Update

Pulmonary Arterial Hypertension. Pathophysiology, Genetic Aspects and Response to the Chronic use of Sildenafil

Alessandra Costa Barreto, Sônia Meiken Franchi, Alexandre C. Pereira, Antonio Augusto Lopes

Instituto do Coração do Hospital das Clínicas - FMUSP - São Paulo, SP - Brazil

In the last decades there has been a growing interest for the understanding of pathophysiological mechanisms of pulmonary hypertension, especially in pulmonary arterial hypertension (PAH). Progresses have been more evident concerning the so-called idiopathic PAH (sporadic and familial, previously called primary pulmonary hypertension). Genes were identified whose changes may be related to the disease unleashing (bone morphogenetic protein type 2 receptor, BMPR2). Other genes have been identified as facilitators (serotonin transporter). Besides, from studies in animal models and cellular culture, a series of biological changes have been identified. Such changes involve endothelial cells, smooth muscle cells, fibroblasts, extracellular matrix, pericellular proteolysis, growth factors and their receptors, among others. The present review aims at identifying the main pathophysiological changes known at PAH so far. Besides, two PAH families are shown with the objective of emphasizing the importance of genetic diagnosis and draw the attention to signaling pathways (nitric oxide/cyclic GMP), currently used in therapeutic interventions (sildenafil, chronic use).

Introduction

Pulmonary hypertension is a clinical and hemodynamic syndrome, which results in the increase of vascular resistance in lungs, generally through mixed mechanisms, involving vasoconstriction and remodeling of arterial (arteriolar)[1] wall. The most recent classification of pulmonary hypertension[2] includes five items, from which the first is called pulmonary arterial hypertension (PAH). In such way of presentation similar entities under the pathophysiological point of view are included, such as, for instance, idiopathic PAH, familial PAH and that associated with congenital heart diseases.

The understanding of pathophysiological mechanisms operating in PAH has significantly progressed in the last decades and has been the reason for the development of new therapeutic resources. So, the objective of the current updating is to show the most recent concepts on PAH pathophysiology. Afterwards two families are shown, with the aim of emphasizing mechanisms connected to disease's genesis and others, implied with response to therapeutic intervention.

Mailing address: Antonio Augusto Lopes – InCor - Cardiologia Pediátrica e Cardiopatias Congênitas do Adulto - Av. Dr. Eneas de Carvalho Aguiar, 44 - 05403-000 - São Paulo, SP
E-mail: aablopes@usp.br

Changes in pulmonary vascular tonus: pulmonary vasoconstriction due to hypoxia

There are studies demonstrating a trend to vasoconstriction in PAH. The increase in production of thromboxane A2, a vasoconstrictor, as well as the insufficient production of prostacyclin, a vasodilator, are found in patients with idiopathic PAH. Another example of that imbalance, related to endothelial dysfunction, is the demonstration of the reduced expression of endothelial nitric oxide synthase (eNOS), and the increased expression of endothelin-1 (ET-1), a powerful vasoconstrictor[3-5].

Most of the events that lead to vasoconstriction in PAH, in last analysis, do it through the increase of calcium in vascular smooth muscle cells. The most studied model of tonus increase in the pulmonary circulation is the vasoconstriction due to hypoxia. The reduction in alveolar oxygen tension promotes a fast and reversible increase of pulmonary vascular resistance, which starts in less than one minute, quickly reversing with the normalization of oxygenation conditions[6]. The mechanism starting vasoconstriction is the inhibition of many potassium channels, designated by K_v, followed by cellular membrane depolarization and entry of calcium through L-type channels. That is a unique response of pulmonary vasculature, as opposed to systemic vasodilation against hypoxia[7]. Pulmonary vasoconstriction is, under normal conditions, an adaptative mechanism with the aim of balancing ventilation and perfusion, restricting blood flow in low ventilated areas (as, for example, in atelectasis) in favor of better ventilated areas. The accurate mechanism of vasoconstriction is not very clear, but it is believed to involve many factors (probably different in acute and chronic hypoxia), always resulting in depolarization of membrane and increase of intracellular calcium. Despite evidences confirming such hypothesis, some authors believe that it is only a facilitating mechanism[7].

A complementary theory for pulmonary vasoconstriction due to hypoxia suggests the participation of redox mechanisms in smooth muscle cells. Under normal conditions, the generation of reactive oxygen species (ROS) in mitochondrias would keep an "oxidated status" of potassium channels, which would stay open. During hypoxia, mitochondrial electron transport is reduced, as well as the generation of ROS. The consequent "reduced status" of cytosol would result in inhibition of potassium channels and depolarization of cellular membrane[7,8]. Alternatively, the oxygen "sensor" system could not be in mitochondrias, but along the plasmatic membrane, represented by NADPH oxidases or similar

enzymes. Another possibility is that hypoxia modulate potassium currents in K_v through cytochrome P450. Studies that involved its inhibition demonstrated a decrease in potassium currents, causing depolarization of cellular membrane[8].

Regarding chronic hypoxia, mechanisms causing pulmonary vasoconstriction can be many. The activity of K_v channels may be limited due to reduction in their genes' transcription. In rats, chronic hypoxia diminishes mRNA expression and protein of α-subunit of potassium channels in smooth muscle cells, causing their functional decrease[8]. Besides, oxygen restriction increases the functional activity of hypoxia-induced transcription factor (HIF-1). Under conditions of normal oxygen tension, the α-subunit of that transactivator is degraded. In hypoxia, however, degradation is inhibited, with accumulation of HIF-1α, dimerization with HIF-1α and formation of functional HIF-1. That is responsible for activation of critical genes, as the vascular endothelial growth factor (VEGF) and ET-1[8].

A particular characteristic in patients with idiopathic PAH is the reduction of the number of functioning potassium channels. Changes in potassium channels were found in those patients' smooth muscle cells, but not in other PAH forms. By comparing normal individuals with carriers of systemic arterial hypertension, PAH associated to other diseases and idiopathic PAH, it was verified that smooth muscle cells of that last group showed a reduced expression of subunits of K_v channels[9]. That would result in a decrease in potassium currents, allowing for the entry of calcium and sustained pulmonary vasoconstriction.

Cellular proliferation and matrix changes in pulmonary vascular remodeling

Next, we start the discussion of some pulmonary vascular remodeling-related mechanisms. Such mechanisms involve growth factors, extracellular matrix, membrane receptors and proteolytic activities.

Among the growth factor and their receptors, the TGF-α (transforming growth factor) super-family is of special relevance in PAH genesis, as it has an important role in physiological processes of inflammation, immunity and cellular differentiation and proliferation. It consists of multifunctional mediators, including TGF-α and BMP (bone morphogenetic proteins). Studies have demonstrated that patients with idiopathic PAH have changes in BMP type 2 receptor (BMPR2), more precisely mutations in the gene that codifies it and that is located in chromosome 2q33[10]. BMPR2 binds cytokines as TGF-α, BMP, activin, inhibin and other growth and differentiation factors. It performs its signaling function through the formation of a heterodimeric complex on the cellular surface, along with type 1 receptor. When activated, that complex starts the signaling inside the cell through specific enzymes, known as Smad. That sign is translocated to the nucleus, where it will regulate the gene transcription. Mutations in BMPR2 would cause a deficient signaling, resulting in the loss of antiproliferative mechanisms, for example, in pulmonary circulation. It has been demonstrated that BMPR2 has its expression dramatically reduced in pulmonary circulation in idiopathic PAH patients, even in individuals who do not have mutations in this receptor[11].

VEGF has been widely studied, more precisely in hypoxia-induced PAH. It highly connects with two tyrosine-kinase-type receptors: VEGFR-1 and VEGFR-2. For being an endothelial cell mitogen, it is involved in their growth, for example, inside the so-called plexiform lesions[12]. Chronic hypoxia, which follows some PAH forms, cruises with the increase of VEGF expression and its receptors[13]. Other growth factors are also influenced by hypoxia, as the platelet-derived growth factor (PDGF), fibroblast-derived growth factor (FGF-2), insulin-like growth factor (IGF-1) and the epidermal growth factor (EGF).

Nitric oxide (NO), an endothelium-derived vasodilator, has also an antiproliferative action over vascular smooth musculature[14], possibly connected to intracellular cyclic GMP generation. In fact, it has been considered that phosphodiesterase 5 enzyme (which degrades cyclic GMP) inhibitors could act on hypoxia-induced pulmonary vascular remodeling[15]. Such protective action of cyclic GMP would be related to the pathway of MAPP kinases and to inhibition of transcription factor AML1 (acute myelogenous leukemia)[16].

Prostacyclin, similar to NO, is a powerful vasodilator and inhibitor of platelet adhesion and cellular growth. It is the main metabolite of arachidonic acid in vascular endothelium, with action on smooth muscle cell, through the increase of cyclic AMP. Besides, prostacyclin inhibits the effect of endothelin-1 (ET-1). Patients with PAH show diminished prostacyclin urinary excretion[4].

The role of serotonin in PAH is related to the induction of hyperplasia and hypertrophy of smooth muscle cells and mitogenic effect also in endothelial cells and fibroblasts. The action of serotonin in pulmonary circulation is wide and also includes vasoconstriction, for instance, in response to hypoxia[17]. Increased plasmatic levels of serotonin are observed in some forms of PAH[18]. Besides, the increased expression of its transporter could be related to accumulation of intracellular serotonin[19].

ET-1 is a powerful vasoconstrictor predominantly produced by the endothelial cell, with mitogenic, inflammatory and proliferative action on the smooth muscle cell. Despite the absence of intracellular storages of ET-1, specific stimuli induce its synthesis and secretion in minutes. There are two types of endothelin receptors: ET_A (found in smooth muscle cells and cardiac myocytes) and ET_B (found in endothelial and smooth muscle cells). The binding of ET-1 to its receptors activates phospholipase C enzyme, increasing intracellular calcium levels, which results in a prolonged vasoconstriction. Diacylglycerol, produced from activated phospholipase C, stimulates protein-kinase C, a mediator enzyme of the mitogenic action of ET-1[20]. Similar to serotonin, the action of ET-1 is potentized in the presence of other growth factors[21]. Other relevant effects of ET-1 are the stimulation for the production of cytokines and growth factors[22], induction of the formation of extracellular matrix proteins[23] and the potentiation of the effects of TGF-â and PDGF[21]. Studies in normal rat lungs demonstrated ET-1 mRNA levels five times higher if compared with any other organ[24]. Under normal conditions, the lungs remove ET-1 from the circulation. In PAH, the lungs synthesize ET-1 in increased amount, which results in an increase of circulating levels, with prognostic implications[3]. Under such conditions, pulmonary circulation becomes a target of this peptide.

Besides the action of growth factors and other signaling molecules in pulmonary vascular remodeling, events involving components of extracellular matrix are extremely relevant (fig. 1). In this way, it is proposed that structural and functional changes of the endothelium, which are consequent to many stimuli, end up

allowing for some factor or factors present in plasma penetrate and accumulate in sub-endothelium. Such factor (possibly apolipoprotein A1 or equivalent) would contribute for the increase of endogenous vascular elastase (EVE) expression, a serine-protease that degrades elastin and collagen and is increased in PAH proportionally to the progression of the disease[25]. It is believed that EVE induction in smooth muscle cells is the result of the interaction of elastin with its binding protein and a serum factor. Signaling process would involve tyrosine-kinases, MAPP kinase pathway and the activation of AML-1 transcription factor. It has been already demonstrated that such signaling, involving AML-1 factor, can be inhibited by the action of NO, through cyclic GMP. Therefore, NO, via cyclic GMP is suggested to be able to inhibit the pulmonary vascular matrix remodeling through the suppression of such signaling pathway. EVE, in addition to its ability of degrade elastin, also degrades proteoglycans that store growth factors such as FGF-2 and TGF-α[25]. Besides, it is able to promote the activation of receptors for such factors, as shown in Figure 1.

The mechanisms described above, in a summarized way, constitute in fact a proposed model of pulmonary vascular remodeling from many experimental studies[1]. It is understood that vascular remodeling in PAH is a complex process that most probably involves serum factors, protease induction, changes in extracellular matrix, availability of growth factors, activation of their receptors, and finally, mitosis. Besides, many changes involving extracellular matrix, integrins and growth factors, contribute to changes in the phenotype of smooth muscle cells from a contractile to a secretory one[1].

Endothelial dysfunction: induction of procoagulant and prothrombotic mechanisms

In addition to expressive participation of the endothelium in changes of tonus and in pulmonary vascular remodeling, which are present in PAH, its involvement in the vaso-occlusive process, strictly speaking, becomes relevant as there is a progressive loss of its anticoagulant and antithrombotic properties, and an unleashing of mechanisms that facilitate coagulation and cellular adhesion, and inhibit fibrinolysis.

Among endothelial substances, which shown to be changed in PAH, with implications in the mechanisms described, there are thrombomodulin, tissue-type plasminogen activator (t-PA), its inhibitor (PAI-1), P-selectin and Von Willebrand factor (FvW), besides NO and prostacyclin. Besides, other prothrombotic changes have been identified, such as the presence of antiphospholipid antibodies and hyperhomocysteinemia.

Thrombomodulin is a plasma membrane proteoglycan. Its extracellular portion is cleaved in many regions, with a consequent release of fragments in blood flow, whose concentration can be easily determined. Those fragments are known as the soluble fraction. Thrombomodulin has thrombin-binding properties, which activates protein C that, for its turn, degrades coagulation factors V and VIII[26]. In PAH patients, thrombomodulin plasma levels are diminished, probably as a result of reduced endothelial
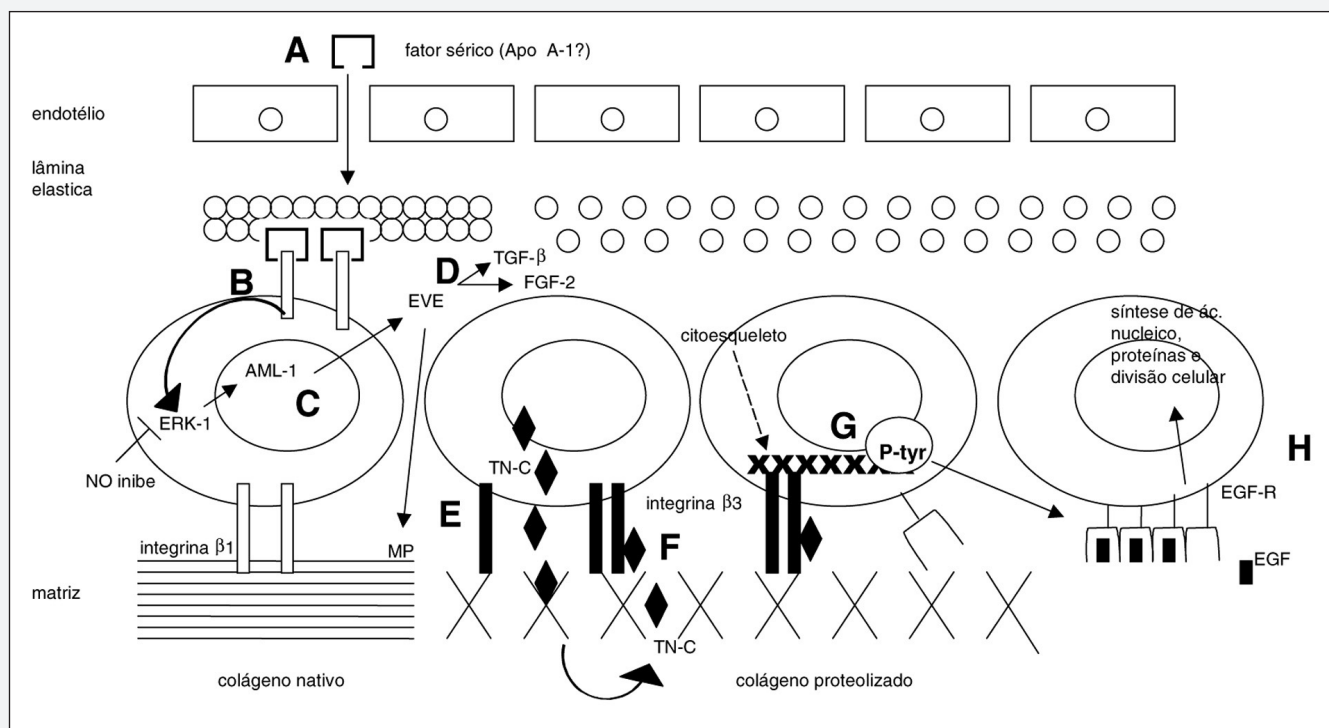

Fig. 1 – A hypothetical scheme of pulmonary vascular remodeling process, from endothelial lesion to the induction of cell division mechanisms. Structural/functional changes of the endothelium result in a loss of barrier function, allowing for the penetration of serum factor (presumably the apolipoprotein A1, Apo A-1) (A). That would facilitate the interaction between elastin and smooth muscle cell's surface, which would result in activation of intracellular kinases (B). Such activity involves the phosphorylation of ERK-1 (extracellular regulated kinase), whose target seems to be the gene AML-1 (acute myelogenous leukemia), a transcription factor that induces the expression of endovascular elastase (EVE) gene (C). In addition, EVE degrades proteoglycans storing growth factors, such as FGF-2 and TGF-β (D). Besides, it activates matrix metalloproteinases, which degrade type I collagen, exposing binding sites for β3-integrins (E), whose signaling inside the cell induces the production of tenascin-C (TN-C). Its secretion and incorporation to collagen result in an "outside-in" signaling, via β3-integrins (F and G). The result is the formation of focal adhesion complexes, organization of the cytoskeleton and tyrosin phosphorylation of many other signaling molecules (P-tyr) (G). Such signaling, involving the cytoskeleton, induces the formation of receptor clusters. Finally, binding of growth factors to their receptors induces cytoplasmatic and nuclear signaling, culminating with protein and nucleic acid synthesis, and cell division (H).

synthesis[26,27]. Besides, there are reports that demonstrate low plasma levels of protein C, as well as its co-factor, protein S[28]. Such changes may reflect a defective synthesis or even consumption due to intravascular coagulation.

Patients with antiphospholipid syndrome, associated or not to systemic *lupus eritematosus*, have a 1.8 to 3.5 % prevalence of PAH. Antiphospholipid antibodies, whose presence in circulation happens through not completely clear mechanisms, seem to promote the appearance of thrombosis through many mechanisms[29,30]. Pulmonary thromboembolism seems to be the main PAH cause in the syndrome[31]. On the other hand, 10 to 20% of the patients with chronic thromboembolism show such antibodies[32]. There are also reports on their presence in individuals with idiopathic PAH, in the absence of other corroborative signs of chronic thromboembolism[33].

Homocysteine is an amino acid that may facilitate coagulation and cellular adhesion in normal individuals. It is known that the presence of high plasmatic levels of homocysteine is associated to endothelial and platelet dysfunction, with a consequent formation of thrombi and vasoconstriction[34]. It is suggested that hyperhomocysteinemia may play some role in PAH pathogenesis, as there are studies showing increased levels of this amino acid in patients with the disease[34].

A particular form of endothelial dysfunction in PAH patients is the one associated with hypoxemia, provided the stressed trend to thrombosis that takes place in the so-called Eisenmenger's syndrome, in which, due to the presence of intracardiac defects with blood deviation from right to left, chronic hypoxemia develops. Changes in coagulation and fibrinolysis in those patients are associated to a high risk of thromboembolic events, with reports of up of 35% of occurrence of pulmonary arterial thrombosis[35]. The fact that hypoxia promotes exocytosis of Weibel-Palade bodies in endothelial cells, which results in FvW and P-selectin release, contributes to that. Besides, it has been shown that hypoxia induces the expression of PAI-1 mRNA and protein in addition to the suppression of thrombomodulin. Therefore, in chronic hypoxemia-associated PAH it is justifiable to imagine that mechanisms as the recruitment of leucocytes and platelets, fibrinolysis inhibition and the loss of control over coagulation, are operative and contribute to the vaso-occlusive event.

In addition to supplying an estimate of thrombotic risk in PAH individuals, endothelial dysfunction markers seem to have prognostic implications. A study in patients with idiopathic PAH and secondary to congenital heart diseases correlated high FvW levels with decreased survival[36]. Patients with the idiopathic form of the disease showed higher levels, associated to a lower survival expectancy[37]. Other biochemical markers are also being studied, with prognostic aim, such as catecholamines, uric acid, and natriuretic peptides (ANP and BNP). However, only BNP seems to have correlation with survival, with high levels in idiopathic PAH patients[38].

Platelets and serotonin

Endothelial dysfunction in PAH leads to a prothrombotic status, in which platelet participation seems unequivocal. They have been the target of studies for many years, as their role in PAH is more than merely thrombogenic, with an involvement also in the vascular remodeling process.

Clinical studies have been bringing about evidences of endogenous platelet activation in PAH patients, suggesting that there is a chronic consumption status. Such suggestion is due to the observation of the reduction of circulating platelet number and high levels of α-thromboglobulin[39], a substance released at secretion stage. It is suggested that such activation favors the formation of cellular aggregates in circulation, involving platelets among themselves or in association with leucocytes and erythrocytes[40], which could contribute to the disease progression in pulmonary vessels. Platelet activation in PAH also promotes the release of dense granules, containing mitogenic agents and vasoconstrictor substances. One of those substances is thromboxane A2, a powerful vasoconstrictor whose production is high in PAH patients[41]. Serotonin and growth factors, such as PDGF, EGF and TGF-α, are also released, with mitogenic effect on vascular smooth muscle cells, fibroblasts and endothelial cells[42].

In different forms of PAH, the platelet involvement (activation) has been regarded as a secondary event to endothelial dysfunction, sub-endothelium exposure and changes in flow conditions. Exception is made to a situation identified as platelet storage pool disease, a rare, familial entity, in which there is a deficit in the ability of storing serotonin in dense granules, with extremely high levels of that substance in the plasma, causing pulmonary vasoconstriction[43]. With the report of PAH associated to that disease[43], a series of studies was started in an attempt to demonstrate a causal association with serotonin.

Serotonin is the main component of platelets dense granules, being released from its activation. The normal response from the endothelial cell to serotonin is NO release, leading to vasorelaxation[44]. However, in endothelial dysfunction, serotonin is unable to stimulate NO release. In pulmonary circulation, such occurrence would favor PAH development through many mechanisms. Serotonin or 5-hydroxytryptamine (5-HT) performs its function through the connection to its receptor 5-HTR, and its transporter 5-HTT. When connected to 5-HTR, serotonin activates IP3 production, inducing the release of intracellular calcium from sarcoplasmatic reticulum, with consequent vasoconstriction[45]. On the other hand, diacylglycerol generation is implied in the induction of protein-kinase C mitogenic pathway. Serotonin connection to 5-HTT starts intracellular events, involving the formation of oxygen reactive species and the activation of MAPP kinase pathway, resulting in the expression of genes involved with hypertrophy and cellular proliferation[46]. Many studies suggest that serotonin has a role in PAH pathogenesis, including the idiopathic form. The presence of polymorphisms in the promoter region of the gene that encodes 5-HTT has already been demonstrated, with its expression increased[19]. So, that gene is today regarded as a "facilitator" in the pulmonary vascular remodeling process.

An important clinical condition, connected to serotonin metabolism and potentially PAH causer, is the use of oral anorexigenic, such as fenfluramine. In central nervous system, the effect would be performed through inhibition of 5-HTT and consequently serotonin reuptake in neurons. In the periphery, also for interfering with the transporter, fenfluramine is able to inhibits serotonin reuptake in platelets and pulmonary endothelial cells. The conse-

quences are the increase of free serotonin, in a similar way to what happens in platelet storage pool disease, and pulmonary vasoconstriction[19,46]. Not every oral fenfluramine user develops PAH, which suggests that disorders in serotonin metabolism and in its transporter functioning are facilitating events that need predisposing factors for the development of the disease.

As previously mentioned, mutations in the gene that encodes BMPR2 change intracellular signaling which, in normal conditions, would suppress proliferation and favor the apoptosis of smooth muscle cells in pulmonary arteries. It is suggested that signaling from BMPR2 could antagonize the effects of serotonin and that mutations in that receptor would allow for an aggravated cellular response to it, especially in patients with polymorphisms of 5-HTT[47]. However, that relation is merely speculative for the time being.

Inflammation

Pulmonary arterial hypertension is complication usually found under many systemic inflammatory conditions, such as scleroderma and the systemic *lupus eritematosus*[48]. In patients with connective tissue disease, pulmonary arterial lesions may be similar to those found in idiopathic PAH patients, including plexiform arteriopathy. Such finding boasted the search for a probable pathophysiological mechanism common to both conditions. It was observed that some patients with idiopathic PAH, similar to connective tissue diseases, show inflammatory infiltrate in pulmonary vessels, composed by macrophages and lymphocytes T and B[49]. That could suggest a role of inflammation in the pathogenesis or progression of the disease.

In addition to inflammatory findings, it is important to emphasize the association of PAH with diseases or conditions of immune character: infection through human immunodeficiency virus[48], POEMS (monoclonal plasma cell dyscrasia with a monoclonal protein present in plasma, polyneuropathy, organomegaly and skin changes) syndrome[50], presence of autoimmune antibodies[51] and Raynaud's phenomenon[52]. The main immunogenetic aspects in those diseases correspond to the presence of autoantibodies and certain alleles from class II major histocompatibility complex (MHC). Similarly, some patients with idiopathic PAH show high levels of antinuclear antibodies[51] and a prevalence of certain alleles present in autoimmune diseases. So, an increased frequency of HLA-DR52 in patients with scleroderma and PAH[53], of HLA-DR3 and HLA-DR52 in infants with idiopathic PAH[54] has been observed. An increase in the frequency of HLA-DQ7 in infants and adults with idiopathic PAH has also been demonstrated. Such finding is important, as the allele is associated to the presence of lupus anticoagulant in patients with systemic *lupus eritematosus*[55].

In an attempt to identify mechanisms that lead to inflammatory conditions found in PAH, an increase of many mediators has been observed. It has been already demonstrated that idiopathic PAH patients have high serum levels of IL-1 and IL-6[56]. Another important finding is the involvement of chemokines, which are chemoattractant cytokines able to direct leucocyte recruiting and migration. One of those chemokines, designated by RANTES (regulated upon activation, normal T cell expressed and secreted), performs the recruitment of monocytes and T cells. Besides, RANTES chemokine is able to induce endothelin-1 converting enzyme, resulting in an increase of mitogenic activity and vasoconstriction[57]. In patients with idiopathic PAH, increased amounts of RANTES mRNA has been demonstrated, with endothelial cells as the main source[58].

Fractalkine is a chemokine that also seems to have an important role in PAH: it promotes leucocyte recruitment through CX_3CR1 transmembrane receptor, with fast capture and integrin-dependent adhesion. Increased fractalkine plasma levels and mRNA, as well as an increased of the expression and function of CX_3CR1 receptor in lymphocites[59] has been demonstrated in patients with PAH.

Another chemokine that may be involved in idiopathic PAH inflammatory process is MCP-1 (monocyte chemoattractant protein), which has a strong chemotaxis power in relation to mononuclear cells. It is produced in many cell types, including monocytes, endothelial and smooth muscle cells. It participates in monocyte activation and migration and also plays a role in proliferation of smooth muscle cells. It is also described that IL-1 and IL-6 cytokines may induce MCP-1 expression in smooth muscle and endothelial cells[60]. Serum concentration of MCP-1 is high in idiopathic PAH patients, a factor that may be related to the progression of the disease[61].

Despite the inflammatory mechanisms established in idiopathic PAH and their association with connective tissue diseases, there is still controversy on their role in congenital cardiopathy-associated PAH. In a recent report on the presence of inflammatory cells in peripheral pulmonary arteries of congenital cardiopathy-associated PAH, a predominance of young macrophages in intimal and medial layers was detected, which suggests an inflammatory reaction in progress[62]. Being macrophages responsible for the production of many cytokines, it is speculated that they may have a relevant participation in events mediated by such substances (for example, cell replication) in patients with PAH.

Familial pulmonary arterial hypertension and its relation with mutation in the gene BMPR2

After presenting the current view on the most relevant pathophysiological mechanisms operating in PAH, we are going to present two families with the disease.

Family 1 – In this family, four cases of PAH were found among 12 members investigated. Two patients (DED and AKD), as indicated in the pedigree below (fig. 2), are under ambulatory follow-up, stable under clinical treatment. In other two cases non-assessed by our institution, with evolution to death, the diagnosis was presumptive, based on family reports.

The genetic study was carried out in five individuals of this family, as demonstrated in the pedigree. The study consisted of the amplification, through polymerase chain reaction (PCR), of 13 exons of the gene that codifies BMPR2 receptor. The product from the reaction was then submitted to sequencing through automatic method. As demonstrated in figure 3, a punctual mutation was identified in exon 9 in patients DED and AKD, but not in healthy family members. The mutation, corresponding to the substitution of a nucleotide, is illustrated in the chromatogram.

Patient DED, 15 years old, was in functional class IV at admission, with mean pulmonary pressure of 62 mmHg, unable to walk

any distance in the 6-minute walk test. After the beginning of treatment with sildenafil orally, in the dose of 225 mg a day, an important improvement in physical capacity was observed, as he started to walk 399m and 471m, respectively, at 6 months and 1 year. An increment of pulmonary flow, estimated through echocardiogram, of 50% at 6 months and 38% at 1 year was also noted. His sister (AKD), 18 years old at admission, had lower intensity manifestations and later beginning of the disease. The treatment was initiated in functional class II, with mean pulmonary pressure of 50 mmHg and a 309m walked distance. Under use of sildenafil (225 mg / day), there was an increase to 504m at 6 months and 1 year. An increment of 26% in pulmonary flow was also noted.

Family 2 – In this family, among 40 members investigated through information given, six potentially PAH ill-taken individuals were identified. From those, the diagnosis was established only for the patient EMB and two of six brothers, who died at 11 and 14 years respectively, as indicated in the pedigree (figure 4). The analysis of mutations in BMPR2 gene resulted negative in this family.

Patient EMB, 33 years old, was in functional class IV at admission, getting to make use of vasoactive drugs. The presence of pulmonary rales, with disappearance by means of diuretic therapy, raised the suspicion of veno-occlusive disease, a diagnosis later confirmed through hemodynamic study and pulmonary biopsy.

The assessment of this patient included acute vasodilatation test with nitric oxide (10ppm) and sildenafil (75 mg orally). The test demonstrated significant pulmonary vasodilatation with both stimuli separately applied, with an expressive decrease of vascular resistance (from 27.9 Wood units/m^2, under basal condition, to 8.3 and 11.1U/m^2, respectively, with nitric oxide and sildenafil). However, the pulmonary capillary pressure was stabilized on a safer level with sildenafil, compared to nitric oxide, with values of 15 and 29 mmHg, respectively. Results from this study allowed for the guidance of chronic use of sildenafil (225 mg/day) associated to diuretics, with a clear clinical improvement after a year, being possible to remove the patient from the waiting list for pulmonary transplantation. The six-minute walked distance was 112m at pretreatment condition and 294m and 408 m at 6 months and 1 year, respectively. Echocardiogram also showed an increase of pulmonary flow of 50% and 120%.

Such observations emphasize some important aspects of PAH. First, the familial form diagnosis, which in clinical practice would make possible the identification of the disease in less advanced stages, in individuals with discreet or absent symptomatology. Second, the importance of mutations in BMPR2 gene in pathophysiological scene, with the possibility of identifying such gene-related changes in at least 50% of ill-taken families. Finally, the pathophysiological role of NO and related pathways, as a rationale for the use of therapeutic resources with ability to increase cyclic GMP intracellular levels in pulmonary arterioles.

Final considerations

In consideration of the mechanisms discussed, it is clear that PAH is a syndrome whose beginning and progression processes in a diverse way, depending on the case. It is not possible to imagine the same pathophysiological mechanism or set of pathophysiological mechanisms explaining vasoconstriction and the evolution of vascular remodeling in all forms of PAH. Besides, even if we consider a single form of the disease, the pathophysiological mechanisms may vary depending on the stage. So, difficulties in the development of therapeutic resources usable in humans are noticed, as it would depend on the clear definition of operative mechanisms in a particular case or even on the identification of changes common to several patients with different forms of the disease. Even so, investigations in PAH pathophysiology have been relevant. Without them, therapeutic acquisitions as prostanoids, endothelin antagonists and phosphodiesterase inhibitors, currently in use, would not have been possible.

Abbreviations

AML-1, acute myelogenic leukemia-1; AMP, adenine monophosphate; ANP, atrial natriuretic peptide; Apo A-1, apolipoprotein A1; BMP, bone morphogenetic protein; BMPR2, receptor 2 of BMP; BNP, brain natriuretic peptide; EGF, epidermal growth factor; ERO,
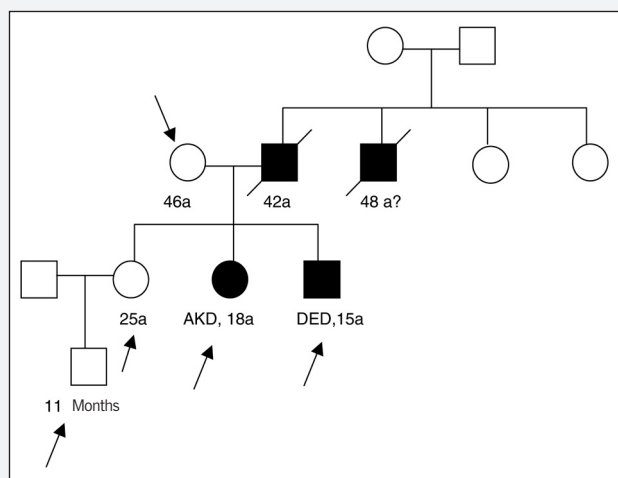

Fig. 2 – Family 1 pedigree showing PAH ill-taken members. The arrows indicate the individuals in whom the genetic study was carried out.
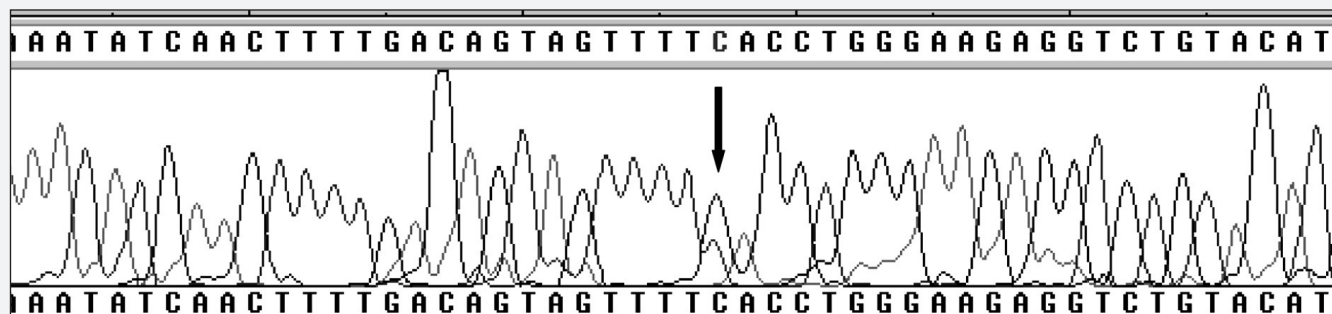

Fig. 3 – Schematic representation of mutation identified in two members of family 1, regarding the gene that codifies BMPR2 receptor. The substitution of a nucleotide in exon 9 was observed. In the chromatogram, the arrow indicates the presence of two alleles, the normal one and the pathologic one, characterizing heterozygosis in the patients.
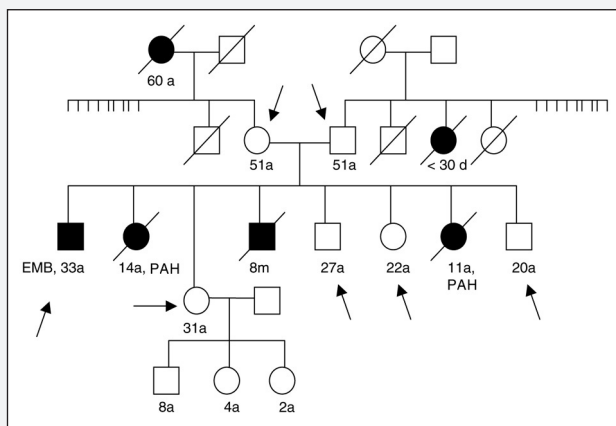
Fig. 4 – Family 2 pedigree, indicating individuals with PAH diagnosis (EMB and 11- and 14-year-old brothers), as well as the other possible ill-taken members. The individuals in whom the genetic study was carried out are indicated by the arrows.

oxygen reactive species; ERK, extracellular signal-related kinase; ET-1, endothelin-1; EVE, endogenous vascular elastase; FGF-2, fibroblast growth factor; FvW, von Willebrand factor; GMP, guanine monophosphate; PAH, pulmonary arterial hypertension; HIF-1, hypoxia-inducible factor; 5-HT, 5-hydroxytryptamine; 5-HTR, 5-hydroxytryptamine receptor; 5-HTT, 5-hydroxytryptamine transporter; IGF-1, insulin-like growth factor; IL-1, interleukin-1; IL-6, interleukin-6; Kv, voltage-dependent potassium channel; MCP-1, monocyte chemoattractant protein; MHC, main histocompatibility complex; NO, nitric oxide; NOS, nitric oxide synthase; PAI-1, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; PKC, protein-kinase C; POEMS (syndrome), plasma cell dyscrasia with polyneuropathy, organomegaly, protein M and skin changes; TGF-β, beta type transformer growth factor; TN-C, tenascin-C; t-PA, plasminogen tissular activator; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

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