Review Article

Physiopathology of Pulmonary Hypertension: from Bio-Molecular Mechanism to Target Treatment

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Abstract

Although the diagnosis and treatment of pulmonary hypertension (PH) is developed constantly in the last decade, PH remains an incurable and difficult to treat disease due to its high life disability and dreadful survival rate. The disease is characterized by sustained vasoconstriction, progressive vascular remodeling, and irreversible right heart dysfunction.

The advanced knowledge in physiopathology and classification of PH in recent years is a useful tool helping physicians to improve the choice of target treatment. In addition, the remarkable progresses in understanding the molecular and cellular mechanisms of PH allow to develop new treatments. Actually, new therapeutic molecules have been discovered and their mechanisms of action are better understood and some are in preclinical and clinical trials. Preliminary results of these molecules with benefit effects on pulmonary arterial pressure and systemic hemodynamic gives a new hope for the future.

Keywords: Pulmonary hypertension; Signaling pathway; Physiopathology

Introduction

The diagnosis and treatment of pulmonary hypertension (PH) have many advances in recent years, which permit to improve the survival of patients with PH [1]. The knowledge of physiopathology of PH is also improved in the last decade with the identification of predisposing genetic factors and molecular mechanisms involved in inflammation, vasoconstriction, and cell proliferation. However, PH remains a severe, complex, and difficult to treat disease. The recent publication of the new international recommendations of PH provide the clarification in its definition, classification and treatment [1,2]. Actually, new therapeutic molecules have been discovered and their mechanisms of action are better understood and some are in preclinical and clinical trials. Preliminary and promising results of these molecules give a new hope for the future.

Overview of Clinical Classification and Pathology of Pulmonary Hypertension

Clinical classification of PH

The new classification of PH is presented in Table 1. Group 1 (PAH) is including idiopathic PAH, heritable PAH (germline mutations of BMPR2, ALK1 or endoglin genes), drugs and toxins induced PAH, PAH associated with other conditions (connective tissue diseases, HIV infection, portal hypertension, congenital heart disease, schistosomiasis. Group1′: PH due to pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary hemangiomatosis (Table 1). Group1″: Persistent pulmonary hypertension of the newborn (PPHN). These conditions have been classified as a distinct category but not completely separated from PAH, and designated as Group 1′ and 1″.

Group 2 includes PH related to left heart disease, including systolic dysfunction, diastolic dysfunction, and valvular disorders. Group 3 is including PH related to lung disease and/or hypoxia, including chronic obstructive pulmonary disease, interstitial lung disease, other pulmonary disease with mixed restrictive and obstructive patterns, sleep-disordered breathing, alveolar hypoventilation disorders, chronic exposure to high altitude, and developmental abnormalities (Table 1).

Group 4 is involved to PH due to chronic thromboembolic disease (CTEPH: chronic thromboembolic pulmonary hypertension) without precise criterion to distinguish between proximal and distal forms and other pulmonary artery obstructions (Table 1). Group 5 includes PH with unclear and/or multifactorial mechanisms, including heterogeneous conditions with different pathological features such as hematological disorders (myeloproliferative disorders, splenectomy), systemic disorders (sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangioleiomyomatosis, neurofibromatosis, vasculitis), metabolic disorders (glycogen storage disease, Gaucher disease, thyroid disorders), and others (tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis).

Pathology of pulmonary hypertension

The pathological changes in Group 1 (PAH) predominantly affect the distal pulmonary arteries with medial hypertrophy, intimal proliferative and fibrotic changes, adventitial thickening with mild to moderate perivascular inflammatory infiltrates and complex lesions (plexiform lesions; Figure 1). For Group 1′ (PVOD), the pathological features are involving septal veins and pre-septal venules with occlusive fibrotic lesions, venous muscularization, pulmonary oedema, occult alveolar haemorrhage, lymphatic dilatation, lymph node enlargement and inflammatory infiltrates. In this group, the distal pulmonary arteries are affected by medial hypertrophy and intimal fibrosis (Figure 1).

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1. Pulmonary arterial hypertension (PAH)
1.1 Idiopathic
1.2 Heritable
1.2.1 BMPR2 mutation
1.2.2 Other mutations
1.3 Drugs and toxins induced
1.4 Associated with:
1.4.1 Connective tissue disease
1.4.2 HIV infection
1.4.3 Portal hypertension
1.4.4 Congenital heart disease
1.4.5 Schistosomiasis
1'. Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis
1". Persistent pulmonary hypertension of the newborn
2. Pulmonary hypertension due to left heart disease
2.1 Left ventricular systolic dysfunction
2.2 Left ventricular diastolic dysfunction
2.3 Valvular disease obstruction and congenital cardiomyopathies
2.4 Congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
2.5 Other
3. Pulmonary hypertension due to lung diseases and/or hypoxia
3.1 Chronic onstructive pulmonary disease
3.2 Interstitial lung disease
3.3 Other Pulmonary diseases with mixed restrictive and obstructive pattern
3.4 Sleep-disordered breathing
3.5 Alveolar hypoventilation disorders
3.6 Chronic exposure to high altitude
3.7 developmental lung diseases
4. Chronic thromboembolic pulmonary hypertension
4.1 chronic throboembolic pulmonary hypertension
4.2 Other pulmonary artery onstructions
5. Pulmonary hypertension with unclear and/or multifactorial mechanisms
5.1 Haematological disorders
5.2 systemic disorders
5.3 metabolic disorders
5.4 Others

Table 1: Clinical classification of Pulmonary hypertension.

The pathology in Group 1″ (PPHN) is characterized by changes in vasoreactivity and wall structure and decreases in pulmonary vascular density with reduced alveolarisation.

In Group 2 (LHD), the pathology features are characterized by enlarged and thickened pulmonary veins, pulmonary capillary dilatation, interstitial oedema, alveolar haemorrhage and lymphatic vessel and lymph node enlargement. Distal PA may be affected by medial hypertrophy and intimal fibrosis. However, in Group 3, the pathological characteristics are involving medial hypertrophy, intimal obstructive proliferation of the distal pulmonary artery (PA) and muscularization of arterioles. In addition, a variable degree of destruction of the vascular bed in emphysematous or fibrotic areas may also be present.

The pathology in Group 4 (CTEPH) are characterized by organized thrombi tightly attached to the medial layer in the elastic pulmonary arteries, replacing the normal intima (Figure 1). A pulmonary microvascular disease can develop in the non-occluded and occluded areas that has similarities with PAH and patchy post-capillary remodelling related to bronchial-to-pulmonary venous shunting [1-3]. For Group 5 (PH with heterogeneous conditions), the pathology patterns are varied and depended on the etiologies.

Hemodynamic Mechanism of Physiopathology of Pulmonary Hypertension

Characteristics of pulmonary circulation

Pulmonary circulation constitutes a vascular system with low pressure, low resistance, and high distensibility. It is located between two ventricles and intrathoracic. Increase of cardiac output induces a recruitment of large number of non-perfused vessels at the basic state. However, due to the low pressure which reigns, in the normal physiological condition, in pulmonary arteries with the thin walls are directly affixed in pulmonary parenchyma, pulmonary hemodynamics might easily be modified by the slightest change in intravascular pressure.

Unlike other circulations, pulmonary circulation does not have an efficacy auto-regulatory mechanism. Therefore, it is important to know the perivascular pressure to assess the active or passive modification of the vascular diameter. In the pulmonary circulation, only the small muscular pulmonary arteries and the arterioles possess the vasoactive properties. They are the resistant vessels or precapillary vessels. The other perivascular contractile elements also take part in the active change of pulmonary vascular resistance (PVR), but their role is negligible. In human, pulmonary circulation is the only circulation being capable of
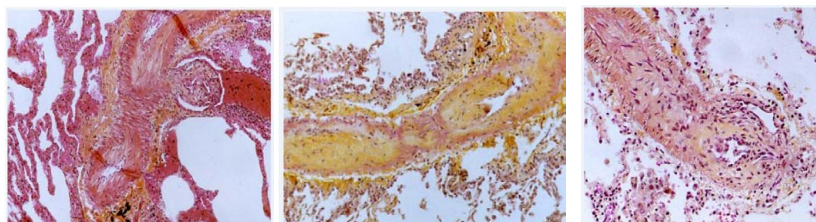
Figure 1: Pathological characteristics of pulmonary hypertension. Left side: Plexiform lesion. In the middle: pulmonary veno-occlusive disease (PVOD). Right side: chronic thromboembolic pulmonary hypertension (CTEPH) [133].

reacting by vasoconstriction with hypoxia characterized by a decline of PaO_2 less than 60 mmHg [4]. Hypoxic vasoconstriction allows to adjust pulmonary capillary perfusion to alveolar ventilation by ensuring a ventilation/perfusion ratio closer to the optimal value [5].

Finally, pulmonary circulation has its particularity by possessing both a function of alveolar capillary gas exchange and a hemodynamic function. The hemodynamic function of pulmonary circulation is related to its role in the regulation of systemic blood pressure via the synthesis of angiotensin II and endothelin by numerous pulmonary endothelial cells.

Hemodynamic mechanism of pulmonary hypertension

PH includes several diseases causing the hemodynamic disorders of pulmonary circulation determined mainly by the increases of mean pulmonary arterial pressure (PAPm) and pulmonary vascular resistance (PVR) at rest. In healthy subjects, PAP varies between 20 mmHg in systole and 5 mmHg in diastole and the PAPm is 10 to 12 mmHg (14 ± 3 mmHg) [6]. PH is defined by PAPm higher or equal to 25 mmHg, measured during a right heart catheterization (RHC). According to the latest recommendation, the different forms of PH are classified in five groups and two subgroup, depending on the clinical and therapeutic characteristics of causal factors [1].

In PH, the increase of PAPm is due to the modification of one of the three determinants of pulmonary circulation, linked by the equation: PAPm=[(PVR × PBFm) + LAP] (where PVR is the pulmonary vascular resistance, PBFm is the mean pulmonary blood flow, and LAP is the left atrial pressure). The LAP is obtained by measuring pulmonary arterial occlusion pressure (PAOP). PH related to the increase of PVR is frequent. The increase of RVP is due to either the vasoconstriction, or the rigidity of vascular walls, or the obstruction of the vascular lumen (pre-capillary PH).

PH associated with increased resistance by hypoxic vasoconstriction: The mechanism of acute pulmonary vasoconstriction inducing by acute alveolar hypoxia is known [7]. The hypoxic pulmonary vasoconstriction assures adequately pulmonary capillary perfusion to alveolar ventilation, thus optimizing gas exchanges. The main cause of hypoxic pulmonary vasoconstriction is the drop of the partial pressure of oxygen in the alveolar gas. When alveolar hypoxia becomes chronic, pulmonary vasoconstriction is more disturbance. It is due to the production of vasoactive mediators from endothelial cells [8]. The chronic hypoxic pulmonary vasoconstriction has been classified in group 3 of PH in the last international guidelines, including COPD, living in high altitude, and respiratory disorders during sleep [1].

PH related to decreased surface of the pulmonary vascular bed: It is usually due to the parenchyma pulmonary fibrosis such as idiopathic pulmonary fibrosis and systemic scleroderma, or to proximal or distal

pulmonary arterial obstruction post-thromboembolic [9]. The cross-sectional area of the arteries could be used to calculate the compliance of the pulmonary vascular bed according to the equation: $\beta=[\ln (Ps/Pd)]/(2 \triangle A/A)$ (where β expresses the vascular rigidity; Ps/Pd ratio is the ratio of systolic pressure to diastolic pressure; $\triangle A/A$ is the variation of the cross-sectional area) [10]. This parameter is correlated with the mortality rate of patients with PH [11].

PH associated with increased pulmonary blood flow: PH due to increased pulmonary blood flow or hyperkinetic PH (Group 1 according to the new classification) [1], is related to an abnormal increase of pulmonary blood flow via the shunt between systemic and pulmonary circulations (left - right extra- or intracardiac). This category of PH includes inter-atrial or -ventricular septal defects, ductus arteriosus, or the atrio-ventricular canal defect. Increased pulmonary blood flow generates the shear forces and circumferential stretching on the vascular walls which stress the intima layer with endothelial cells [12] and promote the increase of the PVR and vascular remodeling (hypertrophy). Due to the remodeling lesions in the pulmonary vascular bed, the PVR is gradually increased during the disease progression, with the possibility of inversion of the shunt at advanced stage (Eisenmenger syndrome).

Although the predominant lesions at the veinular level and pulmonary capillary, veno-occlusive disease (VOD) and pulmonary capillary hemangiomatosis (PCH) have the hemodynamic characteristics identical to those of the pre-capillary PH [9]. They have been integrated into the Group 1' (Table 1).

PH associated with increased post-capillary pressure: Another mechanism responsible for increasing PAPm is the elevation of post-capillary pressure (passive PH). It results from an increase of pulmonary capillary pressure, consequential a rise in the pulmonary venous pressure secondary to left heart failure. Ischemic heart disease, mitral valve stenosis, and myxoma of the left atrium (Group 2) may be the cause [1]. In this group, the gradient between trans-pulmonary pressure (TPp) and the PVR is normal: TPp=PAPm - PAOPm. But when PAP becomes more important than PAOP (with high TPp), PVR is increased and leaded to post-capillary reactive or non-proportional PH. This is due to the increase of the vascular tone of arteries and/or the remodeling of resistant vessels possibly by vasoconstrictor reflex of the pulmonary arteries. It is mediated by baroreceptors of left atrium and pulmonary veins and by endothelial cell dysfunction [13].

The Molecular Mechanisms of Pulmonary Hypertension

Progress in understanding the molecular and cellular mechanisms of PH (Figure 2) allows to develop the new treatments [14].

Role of endothelial dysfunction in pulmonary hypertension

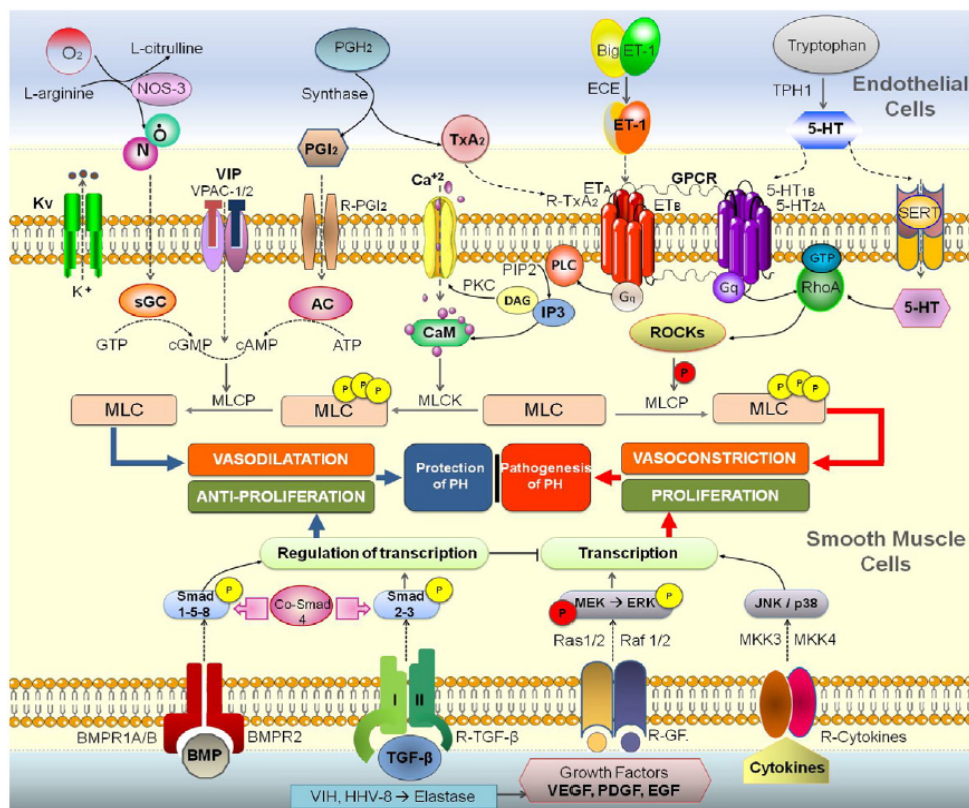The endothelial cells play a key role in modulating vascular tone and

Figure 2: Potential mechanisms involved in the development of PH. Top half: Relaxation of smooth muscle cells induced by NO, prostacyclin, and VIP is mediated by cGMP and cAMP whereas constriction induced by TXA2, ET-1 and 5-HT is mediated by membrane receptors coupled to G proteins and intracellular calcium modifiers such as IP3 (inositol triphosphate) or mediators of RhoA/Rho-kinase pathways. Bottom half: Proliferation of smooth muscle cells is induced by cytokines, growth factors and TGF-β. Control of transcription is regulated by the BMP proteins and its membrane receptors via Smad signaling effectors, and MAP kinase pathways. Abbreviations: 5-HT1B/2A, serotonin receptors 1B or 2A; BMPR2, bone morphogenetic protein receptor 2; ETA/B, endothelin receptors A or B; GF, growth factors; GPCR, G protein-coupled receptors; HHV-8, human herpesvirus 8; JNK, c-Jun N-terminal kinase; Kv, potassium voltage-gated channels; P, phosphorylation; PGH2, prostaglandin H2; PGI2, prostaglandine I2 (prostacyclin); PGI2-R, prostacyclin receptors; R-TXA2, thromboxane receptors; VPAC-1/2, vasoactive intestinal peptide receptors 1 or 2; TGF-β, transforming growth factor-β; TPH1, tryptophan hydroxylase 1; VIP, vasoactive intestinal peptide [131].

endothelial dysfunction is present in all forms of PH [8]. Endothelial dysfunction induces an imbalance between vasoconstrictors (thromboxane A_2 [TxA_2], endothelin-1 [ET-1] and serotonin [5-HT]) and vasodilators (nitric oxide [NO] and prostacyclin [PGI2]). These mediators have direct effects on smooth muscle cells (SMCs) of pulmonary vessels by stimulating (or inhibiting) contraction, migration, and cell proliferation. They also have the ability to alter vascular remodeling by interaction with fibroblasts, coagulation factors and/or inflammatory process.

Nitric oxide (NO) signaling pathway

In endothelium, NO is synthesized by the conversion of L-arginine to L-citrulline via endothelial NO synthase (eNOS or NOS-3) in the presence of its cofactors (NADPH, BH4) [15]. NO activates soluble guanylate cyclase (sGC) which catalyzes the formation of cyclic guanosine monophosphate (cGMP) and the activation of protein kinase G (PKG) by cGMP. The NO/cGMP/PKG signaling cascade causes vasodilation by decreasing the calcium concentration inside of the SMCs (Figure 2). NO also has an anti-proliferative effect on SMCs and antiplatelet agents [16]. However, the production of NO by endothelial cells depends on several factors such as the activity of NOS-3 and that of arginase which constitutes the availability of L-arginine, vascular blood flow, and the resulting shear forces.

In patients with PH, the expression of NOS-3 in the pulmonary arteries is critically diminished [17]. Especially, the expression of NOS-3 is inversely correlated with PVR and the severity of vascular plexiform lesion [18]. Indeed, in idiopathic and familial PH (according to the previous classification), the production of NO, measured after the injection of radioactive L-arginine, was significantly reduced in comparison with healthy subjects [19]. The other mechanisms involved in the decrease of the production of NO are the reduction of L-arginine synthesis by decreasing the activity of dimethyl-aminhydroline dimethyl-aminohydrolase (DDAH) [20] and the negative regulation of NOS-3 by RhoA/Rho-kinases [21,22].

There are several mechanisms which control and modify the intracellular concentration of cGMP. The phosphodiesterase of type 5 (PDE-5), which hydrolyzes cGMP into inactive 5'-GMP, is a target molecular of choice in the treatment of PH. The efficacy of specific PDE-5 inhibitors, such as sildenafil or tadalafil in the treatment of PH is clearly known (Figure 3) [23,24]. In addition to PDE-5 inhibitors, the role of GCs stimulators, cGMP producing enzyme, in patients with PH is currently in clinical use [25].

Prostacyclin (PGI_2) and thromboxane A_2 (TxA_2) signaling pathway

PGI_2, produced by endothelium, is an endogenous vasodilator

which acts on the synthesis of cyclic adenosine monophosphate (cAMP). As the same with NO, PGI_2 inhibits the proliferation and platelet aggregation (Figure 2) [26]. In PH, the activity of PGI_2 synthase and production of PGI_2 are significantly decreased in plexiform lesions (Figure 1) [27]. In transgenic mice, the excessive expression of PGI_2 had a protective effect for hypoxia-induced PH [28]. Inversely, mice with a deficit of PGI_2 receptors had a very severe PH [29]. Developed for more than 20 years in the treatment of PH, the analogues of PGI_2 are currently used for patients with severe form of PH (Figure 3). They are indicated as first-line or in combination with other drugs for patients with clinical deterioration [30].

TxA_2, a potent platelet aggregator also produced by endothelial cells, is a vasoconstrictor of the cyclooxygenase pathway. In patients with idiopathic PH, there is a decrease of PGI_2 metabolic products in urine, associated with an increase in metabolic products of TxA_2 [31]. In addition, the expression of TxA_2 receptors is significantly elevated in patients with PH [32].

In summary, in PH, the imbalanced modulation of vascular tone caused by deficiency of PGI_2 production and increase of TXA_2 activity induces the vasoconstriction and pulmonary arterial remodeling.

Endothelin-1 signaling pathway

The endothelial cells also synthesize endothelin with its three isoforms (ET-1, endothelin-2 [ET-2] and endothelin-3 [ET-3]) are all vasoconstrictors. The main differences between these three isoforms are due to their tissue expression and affinity for both membrane endothelin receptors: ET_A receptors and ET_B receptors [33]. The binding of ET-1 to the receptors ET_A and ET_B activates G protein coupled to phospholipase C, resulting an increase of intracellular calcium concentration, responsible for the contraction of the vascular smooth muscles (Figure 2) [34]. Activating ET_B receptors in endothelial cells stimulates the vasodilation via NO and PGI_2 production (Figure 3). ET-1 is a potent vasoconstrictor possessing the mitogenic effect, pro-platelet aggregation, pro-fibrosis, and pro-inflammatory effect [35].

ET-1 has a major role in the pathogenesis of PH. In patients with idiopathic or familial PH and Eisenmenger syndrome-related PH, the plasmatic concentration of ET-1 is very high and correlated with PVR and patients survival [36-38]. Particularly, the ET-1 expression in endothelial cells is increased only in the pulmonary arteries with hypertrophy of the media or fibrosis of the intima [39]. In the treatment of PH, receptor antagonists of ET-1 (bosentan, ambrisentan) constitute an important therapeutic class (Figure 3). They are prescribed as the first choice for patients with functional stage II of the WHO classification [1,2].

Serotonin signaling pathway

Serotonin (5-hydroxytryptamine [5-HT]) is a neuronal vasoconstriction mediator, promoting SMC proliferation and the formation of local micro-embolisms [40]. 5-HT is synthesized by catalysis of tryptophan to the enzyme tryptophan hyroxylase-1 (TPH1). After its synthesis, 5-HT is captured by SMCs by a specific membrane transporter (SERT). 5-HT may also be linked to 5-HT_{1B} and 5-HT_{2A} membrane receptors. In SMC, 5-HT activates the mitogen-activated protein kinase (MAPK) and Rho-kinases, thus causing vasoconstriction (Figure 2). 5-HT also has a proliferative effect on SMC by activating nucleotide transcription factors (GABA4) via the pERK1/2 proteins and by the formation of reactive oxygen species (ROS) dependent on 5-HT [41].

The increase of serotonergic activity in patients with PH is related to the hyperactivity of SERT and/or TPH1 [42,43]. SERT is encoded by a single gene, localized on chromosome 17q11.2, its polymorphism is associated with an increase in expression and activity of SERT [42]. In mice, the excessive expression of SERT aggravates PH induced by hypoxia and/or consecutive to monocrotalin intoxication (MCT) [44,45]. Inhibition of SERT activity reduces PH induced by MCT in rats [46,47]. In humans, anorectic agents (Aminorex and Fenfluramine) are responsible for the development of PH by acting as substrates of SERT and by inducing the recapture of 5-HT by SMC of pulmonary vessels [48].
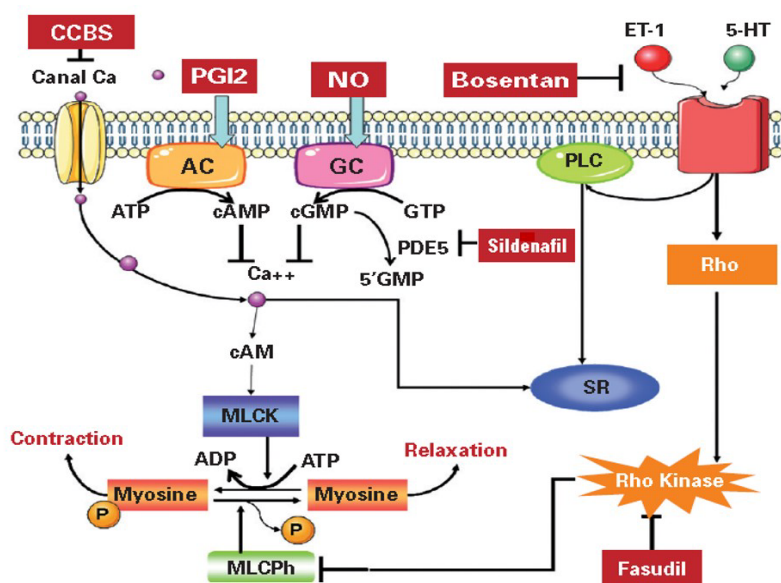

Figure 3: Mechanism of vasoconstriction and target treatment with vasodilators in pulmonary hypertension. CCBS: calcium channel blockers; PGI_2: prostaglandine I_2 (prostacycline); NO: Nitric oxide; PDE5: type 5 phosphodiesterase; ET-1: endotheline-1; 5-HT: serotonine [134].

There is a hyper-expression of TPH1 gene in pulmonary tissue and pulmonary endothelial cells in idiopathic PH [49]. Mice with invalidated coding gene for TPH1 (TPH1-/-) were saved and did not get PH when exposed to hypoxia and/or anorectics (Dexfenfluramine) [50,51].

The other mechanism involved in PH related to 5-HT is the increase in plasma concentration of 5-HT, abnormal storage of 5-HT in platelets [52], or excessive production of 5-HT by endothelial cells [49]. This phenomenon has been found in patients with idiopathic or familial HTP. The role of $5-HT_{1B}$ and $5-HT_{2A-B}$ receptors of 5-HT was also investigated in PH [53-55].

Vasoactive intestinal peptide (VIP) signaling pathway

Known as a neuroendocrine mediator with systemic vasoactive properties, VIP has a significant role in PH. Its biological effect is mediated by its specific membrane receptors that are coupled to G-proteins (VPAC1 and VPAC2) and localized in the different tissues. The stimulation of VPAC receptors causes vasodilatation mediated by the classic signaling cascade: stimulation of adenylate cyclase by the coupled sG protein VPAC (AC) receptors; synthesis of cAMP by adenylate cyclase; activation of protein kinase A by cAMP (Figure 2).

However, it has been suggested that the vasodilation effect of VIP might partly be mediated through NO/cGMP/PKG signaling pathway [56]. The decrease in pulmonary tissue expression and serum concentration of VIP was found in patients with PH [57]. The VIP also has an inhibiting effect on SMC proliferation of pulmonary arteries in patients with idiopathic PH [58]. Moreover, the alteration of gene encoding VIP is also found in idiopathic HTP [59]. The deletion of this gene promotes the development of PH in a murine model, resulting in diffuse pulmonary vascular remodeling, right heart hypertrophy and pulmonary inflammation [57,58].

VIP is a neurotransmitter of the autonomic non-adrenergic non-cholinergic nervous system having a powerful pulmonary vasodilator effect [60]. However, many undesirable effects observed during intravenous administration of VIP constitute a limit for its therapeutic use in PH. In humans, a recent study [61] showed a significant decrease of PAPm and PVR in patients with PH treated by inhaled VIP. Inhalation of VIP does not cause of systemic hypotension, while improving significantly hypoxemia of patients with PH associated with COPD and interstitial lung disease. However, the role of VIP in pulmonary vascular remodeling and its long-term therapeutic benefit are not yet clarified.

Voltage-gated potassium (Kv) channel pathway

Kv channels play an important role in the regulation of membrane potential of SMC and in hypoxic pulmonary vasoconstriction [62]. Decreased expression or dysfunction of Kv channels induces membrane depolarization promoting the calcium influx through of calcium channels dependent on Kv and an increase of intracytosolic Ca_2+ concentration and vasoconstriction.

Kv channel dysfunction in SMC of subjects with idiopathic and familial PH was highlighted (Figure 2) [63]. Hypoxemia and fenfluramine derivatives inhibit the Kv channels [64]. The gene transfection of Kv 1.5 channels attenuates the development of PH and restores the hypoxic vasoconstriction response in PH induced by chronic hypoxia in rats [65]. Previous study has shown that direct inhibition of Kv channels in SMCs causes pulmonary arterial vasoconstriction [64]. The link between receptor mutation (BMPR2) and reduction of Kv channel expression in vasoconstriction of pulmonary arteries has

also been demonstrated [66]. However, the role of this link in vascular remodeling has not yet been established.

RhoA-GTPase/Rho-kinase signaling pathway

Recently, the role of the RhoA-GTPase/Rho-kinase pathway in PH has been demonstrated (Figure 2) [67,68]. The main effectors of the RhoA-GTPase are Rho-kinases or ROCK (ROCK-1 or ROCK-β and ROCK-2 or ROCK-α). The ROCK regulate several functions of SMC: contraction, proliferation and apoptosis [69]. In addition, the role of RhoA-GTPase signaling system and its effectors, Rho-kinases (ROCK-1 and ROCK-2), in endothelial dysfunction has been recently demonstrated (Figure 4) [70].

Many experimental elements report in favor of the involvement of the RhoA-GTPase/Rho-kinase pathway in vasoconstriction and pulmonary vascular remodeling. This signaling pathway has been studied in chronic hypoxia and MCT-induced PH [68,69]. In addition, Rho GTPase/Rho-kinase signaling pathway is also mediated by ET-1 and 5-HT [71,72]. The increase of the activity of the RhoA/Rho-kinase mediated by ET-1 in hypoxic condition has been demonstrated in animal models [73]. The role of 5-HT in constitutive activation of RhoA-GTPase/Rho-kinase in PH was demonstrated [74]. Recently, the interaction between 5-HT, mutation of the bone morphogenesis proteins receptor (BMPR2), and RhoA/Rho-kinase signaling pathway in PH was also established [56,57].

In vivo, the beneficial effect of Rho-kinase inhibitors in the treatment of PH has been confirmed by several studies. Rho-kinase inhibitors reduce PAP, right ventricular hypertrophy and pulmonary vascular remodeling in experimental models [75,76]. Preliminary results showed that fasudil, a selective inhibitor of Rho-kinases, administered intravenously or by nebulization reduced significantly PAPm and PVR in patients with PH (Figure 3) [77-80]. However, the effect of fasudil in long-term treatment of patients with PH is still under evaluation.

The Role of Genetic Factors in Pulmonary Hypertension

Mutation of the bone morphogenetic protein receptor type 2 (BMPR2) in PH

Role of the BMPR2: More than 20 BMP molecules have been identified at now [81]. BMPs are secreted in the form of homodimers or heterodimers and bind to heterodimeric receptors having serine/threonine activity kinase. A distinction is made between type 1 receptors (BMPR1) and type 2 (BMPR2). Like all other ligands belonging to the transforming growth superfamily factor-β (TGF$_β$), BMPs induce the binding of type 1 receptor to type 2 receptor, causing the phosphorylation of the first one (BMPR1). Activation of the BMPR1 then induces the phosphorylation of the cytoplasmic proteins involved in the signaling pathway of the TGF superfamily, known as the TGF Smads [82,83]. The attenuation of BMPR2 receptor expression increases the signal strength of TGF-β and stimulates cell proliferation.

Mutation of BMPR2 in PH: The best characterized genetic defects in heritable pulmonary arterial hypertension are mutations of the gene encoding bone morphogenetic protein receptor type 2 (BMPR2), a member of the transforming growth factor-β signaling family (Figure 2). BMPR2 modulates the growth of vascular cells by activating the intracellular pathway of Smad and LIM kinase [84]. The germline mutations in BMPR2 gene are detected in at least 70% of cases of heritable pulmonary arterial hypertension [85,86]. BMPR2 gene mutations are also detected in 11%–40% of apparently sporadic cases, thus representing the major genetic predisposing factor for PAH
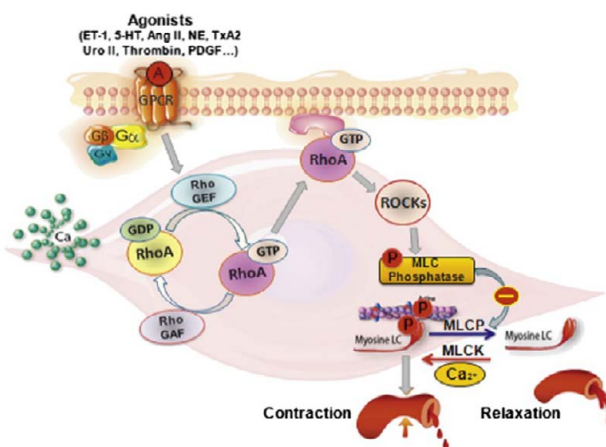
Figure 4: Ca²⁺ sensitization of VSMC mediated by RhoA/Rho-kinase signaling pathway. In basal state of VSMCs, there is a balance between vasoconstriction and vasodilatation that is controlled by the activity of MLCK and MLCP. When Rho-kinase agonists are coupled on GPCR, it converts GDP-RhoA (inactive form) to GTP-RhoA (active form). GTP-RhoA translocates into the membrane and activates its downstream effectors: Rho-kinase or ROCKs. ROCKs increase the vasoconstriction by inhibiting the activity of MLCP via the phosphorylation of this enzyme [131].
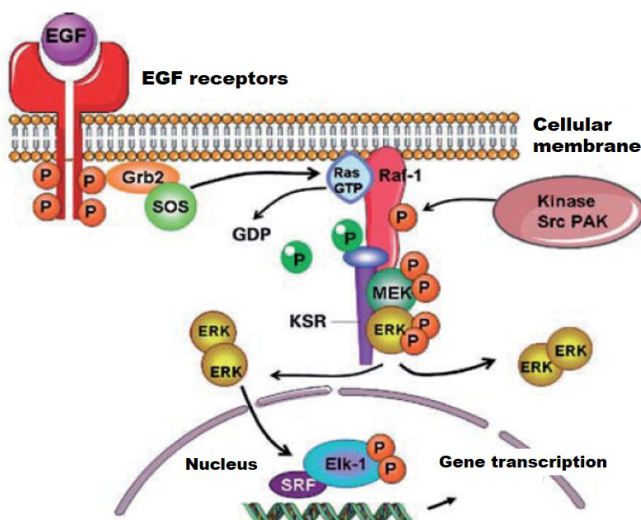

Figure 5: Mechanism of action for Raf-Ras-MEK/ERK. EGF: epidermal growth factor; ERK: extracellular signal regulated kinase [135].

[87]. More than 45 different mutations of BMPR2 gene have been identified in patients with heritable PAH [88,89]. Functional studies have shown that point mutations and truncations in the kinase domain exert dominant negative effects on receptor function [90], resulting in incomplete penetrance and genetic anticipation.

Mutations of activin receptor-like kinase 1 (ALK1): Mutations of other receptors such as activin receptor-like kinase 1 (ALK1) and endoglin have also been identified in PAH patients usually from families with coexistent hereditary hemorrhagic telangiectasia [91]. Mutations in ALK1 are believed to result in cellular growth-promoting via Smad-depending signaling. Genetic mutations of the serotonin transporter (5-HTT) are more frequent in idiopathic PAH than control subjects [42]. The L-allelic variant of the 5HTT gene is associated with an increased expression of the transporter and increased proliferation of vascular SMC. Serotonin gene polymorphism has also been found

in PH patients with hypoxemic chronic obstructive pulmonary disease (COPD) [92].

Role of Growth Factors in Pulmonary Hypertension

Receptor tyrosine kinases (RTKs)

Several growth factors are involved in the pathogenesis of PH (Figure 2). Most of these factors bind to membrane receptors having an enzymatic activity belongs to receptor tyrosine kinases (RTKs) except TGF$_\beta$ links to a family of membrane receptors having an enzymatic activity of serine/threonine kinases. Intracellular signaling of RTKs is relayed by activation of enzymes of the serine/threonine kinase family: Raf kinase. Raf kinase phosphorylates and activates MEK1/2, then the ERK1/2. When activated, the extracellular signal-regulated kinase (ERK) will phosphorylates the effectors that control the genetic transcription in the nucleus [93]. In pulmonary arteries, the

Raf protein is involved in apoptosis, proliferation of vascular cells, and angiogenesis.

Vascular endothelial growth factor (VEGF)

The vascular endothelial growth factor (VEGF), binding to its VEGFR-2 receptor, is involved in many endothelial biological processes such as proliferation, synthesis of NO and PGI2, angiogenesis, and control of vascular permeability (Figure 2) [94]. The increase of VEGFR-2 expression is also found in idiopathic PH [95]. VEGF is also involved in PH associated with systemic diseases [96] or viral infections [97].

Platelet-derived growth factor (PDGF)

Recent data demonstrated that the platelet-derived growth factor (PDGF) has been involved in PH induced by chronic hypoxia or after injection of MCT [98]. This growth factor regulates the proliferation and migration of SMC, angiogenesis, and apoptosis (Figure 2). The PDGF receptor antagonists (PDGFR-) limit the evolution of PH induced by systemic shunt (by ligation of the ductus arteriosus) in the fetuses of sheep [99]. It also decreases the evolution of vascular plexiform lesions in PH [98]. Otherwise, the beneficial effect of imatinib, a PDGFR antagonist, in treatment of patients with PH who have no response to conventional treatment has been demonstrated [100].

Transforming growth factor-β (TGF$_\beta$)

The growth factors of TGF family, including TGF$_\beta$ but also the BMP, are potentially involved in vascular remodeling in PH via Smad (Smad 2 and 3) signaling pathway (Figure 2). TGF$_\beta$ has opposite effects on proliferation and cell migration, depending on its concentration in situ: activating effect at low concentration and inhibiting effect at high concentration. SMC of pulmonary arteries of patients with PH express three isoforms of TGF$_\beta$. There are anomalies in the signaling path of TGF$_\beta$/Smad2-3 in experimental MCT-induced PH [101]. The discovery of new receptors of TGF$_\beta$ and intracellular Smad inhibitors adds a new treatment in PH [102].

Epidermal growth factor (EGF)

Epidermal growth factor (EGF), induced by oxidant stress or by inflammatory mediators, is also involved in PH (Figures 2 and 5). The inhibition of its membrane receptor attenuates the development of MCT-induced PH [103].

Role of Extracellular Matrix and Metalloproteases in Pulmonary Hypertension

Role of extracellular matrix (ECM) and metalloproteases (MMP) in PH

In PH, ECM and MMPs play a preponderant role in pulmonary vascular remodeling. A recent study showed that ECM and MMPs such as tenascin-C (Tn-C) and MMP-2 have been new important markers of pulmonary vascular remodeling in PH [104].

Tenascin-C: Tn-C is an extracellular matrix glycoprotein and its role in pulmonary vascular remodeling has been reported [105]. The increase of Tn-C expression in the medium layer of SMCs of pulmonary arteries in patients with familial or congenital heart disease-induced PH has been found more than 15 years ago [106,107]. Under the effect of soluble growth factors, Tn-C increases the proliferation of SMCs via the activation of the receptors which mediates tyrosine kinase activity (receptors of EGF) [105,108]. In the animal model of MCT-induced PH, the inhibition of Tn-C increases the apoptosis of SMCs and decreases the hypertrophic lesions of the pulmonary arteries [109,110].

Elastase: Serine elastase (SerE) plays an important role in the production of Tn-C [108] and activation of MMP [111]. The increase of SerE expression has been demonstrated in experimental hypoxia-induced PH or by injection of MCT in rats [111]. In this model, either the use of the serine protease inhibitor or the excessive expression of the serine elafine protease could limit the pulmonary vascular lesions in PH.

Metalloproteases of the extracellular matrix: The role of MMP in PH has recently been demonstrated. The expression and activity of MMP-2 are increased in pulmonary tissue of rats having MCT-induced PH [112]. In humans, expression of MMP-1 and MMP-2 is increased in the SMCs isolated from the arteries of patients with idiopathic PH [113]. However, there are currently no therapeutic experiences of the use of specific inhibitors of ECMs and MMPs in PH.

Role of Inflammation in Pulmonary Hypertension

Role of inflammatory cells in PH

The role of inflammation and autoimmunity in vascular remodeling seen in idiopathic PH and PH associated with systemic diseases have been recently highlighted. First, circulating autoantibodies directed against endothelial cells and cell nuclei are frequently found in PH [114]. Secondly, perivascular infiltration of inflammatory cells including lymphocytes (T and B), macrophages, and dendritic cells, is a constant feature of plexiform vascular lesions [115].

Role of cytokines and pro-inflammatory cytokines in PH

Cytokines and inflammatory chemokines are also involved in the pathogenesis of PH. Increased concentrations and expressions of pro-inflammatory cytokines (IL-1β, IL-6) were found in plasma and lung tissue of patients with severe idiopathic PAH (Figure 2) [116]. In mice, high expression of IL-6 was associated with increased pulmonary vascular resistance, and extensive pulmonary vascular lesions [117]. Efficacy of tocilizumab, a monoclonal antibody directed against IL-6 receptors, has been recently reported in a patient with PH [118]. Plasma concentrations and expressions of fractalkine and its receptors (CX3CR1 and CX3CL1) are increased in circulating lymphocytes (CD4+ and CD8+) and lung tissue [119], and the role of the chemokines CCL5 (RANTES) and CCL2 (chemokine ligand 2) studied in PH patients [120].

Role of viral infection in PH

Human immunodeficiency virus (HIV-1): Viral infections and inflammation resulting from these infections can induce PH. Indeed, the association between infection with human immunodeficiency virus (HIV-1) and the occurrence of severe PH is known (Figure 2) [121,122]. Result of a recent study has shown that the prevalence of PH in patients with HIV-1 is approximately 0.46% [123]. This prevalence has not changed in the last 30 years (0.5% in 1991) [121].

Human herpes type 8 (HHV-8): Recently, the role of infection with human herpes type 8 (HHV-8) has been documented in idiopathic and familial PH [124]. In these patients, the expression of HHV-8 was found in lung tissue and in the plexiform lesions of pulmonary arteries [125]. The role of HHV-8 in the development of elastin production-related PH has also been studied in transgenic Mts-1 mice [126]. It suggests that the gene expression of HHV-8 playing an important role in the etiology of PH induced by viral infection via receptors coupled to G proteins [127]. However, the role of HHV-8 infection in PH is still controversial (Figure 2) [128,129].

Hepatitis C: PH is also found in hepatitis C with a prevalence of 1% to 5% according to the previous study [130]. The pathogenesis of PH in this condition is not yet understood (Figure 2) [131]. PH associated with hepatitis C-induced is preceded by portal hypertension [132-135].

Conclusion

Until now, despite the combination of available vasodilator drugs at early stage of disease, the prognostic of PH remains unfortunately the same as some progressed cancer. The development in recent years of new bio-molecular techniques association with the identification of different signaling pathways improve the understanding of the molecular and genetic mechanisms of PH. The new findings on pathophysiology and pathobiology of PH also help physicians to improve advanced knowledge on pathogenesis of this disease and to develop new approaches therapeutics.

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