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Interleukin-6 in pulmonary artery hypertension

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Abstract: Interleukin (IL)-6 is a pleiotropic cytokine, playing an important role in various pathological conditions, such as inflammatory, infectious, and neoplastic disorders. The casual relationship between IL-6 levels and development of pulmonary artery hypertension (PAH) has been elusive. Based on comprehensive retrieval of pertinent literature of recent two decades, this article aims to give an overview of the impact of IL-6 on PAH development in view of both clinical and experimental aspects. Results showed that IL-6 was overexpressed in all types of PAH in both human and animal models. The elevated IL-6 levels were closely related to right ventricular (RV) dysfunction and predicted poor prognosis and mortality of PAH patients. Several IL-6-regulated signaling pathways including transforming growth factor (TGF)- β /bone morphogenetic protein signaling pathway are involved in PAH development. IL-6 antagonizing agents are effective in ameliorating the symptoms and improving the RV function of PAH patients.

Keywords: cytokines; hypertension; interleukin-6; predictive value of tests; pulmonary; signaling transduction; therapeutics.

Introduction

Pulmonary artery hypertension (PAH) is a condition characterized by elevated pulmonary arterial pressure and resistance. According to the updated classification of the Fifth World Symposium on Pulmonary Hypertension (Nice, France 2013), PAH is categorized into heritable, idiopathic, drug- and toxin-induced, and other disease (connective tissue disease, human immunodeficiency virus infection, portal hypertension, congenital heart

disease, or schistosomiasis)-associated types [1]. The typical pathological changes of the pulmonary vasculature in PAH patients were vasoconstriction, pulmonary vascular proliferation, apoptosis, and angiogenesis [2, 3]. It has been proved that the pathogenesis of PAH and the pulmonary vascular remodeling is involved in genetic predispositions, dysregulated cellular signaling pathways, and altered cytokine expressions [4]. The genetic backgrounds of PAH in children with regard to transforming growth factor (TGF)- β superfamily genes have been described [5]. Interleukin (IL)-6 is a pleiotropic cytokine in charge of immune regulation, hematopoiesis, inflammation, and oncogenesis [5]. It has been proposed that casual relations may exist between pathogenesis of PAH and excessive production of IL-6 [6]. However, the implications of upregulated IL-6 in the development of PAH have not been sufficiently elucidated. The purpose of this article is to give an overview of the impact of IL-6 on PAH development in view of both clinical and experimental aspects.

Clinical research

IL-6 profiles in PAH

IL-6 levels were found to be elevated in patients with PAH [7, 8] and animal models of PAH [9, 10]. Selimovic et al. [11] reported that IL-6 levels were significantly higher in PAH patients than in controls (3.98 vs. 0.7 pg/mL; $p < 0.001$). However, they did not find any difference in IL-6 levels detected in blood samples taken either from the right atrium or from the pulmonary artery between patients with idiopathic PAH and those with collagen vascular disease-associated PAH (Figure 1) [11]. A prospective review including 37 children with congenital heart defect-related PAH, 21 children with congenital heart defects only, and 22 healthy children revealed that serum IL-6 levels were significantly higher in children with congenital heart defect-related PAH than in other group patients (Figure 2) [9]. Kalambokis et al. [10] detected serum IL-6 levels in patients with cirrhosis, with and without PAH: 15 cirrhosis patients with a pulmonary vascular resistance (PVR)

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Figure 1: No difference was found in interleukin-6 levels between patients with idiopathic pulmonary artery hypertension (PAH) and those with PAH and collagen vascular diseases (CVD) [11]. PA, pulmonary artery; RA, right atrium.

Figure 2: Serum interleukin-6 levels were significantly higher in the children with congenital heart defect-related pulmonary artery hypertension (PAH) [9].

$<120 \text{ dyn} \cdot \text{s}/\text{cm}^5$ vs. 8 patients with a $\text{PVR} >120 \text{ dyn} \cdot \text{s}/\text{cm}^5$. As a result, serum IL-6 levels were higher in those with a higher PVR compared with those with a lower PVR (43.2 ± 10.7 vs. $16.1 \pm 3.7 \text{ pg/mL}$; $p = 0.008$). Zorzanelli et al. [12] compared serum cytokine levels between pediatric patients with PAH and those without. They found that serum IL-6 levels remarkably decreased in proportion to a significant increase of the pulmonary-to-systemic flow ratio and improvement of peripheral oxygen saturation.

Several studies disclosed that patients with idiopathic PAH had consistently elevated IL-6 levels in blood circulation [7, 11] and in the lung tissues [13]. In patients with systemic lupus erythematosus, mixed connective tissue disease, or systemic sclerosis, an elevated serum IL-6 was noted in patients with PAH than in those without [14]. In addition, elevated serum IL-6 was reported in patients with polyneuropathy, organomegaly, endocrinopathy,

monoclonal gammopathy, and skin changes (POEMS) syndrome owing to the complicated PAH [15].

Heresi et al. [16] recruited in their study 76 PAH patients, including 40 idiopathic, 10 heritable, 19 connective tissue disease-associated, and seven congenital heart defect-associated types of PAH. Targeted therapies were applied in 63 of these PAH patients, 32 of whom received parenteral prostacyclin, and their IL-6 level was 3.82 (range, 1.79–9.87) pg/mL. Furuya et al. [5] reported that a female patient with multicentric Castleman's disease associated with severe PAH and a significantly elevated serum IL-6 of 41.8 pg/mL was treated with tocilizumab, an IL-6 antagonizing immunosuppressive agent, at a dose of 8 mg/kg every 2 weeks. Tocilizumab was proved to be a promising agent for ameliorating the patient's symptoms including low-grade fever, anorexia, and fatigue. A 3-month tocilizumab treatment led to a gradually decreased serum IL-6 level (Figure 3) and decreased pulmonary artery pressure, PVR, and right atrial pressure as well [5].

Casual relations between IL-6 and PAH

IL-6 levels in PAH patients were closely related to PVR [10]. Increased serum IL-6 levels are associated with greater symptomatic burden and increased mortality in PAH, and this was interpreted as the adverse effects of IL-6 on right ventricular (RV) function [17]. Prins et al. [17] analyzed the relationship between IL-6 levels and RV function in 40 PAH patients, and found that patients with an increased IL-6 level had remarkable RV dysfunction, impaired RV-pulmonary artery coupling, higher right atrial pressures,

Figure 3: Serum interleukin-6 decreased gradually after a 3-month treatment with tocilizumab in patient with multicentric Castleman's disease associated with secondary pulmonary artery hypertension [5]. m, months.

reduced cardiac index, and lower stroke volume in comparison to those with a lower IL-6 level. However, IL-6 did not correlate with mean pulmonary artery pressure, PVR, and pulmonary arterial compliance [17]. Oz-Tuncer et al. [9] demonstrated that serum IL-6 levels negatively correlated with systemic flow ($r = -0.572$, $p = 0.021$), positively with systemic vascular resistance ($r = 0.774$, $p = 0.001$), and positively with pulmonary wedge pressure ($r = 0.796$, $p = 0.006$) in PAH patients. Other authors [8, 18] also observed a relationship between elevated IL-6 levels and quality of life-related symptoms including pain, fatigue, anxiety, and depression.

Predictive values of IL-6

In a multivariate analysis, increased IL-6 levels predicted mortality, and elevated IL-6 levels in PAH patients were taken as an independent predictor of adverse outcome [11]. Soon et al. [8] detected serum cytokines including IL-6, and disclosed an elevated IL-6 in patients with idiopathic PAH, which correlated closely with the severity of disease and mortality [8]. Heresi et al. [16] found that both IL-6 and high sensitivity C-reactive protein (CRP) were univariate predictors of mortality. A cut-off value of IL-6 ≥ 4.7 pg/mL had a sensitivity of 86% and a specificity of 72%, predicting a 9-fold increase of mortality [16]. The analysis showed that combined 6-min walking distance (cut-off: 450 m), right atrial pressure (cut-off: 8 mmHg), and IL-6 (cut-off: 5.6 pg/mL) were a sensitive indicator of patient survival. Humbert et al. [7] in a proinflammatory cytokine study in 29 severe PAH patients illustrated that serum IL-6 was significantly higher in these patients than in patients with PAH secondary to chronic obstructive pulmonary disease (COPD) (118 ± 36 pg/mL vs. 3 ± 1 pg/mL, $p < 0.001$) or with idiopathic PAH than in controls (118 ± 36 pg/mL vs. 3 ± 1 pg/mL, $p < 0.001$) (Figure 4). In general, IL-6 is attributable to the pathogenesis of PAH of different types. However, the distinctive effects of IL-6 on each type of PAH warrant further elucidations.

Experimental research

Endogenous IL-6 mediates hypoxic PAH and lung inflammatory process [19]. IL-6 overexpression in the transgenic mice led to spontaneous development of pulmonary vascular remodeling and PAH [20]. A lung-specific overexpression of IL-6 in mice resulted in increased PVR and pathological changes similar to those observed in PAH

Figure 4: Patients with severe idiopathic pulmonary artery hypertension (PAH) had higher serum interleukin-6 levels than controls [7].

patients [6]. PAH shows an inflammatory phenotype of macrophage CD68⁺ infiltration in the lung vasculature associated with reduced T cells and dysregulated natural killer cells [17]. Pulmonary IL-6 production is increased in experimental PAH and is thought to reflect increased production by both inflammatory and vascular cells [19]. There is strong evidence that IL-6 overproduction triggers PAH and lack of IL-6 in knockout mice prevents PAH [6, 17, 19]. Experimental studies revealed that IL-6 overexpression induced muscularization of the pulmonary vascular bed. Knockout of IL-6 leads to partial protection against chronic hypoxia-induced PAH [19]. In lung-specific IL-6-overexpressing transgenic mice Tg⁺ mice, the number of the elastic lamina and the percentage of medial wall thickness of the main pulmonary artery branch were increased in comparison to the wild-type Tg⁻ control under either normoxic or hypoxic conditions. IL-6 overexpression also induced arteriolar neointimal occlusive lesions [6]. Steiner et al. [6] found, in an experiment of pulmonary remodeling in Tg⁺ in comparison to Tg⁻ controls, partial ($27 \pm 5\%$) or complete occlusion ($4 \pm 2\%$) of the arterioles in Tg⁺ mice, but patent arterioles and unthickened intima in the lung tissues of Tg⁻ control mice. They also observed that IL-6 also induced distal pulmonary vascular wall endothelial cellular growth and proliferation represented by an elevated vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2) expression in pulmonary artery endothelial cells of Tg⁺ mice and minimal expression of VEGFR2 in Tg⁻ mice. Savale et al. [19] proved, in hypoxia exposure of IL-6^{+/+} mice, marked increases of IL-6 messenger RNA (mRNA) and protein in the first week, with positive IL-6 immunostaining in the pulmonary vascular walls. Lung IL-6 receptor and glycoprotein 130 (the IL-6 signal transducer) mRNA levels increased after 1- and 2-week hypoxic

insult. Transgenic overexpression of a mutant bone morphogenic protein (BMP) receptor 2 (BMPR2) increases IL-6 levels in the lungs and renders mice susceptible to PAH [21]. IL-6 showed an acute negative inotropic effect on isolated hamster and chicken left ventricular cardiomyocytes mediated by nitric oxide overproduction [22, 23]. Furthermore, neonatal rat ventricular cardiomyocytes cultured in the presence of IL-6 revealed reduced mRNA and protein levels of SERCA2a, a sarcoplasmic endoplasmic reticulum calcium-adenosine triphosphatase found in the membrane of the sarcoplasmic reticulum [24].

IL-6 level is an independent predictor of RV dilation in the Multi-Ethnic Study of Atherosclerosis (MESA)-RV study [25]. Hagen et al. [21] discovered in *in vitro* and *in vivo* studies IL-6 upregulation in both transgenic mice and in cultured human pulmonary artery smooth muscle cells. An experimental study demonstrated that 2-week hypoxia-exposed IL-6-deficient (IL-6^{-/-}) mice showed decreased RV systolic pressure, RV hypertrophy, and number and medial thickness of muscular pulmonary vessels, and less inflammatory cell recruitment in the lungs, compared with hypoxic wild-type IL-6^{+/+} mice, whereas hypoxic IL-6^{+/+} mice had increased mRNA and protein levels of IL-6 within the first week, with positive IL-6 immunohistochemical stainings in the pulmonary vascular walls [19].

Several animal models of PAH, including chronic hypoxia [19] and monocrotaline treatment [18], are also associated with an increased production of IL-6. Moreover, daily subcutaneous injection of recombinant IL-6 in rats induced medial thickening of the pulmonary arterioles, leading to PAH [26]. Young male SV129 mice with a 3-week hypoxic exposure receiving SU5416, a VEGFR inhibitor, 3 times a week, showed pathological changes of characteristic PAH, including concentric neointimal wall thickening, plexogenic lesions, recruitment of macrophages, and distal arteriolar wall muscularization [27]. In the arteriolar adventitia, IL-6 level was increased with stronger inflammatory cell recruitment, and in lung tissue lysates and arteriolar media, endothelin (ET)-1 and ET receptor A were upregulated. The results were in line with the increasing ET-1 and decreasing ET receptor B levels in the recombinant human IL-6 provoked endothelial cells, indicating that IL-6 regulates ET signaling in PAH progression [27].

Experiments on C57BL6 mice at 8 weeks of age exposed to 10% oxygen hypoxia for 4 weeks resulted in upregulation of IL-6 in the lung tissues. After 2 mg of MR16-1 or rat immunoglobulin G was given to the animals by intravenous injection just before the insult and also hypoxic animals by intraperitoneal injection with 0.5 mg of MR16-1, a monoclonal antibody to mouse IL-6 receptor, or rat immunoglobulin G once a week, MR16-1 showed

Figure 5: Schematic illustration of the development of pulmonary artery hypertension (PAH) through the interleukin (IL)-6/Th17/IL-21-signaling axis [29].

PASMC, pulmonary artery smooth muscle cell; TGF, transforming growth factor.

much better RV function than control in terms of improved RV systolic pressure and RV hypertrophy, and significantly decreased medial wall thickness of the pulmonary vessels. It illustrated that the antagonism of IL-6 signaling might become a promising strategy for the treatment of PAH [28]. Hashimoto-Kataoka et al. [29] conducted an experiment in mice, and found that IL-6 blockade by the monoclonal anti-IL-6 receptor antibody, MR16-1, ameliorated hypoxia-induced PAH and prevented the hypoxia-induced accumulation of Th17 cells and M2 macrophages in the lungs. In addition, dexamethasone was observed to effectively reverse the pulmonary IL-6 overexpression and IL-6-expressing adventitial inflammatory cell infiltration in monocrotaline-induced PAH rats [30]. PAH develops via the IL-6/Th17/IL-21-signaling axis incorporated by TGF-β and MR16-1 (Figure 5) [29].

Discussion

IL-6 overexpression increases pulmonary artery pressure and RV hypertrophy. In animal models, IL-6 regulates many pathways of inflammatory disease

progression, mimicking the pathology of human PAH [31]. IL-6 contributes to the development and progression of cirrhosis-associated PAH. IL-6 may also cause vasoconstriction via inhibition of nitric oxide-mediated relaxation, or via the adrenergic or muscarinic receptor pathway [10]. Moreover, IL-6 may promote thrombosis of the lung microarteries [10]. Perivascular infiltration of inflammatory cells, consisting of T cells, B cells, and macrophages, are often present in the affected pulmonary arteries of PAH patients, suggesting that cytokines and growth factors are released from the inflammatory cells and involved in the proliferation of the pulmonary artery smooth muscle and endothelial cells [32]. By this means, a lung-specific overexpression of IL-6 in mice resulted in increased PVR and pathological changes similar to those in PAH patients [33]. IL-6 promotes proliferation of smooth muscle and endothelial cells, thereby dysregulating cellular growth and apoptosis, resulting in pulmonary vascular remodeling in PAH, potentially mediating proliferative, prosurvival, and anti-apoptotic processes. By this means, IL-6 brings about vascular smooth muscle cell proliferation through upregulation of VEGF and its receptor VEGFR2 [32]. In scleroderma-associated PAH, predominant obliterative-concentric lesions with fewer plexiform lesions were observed [34]. Eddahibi et al. [35] reported, based on a study in 50 patients with COPD-related PAH, the IL-6 G/G polymorphism (-174G/C) correlated with a higher level of IL-6 among patients with COPD but not among control subjects. Patients carrying the IL-6 GG genotype showed higher serum levels of IL-6 and higher pulmonary artery pressure than those carrying the CC or CG genotype. These results indicated that the inflammatory cytokine IL-6 might contribute to PAH in COPD and that IL-6 gene polymorphism endorses susceptibility to patients with PAH secondary to COPD. *BMPR2* gene mutation might boost cell division and prevent cell death, leading to pulmonary vascular remodeling, and one-fifth of individuals with a *BMPR2* gene mutation might progress into PAH [36]. Thus, *BMPR2* gene mutations constitute the background of the pathogenesis of heritable PAH [37]. Loss of *BMPR2* causes proliferation of pulmonary artery smooth muscle cells in response to TGF- β_1 and BMP2 [38].

It is well-known that the TGF- β /BMP signaling pathway adjusts vascular smooth muscle and endothelial cell growth by inhibiting excessive proliferations. IL-6-induced arteriopathic changes are accompanied by activation of growth factors, proliferative kinase extracellular signal-regulated kinase, proliferative transcription factors, and the antiapoptotic proteins. [6]. These findings suggest that IL-6 promotes the

development and progression of pulmonary vascular remodeling and PAH through proliferative antiapoptotic mechanisms [6].

Angiopoietin (Ang)-1-Tie2 pathway is essential for both embryonic and postnatal angiogenesis and involves in a protective action on endothelial cells by suppressing inflammation and apoptosis [39]. An increased Ang-1-Tie2 activity may play a critical role during PAH progression [40]. Measures to increase Ang-1-Tie2 signaling may protect against lung endothelial cell injury and development of PAH [40]. During the development of PAH with vascular remodeling, expressions of Ang-1, Tie2, and VEGF in the pulmonary arteries significantly decreased, and Tie2 receptor was inactivated in the lung tissues. Stimulation of macrophage Tie2 with Ang-1 promotes tumor necrosis factor-induced proinflammatory cytokine and chemokine expressions [41]. However, Ang-3, an endogenous antagonist of Ang-1, was upregulated in the intima under a hypoxic condition. As both Ang-1-Tie2 and VEGF promote angiogenesis and vascular survival, and play a protective role in the adaptation of microvascular changes during the onset of PAH, the downregulation of both Ang-1-Tie2 and VEGF and upregulation of Ang-3 appear to be associated with vascular rarefaction and development of hypoxic PAH [42].

The terminal elimination half-lives of IL-6 was 15.5 h. The results suggested that IL-6 synthesized in response to inflammatory stimulation was rapidly eliminated [43]. IL-6 in plasma is very unspecific and volatile, and several investigators have identified this cytokine to be involved in the pathogenesis of various diseases [44]. PAH patients often have concurrent RV dysfunction, with the late condition being a co-factor of inducing the upregulation of IL-6. In patients with connective tissue disorder-related PAH, both PAH and the connective tissue disorders *per se* stimulate IL-6 release [5, 14, 45]. In human immunodeficiency virus-infected patients, glycoprotein 120 activates the monocytes and macrophages to release ET and IL-6 [46]. Inflammation in human immunodeficiency virus infection is due to the lack of gut CD4⁺ T cell restoration, which favors lipopolysaccharide translocation and chronic systemic IL-6 overproduction [46]. Therefore, the diagnostic value of IL-6 can be less specific when PAH is in association with inflammatory disorders. Moreover, as indicated by Chaouat et al. [47] in a multiple stepwise regression analysis, with mean pulmonary artery pressure as the dependent variable, three independent variables, including pulmonary wedge pressure, partial pressure of oxygen, and IL-6, contributed to mean pulmonary artery pressure by 25%, 15%, and 9%, respectively.

Conclusions

Elevated IL-6 levels in PAH patients are associated with RV dysfunction and impaired RV-pulmonary artery coupling, and predicted poor prognosis and mortality of PAH patients. IL-6 is thus a key factor leading to PAH and vascular remodeling process. Accordingly, IL-6 antagonizing agents are effective in ameliorating the symptoms and improving the RV function of PAH patients by their antiproliferative and reversing vascular remodeling effects.

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