Association of Environmental Pollutants Exposure with Pulmonary Fibrosis: A Mini Review of Molecular Mechanism Mediated

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ABSTRACT

Pulmonary fibrosis is a specific form of chronic progressive interstitial lung disease. Deposition of extracellular matrix, mainly collagen, is the pathogenic characteristic of pulmonary fibrosis. Many reports show that environmental pollutants, particularly asbestos, silica, mercury, cadmium, and benzo(a)pyrene, are contributed in the etiology of lung injury and a risk factor in the development of idiopathic pulmonary fibrosis (IPF) in humans. Based on its physicochemical properties, environmental pollutant-induced pulmonary fibrosis can be developed after a particular type or dose of exposure. To date, some studies have focused on variant pollutants that are induced. However, the molecular mechanism of various pollutants to cause lung injury, which leads to pulmonary fibrosis, remained unexplored. Hence, this narrative review articles describe its molecular mechanism in generating pulmonary fibrosis comprehensively. It is helpful to portray the IPF pathogenesis and its drug discovery and development. Collectively, this article also revealed animal models which can be useful for IPF drug development research.

Keywords: idiopathic pulmonary fibrosis; animal models; lung disease; drug discovery; drug development

INTRODUCTION

Pulmonary fibrosis is a form of interstitial lung disease characterized by chronic, progressive, and fibrosing lung (Raghu et al., 2018). In lung function assessment, pulmonary fibrosis is characterized by reduction of vital capacity (VC), forced vital capacity (FVC), and forced expiratory volume in 1 second (FEV1) (Janssens & van Bleyenbergh, 2020). The pathogenic characteristic of fibrosis is marked by the extracellular matrix, mainly collagen, which is expressed by myofibroblast deposition in the lung interstitial (European Respiratory Society, 2013). As shown in Figure 1, the pathogenesis of pulmonary fibrosis starts with lung injury followed by subsequent pathways that cause profibrotic and proinflammatory imbalance, which drive cellular differentiation, that leads to lung fibrosis (Berngard & Afshar, 2016; Drakopanagiotakis et al., 2018; Meyer et al., 2012; Piersma et al., 2015; Sakai & Tager, 2013; Wick et al., 2013). The etiology of pulmonary fibrosis can be classified as idiopathic and known causal (Raghu et al., 2011, 2018). One known risk factor is environmental pollutants-induced idiopathic pulmonary fibrosis (Raghu et al., 2011, 2018).

Several environmental factors have been described to cause pulmonary fibrosis. Previously, air pollution has been reported to cause several lung diseases, including pulmonary fibrosis (Bala et al., 2021; Harari et al., 2020). Agricultural workers also develop pulmonary fibroses due to pesticide exposure (Park et al., 2021). Metal workers are prone to develop pulmonary fibrosis due to metal dust inhalation (Assad et al., 2018). Moreover, several other pollutants such as silica and benzo(a)pyrene also have been reported to induce pulmonary fibrosis (Vetrano et al., 1992; Bo et al., 2020; Byczkowski & Kulkarni, 1990).

This article aims to do a narrative review regarding the association of environmental pollutant exposure, domestic or occupational to pulmonary fibrosis and its molecular mechanism. This article will also describe the animal models’ similarity to the clinical feature of pulmonary fibrosis.

Environmental Pollutant

Silica

Occupational and non-occupational silica dust has become a public concern due to its relationship with lung diseases. A wide range of industrial activities has been associated with silica dust pollutants. Silica-based industries such as quartz crushing, agate grinding, ceramics, slate pencil, mining and milling of sandstones, silica flour milling, and granite have been reported to release silica dust. People or workers who work or stay in the vicinity of these industries are affected (Bhagia, 2012).
Silica occurs in two forms, namely amorphous and crystalline silica. Despite its occurrence in nature, amorphous silica is developed synthetically as a functional nanoparticle for therapeutics application (Jafari et al., 2019), and its toxicity has been reviewed previously (Croissant et al., 2020). Crystalline silica inhalation can induce lung fibrosis, usually after long-term exposure (Brown & Rushton, 2005). At the initial stages of silicosis, it is difficult to be detected by chest radiography. But the linear and nodular opacities distributed in both lungs can be found by high-resolution computed tomography scans in both lungs (Song et al., 2013). In addition to the clinical presentation, this finding is an essential tool for the diagnosis of silica-induced pulmonary fibrosis (Cedillo-Pozos et al., 2020).

Several pathogenesis have been proposed regarding the mechanism of silica-induced pulmonary fibrosis. The mechanisms of silica-induced pulmonary fibrosis are DNA damage (Daniel et al., 1993), oxidative stress (Daniel et al., 1995), and epithelial-mesenchymal transition (Guo et al., 2019). In oxidative stress pathogenesis, several oxidative markers, including superoxide dismutase (SOD), gluthathione (GSH), and lipid peroxide, are altered in the rat lung (Yamano et al., 1995). The redox imbalance is reported to be associated with the change of TGFβ1/SMAD signaling (Feng et al., 2019).

Various lung cells, including macrophages, lung epithelial cells, and fibrocytes, have been reported to take part in silica-induced lung fibrosis. Intratracheal instillation of silica induces a cellular change in bronchoalveolar lavage (Feng et al., 2019). Macrophages, one of the cell types that reside in alveolar, regulate several phenotypes, including fibrotic phenotypes (Wynn & Vannella, 2016). In an in vitro study, silica-induced DNA damages and oxidative stress in alveolar macrophage (Zhang et al., 2000). Rat lungs reported developed lung fibrosis 28 days after intratracheal instillation of silicon dioxide. In this study, silica-induced pulmonary fibrosis is regulated by the change of IncRNA expression in the lung (Sai et al., 2019). Change in IncRNA expression can regulate the epithelial-mesenchymal transition of lung epithelial cells that leads to lung fibrosis (Liu Y. et al., 2018). Fibrocyte differentiation to fibroblast also has a role in silica-induced pulmonary fibrosis (Li et al., 2017).

**Asbestos**

Asbestos is a mineral compound that naturally occurs in the environment. There are six naturally occurring fibrous magnesium silicate minerals that are referred to as asbestos fibers, namely: chrysotile, crocidolite, amosite tremolite, anthophyllite, and actinolite (Lazarus and Phillip, 2011). The naturally occurring asbestos, which appears as a component of soils or rocks, can be released into the environment because of human activities. The release of asbestos from its deposit to the environment representing a potential risk for human health (Campopiano et al., 2020).

Asbestosis is pneumoconiosis, defined as diffuse interstitial fibrosis of the lung. Detection of retained mineral particles in lung tissue provides valuable information for asbestosis (Gibbs et al., 2014). Differential diagnosis with IPF will be more difficult in the more advanced stages than in the early stages of the disease because it may have the same anamnestic, clinical, functional respiratory, and radiographic features (Stufano et al., 2020).

Asbestosis has been described as a dose-dependent disease (Walters, 2020). In an animal study, prolonged exposure until 56 days (about two months) after asbestos installation induces more severe fibrosis in mice (Yamamala et al., 2018). The exact mechanism of asbestosis is not fully understood. Several mechanisms of asbestos-induced pulmonary fibrosis have been
reported, including oxidative stress (Cui et al., 2019), altered cell’s function (Nishimura et al., 2013), and epithelial cells damages which lead to the recruitment of fibrogenic cells (Cheresh et al., 2020), autophagy (Lin et al., 2014).

Seven days after crocidolite installation into mouse lungs, several cells reported an increase in bronchoalveolar lavage (Yanamala et al., 2018). In asbestos-induced pulmonary fibrosis, several cells were also reportedly involved in the development of pulmonary fibrosis. Profibrotic polarization of monococytes-derived macrophages contributes to pulmonary fibrosis mediated by NADPH oxidase 4 (NOX4) (He et al., 2019). Immune cells, such as neutrophils and macrophages, also contribute to the lung’s oxidative stress (Funahashi et al., 2015). Lung epithelial cells undergo apoptosis after amosite asbestos perturbation is mediated by endoplasmic reticulum stress (Kamp et al., 2013). Moreover, lung epithelial cells undergo autophagy after chrysotile asbestos treatment through AKT/mTOR and the c-JUN signaling pathway (Lin et al., 2014).

**Mercury**

In nature, mercury exists in different oxidation states. The most abundant one is the oxidized mercuric Hg²⁺ state (O’Connor et al., 2019). Coal combustion is one of the largest mercury emission sources to the air (Liu K. et al., 2018). Among others, mercury can lead to human lung impairment (Pan et al., 2020; Pateda et al., 2018). In the study of oral ingestion of mercury in rats, pulmonary fibrosis is observed in the rat lung. Moreover, airway remodeling was also observed in this study (Naidoo et al., 2019). This lung impairment is similar to the impairment of human lungs after acute inhalation of mercury vapor (Lilis et al., 1985; Lim et al., 1998).

Mercury exposure induced oxidative stress and various lung cells toxicity. Oxidative stress occurs in rat lungs after intraperitoneal treatment of mercury-chloride. In this study, malondialdehyde was increased and glutathione was reduced in rat lungs. In this study, oxidative stress not only occurred in the lung, but also several organs including brain, liver, and kidney (Şener et al., 2007). In another report, the redox imbalance was accompanied by increased neutrophil activation, increased inflammatory cytokines, and increased cell apoptosis in the mercury-induced pulmonary injury in mice (Liu B. et al., 2018). Mercury-induced lung cell toxicity also has been demonstrated in an in vitro study using human lung cell line WI-38 (Ali, 2018). In addition, mercury-induced lung fibroblast apoptosis regulates cell cycle progression (Kim et al., 2021).

**Cadmium**

Cadmium is one of the heavy metals and is widely used for electroplating in a battery or in the solar cell (Fiducia et al., 2019). In nature, cadmium is closely related to lead and zinc. This naturally occurring cadmium is released to the environment during mining activity (Hamoudah et al., 2002). Cadmium exposure can be mediated through inhalation of polluted air or through smoking (Ganguly et al., 2018). Acute or chronic cadmium exposure can induce lung impairment in a dose-dependent manner (Ganguly et al., 2018).

In the experimental lung toxicity induced by cadmium, interstitial thickening by cellular infiltration and collagen deposit are observed in rat lungs (Hamoudah et al., 2002; Naidoo et al., 2019). In another study, prolonged inflammation is observed in mice lungs after intraperitoneal injection of cadmium chloride (Kundu et al., 2009). Lung inflammation is mediated by the expression of matrix metalloproteinase-2 (MMP2) (Kundu et al., 2009). The role of oxidative stress in cadmium-induced lung toxicity also has been previously reviewed (Cuypers et al., 2010).

Cadmium was demonstrated to induce lung epithelial cells dead mediated by reactive oxygen species production (Kimura et al., 2019; Kumar et al., 2016). Neutrophils are reported to be involved in promoting lung injury through the formation of neutrophils extracellular traps (Wang et al., 2019). Fibroblast differentiation to myofibroblast was also observed after cadmium treatment (Hu et al., 2017).

**Benzo(a)pyrene**

Benzo(a)pyrene (BaP) is one of the most studied polycyclic aromatic hydrocarbons due to its risk to human health (Carrell et al., 1997; Reizer et al., 2019). The formation of BaP is caused by incomplete combustion from both nature and human activity (Reizer et al., 2019). In the atmosphere, BaP can be bound to particulate matter (PM) and inhaled into the lung (Majewski & Piotrowski, 2020). The volatile organic compound such as BaP association with several health problems in humans, including pulmonary fibrosis, has been described in a previous review (Bala et al., 2021).

Animal studies of lung fibrosis induced by BaP also have been demonstrated. Collagen deposition, redox imbalance, and increased expression of inflammatory markers were observed in rat lungs after oral treatment of BaP (Alzohairy et al., 2021). Oxidative stress and collagen deposition were also observed in the mice’s lungs (Barnwal et al., 2018). Another report demonstrated that collagen deposition in the lung of mice treated with BaP and in mice treated with bleomycin is similar (Huang et al., 2020).
Table 1. Route of exposure of environmental pollutants in experimental animal

<table>
<thead>
<tr>
<th>Pollutants</th>
<th>Route of Exposure</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Silica</td>
<td>Intratracheal instillation</td>
<td>(Feng et al., 2019)</td>
</tr>
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<td>Asbestos</td>
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<td>(Barnwal et al., 2018)</td>
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Several lung cells were reported to play a role in BaP-induced lung fibrosis, such as fibroblast, immune cells, and epithelial cells (Azari et al., 2019; Chen et al., 2005; Suzuki et al., 2020). Aryl hydrocarbon receptor (AhR) is a receptor located in the cytoplasm (Shi et al., 2020). This receptor-mediated lung epithelial cell reprogramming (Ye et al., 2021). Activation of epithelial-mesenchymal transition of lung epithelial cells was reported dependent on p21 (Hýžďalová et al., 2021).

Animal Model for Drug Development of Pulmonary Fibrosis

The concern of environmental pollution is growing continuously because of more evidence in the association with poor health conditions in humans. Environmental pollutants increase alongside human activities, which depend on various natural resources. Those pollutants increase the exposure risk to humans, and some may induce serious health problems. Lung injury that leads to lung fibrosis in humans is induced mainly through the inhalation of environmental pollutants. Many studies have been conducted on the association of environmental pollutants with human lung fibrosis.

The experimental animal model is necessary to confirm the association of environmental pollutants and lung fibrosis and for developing drugs. The lung exposure to environmental pollutants is not necessarily through inhalation. Oral exposure to particulate pollutants leads to lung fibrosis in animal experiments. The exposures of environmental pollutants to animals described in this article are summaries in Table 1. The different exposure routes can give the option for researchers to use animal models of lung fibrosis.

DISCUSSION

There are several lung cells that play a role in the development of environmental pollutants-induced lung fibrosis. Several immune cells, such as neutrophils and macrophages, mediated inflammatory response. Persistent inflammatory response leads to lung fibrosis (Suzuki et al., 2020). M2 macrophages are dominating lung alveolar in IPF. It is altered by interleukin-4, interleukin-13, transforming growth factor (TGF-β), and interleukin-10 (Yanagihara et al., 2019). Depleting alveolar macrophages using clodronate also led to less fibrosis (Murray et al., 2011). Moreover, macrophages depletion increases epithelial cells proliferation and increases lung function (Hung et al., 2018).

Except for the pollutants here, the IPF can be triggered by cigarette use, drinking habits, and pathogenic infections that cause recurrent and long-lasting (chronic) lung injury. In our best knowledge, it is difficult to differentiate the mechanism of IPF based on the cause. However, Tao et al (2019) has been shown that inhalation of cadmium in different doses can affect the lung microenvironmental lung thereby changing the balance of lung microbiota.

Environmental dust pollutants such as cadmium, silica, asbestos, and BaP induced repeated lung injury chronically. Simultaneously, it induced activation of macrophage in innate immunity response in the alveolus, as well as redox imbalance in alveolar. These conditions generate enhancement of TGF-beta secretion. Further, it augments autocrine and paracrine profibrogenic signaling pathways. As a result, recruitment and proliferation of fibroblast increases, followed by differentiation and proliferation of myofibroblast. It is due to secretion and accumulation of excessive extracellular matrix, which lead to epithelial mesenchymal transition (EMT) and mediation to IPF (Velagacherla et al., 2022; Raghun and Luca, 2017).

Further, collagen deposition is the main characteristic of lung fibrosis. Apparently, fibrocytes-derived collagen does not contribute to lung fibrosis (Kleaveland et al., 2014). The generation of myofibroblast is crucial in the development of pulmonary fibrosis. In addition to fibroblasts, pericytes contribute to myofibroblast differentiation in the lungs (Rahaman et al., 2014).

In IPF, alveolar becomes stiff and loses its elasticity (Glass et al., 2019). Naturally, individu with IPF become harder to breathe. Other symptoms are chronic cough, fatigue, weight loss, and feeling uncomforted in the chest (Raghun et al., 2017). These symptoms usually occur 2 years before IPF is successfully diagnosed by physicians (Glass et al., 2019; Raghun and Luca, 2017).
Association of Environmental Pollutants Exposure

Treatment of IPF is aimed to refrain the progression, maintain the quality of life, and prolong patient’s survival. Pharmacological intervention was limited to nintedanib and pirfenidone as antifibrotic agents (Pleasant and Tighe, 2019). In addition, Raghu and Luca (2017) mentioned symptom management burdensome and oxygen therapy to overcome shortness of breathing. It also classified others as acid suppressive therapy palliative care, management of comorbidities, pulmonary rehabilitation, vaccination for prevention of respiratory infection, as well as patient education and psycho-emotional support (Arya et al., 2018; Raghu and Luca, 2017).

Nintedanib and pirfenidone are FDA approved and strongly recommended for inhibiting progression of fibrosis (Raghu and Luca, 2017). Nintedanib is well known as a tyrosine kinases inhibitor for fibroblast growth factor receptor, platelet-derived growth factor receptor, and vascular endothelial growth factor receptor. It disrupts proliferation, migration, and differentiation of fibroblast, at once suppressing the secretion of extracellular matrix components in lung fibrosis (Wollin et al., 2015). While the mechanism of pirfenidone is not fully revealed. However, pirfenidone has been promising to inhibit proliferation and differentiation of fibroblast, at one attenuated synthesis collagen in animal lung fibrosis model (Ahluwalia et al., 2014).

The standard for management therapy in IPF patients has been growing rapidly recently along with the increasing incidence of patients. The further research in treatment progresses to gain longer life expectancy and greater quality of life. Both approved FDA drugs for IPF can slow the progression but not ultimately stop and refine the lung fibrosis. The future research might be targeted extracellular matrix deposition and immune response modulation using environmental pollutants as an idiopathic lung fibrosis inducer to generate the in vivo or in vitro models.

CONCLUSION

Environmental pollutants are risk factors in the incidence of IPF. To date, IPF is one of the lung diseases that has a low survival rate. Therefore, the environmental pollutants can be used to induce lung fibrosis in animals in a drug development process with the characteristic of the model being robust inflammation followed by fibrosis.

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