CHAPTER II

LITERATURE REVIEW

Structure and function of blood vessel

Blood vessel is the transporting system of the blood throughout the body. The major types of blood vessel are classified as the arteries, which carry the blood away from the heart, the capillaries, which enable the actual exchange of water, nutrients and chemicals between the blood and tissues, and veins which return blood to the heart.

Blood vessels consist of a three-layered structure (Figure 1) including; Tunica intima (the thin inner layer), is a single layer of simple endothelium cells, a polysaccharide intercellular matrix and connective tissue. Tunica media, the thick middle layer, is separated from the intima by the internal elastic lamina. The media contains rich smooth muscle cells, which control blood vessel. Tunica adventitia is composed of a lot of connective tissue, sympathetic and sensorimotor nerves depending on the vessel.

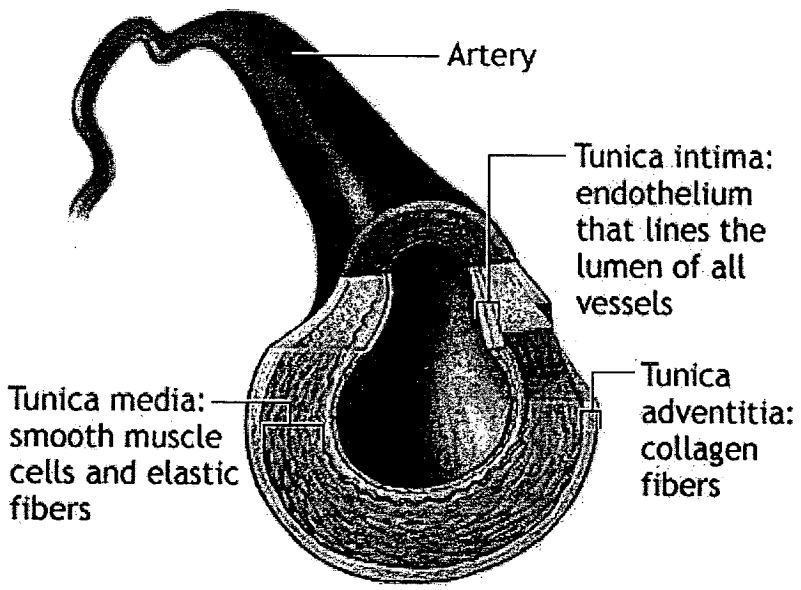

Figure 1 Structure of blood vessel

Source: Modified from http://www.umm.edu/patiented/articles/artery

Blood vessels do not only function in the transportation, but arteries and veins also possess smooth muscle in the media which regulates their inner diameters by contraction of the muscular layer (tunica media). This changes the blood flow to downstream organs and determined by the autonomic nervous system and circulating humoral agents. Arteriolar smooth muscle cells are much smaller and regulate local blood flow as well as having the great effect on systemic blood pressure.

Mediators involved in vasoconstriction and vasodilation

The mechanisms of smooth muscle cell contraction and relaxation involve changes in level of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The increase in $[Ca^{2+}]_i$ leads to vasocontraction, while the decrease in $[Ca^{2+}]_i$ causes vasorelaxation. Both vasoconstrictors and vasodilators are involved in regulation of vascular tone. In pulmonary circulation, ET-1 and angiotensin II are among the important mediators producing vasoconstriction [20]. The endogenous compounds that cause vasodilation include NO, and PGI_2, and C-natriuretic peptides (CNP), (Figure 2). Here I will focus on the effect of NO since it will be most related to the present study.

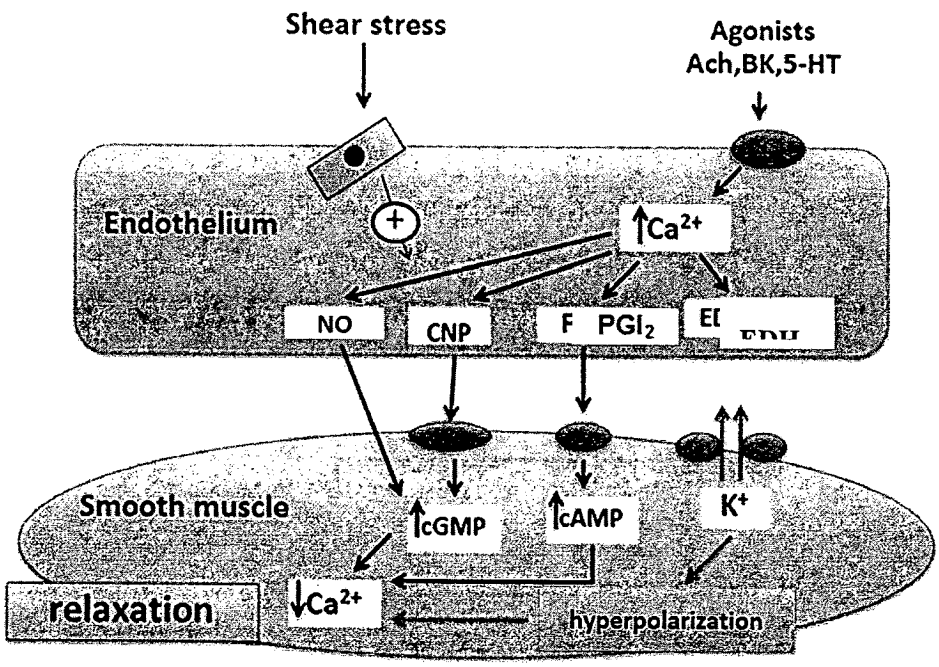

Figure 2 Mediators involved in vasoconstriction and vasodilation

NO and its role in vascular tone regulation

NO is mediated via second messenger, cGMP [21], which is degraded by PDE. PDE5 is the predominant PDE isoform in the lung that metabolizes cGMP [22].

NO is in the pulmonary endothelium and released to the smooth muscle cells. It activates GC and produces cGMP, which leads to vasodilation. NO production can be increased by eNOS activity. eNOS is activated by level of Ca^{2+} increase and mechanical force (shear stress). Moreover, endothelium can be activated by several endogenous substances such as acetylcholine (ACh), bradykinin (BK), 5-HT which act on their respective receptor on the endothelium [23].

There are 3 isoforms; 1) n-NOS (constitutive form) is found in most normal body which can be activated by Ca^{2+}-calmodulin (CAM). It is found in neurons of the central nervous system and non-noradrenergic and non-cholinergic system. 2) i-NOS (inducible form) which is activated during inflammation. This does not need Ca^{2+}-CAM for activation. 3) e-NOS is Ca^{2+}-CAM dependent and is found in the endothelium, cardiac myocytes, renal mesangial cells, osteoblasts, airway epithelial cells and platelets.

L-Arginine is a primary source of NO for all NOS isoforms. It is an amino acid found in cytoplasm of endothelial cells. L-Arginine is converted into NO via e-NOS, thus its dysfunciton leads to attenuated vessel relaxation. Arginine can be depleted by L-arginase, which is metabolized enzymes of L-arginine could decrease NO [24]. Therefore, L-arginase could induce vasoconstriction and pathology of cardiovascular system such as hypertension, ischemic stroke, and ischemia heart disease. In addition, Frostell (1991) reported effect of inhaled NO at dose of 5-80 ppm in lambs. The result showed pulmonary vasodilation and in hypoxia condition, where vasoconstriction occurred. In persistent pulmonary hypertension of the newborn, which occurs in infants, the blood flow of pulmonary is less than 10% of the fetal cardiac output [25] include hypoxemia as well as hypertension were also present. This is a serious disorder in infants [26]. NO decreases hypertension via increasing of arginine level which is one of the substrate to synthesis of NO [27]. NO also can help in hypoxemia [28]. The study of the vasodilation effect of NO inhalation at concentration 10, 20 and 40 ppm in the 35 primary hypertension patients compared

with PGI_2 showed that both drugs produced similar individual vasodilation response [29].

Mechanism of chemicals used as pharmacological tool in the present study

Acetylcholine (ACh)

ACh acts through both nicotinic and muscarinic receptors. The principal ACh receptor of the endothelial is the muscarinic M_3 receptor which is coupled to phospholipase C whose substrate is membrane PIP_2. The products are diacyl glycerol which activates protein kinase C (a modulator of membrane ion channels) and inositol 1, 4, 5 triphosphate (IP_3). IP_3 binds to IP_3 receptor on endoplasmic reticulum (ER) membrane causing flow of Ca^{2+} from the ER and increase cytosolic $[Ca^{2+}]$. Increase in $[Ca^{2+}]_i$ in endothelium stimulates e-NOS thus converting L-arginine into NO. NO activated GC in smooth muscle cell, which then change guanosine tri-phosphate (GTP) into to cGMP. The cGMP is stimulating protein kinase G (PKG), which inactivate the Ca2+ channel on membrane of smooth muscle cell and lead to decreasing of $[Ca^{2+}]_i$. Moreover, PKG increases Ca^{2+} influx into SR by activation of SR Ca^{2+} ATPase (SERCA) and it also stimulates myosin light chain phosphatase. Both of these effects cause decreasing of $[Ca^{2+}]_i$ and vasodilation. (Figure 3).

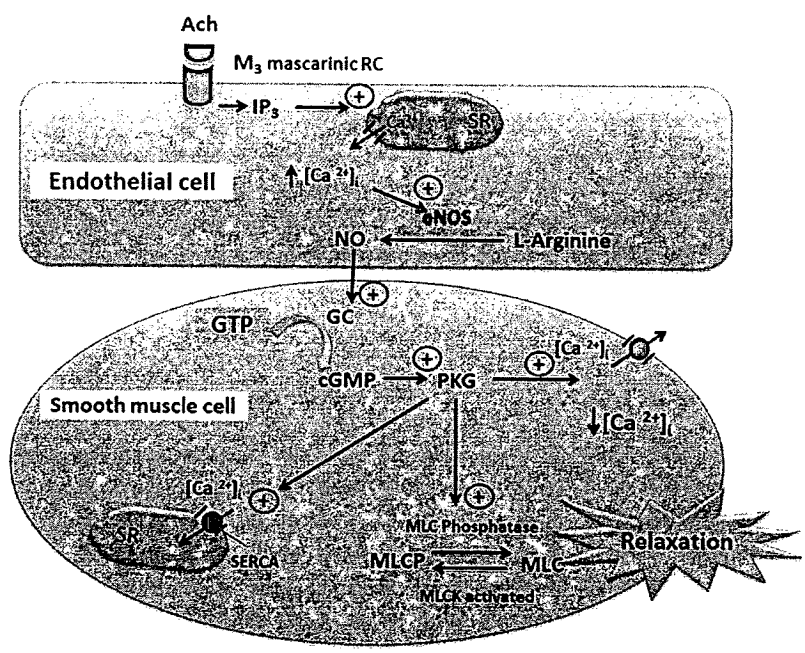

Figure 3 Mechanism of ACh induced vasodilation

Phenylephrine (PE)

Phenylephrine (PE) causes vasoconstriction of smooth muscle cell. PE binds with α1 adrenergic receptor, Gq-protein couple receptor (GPCR). Upon its stimulation, Gq protein activates another isoform of phospholipase-C, which change inositol 1, 4, 5 diphosphate (IP_2) into IP_3. IP_3 receptor is stimulated in SR by IP_3 causing Ca^{2+} release from SR and increases $[Ca^{2+}]_i$ in the smooth muscle cell. Ca^{2+} then forms Ca^{2+}-CAM complex and stimulates myosin light chain phosphorylation (MLCP) leading to myosin and actin cross bridge resulting in contraction of smooth muscle cell. Diacylglycerol (DAG) is a product from IP_2 which cause vasoconstriction by stimulating protein kinase-C (PKC). Activated PKC opens Transient receptor protein type C3 (TRPC) channels and voltage dependent L-type Ca^{2+} channel. The increased cytosolic Ca^{2+} stimulates the contraction of the smooth muscle cells. PKC also inhibits and closes potassium channels leading to depolarization and further increase Ca channel opening (Figure 4).

High potassium solution

High potassium (80 mM K+) solution can cause vascular contraction. The mechanism of high K^+ solution is via producing depolarization of the smooth muscle membrane and opening L-type Ca^{2+} channels. This also leads to the influx of Ca^{2+} causes vascular smooth muscle contraction (Figure 4).

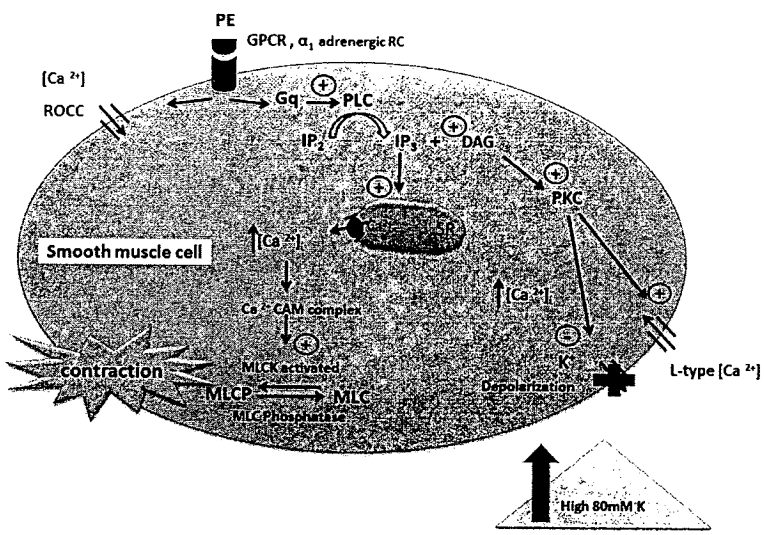

Figure 4 Mechanism of PE and high K^+ solution induced vasoconstriction

Rationale and background

Pulmonary arterial hypertension (PAH) is a rare lung disorder in which the arteries from the heart to the lungs become narrowed, which not only reduces pulmonary blood flow but impacts on systemic cardiac output in general. Mean pulmonary arterial blood pressure is normally ~15 mmHg [1] compared to 70-90 mmHg in the systemic circulation. PAH is classified when pulmonary pressure exceeds 25 mmHg.

Diagnosis and symptoms of PAH

Diagnosis of PAH may be delayed because of the subtlety and non-specificity symptoms. Diagnosis relies on clinical signs and is often found in the late stages of the disease, where the severe symptom such as right heart failure is present. The first of diagnosis is a biography question such as symptom, risk factor and participate disease. The symptoms reported in PAH patients are exertional dyspnea, which is indicative of an inability to increase pulmonary blood flow with exercise [30], fatigue, chest pain, cyanosis, clubbing of fingers, syncope and lower extremity edema [3].

Hemodynamics of pulmonary circulation

Lung function including blood flow in the pulmonary circulation is primarily for oxygen (O_2) exchange. The right ventricle receives hypoxia blood from body via the vena cavae which it pumps into the pulmonary arteries and the pulmonary circulation. Carbon dioxide (CO_2) and O_2 are exchanged across the pulmonary capillary and alveolar walls. The reoxygenated blood flow through the pulmonary veins into the left atrium, then into left ventricle and to the body [31] (Figure 5).

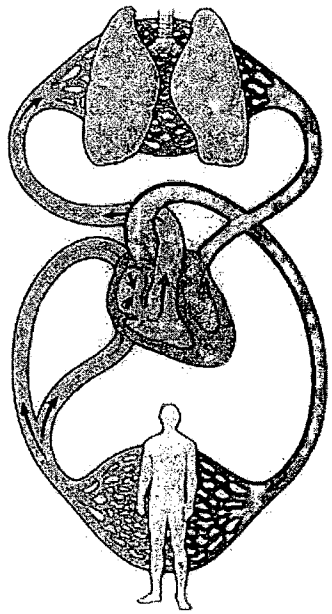

Figure 5 Pulmonary circulation

Source: Modified from www.accindia.org/.../daa724753ef0cb

The 7-fold lower pulmonary arterial pressure compared to the systemic circulation [32] arises from the forces found across the capillary/alveolar walls not seen in other circulations. In most capillaries there is an outward fluid movement (plasma without the protein) due to the hydrostatic pressure of 20-30 mmHg that is in most capillaries. Another force pulls this fluid back into the capillary due to the colloid osmotic pressure of plasma which is roughly equal to the hydrostatic pressure. If capillary pressure exceeds the colloid osmotic pressure, then the net out flux of fluid creates edema. In contrast, the lungs has an additional force due to the surface tension of the air-water interface which pulls the plasma towards the alveolae. Therefore, in order to avoid pulmonary edema, the capillary hydrostatic pressure needs to be correspondingly lower (normally 10 mmHg).

When there is left heart failure, the heart cannot remove the pulmonary venous blood fast enough and as a consequence, an increased pulmonary venous pressure which backs up into the capillary and pulmonary artery resulting in pulmonary hypertension. Besides reducing pulmonary blood flow, there is pulmonary edema due to this increased capillary back pressure. This edema increases

the diffusion distance for respiratory gases across the alveolar wall and the oxygenation and the effective O_2 carrying capacity of the blood is reduced. This poor hemoglobin oxygenation appears as the patient being cyanosed and having the sensation of shortness of breath and other symptoms associated with poor O_2 delivery to tissues [33]. In this condition, the primary disorder is weakening of the right heart failure [34]. Normally, in systemic hypertension, the heart muscle hypertrophies because the right heart is performing an increased workload to maintain the higher pressure (>140/90 mmHg) in systemic circulation [35].

Pulmonary artery hypertension (PAH)

Here, the primary pathology appears to be within the pulmonary artery and its branches which become narrowed. As a result, the right ventricle needs to contract more forcibly to maintain the same blood flow by a combination of the Frank-Starling mechanism and sympathetic stimulation [36]. Blood pressures in pulmonary arteries rise above normal levels (25 mmHg at resting or more than 50 mm Hg with exercise). This persistently raised pressure on right ventricle of the heart, leads within 1-5 years to right heart failure in 30% of patients and death [2]. Nevertheless, an early lung transplant can prevent this, which demonstrates that the primary cause is originates from within the lung arteries themselves rather than by an external influence. The symptoms of PAH include pain in the chest, shortness of breath, fatigue and fainting [3].

The causes for such symptoms are because PAH leads to right ventricle pressure overload, thus reducing pulmonary blood flow and affecting CO_2 and O_2 gas exchange efficacy and levels of arterial blood O_2 decrease. However, pulmonary hypertension describes a symptom and the World Health Organisation have classified it as shown in Table 3.

Table 3 Classification of pulmonary hypertension from WHO classification 2003

1. Group 1-Primary pulmonary arterial hypertension (PAH)
1.1. Idiopathic (iPAH)
1.2. Familial (FPAH)
1.3. Associated with (APAH):
1.3.1. Connective tissue disorder
1.3.2. Congenital systemic-to-pulmonary shunts
1.3.3. Portal hypertension
1.3.4. HIV infection
1.3.5. Drugs and toxins
1.3.6. Other (thyroid disorders, glycogen storage disease, Gaucher's disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, splenectomy, chronic myeloproliferative disorders,)
1.4. Associated with significant venous or capillary involvement
1.4.1. Pulmonary veno-occlusive disease (PVOD)
1.4.2. Pulmonary capillary hemangiomatosis (PCH)
1.5. Persistent pulmonary hypertension of the newborn
2. Group 2 - Pulmonary hypertension associated with left heart disease
2.1. Left-sided atrial or ventricular heart disease
2.2. Left-sided valvular heart disease
3. Group 3 - Pulmonary hypertension associated with lung diseases and/or hypoxemia
3.1. Chronic obstructive pulmonary disease
3.2. Interstitial lung disease
3.3. Sleep disordered breathing
3.4. Alveolar hypoventilation disorders
3.5. Chronic exposure to high altitude
3.6. Developmental abnormalities

Table 3 (cont.)

4. Group 4 - Pulmonary hypertension due to chronic thrombotic and/or embolic disease (CTEPH)
4.1. Thromboembolic obstruction of proximal pulmonary arteries
4.2. Thromboembolic obstruction of distal pulmonary arteries
4.3. Nonthrombotic pulmonary embolism (tumor, parasites, foreign material)
5. Group 5 - Miscellaneous: Sarcoidosis, histiocytosis X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumor, fibrosingmediastinitis)

Source: Modified from Simonneau G, et al. [37]

This has ungone some medication so that the term PAH proper has been reserved for group 1 forms of the disease[38] Various reports have given the incidence as 5 to 52 cases per one million persons [39], higher in women than men by 2 to 1 and tending to affect middle-age. Without treatment, the average survival was around 2.5 years [40].

Cause of PAH

The etiology of the disease is unclear, there but there are several current hypotheses including bone morphogenetic protein (BMP), endothelin-1 (ET-1), arterial wall sclerosis, vascular smooth muscle hypertrophy. In PAH patients, low PGI_2 and high thromboxane A_2 (TXA_2) are found [8]. TXA_2 leads to vascular constriction. ET-1 causes pulmonary artery constriction and increases smooth muscle cell proliferations. Serotonin as a vasoconstrictor may also mediate PAH [7].

Nevertheless, no clear mechanism has emerged which would provide a rational target in the treatment which could provice a cure for PAH. Therefore, we need to consider other processes in the vessel, which might at least provide symptomatic relief from the condition. The severity and continued poor outcomes from the disease [41] continues to stimulate the search for efficient therapeutic strategies including new drug development.

Role of endothelium in controlling vascular contraction

Arteries consists of 3 layers i.e. intima, media and adventitia. The endothelial cell is the main cell of the intimal layer and has ability to respond to signaling such as hypoxia, shear stress and inflammation. It responds by releasing several mediators which result in vascular smooth muscle relaxation and vasodilation. These include the following:

Nitric oxide (NO) which is produced from the amino acid L-arginine by the enzymatic action of endothelial nitric oxide synthase (eNOS) in endothelial cell. It is activated by Ca gaining entry into the endothelial cell via a variety of Ca channels in the membrane facing the vessel lumen. The resultant and highly diffusible NO acts on soluble guanylyl cyclase (GC) within the vascular smooth muscle cytosol and converts guanosine triphosphate (GTP) to cGMP which serves as a second messenger for many important cellular functions, particularly for signaling smooth muscle relaxation via several effectors (figure 6). Other actions of NO are anti-thrombotic effect and anti-inflammatory acting on platelets and endothelial cells and anti-proliferation also acting on smooth muscle. A common problem in vascular disease is reduced NO bioavailability and this is especially so in PAH through a several mechanisms [42].

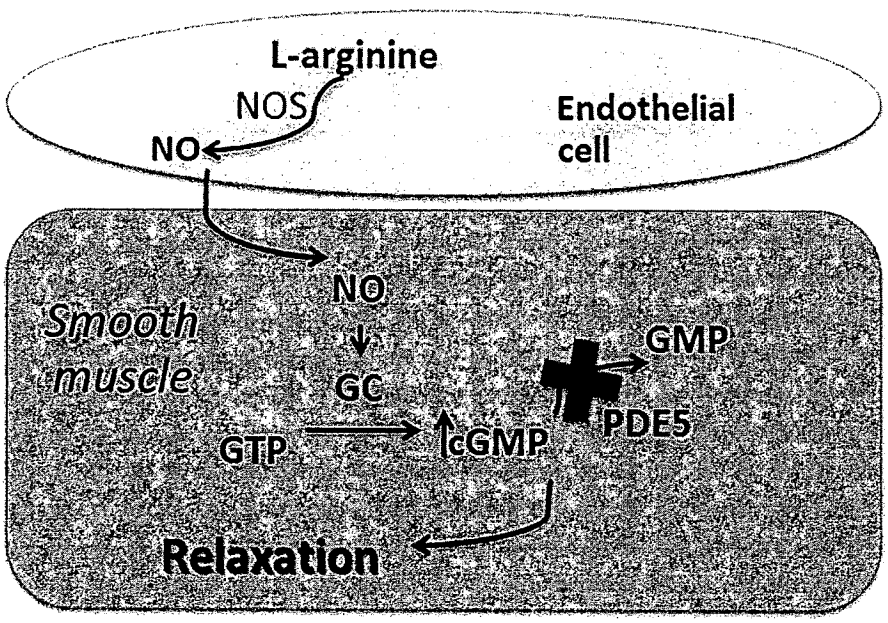

Figure 6 Nitric oxide pathway to increase cGMP for smooth muscle cell relaxation

PGI_2 is synthesized from arachidonic acid via cyclooxygenase-1 in the endothelial cell. It is an inhibitor of platelet aggregation and in the smooth muscle cells via PGI_2 synthase also causing smooth muscle relaxation and thus reduced blood pressure [43].

Carbon monoxide is produced in pulmonary artery vessels via cytochrome P450 and acts by blocking the cytochrome P450 mediated production of the vasoconstrictor, ET-1. It then binds to GC leading to GTP conversion to cGMP and relaxation. The endothelium also contains the sulphydryl amino-acid L-cysteine which is use as the source of hydrogen sulfide (H_2S) [28] which also diffuses out to the smooth muscle layer. H_2S induces pulmonary artery relaxation and lowers blood pressure by opening ATP-sensitive K channels in vascular smooth muscle cells [44]. H_2S releasing from endothelial cell to smooth muscle cell inhibits activity of PDE5 leading to decrease of intracellular Ca^{2+} and activation of K^+ channel.

H_2O_2 (Hydrogen peroxide) is an important damaging reactive oxygen species leading to vasoconstriction [45] but at lower acute concentrations it is also an important physiological relaxant in some vessels including those in the heart [46]. In the physiological context, it is produced by endothelial nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) either directly or from superoxide dismutase [47].

EDHF (Endothelium-Derived Hyperpolarizing Factor): In addition to the above, there is evidence for other hyperpolarizing (hence vasodilator) substances acting on vascular smooth muscle but the chemical nature of this continues to be uncertain and subject to disagreement [48]. In the pulmonary artery EDHF (none NO, H_2S and PGI_2) appears to be important both in the normal and in those from patients who have PAH.

Another apparently important but overlooked influence on vascular smooth muscle is the perivascular adipocytes which include many of those dilator substances which classically come from endothelial cells but also the peptide adiponectin [49]. Furthermore, in obesity these cells become inflammatory and along with more inflammatory immune invading the tissue now produce mediators which are predominantly vasoconstrictor. However, since we remove the fat surrounding the vessels, these influences are removed.

Role of cGMP in contraction/relaxation

cGMP targets in the vascular smooth muscle cell are BK, K_{ATP} and K_V K-channels thereby hyperpolarizing the membrane and preventing the opening of depolarization dependent L-type Ca-channels. Normally, depolarisation through these Ca-channels usually initiates muscle contraction. This is important in pulmonary artery. In addition, cGMP also acts on Rho kinase which cGMP dependent on protein kinase G (PKG) which prevents Rho-mediated inhibition of myosin light-chain (MLC) phosphatase, thus also promoting vasorelaxation.

Because of these multiple actions of the cGMP system, it could provide a useful target to improve pulmonary artery relaxation. That mechanism expresses the Ca^{2+} channel activated and increase intracellular Ca^{2+}.

There are many causes of PAH, some of which still have unclear explanation. Often more than one mechanism is involved in this specific disease process. This can also change as the disease progresses. Hypoxic pulmonary vasoconstriction is the process in which the lung vessels narrow in attempt to divert blood from poorly ventilated and hence hypoxic parts of the lung. This process have a augmented in, for example pneumonia, where the local inflammation contributes the diversion of blood. However, if the whole of the lungs become hypoxic, the entire pulmonary circulation becomes constricted [50].

Vascular remodeling is a consistent feature of PAH patients where the vessel wall becomes fibrotic, thickened, stiffer and smooth muscle hyperplasia through a several several growth factors [42]. As a consequence, there is increased vasoconstriction, inflammation and thrombosis all leading to reduced blood flow and PAH [51]. However, systemic sources of inflammatory cytokines are also strongly associated with PAH [42] further adding to the inflammation endogenous to the vessel [42]. This probably accounts for portal PAH seen in liver failure. When these individuals receive a liver transplant, the PAH disappears which supports this hypothesis [52].

Some drugs can trigger the onset of PAH especially those based on amphetamine which are used to reduce obesity, such as dexfenfluramine. Although such medications are no longer licensed, ilicit cocaine and methamphetamines still pose to threat to the development of PAH .

Thus although PAH is often described as a disease with no known origin, it is more likely there are a series of risk factors all making a varying contributions to the onset of the disease. Likewise, the established disease involves several pathological processes and no one of these is prominent enough to form a target for specific drug treatment.

Current treatments for PAH

Nowadays, there are 3 groups of drugs for the treatment of PAH i.e. 1) inhibitors phosphodiesterase-5 (PDE5) 2) selective pulmonary vasodilators such as PGI_2 analogs 3) ET-1 receptor antagonists [9].

PDE5 Inhibitors

PDEs are a family of enzymes which hydrolyze adenosine 3′, 5′-cyclic monophosphate (cAMP) and cGMP [53]. PDE is classified into various subtypes according to 11 gene families in mammalian cells [54]. PDE5 is found in smooth muscle cell especially in lung and erectile tissue [55, 56]. It is also found in platelets. A high level of PDE5 can decrease cGMP leading to smooth muscle constriction. PDE5 inhibitors can decrease PDE5 thus augmenting the cytosolic cGMP concentration [57]. Such inhibitors have been widely used in PAH to increase the level of cGMP [55, 58]. The selective and competitive PDE5 inhibitors such as sildenafil inhibit cGMP which change to GMP and cause increase cGMP level [59] (Figure 7). Sildenafil was firstly registered by Pfizer as an oral drug for erectile dysfunction under the name "Viagra®". In 2005, it was approved by the US-FDA for the treatment of PAH under the name of Revatio® to avoid association with Viagra® and erectile dysfunction [58]. While erectile disfunction is an occasional acute use, PAH requires long term medical where side effects are more serious. Sildenafil and the other PDE5 inhibitor, vardenafil(Levitra®) have some inhibitory action on PDE6 which is found in retinal photoreceptors and this action causes visual disburbances . However, these are not seen with tadalafil (Cialis®) which has a much smaller effect on PDE6 than on PDE5 [60] and this high-lights the importance of selectivity. Sildenafil consistently improves pulmonary hemodynamics but has little effect on mortality [41] suggesting it does not treat the underlying cause.

The advantage of sildenafil is due to its excellent safety and tolerability profile [61]. It has high efficacy and low adverse effect when compared with vardenafil and tadalafil [62].

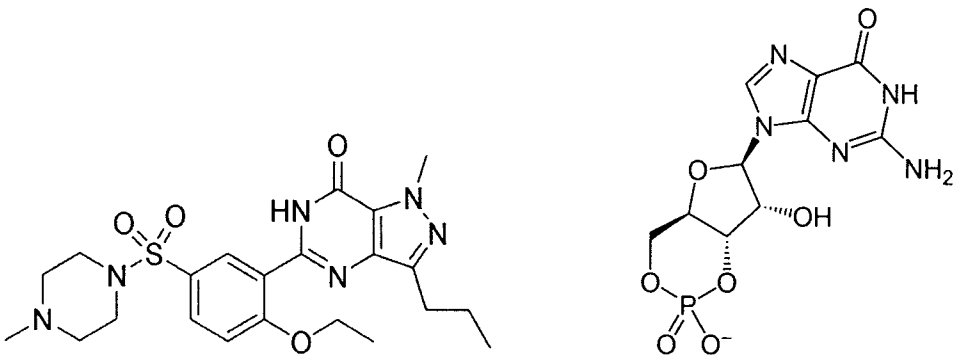

Figure 7 Chemical structure of the PDE5 inhibitor structure of sildenafil and cGMP

PGI_2 Agonists

PGI_2, a member of the endogenous prostanoid family, is produced from arachidonic acid in a multistep process involving the enzymes PGI_2 synthase and cyclooxygenase (COX) [63]. The biological functions of PGI_2 in the pulmonary circulation are mediated by a specific cell-surface GPCR class receptor [64] and PGI_2 is found in platelets and endothelial cells. The binding of PGI_2 to its receptor increases intracellular cAMP, which activates protein kinase A. This causes inhibition of platelet aggregation, relaxation of smooth muscle, and vasodilation of the pulmonary arteries. PGI_2 substantially increases vascular capacity and has direct effect on vascular smooth muscle [65]. The example drug in PGI_2 group is epoprostenol. It was approved by FDA for treatment of PAH. It has very short half-life and must be given intravenously [66]. Epoprostenol has many side effects [67] but could significantly improve exercise capacity, quality of life, exercise capacity, hemodynamics and even survival in PAH [68]. Stable analogues and inhalation forms have received various approvals for symptomatic relief but the long term prognosis shows minimal improvement [42].

ET-1 Antagonists

ET-1 which is produced from a 39–amino acid precursor from big ET-1 through a proteolysis processing, produces potent systemic, renal, and coronary vasoconstriction at pharmacological and pathophysiological concentrations by binding to specific receptors on the vascular smooth muscle [69, 70]. ET-1 is present in normal plasma. It's circulating tissue concentrations are elevated in cardiovascular disease associated with endothelial dysfunction [71]. ET-1 expression in endothelial cells is regulated by complex signals that involve retinoic acid (RA); leptin, prostaglandins, thrombin, TNF-β, IL-1, NO and especially hypoxia,which is a particularly effective potent inducer of ET-1 gene expression in endothelial cells. This mechanism is important during myocardial ischemia [72]. The ET receptor antagonists approved for the use in the PAH patient include bosentan and ambrisentan can significantly improve exercise capacity and hemodynamics in PAH [73];[74]. Bosentan is a nonselective in ET receptor blocker and ambrisentan blocks only ET-A receptors [75].

Other drug classes

Ca-channel blockers: These block the L-type Ca-channel subtype found in vascular smooth muscle and are widely used for systemic hypertension. However, they are effective in <10% of PAH patients.Soluble guanylate cyclase (sGC) stimulator, riociguat showed some promise in PAH and a large number of other potential agents but none show remarkable efficacy [42]. It is clear that current treatments do produce produce an improved pulmonary blood flow and exercise tolerance, but have little effect on long term survival. Because of the complex pathology of PAH, polypharmacy is a rational treatment and such a strategy is routine for hypertension, diabetes and heart disease. Thus using two drugs to treat PAH is meeting with some success.

***Curcuma longa* L.**

Curcuma longa L. is a perennial herb and member of the Zingiberaceae (ginger family), grows to a height of 1-1.5 m. It is commonly found in the tropical regions of Asia and Africa. Common name in English is turmeric. The yellow rhizome is extensively used as a condiment and spice, flavoring agent and colorant for food [76]. The major constituents of *C. longa* are curcuminoids i.e. curcumin,

demethoxycurcumin and bisdemethoxycurcumin [77]. *C. longa* has various pharmacological properties such as anti-inflammatory, anti-oxidant, antiviral and fungal, anti-platelet aggregation and anti-cancer, etc.[78].

Vasorelaxation effect:

A methanolic extract of *C. longa* showed a concentration-dependent relaxant effect on PE or KCl (80mM) pre-contracted mesenteric artery in rings. The relaxation was not observed when the endothelium was removed [79]. In aorta precontracted by prostaglandin ($PGF_{2\alpha}$) the relaxation was prevented by the eNOS blocker N(G)-nitro-l-arginine methyl ester (L-NAME) [80]. Curcumin similarly relaxed goat ruminal arteries only with intact endothelium which also activating sGC [81]. Moreover, curcumin at 10 μM relaxed porcine coronary arteries precontraction with $PGF_{2\alpha}$. via NO and cGMP while the COX blocker, indomethacin, had no effect demonstrating the absence of any prostaglandin mediation [82]. Hamster cheek pouch metarterioles were also relaxed with curcumin (EC_{50}=0.1nM) by a direct beta-adrenoreceptor action on smooth muscle cells and via the endothelium/NO pathway and a direct additional constrictor action via alpha receptor but at higher concentrations (EC_{50}=10nM) [83]. Curcumin reduced Kv potassium currents (Kd=1uM) suggests that some of the contractile effect is through this channel [84]. These results suggest that the therapeutic possibility of curcumin is in idiopathic pulmonary artery.

Anti-oxidant activity:

Curcumin has a comparable anti-oxidant activity to vitamins C and E [85]. A study of ischemia in the feline heart demonstrated that curcumin pretreatment decreased ischemia-induced changes in the heart [86]. An *in vitro* study measuring the effect of curcumin on endothelial heme oxygenase-1, an inducible stress protein, was conducted utilizing bovine aortic endothelial cells. Incubation (18 hours) with curcumin resulted in enhanced cellular resistance to oxidative damage [87].

Anti-microbial activity

Turmeric extract and the essential oil of *C. longa* inhibited the growth of a variety of bacteria, parasites, and pathogenic fungi. A study of chicks infected with the caecal parasite *Eimera maxima* demonstrated that diets supplemented with 1-percent turmeric resulted in a reduction in small intestinal lesion scores and improved weight gain [88]. Another animal study, in which guinea pigs were infected with either

dermatophytes, pathogenic molds, or yeast, found that topically applied turmeric oil inhibited dermatophytes and pathogenic fungi, but neither curcumin nor turmeric oil affected the yeast isolates. Improvements in lesions were observed in the dermatophyte- and fungi-infected guinea pigs, and at seven days post-turmeric application the lesions disappeared [89]. Curcumin has also been found to have moderate activity against *Plasmodium falciparum* and *Leishmania* major organisms [90].

Anti-inflammatory activity [91]:

The volatile oils and curcumin of *C. longa* exhibit potent anti-inflammatory effects [92]. Oral administration of curcumin in instances of acute inflammation was found to be as effective as cortisone or phenylbutazone, and one-half as effective in cases of chronic inflammation [93]. In rats with Freund's adjuvant-induced arthritis, oral administration of *C. longa* significantly reduced inflammatory swelling compared to controls [94]. In monkeys, curcumin inhibited neutrophil aggregation associated with inflammation [95]. *C. longa* anti-inflammatory properties may be attributed to its ability to inhibit both biosynthesis of inflammatory prostaglandins from arachidonic acid, and neutrophil function during inflammatory states. Curcumin may also be applied topically to counteract inflammation and irritation associated with inflammatory skin conditions and allergies, although care must be used to prevent staining of clothing from the yellow pigment [93]. In PAH, there are several pathologies on which curcuminoids may affect especially inflammation is one choice for anti-inflammatory effect by inhibiting the key inflammatory transcription factor, NF-κB (nuclear factor κB) [96]. Thus curcuminoids may provide a multipronged attack on the disease.

Anti-cancer activity:

Curcuminoids have this very wide array of biological actions including cytotocity, at least to cancer cells [82] and thus lack the central requirement of therapeutic drug action specificity. Thus it is difficult to envisage how they could act on a single target without multiple off-target actions. Very low bioavailability, poor water solubility and metabolism are also hurdles to clinical application. One way used by several authors to circumvent specificity has been to take curcuminoids as lead compounds to synthesise more potent analogues. Thus in precontraced isolated rat

basilar arteries a screen of various sulfonyl curcumin mimetics yielded a compound having double the potency of curcumin [97]. For hydrazine- substituents, 30- fold increased potency was achieved for anti-angiogenesis [98]. This strategy has achieved some progress in cancer chemotherapy in creating derived curcuminoids which are selectively taken up by target tumours [99]. carcinogenic [100, 101]; activities test in animal studies involving rats and mice, as well as in vitro studies utilizing human cell lines, have demonstrated curcumin's ability to inhibit carcinogenesis at three stages: tumor promotion, angiogenesis and tumor growth [102]. In two studies of colon and prostate cancer, curcumin inhibited cell proliferation and tumor growth. Turmeric and curcumin are also capable of suppressing the activity of several common mutagens and carcinogens in a variety of cell types in both *in vitro* and *in vivo* studies [103]. The anticarcinogenic effects of turmeric and curcumin are due to direct antioxidant and free-radical scavenging effects, as well as their ability to indirectly increase glutathione levels, thereby aiding in hepatic detoxification of mutagens and carcinogens, and inhibiting nitrosamine formation [104]. Additionally, it variety effect sush as hepato- and nephro-protective [105] thrombosis supressing, myocardial infarction protective [106].

Cardiovascular effects:

Turmeric's protective effects on the cardiovascular system include lowering cholesterol and triglyceride levels, decreasing susceptibility of low density lipoprotein (LDL) to lipid peroxidation and inhibiting platelet aggregation [107]. These effects have been noted even with low doses of turmeric. A study of 18 atherosclerotic rabbits given low-dose (1.6-3.2 mg/kg body weight daily) turmeric extract demonstrated decreased susceptibility of LDL to lipid peroxidation, in addition to lower plasma cholesterol and triglyceride levels. The higher dose did not decrease lipid peroxidation of LDL, but cholesterol and triglyceride level decreases were noted, although to a lesser degree than with the lower dose [108]. Turmeric extract's effect on cholesterol levels may be due to decrease of cholesterol uptake in the intestines and increased conversion of cholesterol to bile acids in the liver. Inhibition of platelet aggregation by *C. longa* constituents is thought to be via potentiation of prostacyclin synthesis and inhibition of thromboxane synthesis.

Tissue distribution: Uptake and distribution of curcumin in body tissues is obviously important for its biological activity, yet only a limited number of studies have addressed this issue. Ravindranath et al. report showed that after oral administration of 400 mg of curcumin to rats only traces of unchanged drug were found in the liver and kidney. At 30 min, 90% of curcumin was found in the stomach and small intestine, but only 1% was present at 24 h [109]. In an *in vitro* study, when everted sacs of rat intestine were incubated with 50–750 μg of curcumin in 10 mL of incubation medium 30–80% of the curcumin disappeared from the mucosal side and no curcumin was found in the serosal fluid. Less than 3% of the curcumin was found in the tissues at the highest curcumin concentration [110]. Another study evaluated the tissue distribution of curcumin using tritium-labeled drug. They found that radioactivity was detectable in blood, liver, and kidney following doses of 400, 80, or 10 mg of [3H] curcumin. With 400 mg, considerable amounts of radio labeled products were present in tissues 12 days after dosing. The percentage of curcumin absorbed (60–66% of the given dose) remained constant regardless of the dose indicating that administration of more curcumin does not result in higher absorption. That is, in rats there is a dose-dependent limitation to bioavailability.

The main curcuminoids isolated from *C. longa* are curcumin (1), demethoxycurcumin (2) and bisdemethoxycurcumin (3) [77]. Recently, Suksamran and co-workers have synthesized various curcumin analogs (compound 4-9). Some of these compounds showed activity on PDE5 inhibition (Temkitthaworn, et al., unpublished data). In this study, we chose a series of curcumin analogs that could inhibit PDE5 (Table 2) for testing on the rat isolated pulmonary artery. The profiles of PDE5 inhibitory activity will be compared with the effects on pulmonary artery relaxation.