experimental	Male C57BL/6 rats, 2 to 3 months, subjected to MCA occlusion	Experimental group (MCAO+PDE-5i, n=17)	Slidenafil 0.3 mg/kg PO, once daily for 6 days, given 24 hours post-reperfusion	**Anatomical** No significant difference in the infarct volume between groups **Functional** Significant improvement in adhesive removal test score in the combination group as compared to atorvastatin and placebo
				Experimental group (MCAO+Statin, n=16)	Atorvastatin 0.3 mg/kg PO, once daily for 6 days, given 24 hours post-reperfusion	
				Experimental group (MCAO+ Combination, n=17)	Sildenafil 0.3 mg/kg + Atorvastatin 0.3 mg/kg PO, once daily for 6 days, given 24 hours post-reperfusion	
				Control group (MCAO+Vehicle, n=17)	H20 0.3 mg/kg PO, once daily for 6 days, given 24 hours post-reperfusion	
Yu, et al., 2022 [29]	Corea	Experimental	Male Sprague-Dawley rats, 8 weeks, subjected to MCA occlusion	Experimental group (MCAO+PDE-5i pre-stroke, n=20)	Sildenafil 20 mg/kg IP, sigle dose, given 30 minutes prior to MCAO	**Anatomical** Significant reduction in infarct volume in both the pre- and post-sildenafil treatment compared to no intervention **Functional** Significant improvement in motor defects on both the pre- and post-sildenafil treatment compared to no intervention.
				Experimental group (MCAO+PDE-5i post-stroke, n=20)	Sildenafil 20 mg/kg IP, single dose, given 30 minutes after MCAO	
				Experimental group (MCAO+No intervention, n=20)	No intervention after MCAO	
				Control group (Naive, non-stroke, n = 20)	Non-stroke, healthy rats	
Zhang, et al., 2002 [37]	China	Experimental	Male Wistar rats subjected to MCA occlusion	Experimental group (MCAO+early PDE-5i, n=10, 9 respectively)	Sildenafil (2/5) mg/kg PO, once daily for 6 days, given 2 hours post-reperfusion	**Anatomical** No significant difference in infarct volume among all groups. Significant increase in numbers of BrdU-immunoreactive cells in the dentate gyrus on all Sildenafil groups Significant increase in numbers of BrdU-immunoreactive cells in the ipsilateral subventricular zone in the 2 mg/kg dose group Significant increase in the numbers of BrdU-immunoreactive cells in the subventricular zone of both hemispheres in the 5 mg/kg dose group **Functional** Significant performance improvement in functional tests (foot-fault test and adhesive removal test) in the early PDE-5i group
				Experimental group (MCAO+late PDE-5i, n=10)	Sildenafil 2 mg/kg, once daily for 6 days, given 24 hours post-reperfusion	
				Control group (MCAO+Water, n=9)	Tap water PO, once daily for 6 days, given 24 hours post-reperfusion	

First Author, Year	Region	Study Design	Population	Groups (intervention, size/n)	Interventions	Outcomes
Zhang, et al., 2005 [38]	China	Experimental	Male Wistar rats, 12 weeks, subjected to MCA occlusion	Experimental group (MCAO+Aged Oral PDE-5i, n=10)	Sildenafil 2 mg/kg PO, given 24 hours after occlusion, once daily for 6 days	**Anatomical** No significant difference in infarct volume among all groups **Functional** Significant performance improvement on NSS and adhesive removal test in aged subcutaneous Sildenafil group Significant performance improvement on NSS, adhesive removal test, foot-fault test, and corner test in both the young oral and subcutaneous Sildenafil group
				Experimental group (MCAO+Aged Subcutaneous PDE-5i, n=8)	Sildenafil 10 mg/kg SC, given 24 hours after occlusion, once daily for 6 days	
				Control group (MCAO+Aged Saline, n=15)	Saline SC, given 24 hours after occlusion, once daily for 6 days	
				Experimental group (MCAO+Young Oral PDE-5i, n=10)	Sildenafil 2 mg/kg PO, given 24 hours after occlusion, once daily for 6 days	
				Experimental group (MCAO+Young Subcutaneous PDE-5i, n=13)	Sildenafil 10 mg/kg SC, given 24 hours after occlusion, once daily for 6 days	
				Control group (MCAO+Young Saline, n=12)	Saline SC, given 24 hours after occlusion, once daily for 6 days	
Zhang et al., 2006 [39]	China	Experimental	Male Wistar rats subjected to MCA occlusion	Experimental group (MCAO+PDE-5i, n=6)	Tadalafil 2 mg/kg PO, given every 48 hours for 6 days	**Anatomical** No significant difference in infarct volume among all groups. Significant vessel density and BrdU immunoreactive endothelial cells within the ischemic boundary area in the Tadalafil group **Functional** Significant performance improvement in functional test (foot-fault test, NSS, and adhesive removal test) in all Tadalafil group.
				Experimental group (MCAO+PDE-5i, n=6)	Tadalafil 10 mg/kg PO, given every 48 hours for 6 days	
				Control group (MCAO+Saline, n=8)	Saline PO, given every 48 hours for 6 days	

*Abbreviations: AUEC, area under the efficacy curve; BrdU, bromo-deoxyuridine; MCA, middle cerebral artery; PDE-5i, phosphodiesterase-5 inhibitor; IP, intraperitoneal injection; IV, intravenous; SNAP, S-nitroso-N-acetyl-penicillamine; L-NAME, L-nitroarginine methyl ester; iPoCo, ischemic postconditioning I/R, ischemia-reperfusion; PO, peroral; SC, subcutaneous injection; SWI, susceptibility-weighted imaging; T2*WI, T2-weighted imaging*
[a]sample size is written in range because different outcome measurements (bean walking and rotarod tests, the dose-response experiment, the therapeutic-time window experiment) had different sample sizes
**number of subjects for each subgroup (different doses, different method of administration)*

Penumbra size was improved in 2 studies with Yonkenafil [6] and Bevacizumab [34]. Yonkenafil 8 to 32 mg/kg was given single intravenous/intraperitoneally, and Bevacizumab 75 mcg/mcL was administered intraperitoneally. The blood flows in the penumbral tissue after stroke was primarily due to the recruitment of collateral circulation in the brain, which involves nitric-oxide (NO)-dependent vasodilatation [33]. Increased cGMP (guanosine 3', 5'-cyclic monophosphate) level by preventing phosphodiesterases (PDEs) from hydrolysing cGMP without further enhancement of NO production was expected to dilate the vascular without causing further brain tissue damage [23].

Cerebral blood flow, angiogenesis, and vascular density were improved in 4 studies, and no changes in 1 study [23]. Among the 4 studies, improvement was observed with Sildenafil [8,9,31], and Zaprinast [23]. All three Sildenafil studies used daily subcutaneous 10 mg/kg administration for seven days. No progress was observed in Verdenafil [35] with 10 mg/kg oral administration. Both Sildenafil and Zaprinast were able to cause a selective increase in CBF through the NO-cGMP pathway in the ischemic penumbra side. Due to the mechanism of CBF increase being located downstream of the NO-mediated cascade, tissue damage caused by NO by acting as a highly toxic radical in combination with the superoxide anion can be avoided [23].

Neuronal loss was significantly reduced in three studies with Yonkenafil [6], Sildenafil [7], and Icariside-II [30]. The Yonkenafil and Sildenafil studies used single intravenous/intraperitoneal doses ranging from 8 to 32 mg/kg, whereas the Icariside-II study used intravenous 4-16 mg/kg twice daily for seven days. Sildenafil inhibits neuronal loss via

TABLE 3. Human studies of PDE-5i in stroke subjects

First Author, Year	Region	Study Design	Population	Groups (size/n)	Interventions	Outcomes	Trial Registry
Cesare et al., 2015 [40]	UK	Double-blind Placebo-Controlled Randomized Clinical Trial	Patients (60% males), aged 18 to 85 years, stroke on-set 1 to 3 days* Antiplatelet/ anticoagulant	PDE-5i group (n=70)	PF-03049423 6 mg PO/NGT[a], once daily for 90 days	No significant impact on neurological (mRS day 90, BI score, NIHSS score) and functional test (Box and Blocks Test, Hand-Grip Strength Test, 10-Meter Walk Test, Repeatable Battery Assessment of Neuropsychological Status Naming and Coding Subtests, Line Cancellation Test, and Recognition Memory Test) in the PF-03049423 group	NCT-01208233
				Placebo group (n=67)	Placebo PO/NGT[a], once daily for 90 days		
Silver et al., 2009 [41]	USA	Clinical Trial	Patients (58% males) aged 18 to 80 years, stroke on-set 2 to 9 days	PDE-5i group (n=12)	Sildenafil 25 mg, once daily for 14 days	Improvement in NIHSS score, BI score, and mRS at day 90 from baseline in the Sildenafil group.	NS-23393
Lorberboym, et al., 2014 [42]	Israel	Randomized Clinical Trial	Males aged 50 to 66 years with previous history of stroke	PDE-5i single-dose group (n=15)	Taldalafil 20 mg, single dose	Significant reduction in regional cerebral blood flow in the affected hemisphere in the Taldalafil group	-
				PDE-5i continuous-dose group (n=15)	Taldalafil 5 mg, once daily for 7 days		

Abbreviations: BI, Barthel Index; mRS, modified Rankin Scale; NGT, nasogastric tube; NIHSS, National Institutes of Health Stroke Scale; MI, Myocardial Infarction; PDE-5i, phosphodiesterase-5 inhibitor; PO, per oral.

**Subjects receiving thrombolytic therapy within 4.5 to 6 hours post-onset of acute ischemic stroke were still enrolled as long as they had been stable. Antiplatelet and antithrombotic agents were allowed. Nitrates (due to the risk of blood pressure low-ering in subjects also taking any PDE5 inhibitor), alpha-blockers, strong cytochrome P450 3A4 inhibitors, cytochrome P450 3A4 inducers, other PDE-5i, and P-glycoprotein substrates (digoxin/digotoxin) were prohibited.*

[a]The treatment was administered via a nasogastric tube in subjects unable to swallow.

several mechanisms: (1) The Nogo-66 receptor (Nogo-R) pathway, as evidenced by increased Nogo-R expression in the cortex and striatum. When Nogo-R is activated, RhoA is activated, activating ROCK, which phosphorylates several substrates involved in the survival pathway. PTEN is a ROCK downstream target that negatively regulates Akt signaling, which is involved in protein synthesis and cell-to-cell modulation of neurite outgrowth and survival. Thus, inhibiting the expression of Nogo-R, RhoA, and p-PTEN expression will increase p-Akt and PI3K levels via a cGMP-dependent pathway, leading to axonal sprouting and decreased neuronal loss [7]. (2) Suppression of S100B and AQP4 co-expression in astrocytic structures, which regulate calcium influxes caused by reactive astrocytes to injury and the regulation of extracellular matrix volume, neuroinflammation, and calcium signaling, respectively [29]. (3) Increasing the expression of synaptophysin and reducing the expressions of uncoupling PSD-95 and nNOS, resulting in reduced synaptic damage and synapse structure protection in the ischemic brain [7]. (4) The cGMP-PKG activation results in lower levels of intracellular calcium ions (Ca^{2+}), which are a significant initiator and activator of apoptotic pathways [29].

Neurological (modified six-point scoring, modified neurological severity score, sensorimotor) and behavioral functions (beam walking test, rotarod test, adhesive removal test) were improved in 11 studies, and no changes in only 1 study. Among the 11 studies, improvement was observed with Yonkenafil [6], Sildenafil [7,8,29,31,32,36-38], Icariside-II [30], and Tadalafil [39]. Sildenafil is administered intravenous/intraperitoneally with doses ranging from 12 to 32 mg/kg, orally with doses ranging from 0.3 to 5 mg/kg, and subcutaneously with 10 mg/kg all showed improvement. No functional improvement was observed using Verdenafil [35] with 10 mg/kg oral administration. This result was surprising because studies with oral/subcutaneous were unable to reduce infarct area size but were able to improve the functional score significantly. This improvement in neurological outcome was suspected to be related to the significant increase in numbers of BRdU-(bromodeoxyuridine) and TuJ1-(bIII-tubulin) immunoreactive cells in the ischemic brain of rats, which are markers for progenitor cell proliferation in the subventricular zone and dentate gyrus [37,39].

Many experimental studies in rodents demonstrated some benefit of PDE-5i, though the extent of activity of these inhibitors and the specific underly-

ing mechanisms remained unknown. Many factors, including the different experimental species and sizes/ages, duration of ischemic induction, the time before reperfusion performed, types of PDE-5i, doses, route of administration (oral, intravenous, or intra-peritoneal), and timing of PDE5 inhibitor administration, were contributed to the inconsistent findings in this study.

In humans, Cesare and colleagues [40] showed the use of PF-03049423, a selective and brain penetrant PDE-5 inhibitor, on acute stroke subjects was safe and well tolerated but did not significantly impact neurological or functional outcome measured by modified Rankin Scale (mRS), Barthel Index (BI), or National Institutes of Health Stroke Scale (NIHSS), along with other secondary assessment. Although the primary efficacy analysis using mRS showed the proportion of subjects with mRS score ≥2 at Day 90 was lower in the PDE-5i group (n = 42.6%, OR 0.74; 0.41 to 1.31 95% CI) compared to the placebo group (n = 46.2%), but the difference is not significant (p-value 0.49). A tiny difference in placebo in the interim was observed; thus, the author decided to terminate early using a futility rule due to a less than 20% probability of showing a statistically significant effect at the end of the study should they have continued. In the preclinical model, the use of PF-03049423 demonstrated a potential impact on functional improvement. Still, in the current clinical trial, the treatment with PF-03049423 did not show any clinical benefits relative to a placebo in a selected population of stroke patients over a limited time window.

Silver and colleagues [41] showed that using Sildenafil on acute-subacute stroke patients was considered safe. This study primarily assessed the safety use of PDE-5i in acute stroke patients but had a secondary outcome of brain function assessment using mRS, BI, and NIHSS. Among the ten subjects observed at day 90 (one death and one suicide due to a history of depression without suicidal attempts), the median NIHSS score was 2, the median BI was 95 (Range 15-100), and the median mRS score was 1.5 (range 0-5) which represented an improvement from baseline in all the survivors. The proportion of subjects with an mRS score ≥2 after Sildenafil use was 50%. Since there is no control group, thus the signifi-cance/power of the study cannot be determined, although this result was higher compared to the previous study by Cesare and colleagues [40] with 42.6% of the population having mRS score ≥2.

Several other studies of PDE-5i on humans had been conducted in non-stroke and healthy individuals. Outcomes assessed included mean blood flow velocity in the middle cerebral artery [43], average maximal velocities in the middle cerebral artery (Vmca) [44], SPECT imaging with Xenon 133 inhalation [44], and fMRI assessment [45]. Three studies showed no improvement in resting cerebral blood flow after receiving Sildenafil compared to a placebo. In contrast, a study with 14 healthy male volunteers found that one hour after administration of 100 mg of Sildenafil improves cerebrovascular regulation (CVR) [46]. Impaired CVR was associated with vascular risk factors and vascular abnormalities in general. Impairment of endothelium-dependent CVR was also postulated as a specific risk factor for small cerebral vessel disease [47,48]. A systematic review by Pauls and colleagues [49] concluded that PDE-5i only affects CBF in certain clinical conditions. PDE-5i might improve CVR measurement, but not basal CBF, especially in disorders characterized by an impaired endothelial dilatory response due to deficient nitric oxide-mediated signaling has disproportionate effects on brain microvascular responsiveness compared to the resting state. There is still a possible action of PDE-5i on resting CBF at the level of small arterioles, as this remains untested up to this date. Further studies using blood-flow-specific cross-sectional imaging techniques like ASL MRI are warranted to explore this hypothesis at the arteriolar level.

Stroke recovery is a complex and multidimensional process in which internal (neurobiological and psychological factors) and external factors (specialized medical care) play a role. Unfortunately, it is evident that these endogenous restorative activities using medications, including PDE-5i, are typically insufficient to promote a full stroke recovery in terms of brain structural damage and dysfunction as well as the patient's functional/behavioral abilities.

CONCLUSIONS

The use of PDE-5i in experimental stroke animal models has demonstrated some advantages in anatomical and functional recovery. There was no improvement in clinical or functional recovery in persons with acute stroke. PDE-5i should be administered with caution in persons with chronic stroke since a drop in regional blood flow was observed. The disparity in the intricacy of stroke pathophysiology between human and animal models was crucial. Further research in a larger population with more consistent stroke onset, medicines, and doses is required to reach a more conclusive result.

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