Chapter 16
Genetic Understanding of Stroke Treatment: Potential Role for Phosphodiesterase Inhibitors

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Abstract Phosphodiesterase (PDE) gene family is a large family having at least 21 genes and multiple versions (isoforms) of the phosphodiesterase enzymes. These enzymes catalyze the inactivation of intracellular mediators of signal transduction such as cAMP and cGMP and therefore, play a pivotal role in various cellular functions. PDE inhibitors (PDEI) are drugs that block one or more of the five subtypes of the PDE family and thereby prevent inactivation of the intracellular cAMP and cGMP by the respective PDE-subtypes. The first clinical use of PDEI was reported almost three decades ago. Studies later found the ability of these compounds to increase the levels of ubiquitous secondary messenger molecules that can cause changes in vascular tone, cardiac function and other cellular events and thus these findings paved the way for their use in various medical emergencies. PDEs are found to be distributed in many tissues including brain. Therefore, new therapeutic agents in the form of PDEI are being explored in neurodegenerative diseases including stroke. Although studies have revealed their use in cerebral infarction prevention, their full-fledged application in times of neurological emergency or stroke in specific has been very limited so far. Nevertheless, recent investigations suggest PDE4 and PDE5 inhibitors to play a vital role in mitigating stroke symptoms by modulating signaling mechanisms in PDE pathway. Further, extensive research in terms of their pharmacological properties like dosing, drug specific activities, use of simultaneous medications, ancillary properties of these compounds and studies on adverse drug reactions needs to be carried out to set them as standard drugs of use in stroke.

Keywords Phosphodiesterases • Phosphodiesterase inhibitors • Stroke • Rolipram • Therapeutic potential

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16.1 Introduction

Stroke is the most common cause of neurological disability and a leading cause of death worldwide. Attack of stroke can result in a variety of symptoms and signs but the most widely seen effect is that of motor impairment that controls the movement of face, arm and leg of one side of body (Lawrence et al. 2001). This clinical syndrome results from a number of different disease processes and is categorized into two types. Approximately 80% of patients have been recorded to suffer from ischemic stroke while the other 20% suffer from hemorrhagic stroke. An ischemic stroke happens when a blood vessel (artery) supplying blood to an area of the brain becomes blocked by a blood clot. A hemorrhagic stroke happens when an artery in the brain leaks or bursts (ruptures). These two basic categories of stroke are further divided into other subtypes depending on several other delineating clinical features. Its prognosis too varies greatly depending on a number of factors like pre-morbid condition, stroke severity, age, and post-stroke complications. Although stroke affects both young and adult individuals, population demographics study has shown that stroke among older people to result in more severe functional loss (Baztán et al. 2007). Stroke rehabilitation has thus focussed on recovery of impaired movement/function to reduce disability resulting from both motor and non-motor impairments post stroke.

Recent reports have focussed on acute management of stroke and a significant amount of progress has been made in this direction. A multitude of studies have assessed novel therapeutic interventions in patients and emerging evidence has revealed the concept of brain repair (neuroplasticity) or endogenous neurorestorative processes that have helped in development of pharmacological and cell-based therapies capable of stimulating neurological recovery after stroke (Hermann and Chopp 2012). Despite novel interventions, drugs have been the main management agents till date that are capable of altering numerous biological processes leading to a stroke attack or worsen the condition of a patient post stroke. Agents such as lipid lowering drugs (statins and recombinant tissue plasminogen activator) and antiplatelet agents (aspirin or ecosprin and clopidogrel) have now long been in use along with management of conventional risk factors like hypertension, diabetes and hyperlipidemia that are the main contributing factors that predisposes one to stroke (Meschia 2007). However, apart from clinical characteristics and environmental factors, genetic factors have been suggested to contribute in the development and worsening of condition. A number of genomic studies in the area of stroke have suggested different candidate genes responsible for stroke. One such widely studied plausible gene is phosphodiesterase 4D (PDE4D) identified by deCODE group in Icelanders. Further, replicative studies among different populations (Gretarsdottir et al. 2003; Munshi et al. 2009; Saleheen et al. 2005; Liu et al. 2013; Staton et al. 2006; Matsushita et al. 2009) and studies on different variants of this gene including various aspects of PDE4 pathway provided a strong evidence for its role in the development of stroke (Das et al. 2016). Interestingly, the Icelandic study by

Gretarsdottir et al. (2003) reported decrease in stroke risk in individuals with PDE4D polymorphisms by PDE4D inhibition using small molecule inhibitors (Gretarsdottir et al. 2003).

With advancement in research, PDE and its inhibitors have been reported to be involved in functional behavior of humans and animals and therefore, PDEI are emerging as therapeutic agents and physiological modifiers (Kuhlenbaumer et al. 2006). Therefore, exploring the use of PDEI in molecular therapeutics for possible diseases would prove to be very helpful.

16.2 General Features of PDE and PDEI

PDE or diester-orthophosphoric-phosphohydrolases are basically a group of enzymes capable of hydrolyzing cyclic nucleotides adenosine 3' and guanosine 3' in their inactive form 5'-cyclic monophosphates (cAMP and cGMP). These molecules are essentially secondary messenger molecules and PDEs essentially degrade the phosphodiester bond in these molecules. In mammals PDEs are classified into 11 families (PDE1-PDE11) depending on amino acid sequences, substrate specificities, regulatory properties, pharmacological properties and tissue distribution. Regulation of localization, duration and amplitude of cyclic nucleotide signaling within subcellular domain is their main function (Beavo 1990). Similarly, there are a number of PDEI having various therapeutic implications that have altered response to specific tissues depending on cyclic nucleotides. Initially it was caffeine that was found to act as an inhibitor of PDE following which a lot of nonselective PDE inhibitors such as theophylline (caffeine analogue) entered into clinical use. Later, several isoenzyme-selective PDEI were developed as therapeutics. As therapeutic agents they have been used in control of pathophysiological changes caused due to cyclic nucleotides in central nervous system, lungs, digestive tract and inflammatory processes (Clarke et al. 1994; Cristina and Nagy 2003; Kanes et al. 2007; Li et al. 1994; Lipworth 2005; Wright 2006). Since PDE have unique tissue distribution, structural and functional properties they have been used as successful targets for pharmacological inhibition during times of cardiac failure, pulmonary hypertension, erectile dysfunction etc. (Barnes et al. 1988; Torphy and Undem 1991; Boolell et al. 1996).

Although the use of PDEI in a number of clinical conditions has been well studied, its use in stroke has only recently been explored. PDE1, PDE4, PDE8, PDE9 and PDE10 have been reported to be distributed in brain tissues and therefore, various PDEI have the potential to be used in the treatment of neurodegenerative diseases (Fig. 16.1) (Boswell-Smith et al. 2006). Apart from their distribution in brain, PDE isoforms found on blood platelets (PDE2, PDE3 and PDE5) also offer a novel strategy to deal with stroke (Fig. 16.2). Further, it is to be noted that PDEI generally has multiplicity of effects due to action of drugs on more than one isoform and many tissues harboring more than one isoform.

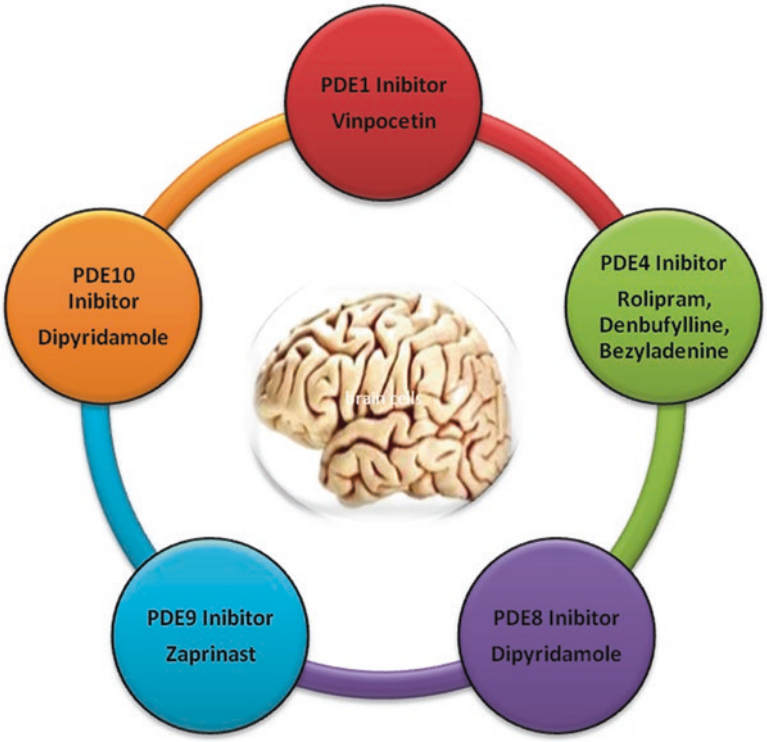

Fig. 16.1 PDE1, 4, 8, 9, 10 inhibitors affect neuronal tissues, brain cortex cells and cerebral blood flow thus finding its use in neurodegenerative diseases and stroke

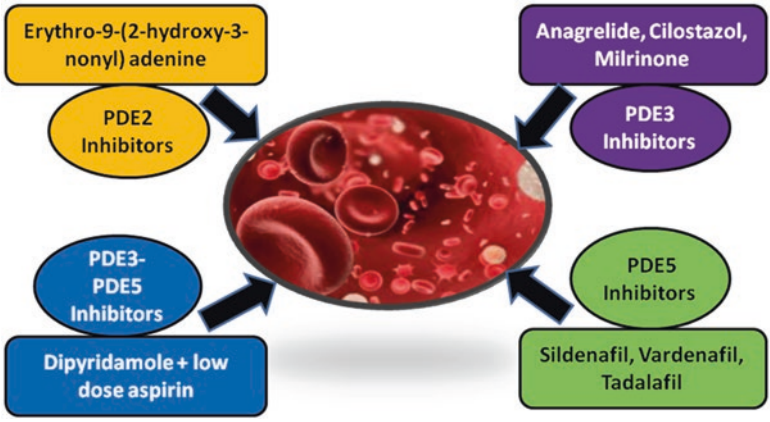

Fig. 16.2 Inhibition of three PDE isoforms (PDE2, PDE3 and PDE5) found on platelets exerts a strong platelet inhibitory effect. These PDEIs have shown great benefit for the treatment and prevention of stroke

16.3 PDEs Localized in Brain Tissues, Their Pharmacology and Inhibitors

PDE1 family is mostly found in cytosolic region and is specifically activated by calcium calmodulin (Ca+2/CaM) and thus named as CaM-PDE (Wells et al. 1975). It hydrolyzes both cAMP and cGMP. PDE1A, PDE1B and PDE1C are the three genes having various splice variants that constitute this family. In brain PDE1A is highly expressed while PDE1B1 mRNA is predominantly found in neuronal cells of the cerebellum, hippocampus, caudate and purkinje cells. Its expression mainly correlates to brain regions having extensive dopaminergic innervations and D1 dopamine receptor mRNA. PDE1C also mainly expresses in brain and heart and is highly expressed in the mouse cerebellar granular cells (Polli and Kincaid 1992; Yu et al. 1997; Loughney et al. 1996; Yan et al. 1996). This family has been implicated in a number of pathological and physiological processes and most likely regulates vascular smooth muscle contraction and induction of apoptosis in human leukemic cells (Jiang et al. 1996). However, the most important feature of PDE1 is the regulation of smooth muscle cells and neuronal signaling. It has been suggested that inhibition of PDE1C can cause beneficial effects by inhibiting proliferation of smooth muscle cell an event that contributes to atherosclerosis one of the main contributing factors for stroke (Sonnenburg et al. 1995). Inhibitor vinpocetin (chemically called as ethyl apovincaminate) is a semisynthetic derivative of vincamine extracted from periwinkle plant that inhibits PDE1 with an IC_{50} of approximately 10^{-5} M and is known to increase cerebral blood flow and improves memory (Sonnenburg et al. 1995).

Similarly, PDE4 family represents the largest of all PDE families and constitutes four genes PDE4A, PDE4B, PDE4C and PDE4D with a number of alternative mRNA splice variants of long and short isoenzymes of PDE4 and with 35 different PDE4 proteins (Swinnen et al. 1989; Livi et al. 1990; Bolger et al. 1993; McLaughlin et al. 1993). Its localization is complex and is found in cytosol or associated with cellular membranes and mainly found in brain, smooth muscle, inflammatory cells and cardiovascular tissues. Among the PDE subfamily, PDE4 alone represents 70–80% of PDE activity in neuronal tissue and specifically hydrolyzes cAMP (Beglopoulos and Shen 2006). Studies mostly have focused on PDE4D and PDE4D deficient mice have been known to display delayed growth, reduced viability and an antidepressant profile which revealed the PDE4D-regulated cAMP signaling to play a role in pharmacotherapy of depression (Jin et al. 1999; Zhang et al. 2002a; Zhang et al. 2002b). Studies on stroke pathogenesis had long suggested the vital role of PDE4 pathway in influencing it via an uncertain mechanism. Thus, Yang et al. (2012) investigated specifically the role of tissue plasminogen activator (tPA) on inhibition of PDE4 with the drug rolipram and reported that inhibition of PDE4 and PDE4D to reduce expression of tPA by Epac pathway (Yang et al. 2012). Rolipram the first generation potent inhibitor of PDE4 was the archetype to synthesize new potent and selective PDE4 inhibitors. Although other PDE4 inhibitors like Denbufylline (xanthine derivative) and Benzyladenine derivatives were synthesized as potent inhibitors they were not found to be that effective due to their adverse

emetic effect. These latter drugs also have broad anti-inflammatory and immunomodulatory actions which makes them applicable in other diseases too. However, in neuronal cultures it has been seen that PDE4 tightly regulates cAMP formed by stimulation of N-methyl-D-aspartate receptors and that rolipram due to its ability to cross blood brain barrier decreases ischemic neuronal damage and administration of it in 1 mg/kg significantly enhances hippocampal neurogenesis (Kato et al. 1995; Suvarna and O'Donnell 2002; Nikulina et al. 2004).

Further, recent reports also showed rolipram to promote axonal regeneration, attenuate glial scar formation, enhancement of functional recovery after spinal cord injury and to improve synaptic and cognitive functions (Nikulina et al. 2004; Gong et al. 2004). Additionally the experiment results by Sasaki et al. (2007) revealed rolipram to enhance survival of newborn neurons due to pharmacological activation of cAMP-CREB signaling that may also provide to be an effective therapy for stroke and post stroke complications (Sasaki et al. 2007). Adding to these findings the study carried out by Kraft et al. (2013) found rolipram to improve stroke outcome by modulation of important mechanism of ischemic neurodegeneration such as blood brain barrier disruption, inflammation and thrombosis. Their study revealed rolipram to show multifaceted mode of action and be an important and effective lead compound in stroke therapies when applied 2 h after stroke (Kraft et al. 2013). However, to comment on the safety and efficacy of the drug is too early and intense efforts are required in this direction to determine the same not only in animal experiments but its applicability in human system too. Despite its experimental effectiveness its limitation lies in specific side effects such as gastrointestinal problems, hypotension, fear or flushing which has forced a number of patients to withdraw from clinical trials. To overcome this problem next generation PDE4 inhibitors with improved side effect profiles have been suggested (Dal Piaz and Giovannoni 2000; Pagès et al. 2009). Apart from this, unresolved critical pharmacological issues related to optimum dosage, time point, delivery of the inhibitor (single vs. continuous application), its long term effect in acute ischemic stroke and additional mechanisms such as modulation of endogenous tPA release from cerebral endothelial cells needs to be successfully addressed (Yang et al. 2012).

The other subfamily found in brain is PDE8 that specifically hydrolyzes cAMP and is encoded by two genes PDE8A and PDE8B and has its least amount of mRNA expression in the brain with PDE8B3 being the most abundant form in the brain (Hayashi et al. 2002). Similarly PDE9 subfamily is encoded by a single gene PDE9A with several variants and specifically hydrolyses cGMP. PDE9A gene is known to have complex regulation of expression and more than 20 variants have been observed to exist but its specific function has still not been elucidated. However, its pattern of mRNA expression in brain closely resembles that of soluble form of guanylyl cyclase that suggests a possible functional association in regulation of cGMP levels that play a vital role in behavioral state regulation and learning. On the other hand PDE10 has been reported to be cGMP-sensitive and cAMP selective and is encoded by PDE10A gene with abundant transcripts found in brain. Huntington's chorea a progressive neurodegenerative disease is reported to be associated with PDE10

family (Hebb et al. 2004). For these above three families only the differential sensitivity to inhibitors has been reported. PDE8A was found to be inhibited by dipyridamole; PDE9A was reported to be sensitive to zaprinast and PDE10A is known to be inhibited by dipyridamole.

16.4 PDEs Localized in Platelets, Their Pharmacology and Inhibitors

Inhibition of platelet aggregation has been shown to be a great benefit for the treatment and prevention of stroke. This can be achieved either by the blockade of the membrane receptors or by interaction with intracellular signaling pathways. Two critical intracellular secondary messengers' cAMP and cGMP are provided with strong inhibitory activity of fundamental platelet functions. The intracellular levels of cyclic nucleotides are limited by PDEs by catalyzing the hydrolysis of cAMP and cGMP that leads to the regulation of platelet functions. Platelets possess three PDE isoforms i.e. PDE2, PDE3 and PDE 5 and inhibition of these PDEs may therefore, exert a strong platelet inhibitory effect. Non-selective or isozyme-selective PDE inhibitors have been developed and some of them are being used as antiplatelet agents in clinical use.

16.4.1 PDE2 Inhibitors

Inhibitors of PDE2 have been investigated for their effectiveness in memory impairment and prevention of endothelial permeability in inflammation (Bender and Beavo 2006). One of the selective inhibitors called as Erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) that inhibits adenosine deaminase (ADA) has reported no direct effect on platelet aggregation but potentiates the inhibition of thrombin-induced platelet aggregation by nitroprusside-a guanylyl cyclase stimulator (Dickinson et al. 1997). A natural product from *Ocotea pretiosa* has also been explored for its antiplatelet activity (Lima et al. 1999). Further, a novel selective compound 9-(6-phenyl-2-oxohex-3-yl)-2-(3, 4- dimethoxybenzyl)-purin-6one (PDP) was recently developed but has not been tested on platelets as of now (Diebold et al. 2009).

16.4.2 PDE3 Inhibitors

PDE3 is known to have two isoforms i.e. PDE3A and PDE3B, of which PDE3B subtype is mainly expressed in platelets (Sun et al. 2007). Anagrelide is a potent and broad-spectrum inhibitor of platelet aggregation but studies involving humans have

shown the drug resulting in thrombocytopenia (Seiler et al. 1987; Thiele et al. 2006) and therefore, it has mainly found its clinical use among patients with essential thrombocythemia (Silverstein et al. 1988). The other well-known specific and strong inhibitor of PDE3 in platelets and smooth muscle cells is drug Cilostazol that causes smooth muscle cell relaxation and inhibition of platelet activation (Shrör 2002). It inhibits primary and secondary platelet aggregation and its use has been suggested over conventional antiplatelet therapy due to its short recovery time of platelet function (Iwamoto et al. 2003). This drug has also been studied for secondary prevention of stroke and studies have shown that use of this drug significantly reduces the recurrence of ischemic stroke, myocardial infarction, transient ischemic attack and intracranial hemorrhage. Data from studies have confirmed a low bleeding risk, fewer hemorrhagic events, significant reduction in risk of cerebrovascular events and prevention of post-stent restenosis (Gotoh et al. 2000; Weintraub et al. 2004; Uchiyama et al. 2009; Shinohara et al. 2010) with adverse effects like headache, tachycardia, palpitations, soft stools and diarrhoea (Sorkin and Markham 1999). Milrinone is another specific PDE3A inhibitor that induces an elevation of intraplatelet cAMP in a dose dependent manner, resulting in inhibition of platelet aggregation but its clinical use has so far been restricted to congestive heart failure (Manns et al. 2002; Colucci 1991).

16.4.3 PDE3-PDE5 Inhibitors

Interestingly enough there are drugs that simultaneously inhibit PDE3 and PDE5. Drug dipyridamole although used initially as a coronary vasodilator, later showed its property of inhibiting platelet aggregation (Born and Cross 1963; Elkeles et al. 1968) and this paved way for its use as antithrombotic agent (Schwartz et al. 1988). It inhibits both PDE3 and PDE5 thus increasing the intraplatelet cAMP and/or cGMP, and it also acts as an antioxidant by scavenging free radicals that inactivate cyclo-oxygenase. Dipyridamole however, inhibits platelet aggregation in whole blood but not in platelet-rich plasma by blocking the reuptake of adenosine (Gresele et al. 1983; Gresele et al. 1986) and the antioxidant property of this drug is known to be better than ascorbic acid, α-tocopherol and probucol (Iuliano et al. 1996; Pascual and Romay 1992). Other pharmacological effects like inhibition of vascular smooth muscle cell proliferation, prevention of endothelium-leukocyte interactions and inhibition of inflammatory gene expression in platelet–monocyte aggregates also help in prevention of atherothrombosis (Kim and Liao 2008; Iimura et al. 1996; Weyrich et al. 2005). However, the clinical evidence of it alone exerting antithrombotic effect is very little and two large studies have shown dipyridamole in combination with low-dose aspirin leads to greater stroke risk reduction in ischemic cerebrovascular disease (Diener et al. 1996; Halkes et al. 2006).

16.4.4 PDE5 Inhibitors

PDE-5 family was originally identified and purified from rat platelets (Coquil et al. 1980) but subsequent studies have showed its distribution in vascular and bronchial smooth muscles, platelets and lungs (coquil et al. 1980; Francis et al. 1980). Its inhibitors result in increased tissue level of cGMP that cause smooth muscle cell relaxation. Three known PDE5 inhibitors sildenafil (Viagra), vardenafil (Levitra) and tadalafil (Cialis) are currently in clinical use. However, the first developed inhibitor of this group was zaprinast that was originally meant for treatment of allergic diseases (Murray 1993). The observation that zaprinast induced elevation of cGMP and caused smooth cell relaxation led to its application in cardiovascular diseases (Rudd et al. 1983) and is known to inhibit human platelet PDE5 with an IC_{50} of 0.3 μM and PDE2A with an IC_{50} of 42 μM. However, this compound was unsuccessful and was modified leading to the identification of sildenafil a 100 times more potent and highly specific drug. Studies found it to have rapid absorption after oral administration with ~40% bioavailability and were shown to significantly increase bleeding time in healthy men 1 h after 100 mg of its intake with a recovery time of 4 h (Berkels et al. 2001). Nevertheless, this drug has relatively low selectivity for PDE5 and thus more potent selective PDE5 inhibitors like vardenafil and tadalafil were later developed (Young 2002; Corbin and Francis 2002).

PDE-5 inhibitors have been suggested to protect the brain against stroke and other neurodegenerative diseases but the mechanisms by which they exert cytoprotective effects are not understood completely. However, it has been hypothesized that the vasodilatory action of PDE-5 inhibitors *in vivo* could release endogenous mediators of pre-conditioning. For example, adenosine and bradykinin (endogenous mediators) from endothelial cells may trigger a signaling cascade activating kinases resulting in phosphorylation of endothelial nitric oxide synthase (eNos), synthesis of eNos and inducible nitric oxide synthase (Rosanio et al. 2006; Das et al. 2005 and Salloum et al. 2003). Further, animal studies on sildenafil report its oral administration for seven consecutive days starting 2–24 h after embolic middle cerebral artery occlusion to enhance neurological recovery without any effect on volume of the infarct (Zhang et al. 2002b). Many other cerebral vascular-protective effects of the drug have been demonstrated in patients suffering from pulmonary hypertension and this drug is also known to affect the cerebral hemodynamics during acute exposure to high altitudes (Rosengarten et al. 2006; Chan et al. 2005).

The first study showing a pre-conditioning like effect of sildenafil against myocardial ischemia/reperfusion therapy was reported by Ockaili et al. (2002). Subsequent studies showed the infract-limiting effect of sildenafil in several models including mouse hearts, infant rabbit hearts and rat hearts (Salloum et al. 2003; Wang et al. 2008; Das et al. 2009; Bremer et al. 2005; Das et al. 2002; du Toit et al. 2005 and Rosanio et al. 2006). The anti-ischemic effects of PDE-5 inhibitors have also been observed against ischemia/reperfusion-triggered ventricular arrhythmias and also the improvement of post ischemic ventricular contractile function

(Das et al. 2002; Nagy et al. 2004; Bremer et al. 2005; Das et al. 2002). Studies have also showed the infarct-limiting effect of sildenafil and vardenafil when these inhibitors were administered just before reperfusion (Elrod et al. 2007; Salloum et al. 2007).

16.5 Discussion and Conclusion

Stroke is a leading cause of serious and long term disability and an estimated 5.7 million people die from stroke worldwide (Lopez et al. 2006; Feigin et al. 2003). With the attack of stroke the problem of recurrence in stroke survivors has been estimated to be around 7.7% at 1 year, increasing to 18.3% in 5 years (Feigin et al. 2003). Effective strategies that can prevent stroke recurrence; stroke related morbidity and mortality are a major issue for the healthcare organizations worldwide. Mostly prevention of stroke is done by the use of antiplatelet drugs and anticoagulants useful in primary or secondary prevention of ischemic stroke (Apostolakis et al. 2013). Administration of drug aspirin within 48 h after stroke has been the recommended treatment of action and the other drug clopidogrel mostly used in secondary prevention has complex pharmacokinetics and consequently its early use after stroke attack has not been recommended (Floyd et al. 2012; NICE Guidelines 2008). Nevertheless, despite their use the pharmacological understanding of these drugs has been poor and sometimes their use has to be withdrawn in patients undergoing surgery to prevent bleeding complications.

Platelets although lifesaving during bleeding but over active platelets pose life threatening situation due to severe ischaemic tissue and other devastating complications. Thus, understanding the pharmacokinetics of antiplatelet agents is important since haemostatic properties of platelets are mediated by different receptors and downstream intracellular mechanisms. It has been found that antiplatelet agents act principally on three target molecules i.e. cyclooxygenase-1 (e.g., aspirin), adenosine 5-diphosphate receptor (e.g., clopidogrel, prasugrel, ticagrelor) and glycoprotein IIb/IIIa antagonists (e.g., abciximab, eptifibatide, tirofiban). Even if a particular receptor engaged in platelet aggregation is blocked, residual platelet activity may take place through an alternative pathway and thus more than one antiplatelet agent may be needed to be used simultaneously to achieve platelet inhibition. Therefore, strategies in such treatments should be a tailor made approach depending on individual patient circumstances (Apostolakis et al. 2013). Apart from this the use of intravenous administration of anticoagulants had been a common practice of treatment in acute phase of ischemic stroke but data on use of anticoagulant heparin has mostly been inconclusive (Jauch et al. 2013). Experimental results by International Stroke Trial (IST) on the use of subcutaneous unfractionated heparin reports, reduction of acute recurrent cardioembolic stroke but increase of intracerebral haemorrhage rate to a similar degree (International Stroke Trial Collaborative Group 1997). Similarly, the secondary analysis by trial of ORG 10172 in Acute Stroke Treatment (TOAST) on the beneficial effect of heparinoid danaparoid in

cardioembolic stroke group also reports negative results (The Publications Committee for the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) Investigators 1998). Another study by Heparin in Acute Embolic Stroke Trial (HEAST) also reports aspirin to be a better compound than low molecular weight heparin in acute phase of cardioembolic stroke due to atrial fibrillation (Berge et al. 2000). Thus, the results of these findings do not recommend the use of anticoagulants in clinical management of ischemic stroke. Nevertheless, studies now have suggested other important pathways that if blocked can mitigate the occurrence of stroke symptoms. Thus, new options of management of stroke risk in patients are an urgent need of the hour and therefore, agents like PDEI that interfere with intracellular signaling pathways have theoretically great potential for platelet inhibition.

In view of this, recent studies have thus explored PDEI after the successful use of drugs like theophylline and papaverine (non-selective inhibitors) in a range of diseases. Their real impact on treatment of various diseases has gained importance in last 10 years and its use in stroke is a relatively new concept and yet to be established. A series of PDEI namely milrinone, enoximone, vesnarinone, pentoxifylline, and cilostazol are in use, each having unique pharmacologic properties but so far the application has mostly been restricted to wide use in cardiovascular failure and asthma. These inhibitors mostly promote reduction in cAMP breakdown with variety of tissue specific effects and vasodilation resulting in hypotension particularly in vasoconstricted and hypovolemic patient. Apart from this, these drugs tend to show inotropic effect that improves functional status, reduces inflammation and oxidative stress. Among the various classes of inhibitors PDE4 inhibitors have been reported to have greater effect on inflammation when compared with PDE3 inhibitors. Drugs like roflumilast, cilomilast, and rolipram have been associated with significant anti-inflammatory effects and have received considerable amount of attention but side-effects such as nausea, vomiting and headache have also limited their use (Feneck 2007).

Most of the studies in stroke have focussed on PDE4 pathway and PDE4 inhibitor (rolipram). Subfamilies like PDE2 although reported to be present in brain cortex but their possible functional role has not been studied. PDE3 family has been reported to be found in platelets, heart and liver and thus its inhibitors find application during heart failure. Similarly PDE5 inhibitors mainly have helped in treatment of pulmonary hypertension and respiratory distress (Hansen et al. 2000). Apart from this PDE5 inhibition has shown to improve early memory consolidation of object information and to reduce neurological deficits and evoke neurogenesis (Prickaerts et al. 2004; Zhang et al. 2002b).

In conclusion, devastating neurological emergency like stroke needs to be measurably improved and treatment through PDEI seems to offer quiet a novel approach. Both non-selective and selective inhibitors have been used in a number of medical conditions. However, with respect to stroke, studies so far suggest inhibitors of PDE4 and PDE5 to be most relevant in post stroke management. With this possible strategy the complications also arise due to widespread distribution of PDE in the body that renders it difficult for an effective antiplatelet action without any significant unwanted effects (Gresele et al. 2008). Further, reversibility of effect of most

clinically used PDEI on their target is a serious limitation on their antithrombotic effectiveness for long term secondary prophylaxis. Therefore, a deeper understanding of physiology of PDEs in platelets and other tissues, targeting of PDE inhibition to platelets and development of long term acting selective PDEI are required for an effective antiplatelet therapy. Their full fledged use in stroke, thus, requires a lot of clinical study not only with respect to their pharmacological properties but also in regards to the adverse side reactions that may result from its administration.

Conflict of Interest The authors declare that they have no conflicts of interest**.**

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