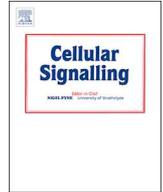




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Cyclic nucleotide phosphodiesterases (PDEs) and endothelial function in ischaemic stroke. A review

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ABSTRACT

Background: Endothelial dysfunction is a hallmark of cerebrovascular disease, including ischemic stroke. Modulating endothelial signalling by cyclic nucleotides, cAMP and cGMP, is a potential therapeutic target in stroke. Inhibitors of the cyclic nucleotide degrading phosphodiesterase (PDE) enzymes may restore cerebral endothelial function. Current knowledge on PDE distribution and function in cerebral endothelial cells is sparse. This review explores data on PDE distribution and effects of PDEi in cerebral endothelial cells and identifies which PDEs are potential treatment targets in stroke.

Method: We performed a systematic search of electronic databases (Medline and Embase). Our search terms were cerebral ischaemia, cerebral endothelial cells, cyclic nucleotide, phosphodiesterase and phosphodiesterase inhibitors.

Results: We found 23 publications which described effects of selective inhibitors of only three PDE families on endothelial function in ischemic stroke. PDE3 inhibitors (PDE3i) (11 publications) and PDE4 inhibitors (PDE4i) (3 publications) showed anti-inflammatory, anti-apoptotic or pro-angiogenic effects. PDE3i also reduced leucocyte infiltration and MMP-9 expression. Both PDE3i and PDE4i increased expression of tight junction proteins and protected the blood-brain barrier. PDE5 inhibitors (PDE5i) (6 publications) reduced inflammation and apoptosis. In preclinical models, PDE5i enhanced cGMP/NO signalling associated with microvascular angiogenesis, increased cerebral blood flow and improved functional recovery. Non-specific PDEi (3 publications) had mainly anti-inflammatory effects.

Conclusion: This review demonstrates that non-selective and selective PDEi of PDE3, PDE4 and PDE5 modulated endothelial function in cerebral ischemic stroke by regulating processes involved in vascular repair and neuroprotection and thus reduced cell death and inflammation. Of note, they promoted angiogenesis, microcirculation and improved functional recovery; all are important in stroke prevention and recovery, and effects should be further evaluated in humans.

1. Background

Endothelial dysfunction is a common feature in small vessel and large artery strokes. Stroke is the third leading cause of death and a major cause of disability [92] in the Western countries. Early initiation of prophylactic treatment is warranted to reduce the impact as well as the risk of recurrence of this often-devastating disease. Though some signalling pathways in endothelial function are well-known, such as the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway, the underlying mechanisms for endothelial dysfunction precipitating a stroke may vary and they have yet to be fully resolved.

Diabetes, hypercholesterolaemia and hypertension are risk factors

which cause both endothelial dysfunction and stroke. In these conditions the cerebrovascular response appears to be decreased and the vessels are more vulnerable to platelet adherence, blood-brain barrier breach, inflammation, and vasospasms [65,75,120]. Modulation of endothelial function is a potential new treatment target, in both primary and secondary prevention of stroke [76,84,115,116]. Current stroke treatments aim to dissolve blood clots and reduce platelet aggregation. However, targeting endothelial function may increase the collateral blood flow [80,81,94]. Also, the blood-brain barrier (BBB) homeostasis, important in reducing an inflammatory or toxic response in the brain tissue, requires an intact endothelial function. With diabetes, hypertension and hypercholesterolemia the endothelium

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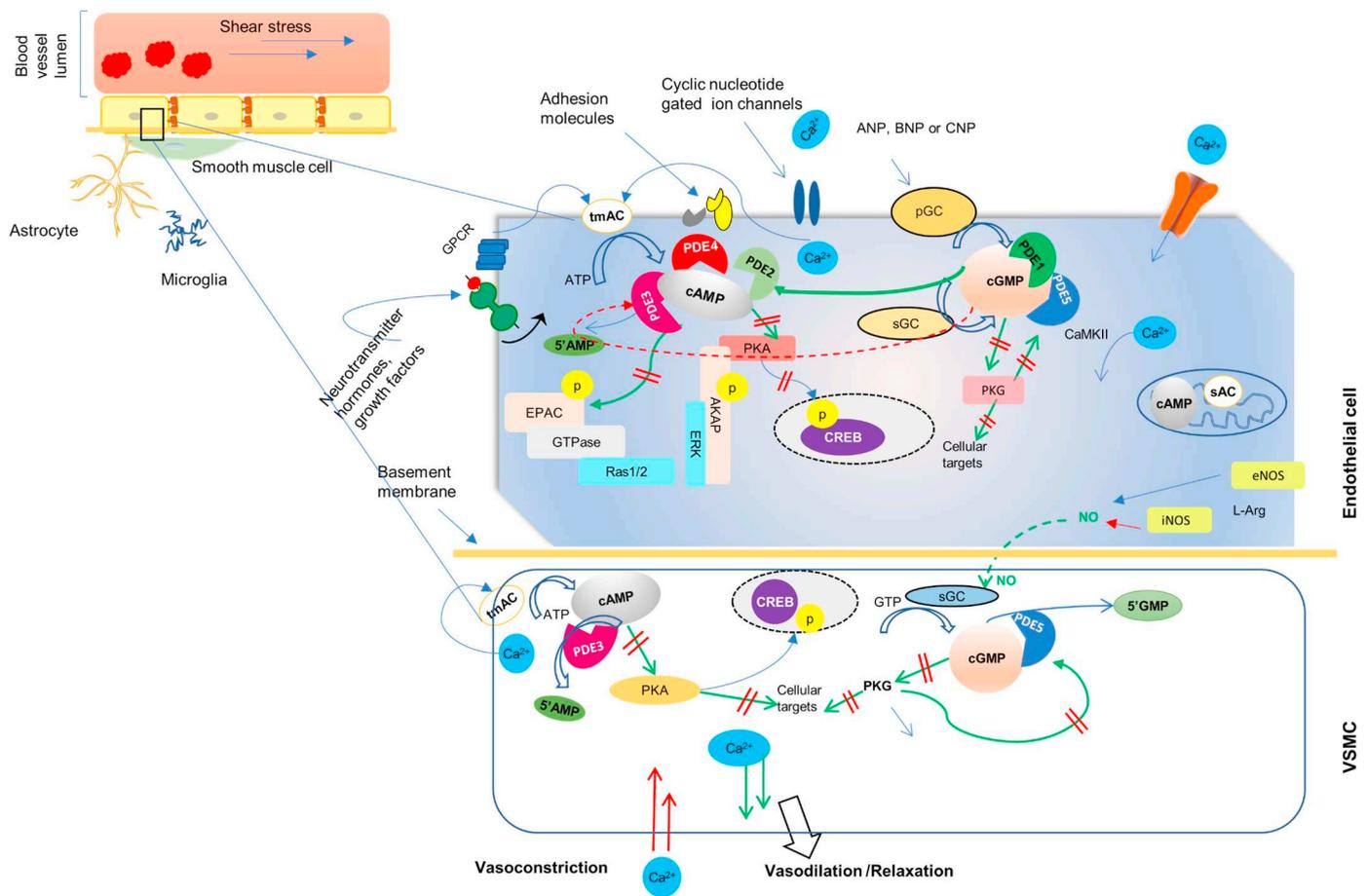


Fig. 1. A schematic of Endothelial function/dysfunction.

Phosphodiesterase enzymes (PDE) are present in vascular cells, both endothelial and smooth muscle cells. The different PDE families differ in cAMP and cGMP specificity and associate with downstream effector proteins forming signalling domains regulating cyclic nucleotide signalling with a downstream impact on a multitude of biological processes, including endothelial cell signalling.

Signalling domains associated with PDEs also include downstream effector proteins such as A-kinase anchoring proteins (AKAP), exchange protein activated by cAMP (EPAC), protein kinase A (PKA), and protein kinase G (PKG), cyclic nucleotide-gated channels (CNGC), protein phosphatases (PP) and cAMP response element binding protein (CREB). G_s protein-coupled cell-surface receptors respond to extracellular stimulation (hormonal/environmental stimuli and catecholamines) and activate the adenylate cyclase (AC) enzyme, expressed as a transmembrane and a soluble form and initiate the synthesis of cAMP [37]. Guanylate cyclases (GC) synthesize cGMP and exist in two forms: a soluble, which is activated by nitric oxide, and a particulate form, which is activated by natriuretic peptides (ANP, BNP and CNP). cAMP and cGMP exert their function by interaction with several cellular effector proteins such as PKA, PKG, AKAP, and EPAC and regulate downstream signalling pathways, such as the GTPase activated Ras1/2 pathway and the extracellular signal regulated kinase pathway (ERKs). The cyclic nucleotide regulation is tightly regulated by formation of distinct microdomains, containing specific effector proteins, cyclases and PDEs, specific to subcellular location, where they facilitate localized signalling and strictly control activation of off-target effector proteins by the cyclic nucleotide pool.

A healthy endothelium is maintained and stimulated by reactions catalyzed by the blue and green arrows. Two red lines on these reactions indicates inhibition or reduction of the process, which leads to a dysfunctional endothelium. A truncated red arrow is inhibition of PDE3, and a thick green arrow is stimulation of PDE2. Grey oval structure in the middle of the cell where CREB is located is the nucleus. The color of the endothelium is yellow, and the smooth muscle cell is colored pink. The purple bar between the cells shows the basement membrane. Abbreviations: AKAP, A-kinase anchoring proteins; CNGC, cyclic nucleotide-gated channel; EPAC, exchange protein activated by cAMP; GPCR, G-protein-coupled receptors; PKG, protein kinase G; PP, phosphatase protein; CREB, cAMP response element binding protein; pGC, particulate guanylate cyclase; tmAC, transmembrane adenylate cyclase; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide and CNP, C-type natriuretic peptide. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

becomes non-responsive to vascular cues, increases the pro-inflammatory response, and may turn pro-thrombotic [124]. The underlying cellular processes which render the endothelium less responsive to stimuli and which reduce endothelial cell integrity need to be explored to identify new treatment targets. These processes are known to involve the cyclic nucleotides cyclic adenosine monophosphate (cAMP) and cGMP, intracellular second messenger molecules ([20,51,73,104]; Stelzner Thomas, Weil, and O'Brien 1989). Cyclic nucleotide signalling contributes to regulation of the expression of endothelial adhesion molecules, activation of smooth muscle cell function, and is involved in receptor mediated signalling [23,117,126] (Fig. 1). Cyclic nucleotide levels are regulated through their production by cyclases, by efflux, and through degradation by cyclic nucleotide phosphodiesterases (PDE)

[4,14,48,95]. Changing the cyclic nucleotide signalling in the endothelial cell affects vasoreactivity (Fig. 1).

Excessive production of reactive oxygen species and low-grade inflammation can precede disease progression and further add to the dysfunction of the cerebral endothelium. Increased chemokine and cytokine secretion, caused by vascular injury, attracts the passage of leucocytes from the blood into the brain and contributes to the development of cellular and vasogenic oedema with sustained inflammation and a disturbed cerebral microcirculation [85,108]. In a stroke, several mechanisms of action may thus be targeted: platelet aggregation, flow changes induced by cardiac arrhythmia or stenosis of the arteries, or changes in the vessel walls [72]. PDEs are potential therapeutic targets in all these mechanisms.

We need to fully uncover the role of PDEs in the cells which are cardinal to the blood-brain barrier function and to the expression of adhesion molecules associated with the prevention and treatment of ischemic stroke and related tissue damage. Several PDE inhibitors (PDEi) are available for clinical use in humans, but so far only two, cilostazol and dipyridamole, are approved for the secondary prevention of stroke and both are considered to mainly inhibit platelet aggregation [36].

In this review, we investigate current literature on the role of PDEs and related cyclic nucleotides in cerebral endothelial function and dysfunction. Further, we disclose the underlying mechanisms of how PDEi may amend endothelial signalling pathways involved in protecting the integrity and function of cerebral endothelium during or after ischemic stroke. We wish to highlight which PDEi may be relevant for both treatment and prevention of endothelial dysfunction and stroke.

2. Methods

We followed Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (Moher et al. 2015). Employing a systematic literature search of Medline and Embase, we identified relevant publications with last search done 31st January 2019. Search terms were cerebral ischemic stroke OR cerebral endothelium AND cyclic nucleotide phosphodiesterase OR phosphodiesterase inhibitor. The selection was based on pre-determined criteria. Two investigators independently screened the titles and abstracts for eligibility (S.Y. and B.H.A.). In cases of disagreement, consensus was achieved by a third investigator (C.K.). A subsequent screening was done by reading the full text to comply with the inclusion and exclusion criteria (S.Y.). Inclusion criteria were studies that investigated the role of PDEi in the cerebral endothelial function in each of the following: ischemic stroke patients, animal ischemic stroke models or *in-vitro* endothelial cell models. Exclusion criteria were studies that investigated the role of PDEi in tissues and cells outside the brain, the role of PDEi in cerebral cells other than endothelial cells, which included non-ischemic stroke types, non-English papers, and reviews.

In addition to the systematic search for PDEi effects on cerebral endothelium, the currently available data on the expression of PDEs in the cerebral microvessels in general was included.

3. Results

With the systematic database search, we retrieved 677 non-duplicate papers. After reviewing titles and abstracts we identified 38 papers eligible for full text screening according to the inclusion and exclusion criteria. Subsequently, 15 papers were excluded for the following reasons: the papers reported the effect of PDEi or cyclic nucleotides in non-cerebral endothelial cells or in non-vascular brain cells or did not apply PDEi. As a result, 23 papers in total met the inclusion criteria for this review. A flow chart for selection of papers is shown in the PRISMA flow chart (Fig. 2).

3.1. Overview of the distribution of PDEs in the cerebral vasculature

So far 11 PDE families have been identified, each with unique cAMP and/or cGMP degrading functions, variable mechanisms of regulation, as well as cell and tissue specific distribution [48]. The expression data in Table 1 summarize which PDEs have been characterized in tissue and isolated cells from human, rat and mouse brain, in addition to the findings in mouse and human cerebral endothelial cell lines, bEND.3 and hCMEC/D3. In contrast to PDEs in brain tissue [50], the distribution of PDE families throughout the cerebral vascular tree is little studied. There is a considerable knowledge gap in how each of the cell types in the neurovascular unit, endothelial cells, smooth muscle cells, astrocytes, and pericytes, contributes to the regulation of endothelial

function and brain microperfusion. Most studies report on brain homogenates rather than isolated cell types. Further, the cerebral endothelial cell layer is fragile, and they rapidly deteriorate *post mortem* or during the preparation for experimental procedures rendering it difficult to identify the function of this specific cell type [78].

PDE activities were initially measured in cerebral microvessels isolated from bovine cortex [103], where both cGMP and cAMP hydrolytic activity was detected.

All PDE families are expressed in the human brain, but differential expression of the PDE families and their isoforms in specific brain areas or cell types contribute to their versatile functions in cell signalling [59]. There is little information on the expression and function of the PDE families specifically in brain microvascular endothelial cells.

PDE1. The PDE1 family was one of the first to be purified from bovine brain homogenate, identifying a PDE enzyme which was activated by Ca^{2+} /calmodulin and with a high affinity for cAMP [71]. PDE1 is now known to be abundantly expressed in the mammalian brain [97] and includes the three isozymes PDE1A, PDE1B and PDE1C. Expression pattern varies between brain regions and even within the same type of Purkinje cells [3,6]. In large cerebral arteries of both guinea pig and man, PDE1A and PDE1B protein and corresponding PDE1 hydrolytic activity was detected [55,56].

PDE2. Expression of PDE2A was detected in the bovine brain [101]. In human brain tissue, PDE2A mRNA was present in the hippocampus and all cortical regions [59]. PDE2A protein was expressed in endothelial cells of capillaries supplying the human cerebellum, and in cell homogenates showed that protein expression was highest in the cerebellar cortex while it was lower in the cerebellum [93]. The most abundant expression of PDE2A mRNA was in the human forebrain [106].

In the human cerebral microvascular endothelial cell line (hCMEC/D3), a widely used *in vitro* cell model for BBB experiments, most PDE isoforms (excluding PDE1, PDE2B, PDE3B, PDE9B, PDE11) were present at the mRNA level (Table 1) [42].

PDE3 and PDE4. Little is known of the specific cellular distribution of PDE3 and PDE4 in cerebral arteries and microvessels [46,47]. The hydrolytic activity of both PDE3 and PDE4 was detected in homogenized guinea pig and human large cerebral arteries; only PDE4 effects appeared endothelial dependent [8]. These findings confirmed the results which showed hydrolytic activity of PDE4 in canine cerebral arteries [119].

PDE5. In the human brain, two PDE5 isoforms, PDE5A1 and PDE5A2, were expressed at the mRNA and protein level. PDE5A1 and PDE5A2 mRNA expression was ubiquitous, PDE5A3 expression was found to be specific to the smooth muscle cells [63]. In human brain tissue sections, expression of PDE5 protein has been detected in cerebral smooth muscle cells [113]. PDE5A protein and hydrolytic activity was reported in homogenates of guinea pig and human large cerebral arteries [55,56].

The effect of the PDE deficiency on cerebrovascular outcome or endothelial function was not investigated in the various studies of PDE knockout animals and cell lines ([2,9,13,16,21,60,62,83,99,107,122,129]), but it is possible that some PDE knockout animals may not be viable due to vascular dysfunction, though such needs to be confirmed.

3.2. PDEi in endothelial function during cerebral ischaemia

Though specific inhibitors for some PDE families such as PDE3i, PDE4i, and PDE5i are currently commercially available and applicable in humans, so far there is a lack of specific inhibitors for the remaining PDE families. Further, the effect of PDEi on cerebral endothelial function are only reported for the inhibitors of these three PDE enzymes.

A key approach to better treatment strategy of stroke injury needs to be combined therapy which inhibits platelet aggregation and enhances blood perfusion in ischemic stroke regions [72]. Briefly, as a reaction to a stroke injury the brain responds with activation of the immune



PRISMA 2009 Flow Diagram

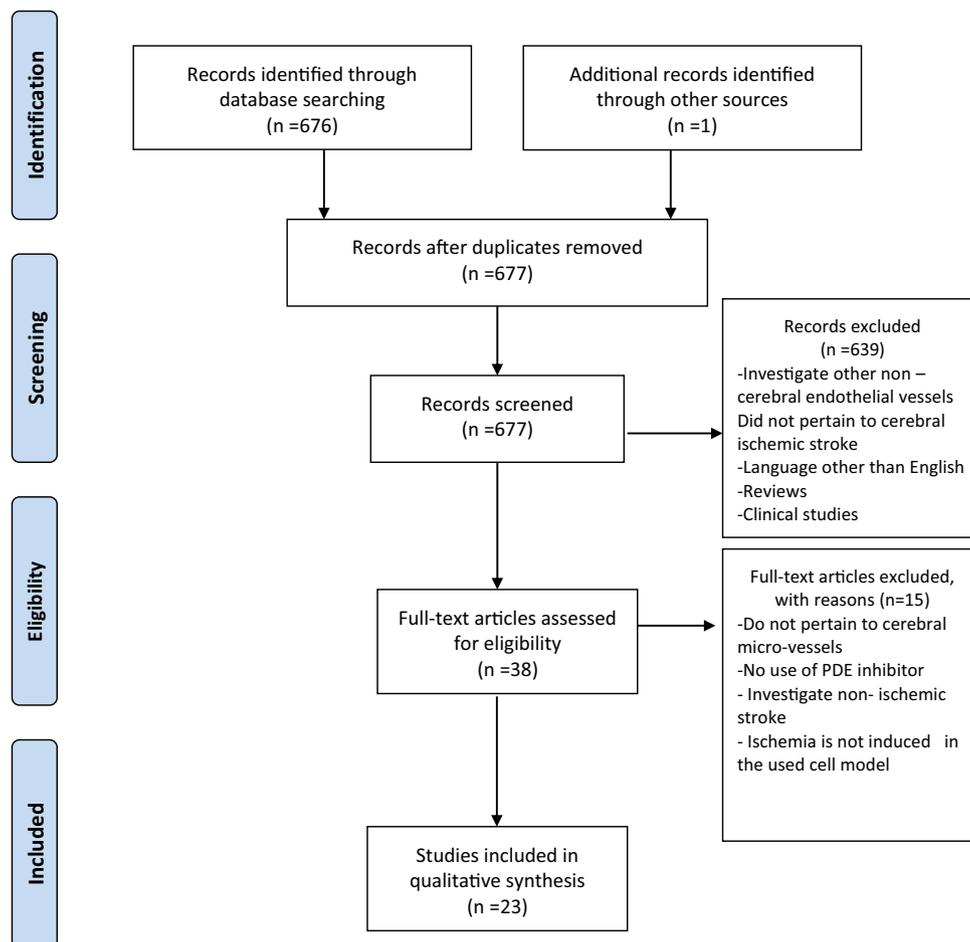


Fig. 2. PRISMA flow chart for selection of studies. Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2009 flow diagram. PDE = phosphodiesterase.

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA [70]

Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:<https://doi.org/10.1371/journal.pmed1000097>. For more information, visit <http://www.prisma-statement.org>.

system, and neuroinflammation is initiated. Damage of the cerebral vascular endothelium, which is a basic element of the BBB, triggers expression of adhesion molecules and mediates infiltration of immune cells into the brain parenchyma [58]. Platelet aggregation and consequent occluded vessels result in insufficient supply of oxygen and nutrients to brain regions, leading to cerebral ischaemia characterized by hypoperfusion and loss of neuronal tissue. Therefore, an optimal therapeutic approach for stroke needs to be tailored to cover anti-inflammatory, anti-apoptotic and pro-angiogenic effects for neuro-reparative and neuroprotective effects.

3.2.1. PDE3 inhibitors

In total, eleven studies investigated the effect of PDE3 inhibition on cerebral endothelial function using cilostazol (listed in Table 2). Two of the studies [33,108] investigated effects of cilostazol by measuring the transendothelial electrical resistance (TEER) in a triple co-culture cell model. The triple co-culture cell model is an *in vitro* blood-brain barrier model that mimics the neurovascular unit which comprises endothelial cells, astrocytes and pericytes. TEER is a surrogate marker for the integrity of the BBB and is used to determine the endothelial tightness in

response to treatment. TEER values were, compared to placebo, significantly higher when cilostazol was applied in a co-culture cell model using primary rat brain endothelial cells, astrocytes, and pericytes, exposed to oxygen glucose deprivation (OGD)/3-h reoxygenation, and treated with cilostazol (1 μ M) [33]. This suggested a preservation of the brain barrier function and integrity of the rat brain endothelial cells after cilostazol treatment during conditions mimicking a stroke [33]. Accordingly, cilostazol (10 μ M) treatment resulted in a significant increase in TEER in a similar co-culture cell model exposed to OGD/24-h reoxygenation, compared to normoxia/reoxygenation control cells in the presence of advanced glycation end products (AGE) indicative of a barrier dysfunction [108]. AGEs form when protein or fat is glycosylated after exposure to high glucose in the blood, and they accumulate in the endothelial cell walls, trigger low-grade inflammation and contribute to diabetic vascular complications such as stroke [118]. In addition, expression of claudin-5 (cld-5), a tight junction protein connecting endothelial cells, was restored in response to cilostazol treatment compared with vehicle treatment cells exposed to OGD/AGE [108]. Thus, PDE3 inhibition helps maintain the integrity of the endothelial barrier function during ischemic conditions in cell models.

Table 1
Expression of PDEs in cerebral vasculature. Expression of PDE mainly in cerebral microvessels from human, rat and mouse are listed. If location of PDE is not described, it is expressed in the microvessels. hCMEC/D3 is a human cell line and bEND.3 is a mouse cell line and the expression of PDEs may not be like primary cells. R = mRNA transcript; P = protein; A = PDE activity.

PDE form	Human	Protein/Transcript/ Activity	Rat	Protein/Transcript/ Activity	Mouse	Protein/Transcript/ Activity
PDE1	PDE1B1 highest in brain regions: Caudate nucleus, hippocampus, cerebellum [125]	R + P + A	PDE1A [31] PDE1B1 [25]	R	PDE1B1 [121,125]	R
PDE2	PDE2A2 (10-fold difference; differentially expressed in human cortex and cerebellum) [59] PDE2A in hCMEC/D3 cell line [96] Highest level of PDE2A found in brain [89] PDE2A in hCMEC/D3 cell line [42] PDE2A in granular layer of cerebellum [93]	R + P R R R R	PDE1B1 and PDE1B [31] PDE2A2 in cerebral cortical microvessels [93] PDE2A [105] PDE2A [87] PDE2A highly expressed in rat habenula and PDE2A in neurons in dorsal root ganglion [105] PDE2 brain [111]	R R R P R R	PDE2A in endothelial cells of capillaries and veins [105] PDE2 expression in mouse endothelial cells from bEND.3 cell line; murine brain endothelium-derived cell line [112]	R R
PDE3	PDE3B in hCMEC/D3 cell line [33] PDE3B in hCMEC/D3 cell line [96] PDE3 in cerebral arteries [8] PDE3A in hCMEC/D3 [42] PDE3A in hCMEC/D3 [123]	A + R R + P A R R + P	PDE3A/B [31] PDE3B [33] PDE3A and PDE3B in middle cerebral arteries + basilar artery [54]	R A + R R	PDE3B in bEND.3 cell line [112]	R
PDE4	PDE4A-D in hCMEC/D3 cell line [42] PDE4 in cerebral arteries [8]	R + P R + P	PDE4A, PDE4B, PDE4D [31] PDE4D [32]	R R	PDE4B in mouse endothelial cells, bEND.3 cell line [112]	R
PDE5	PDE5A1 and PDE5A2 in brain [63] PDE5A in middle cerebral arteries [55] PDE5A in hCMEC/D3 cell line [42,96]	R + A R R + P + A R + P	PDE5A expressed in microvessel and pericytes [32] PDE5 in brain [111] PDE5A in arteries and arterioles [31] PDE5A in middle cerebral arteries, basilar artery [54]	R R R R + P		R
PDE6	PDE6A and PDE6B in hCMEC/D3 cell line [42]	R				
PDE7	PDE7A and PDE7B in hCMEC/D3 cell line [42]	R				
PDE8	PDE8A and PDE8B in hCMEC/D3 cell line [42]	R			PDE7A expression in mouse endothelial cells, bEND.3 cell line [112] PDE8A expression in mouse endothelial cells, bEND.3 cell line [112]	R
PDE9	PDE9A, PDE10A, PDE11a in hCMEC/D3 cell line [42]	R	PDE9A in middle Cerebral arteries, basilar artery, cerebellar cortex [53]	R + P		
PDE10A	PDE10A in hCMEC/D3 cell line [42]	R	PDE10 A in middle Cerebral arteries, basilar artery, cerebellar cortex [53]	R R + P		
PDE11A	PDE11A in hCMEC/D3 cell line [42]	R	PDE11A in middle Cerebral arteries, basilar artery, cerebellar cortex [53]	R + P		

Table 2

Overview of studies with PDE3, PDE4 and PDE5 inhibitors in cerebral ischemic stroke animal/cell models on which the review is based. Studies with patients and non-specific PDEi are not included in the table. Age of the animals is indicated, where the information was available from the original study. Abbreviations used in the table. AGE: advanced glycation end products; CCA: common carotid artery, SHR: spontaneously hypertensive rats; tMCAO: transient middle cerebral artery occlusion; VEGF: vascular endothelial growth factor, HFC: High fat cholesterol diet; CBF: cerebral blood flow; NO: nitric oxide, cGMP: cyclic guanosine mono phosphate, i.p.: intraperitoneal; s.c.: subcutaneous; p.o.: *per os*; ↑ = increase; ↓ = decrease and ↔ = no change.

Material/species	Stroke model	Treatment/inhibitor	Outcome	Reference
PDE3 inhibitor Triple cell co-culture of rat brain cells (BBB kit™)	OGD 6 h	Cilostazol 1 μM 6 h after OGD	↑Cell tightness	Horai et al.
Triple cell co-culture rat brain cells (Wistar rats) Cells from 3-week-old rats	OGD 3 h + AGE	Cilostazol 10 μM before and after OGD	↔Cld-5 ↔cell tightness	Takeshita et al.
SHR-SP rats 7-week-old	Risk factor induction: HFC diet	Cilostazol, 100 mg/kg. Daily <i>i.p.</i> for 14 days after start of HFC diet + 1% NaCl until 10 weeks	↓MMP-9 ↓infarct size ↑ VEGF-R	Omote et al.
CB17/Icr mice 7-week-old	tMCAO 90, 120, 180 and 240 min	Cilostazol 0.3% in diet For 7 days before induction of ischemia	↓MMP-9 ↓bleeding ↔microvessel density	Kashara et al.
C57BL/6 J mice 10–12 weeks old	tMCAO 45 min and 90 min	Cilostazol 0.3% in diet For 3 h or 7 days before induction of ischemia	↓bleeding, oedema ↓MMP-9 ↑angiogenesis ↑Neurological outcome ↓Leukocyte migration	Hase et al.
ddY mice 4-week-old	tMCAO 2, 3 and 6 h	Cilostazol <i>i.p.</i> 10 mg/kg After tPA injected 2, 3 and 6 h after ischaemia	↓MMP-9 ↓HT, oedema ↔Cld-5 ↔ microvessel density retained	Ishiguro et al.
ddY mice 4-week-old	tMCAO 3 h	Cilostazol <i>i.p.</i> 1 or 3 mg/kg 21 h after reperfusion	↑VE-cadherin Cld-5, ZO-1 ↓bleeding ↑survival	Kitashoji et al.
C57BL/6 J mice 8–10 weeks old	BCCA 15 min	Cilostazol 30 mg/kg p.o. 30 min before induction of ischaemia	↓ bleeding ↓Leukocyte migration ↓Platelet adherence	Fukuoka et al.
SHR-SP rats 5-week-old	tMCAO 80 min	5-week treatment with either 0.1% or 0.3% cilostazol before induction of ischemia	↓ infarct size ↔ rCBF ↑eNOS phosphorylation	Oyama et al.
PDE4 inhibitor Male Wistar rats Age N.A. Age unknown	tMCAO 2 h	Rolipram 3 mg/kg After ischaemia and treatment continued for 3, 7 or 14 days	↓Apoptosis, infarct size ↑Angiogenesis, ↑functional outcome ↑EPC	Hu et al.
Sprague–Dawley rats Age unknown	tMCAO 2 h	BBB022- PDE4 inhibitor, was infused after 5 h or 48 h from ischaemia induction	↑ BBB integrity ↑Functional outcome	Belayev et al.
C57BL/6 mice 6–8 weeks old	tMCAO 2 h	Rolipram, <i>i.p.</i> 2 or 10 mg/kg administered 2 h after induction of ischaemia	↓ infarct volume, oedema ↓leukocyte migration ↑BBB integrity, tight junction ↓ TNF-α and IL-1β ↓thrombotic vessels	Kraft et al.
PDE5 inhibitor Sprague–Dawley rat pups (P7) Age unknown	Occlusion of right CCA followed by 120 min hypoxia	Sildenafil, <i>i.p.</i> 5 or 10 mg/kg after hypoxia-ischemia start. Animals were euthanized 72 h and 7 days after hypoxia-ischemia (P14)	↑ motor coordination ↑CBF ↓microglia activation ↓ apoptosis ↑vessel density	Charriaut et al. 2014
Male Wistar rats Age unknown	Embolic MCA	Sildenafil 2 mg/kg in water 24 h after induced stroke and for 6 days	↑ VEGF↑ angiogenesis	Zhang et al.2003
Male Wistar rats Aged Rats (18 month)	Embolic MCA	Tadalafil, 2 or 10 mg/kg, every other day, starting 24 h after stroke onset	↑Neurogenesis ↑Angiogenesis ↑cGMP ↑Functional recovery	Zhang et al.2006
SHR-SP rats 6-week-old	High salt diet and fed with inhibitor	PDE5 inhibitor (DA-8159) p.o., 1, 3 and 10 mg/kg, once a day until nearly half of the vehicle-treated animals had died	↑NO ↑cGMP ↑anti-oxidative level ↓cerebral lesion size	Choi et al.
Male Wistar rats Aged Rats 8–12 weeks Young rats 18 month	Embolic MCA	Sildenafil p.o. 3 mg/kg from day 7 and for 7 days after induced ischemia	↑Synaptogenesis ↑Functional recovery ↑cGMP ↑Angiogenesis ↑vessel density	Zhang et al.2005
Male Wistar rats 12–16 weeks	Embolic MCA	Sildenafil s.c. 10 mg/kg, 24 h after ischaemia and daily for 6 days	↑CBF ↑vessel density ↑functional recovery	Li et al. 2007

Further to this, the effects of cilostazol on the integrity of endothelial cells in the neurovascular unit were investigated in stroke-prone spontaneously hypertensive rats (SHR-SP) [77]. Animals were allocated to four different groups and treated for 14 days with vehicle, cilostazol, the more widely used antiplatelet agents in stroke [18,90] acetylsalicylic acid, or clopidogrel. Cilostazol treatment reduced the expression of matrix metalloproteinase-9 (MMP-9), an enzyme which degrades extracellular matrix, and inhibited the detachment of astrocytes and pericytes from endothelial cells, reducing the infarct volume [77]. Matrix metalloproteinases disrupt tight junctions and increase BBB permeability post stroke [15]. Histological analysis of brain tissue from rats treated with cilostazol showed an increased expression of vascular endothelial growth factor receptor 2 (VEGFR-2) compared to tissue derived from rats receiving acetylsalicylic acid, clopidogrel, or vehicle. Increased VEGF expression is correlated with local brain angiogenesis and a corresponding up-regulation of VEGFR-2 on cerebral endothelial cells post stroke [131].

Patients with diabetes and cerebral ischaemia who were treated with cilostazol responded with an increased level of circulating endothelial progenitor cells (EPC), [110] which are important for maintenance and repair of injured vessels [86].

Haemorrhagic transformation in the infarcted area is a potentially devastating side effect of thrombolytic therapy. Thrombolysis, performed by infusion of tissue plasminogen activator (tPA) which dissolves the embolus, can induce hemorrhagic transformation due to disintegration of cerebral microvessels over time. It is required that tPA is administered as early as possible, preferably within 4.5 h from stroke onset to reduce the risk of hemorrhagic transformation and intracerebral bleeding. If cilostazol protects endothelial integrity, the time window for thrombolysis could be expanded by concomitant administration of cilostazol and tPA, and more patients may be treated [26]. Mice subjected to both transient middle cerebral artery occlusion (tMCAO) and reperfusion were fed with food containing cilostazol, acetylsalicylic acid or normal diet for 7 days prior to induction of ischaemia. Hemorrhage lesions in the ischemic area were assessed 24 h after induction of ischaemia. tPA was administered immediately before the reperfusion [44]. Cilostazol significantly reduced the tPA-induced cerebral hemorrhagic transformation in the ischemic area after 90 min and 120 min of ischaemia [44]. Further, in cilostazol-treated mice the platelet endothelial cell adhesion molecule 1 (PECAM), which is essential for the function, structure and survival of endothelial cells [82], was not reduced. Also, pretreatment with cilostazol, compared to acetylsalicylic acid, protected the tight junctions and basement membranes in the ischemic area. Thus, cilostazol prevented degradation of the cerebral microvessels and protected the structural integrity through reduction of MMP-9 after cerebral ischaemia [44].

In a murine model of transient focal cerebral ischaemia of 45 or 90 min, mice were treated with cilostazol or vehicle for 7 days. tPA or placebo was administered prior to a 24-h reperfusion [30]. Treatment with cilostazol without tPA improved blood flow and reduced expression of endothelial adhesion molecules involved in leukocyte infiltration and platelet adhesion after acute ischemia. Further, brain oedema and hemorrhagic transformation was significantly reduced after 45- and 90-min ischaemia with a decreased density of MMP-9 positive microglia. Cilostazol, both with and without tPA treatment significantly improved neurological outcome, suppressed the local re-flow, and improved microvascular integrity [30].

Inducing tMCAO for two, three, and six hours in a similar murine model, followed by tPA and cilostazol or vehicle infusion prior to reperfusion, the combined cilostazol/tPA treatment [38] ameliorated the hemorrhagic transformation at six hours. Cilostazol further reduced brain oedema, decreased MMP-9 activity and prevented a reduction in claudin-5 expression compared to tPA therapy alone [38].

Application of tPA to a co-culture of endothelial cells, pericytes and astrocytes induced NVU damage, which was reversed by a high dose of cilostazol (100 μ M). Cilostazol also significantly reduced cellular injury

of the pericytes but not the astrocytes. Addition of a cAMP analogue at concentrations of 300 and 1000 μ M to the cell co-culture showed that the tPA-induced vascular damage was mitigated [38].

In patients with atrial fibrillation, a major risk factor for stroke, the use of anti-coagulation therapy (warfarin or new oral anticoagulation, NOAC) for stroke prevention is associated with a higher risk of intracerebral hemorrhage or hemorrhagic transformation of ischemic brain tissue. Cilostazol, administered immediately after reperfusion, alleviated the warfarin-induced hemorrhagic transformation in mice treated with warfarin prior to three hours of tMCAO followed by 21 h of reperfusion [49]. Cilostazol reduced bleeding time and increased the survival rate in the group receiving the dual therapy. Further to this, cilostazol increased the expression of tight junction proteins: zonula occludin (ZO-1), claudin-5, and the vascular endothelial cadherin (VE-cadherin). This suggested that cilostazol protected the vascular endothelial integrity [49].

A key feature in endothelial dysfunction related to ischaemia is the adherence of platelets and leucocytes to endothelial cells in cerebral microvessels. One group investigated whether cilostazol inhibited the rolling and adhering of platelets in cerebral microvascular endothelial cells after transient ischemia. Mice were subjected to transient cerebral ischaemia by occlusion of bilateral common carotid arteries (BCCA) for 15 min followed by reperfusion. Cilostazol was administered 30 min prior to the BCCA occlusion and compared to a control group with no ischaemia and no reperfusion [24]. At three and six hours post ischemia, the group of mice pretreated with cilostazol showed a reduced number of rolling and adhering of platelets to endothelial cells, compared to what was observed in the ischaemia reperfusion group and the control group [24]. This showed that cilostazol inhibited platelet adherence to vessel walls, thus reducing an inflammatory response after ischemia. Hemorrhagic transformation was not observed in the ischemic area when treated with cilostazol.

Patients with non-cardioembolic ischemic stroke, enrolled within a week from stroke onset, were evaluated to determine if acetylsalicylic acid with or without cilostazol co-medication reduced platelet aggregation and endothelial markers in the blood. Platelet aggregation, induced by collagen, adenosine diphosphate or arachidonic acid, decreased significantly in pre-treatment samples, with no change between the groups receiving acetylsalicylic acid alone or in combination with cilostazol. There were no investigations into the effect on endothelial function [74].

In SHR-SP rats fed on chow with acetylsalicylic acid, cilostazol, or vehicle for five weeks prior to induction of transient ischemia, the cilostazol-treated rats showed a higher regional cerebral blood flow (rCBF), and a reduced infarct size compared to rats fed on acetylsalicylic acid or vehicle [79].

3.2.2. PDE4 inhibitors

Three studies explored the role of PDE4 inhibition on endothelial function in cerebral microvessels and PDE4 inhibitor effects during cerebral ischemia. *CD34*, a transmembrane phosphoglycoprotein, is a marker for the EPC involved in angiogenesis [43]. The change in EPCs was investigated in rats subjected to tMCAO for two hours followed by reperfusion and treatment with the PDE4 inhibitor, rolipram, from the first day of ischaemia and continued for three, seven or 14 consecutive days. Analysis of tissue sections from the ischemic brains showed that rolipram attenuated apoptosis of neuronal cells in the peri-infarct region, and enhanced the cell proliferation of microvessels by recruiting CD34-positive cells to the ischemic tissue, thereby contributing to angiogenesis [35]. Furthermore, the infarct size was reduced, and the functional outcome was improved in rats treated with rolipram compared to vehicle-treated rats.

The involvement of PDE4 in the BBB and the increased vascular permeability induced by ischemic stroke has been a focus of attention. In rats subjected to transient ischaemia and treated with the PDE4 inhibitor BBB022 or vehicle [5], the neurological scores and thus

functional outcome was significantly improved by BBB022 compared to vehicle-treated animals [5]. Animals treated with BBB022 showed significantly reduced BBB vascular leakage compared to vehicle-treated animals. In addition, treatment of porcine and rat brain endothelial cell monolayers with PDE4 inhibitor rolipram or BBB022 increased the cAMP levels and improved cellular tightness. Thus, inhibition of PDE4 protected the blood-brain barrier integrity by lowering the vascular permeability after cerebral ischaemia [5].

When injured, the endothelium releases pro-inflammatory mediators, which activate migration of immune cells into the cerebral vasculature augmenting the tissue damage [1]. In mice with induced transient ischemia, rolipram caused higher expression of claudin-5 and occludin. In addition, they observed a reduced leukocyte infiltration through the endothelial cell layer and a decrease in the pro-inflammatory cytokines IL-1 β and TNF α [52]. Further, a reduction was noted in infarct volume and oedema formation in rolipram-treated compared to non-treated mice.

3.2.3. PDE5 inhibitors

The impact of PDE5 inhibition on endothelial function was investigated in six studies: four studies [10,130] ([61], Zhang R. 2005) applied sildenafil; one study, a novel PDE5 inhibitor, DA-8159 [12]; and one study used tadalafil [128].

In rats subjected to one-sided common carotid artery occlusion and hypoxia for 120 min, followed by administration of either sildenafil or vehicle, brain sections were analyzed for integrity of microvessels, cell death and endothelial vessel density in addition to blood flow measurements [10]. The glucose transporter 1 (Glut-1) is responsible for entry of glucose into endothelial cells and is used as a marker for changes in BBB capillary density. Sildenafil-treated rats had increased Glut-1 staining after ischaemia induction, indicative of preserved capillary density and branching. Sildenafil treatment was able to increase the mean blood flow velocity in rats with significant lesions after ischaemia and the effects were associated with improved motor function. In addition, sildenafil treatment resulted in reduction of astrocyte and microglial activation during ischemia. [10].

In a rat model of embolic MCA stroke, either sildenafil or a NO donor was administered 24 h after stroke onset [130]. They demonstrated that rats treated with either sildenafil or NO donor showed significant increase in VEGF levels in tissue homogenates and induced capillary-like tube formation. They showed that NO enhanced angiogenesis in the ischemic brain via a NO/cGMP dependent pathway and that inhibition of PDE5 with sildenafil promoted the angiogenic process [130]. Supplementary to this, using the rat embolic stroke model with tadalafil administered 24 h after stroke onset [128], an increased proliferation of endothelial cells and vascular density was detected in the ischemic penumbra when compared to saline-treated rats. Tadalafil-treated rats also had significantly increased proliferation of sub-ventricular zone cells, the neural progenitor cells, which suggested an increased neurogenesis. This effect may contribute to the improved functional recovery reported in this study after tadalafil treatment [128].

The effects of a novel PDE5 inhibitor (DA-8159) were investigated on the endothelial function in.

SHR-SP rats. [12]. The animals were allowed free access to a high-salt diet (containing 4% NaCl) to accelerate induction of hypertension, which is a risk factor for stroke. DA-8159 was administered daily and endothelial function was assessed: nitric oxide (NO), nitric oxide synthase (NOS), cGMP, P-selectin, endothelin-1, and the total anti-oxidative status. This study reported a significant increase in cGMP and NO levels, along with an improvement of the total anti-oxidative status and a decreased stroke lesion size in DA-8159-treated rats, compared to vehicle-treated rats [12].

Following induction of embolic stroke, aged and young rats were treated with sildenafil or vehicle [127]. Aged rats treated with sildenafil responded with a significantly improved functional recovery

concomitant to higher cGMP levels, increased synaptogenesis and angiogenesis of endothelial cells compared to vehicle-treated controls. In general, vascular density and endothelial cell proliferation after stroke was attenuated in aged compared to young rats. In the embolic stroke model, sildenafil treatment resulted in long-term effects on stroke recovery, which were reported even after 6 weeks [61]. Sildenafil treatment of rats with embolic stroke partly enhanced the level of CBF, which, related to increased angiogenesis in the ischemic boundary area and, significantly improved functional recovery, compared with vehicle-treated animals [61].

Other selective PDEi: Expression of the remaining PDE families other than PDE 3, 4 and 5 in cerebral vascular cells (Table 1) opens the possibility that they may also possess therapeutic potential to amend endothelial function. Though specific inhibitors are being developed, none are yet commercially available for human use and none have been reported to have effects on cerebral endothelial cells. Further studies are warranted to explore this interesting area of PDE effects.

3.2.4. Non-specific PDEi

The non-selective and non-specific PDEi, dipyridamole (DP), used in secondary prevention of stroke based on its antithrombotic effects, was evaluated for effects on endothelial function in three studies [27,29,132]. The main outcome measured in the studies was the adhesion of neutrophils to an endothelial cell line and the expression of adhesion molecules on the endothelial surface, which contributes to leukocyte extravasation.

Neutrophils are a subclass of leucocytes which play an important role in the innate immune response [88]. Blood was retrieved from 12 stroke patients within 48 h of stroke onset: from six patients with previous stroke, and from six healthy controls. Neutrophils were isolated from the blood and subsequently incubated with dipyridamole, the antihypertensive drug candesartan, an angiotensin II antagonist [19], or a mix of dipyridamole and candesartan [29]. Brain microvascular endothelial cells were incubated with the drug-treated neutrophils. The neutrophils from acute stroke patients treated with any of the drugs, alone or in combination, inhibited adhesion to the endothelial cells, and additional inhibition of neutrophil-adhesion to endothelial cells was observed with the dual treatment regime. Incubation of neutrophils from patients with chronic stroke or healthy controls, with dipyridamole or candesartan, did not reduce the adhesion of neutrophils to endothelial cells, though the authors reported a small non-significant effect of the combined treatment in both groups.

Furthermore, expression of the adhesion molecule macrophage antigen 1 (Mac-1) on neutrophils from patients with acute ischemic stroke after treatment with dipyridamole alone or combined with candesartan was reported to be significantly reduced, when compared to vehicle-treated neutrophils [29]. This suggests that dipyridamole may have more than just anti-platelet effects in stroke prevention.

Effects of dipyridamole were evaluated in a human brain microvascular endothelial cell line exposed to 1) inflammatory stress by incubation with tumor necrosis factor alpha (TNF- α) after serum deprivation and 2) metabolic stress by OGD for 6 h, followed by reperfusion for 18 h [27]. Dipyridamole treatment (1–5 μ M) reduced the TNF- α induced expression of ICAM-1 and MMP-9. Also, dipyridamole significantly reduced cell death and MMP-9 levels after the metabolic insult. In the *in vitro* experiments, dipyridamole was reported to show both anti-apoptotic and anti-inflammatory effects in the endothelial cells [27].

The effect of dipyridamole, clopidogrel and acetylsalicylic acid on endothelial function was evaluated by measurements of NO, cGMP and von Willebrand factor (vWF) in plasma samples from ischemic stroke patients and healthy controls [132]. Also, C-reactive protein and platelet derived growth factor was measured in serum. A small cohort of patients ($n = 11$) and healthy controls ($n = 11$), with a history of ischemic stroke, were included within five years from the stroke incident. Both patients and controls received the drugs daily over a 2-week

period, either as single medications, in pairs (acetylsalicylic acid/clopidogrel, acetylsalicylic acid/dipyridamole, or clopidogrel/dipyridamole) or altogether. No changes in cGMP or NO levels in plasma were reported using any drug or the subject groups. However, a significant decrease in vWF was observed in both controls and patients when treated with dipyridamole. vWF is a pro-coagulator glycoprotein released from injured endothelial cells, which promotes thrombus formation and platelet adhesion on the endothelial surface [17] and is a marker of endothelial dysfunction. Recent studies suggest a role for vWF in regulating angiogenesis [102]. The C-reactive protein level was reduced in patients treated with dipyridamole. Clopidogrel reduced plasminogen activator inhibitor-1 in patients (non-significantly) and in controls (significantly). The platelet-derived growth factor level was reduced in controls but not patients when treated with acetylsalicylic acid. Treatment with triple anti-platelet treatment did not result in any additive effect on the vascular parameters measured, compared with mono or dual treatment. Triple anti-platelet treatment reduced levels of vWF in both patients and controls, which was not reported with either clopidogrel or acetylsalicylic acid.

Another non-selective PDEi, theophylline, has recently been tested as an add-on agent to thrombolysis, as it is assumed to reduce the reperfusion damage and improve the final outcome in stroke patients [69], and clinical results are underway.

4. Discussion

We explored, through a systematic search, studies investigating the effects of PDEi on endothelial function and dysfunction in ischemic stroke. The non-selective PDEi dipyridamole and the selective PDE3 inhibitor cilostazol are both currently used in secondary prevention of stroke based on their anti-platelet effects. However, the included studies reveal that other important mechanisms of action of PDEi, including effects on endothelial function, may apply to prevent strokes or reduce stroke outcome. PDEi are thus likely candidates to further target vascular changes related to ischemic stroke.

A crucial strategy for a better stroke treatment requires a multimodal approach which inhibits platelet aggregation and enhances blood perfusion in ischemic stroke regions [64,72]. Briefly, as a response to the endothelial damage and cerebral ischaemia related to a cerebral vessel occlusion by an embolus or local thrombus, the brain tissue reacts by activating the immune system and neuroinflammation [58]. Injury to the cerebral vascular endothelium, affecting the BBB, triggers expression of adhesion molecules and mediates infiltration of immune cells into the brain parenchyma [58]. Thus, the optimal therapeutic approach in stroke treatment needs to be tailored to improve microcirculation and modulate anti-inflammatory, anti-apoptotic, and pro-angiogenic processes to achieve neuro-reparative and neuroprotective effects.

PDEi are promising tools to amend endothelial dysfunction and prevent cellular damage in ischemic stroke, though most data derive from *in vitro* experiments based on cells and animal studies, with inherent translational problems to humans.

The key PDE families currently shown to be involved in endothelial signalling in stroke are PDE3, PDE4 and PDE5, which are involved in promoting vessel formation, anti-apoptotic and anti-inflammatory pathways. The major PDEi tested in stroke models are the inhibitors of PDE3 (cilostazol), PDE4 (rolipram) and PDE5 (dipyridamole, sildenafil, tadalafil). In patients, only one PDEi with an effect on clinical outcome in stroke patients been tested. Administration of cilostazol combined with other conventional anti-thrombotics in acute stroke patients was associated with good clinical outcome and recurrence three months after stroke onset [22,98], but additional clinical studies are warranted.

PDE4 inhibitors have a narrow therapeutic window due to the associated side effects such as headache, nausea and vomiting [91]. Mutations in the PDE4D gene have been associated with a higher susceptibility to ischemic stroke, in particular the cardioembolic stroke

subtype, though results are ambiguous [7,41,57,68,100,114]. A recent *in vivo* study in rats [11] used a modified PDE4 inhibitor with strong anti-inflammatory effects. The same drug showed no emetic side effects in beagle dogs, which warrants further studies to support a possible role of this modified PDE4 inhibitor in human stroke treatment. The PDE4D gene or associated pathways may still be an interesting therapeutic target in stroke, but the complexity of the physiological effects needs to be investigated.

The use of PDE3 inhibitors (e.g. cilostazol) to improve endothelial function and increase BBB tightness in animal models of stroke or rat/human endothelial cell models (triple co-culture to mimic neurovascular unit) show promise for more targeted treatment in humans with altered BBB function. In addition, cilostazol inhibited adhesion of platelets to endothelium post-ischaemia, mitigated the inflammatory reaction, and reduced expression of adhesion molecules and MMP-9. Of major clinical importance is that cilostazol seems to attenuate the tPA-induced hemorrhagic transformation, thus opening a possibility for increasing the time window for tPA treatment if combined with PDE3 inhibition.

The protective effect of cilostazol on hemorrhagic transformation may be through the expression of tight junction proteins that connect the ECs and maintain their integrity. AGE products are involved in diabetic vascular injury and contribute to ischaemia-reperfusion injury. As cilostazol was shown to alleviate the damage incurred by AGE, cilostazol may eligible as an add-on therapy in stroke patients with diabetes. Cilostazol also restored expression of tight junction proteins and ameliorated the damaging effects of AGE on integrity of the BBB, though these data need to be confirmed. The preclinical studies also suggested that the therapeutic time window of tPA could be extended by prior or concomitant administration of cilostazol.

The effect of PDEi on angiogenesis is interesting. Application of PDE4i in rats subjected to tMCAO showed enhanced angiogenesis reflected by differentiation of CD34+ cells to vascular endothelial cells, thus contributing to endothelial renewal/rejuvenation. Further, a reduction in neuronal apoptosis was observed in response to PDE4 inhibitor treatment [35]. The regenerative capacity of EPC can propagate angiogenesis post stroke or in patients with major risk factors for stroke [109]. This is supported by studies on traumatic brain injury where transplanted EPC promoted neurogenesis and amended the endothelial cell integrity [28]. A higher level of circulating EPC was reported in diabetic patients with cerebral ischaemia when treated with cilostazol [110], which suggests that cilostazol stimulated the endothelial restorative processes. Other studies confirm that treatment with the PDE3 inhibitor cilostazol and the PDE4 inhibitor rolipram had a positive effect on neovascularization in response to ischemic events [11,34].

The beneficial effects of PDE5 inhibition in stroke is associated with enhanced NO-cGMP signalling, improved cerebral blood perfusion, augmented vascular repair processes, and increased angiogenesis in addition to improved functional recovery in response to ischaemia [10,12,61,127,128,130]. Application of PDE5 inhibitors may be useful both in acute stroke and in secondary stroke prevention since PDE5 inhibitors reduce endothelial cell death and possibly increase angiogenesis, which contributes to neuro-repair, restoration of cerebral blood flow after cerebral ischemia and improves functional recovery after stroke [66,67,128,130]. In the recovery phase, post-stroke formation of blood vessels will assist in improving blood supply in the vulnerable hypoperfused brain tissue. VEGF stimulates neurogenesis [40] and is shown to possess neuroprotective effect in *in vitro* ischaemia [39]. Treatment of rats with sildenafil resulted in increased plasma nitric oxide levels inducing angiogenesis via synthesis of vascular endothelial growth factor (VEGF) and cGMP [130]. Also, cilostazol was reported to stimulate production of VEGF and its receptor, VEGFR, but whether these effects are cAMP- or cGMP-mediated is not fully understood [77].

Ischaemic stroke, which induces formation of apoptotic cells, cell debris and increased reactive oxygen species, triggers an inflammatory response in the vessel walls. The neuro-inflammatory process activates

microglia and the infiltration of leucocytes from the bloodstream to the site of ischemic injury, which can result in larger infarcts in the post-ischemic tissue [45]. Dipyridamole reduced the plasma levels of vWF and CRP in patients with ischemic stroke [132], improved the endothelial reactivity, and inhibited the inflammatory response post stroke [29,132]. Dipyridamole appears to not only prevent platelet aggregation, but it also reduces the impact of ischaemia by reducing the inflammatory response as an add-on effect.

This review assessed the current literature for expression of PDEs in the brain with a focus on PDEs in cerebral microvascular endothelial cells as a therapeutic target to amend endothelial dysfunction in cerebral ischemic stroke. Modulation of both cAMP and cGMP signalling by PDEi possesses potential therapeutic value for BBB repair and protection to alleviate the ischemic stroke injury. Studies which explore the cAMP, cGMP or the cross-talk signalling in endothelial dysfunction are still warranted.

PDEi have been used to treat vascular diseases for decades, but many PDEi are associated with considerable side effects, which leaves a narrow therapeutic window. Having said that, in virtue of the differential tissue and subcellular distribution of the PDE families and their numerous isoforms, PDEs are highly potential drug targets. This diversity allows modulation of more specific molecular and tissue-related signalling pathways, hence drugs with better tolerability and improved clinical efficacy can be developed for treatment. In this review, we found that modulation of either cAMP or cGMP levels with specific PDEi can amend the endothelial function and perhaps improve treatment for ischemic stroke and cerebral endothelial dysfunction at least in *in vitro* models, though human studies are needed to confirm these findings.

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