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Pirfenidone protects against paraquat-induced lung injury and fibrosis in mice by modulation of inflammation, oxidative stress, and gene expression

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ABSTRACT

In this study we investigated the protective effects and possible mechanisms of pirfenidone (PF) in paraquat (PQ)-induced lung injury and fibrosis in mice. Lung injury was induced by injection of PQ (20 mg/kg). Thereafter, mice orally received water and PF (100 and 200 mg/kg) for four weeks. After 28 days, the inflammation and fibrosis were determined in the lungs by analysis of histopathology, bronchoalveolar lavage fluid (BALF) cell count, lung wet/dry weight ratio, hydroxyproline content, and oxidative stress biomarkers. Expression of several genes involved in fibrogenesis and modulation of reactive oxygen species (ROS) production, such as TGF-β1, α-SMA, collagen Iα and IV, NOX1, NOX4, iNOS, and GPX1 were determined using RT-qPCR. PF significantly decreased the lung fibrosis and edema, inflammatory cells infiltration, TGF-β1 concentration, and amount of hydroxyproline in the lung tissue. PF dose-dependently improved the expression level of the studied genes to the near normal. Decreasing of lung lipid peroxidation and catalase activity, and increasing of SOD activity in the treated mice were significant compared to the control group. Pirfenidone ameliorate paraquat induced lung injury and fibrosis partly through inhibition of inflammation and oxidative stress, and downregulation of genes encoding for profibrotic cytokines and enzymatic systems for ROS production.

1. Introduction

Paraquat poisoning is the most common cause of fatal herbicide intoxication and most cases result from deliberate ingestion. It has been observed in patients who ingest the pesticide either accidentally or intentionally as a suicide attempt (Dinis-Oliveira et al., 2008). PQ has detrimental effects on many organs namely, the central nervous system, gastrointestinal tract, kidney, liver, and heart. Pulmonary toxicity is the most common complication in treatment of paraquat poisoning (Gawarammana and Buckley, 2011). It has been shown that generation of redox cycling and free radicals is the main culprit in progressive inflammatory responses and pulmonary fibrotic process in lungs (Blanco-Ayala et al., 2014; Cheresh et al., 2013; Dinis-Oliveira et al., 2008; Mohammadi-Bardbori and Ghazi-Khansari, 2008).

Growing strategies for decreasing the toxicity of PQ have been proposed, including prevention of absorption, hemoperfusion, prevention of accumulation in the lungs, scavenging oxygen free radicals and prevention of lung fibrosis (Blanco-Ayala et al., 2014; Dinis-Oliveira et al., 2008; Gawarammana and Buckley, 2011). However these interventions have no confirmed effects for improving survival (Loveman et al., 2014). Among the therapeutic drugs available for pulmonary fibrosis, pirfenidone has opened a new promising window for both patients and physicians, suggesting that we could be able to modify and improve the prognosis for this devastating disease in the future (Rasooli et al., 2017b; Selvaggio and Noble, 2016). Many cases of idiopathic pulmonary fibrosis (IPF) have been improved by pirfenidone (Cottin

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and Maher, 2015; Hilberg et al., 2012; Inomata et al., 2014; Noble et al., 2016) and it may be effective against PQ induced lung fibrosis (Rasooli et al., 2017b; Seifirad et al., 2012).

Pirfenidone (PF) [5-methyl-1-phenyl-2-(1H)- pyridone] as an agent that had antifibrotic, analgesic, antipyretic and anti-inflammatory actions, is an orally available synthetic small molecule which is easily absorbed from the gastrointestinal tract after oral administration and is able to move through cell membranes without requiring a receptor (Selvaggio and Noble, 2016). The exact cellular mechanism whereby PF modulates fibrogenesis is still unclear in details, but its effects are attributed to antioxidant, anti-transforming growth factor-beta (*anti*-TGF-β) anti-tumor necrosis factor (TNF)-alpha, upregulation of RGS2, and antiplatelet derived growth factor (Iyer et al., 1999; Macias-Barragan et al., 2010; Nakazato et al., 2002; Salazar-Montes et al., 2008; Selvaggio and Noble, 2016; Xie et al., 2016). Although multiple different mechanisms of action of PF in IPF have been suggested, studies on PF and novel analogues are continued and further work in this field will possibly contribute to a better understanding of the mechanisms behind IPF and treatment.

Considering the possible antifibrotic effects of PF in PQ-induced lung fibrosis (Rasooli et al., 2017a, 2017b; Seifirad et al., 2012), in the present study we hypothesized that PF elicits its pharmacological effects by modulating the expression of some genes contributed to the enzymatic production of reactive oxygen species (ROS) and fibrogenesis, and oxidative stress inhibition. To test this hypothesis, we administered PF to PQ treated mice and examined the effect of PF on morphological changes, wet/dry weight ratio, differential cell count in bronchoalveolar lavage fluid (BALF), hydroxyproline content, and tissue oxidative stress parameters in lung tissue. In addition, expression of several genes contributed in fibrogenesis (TGF-β1, α- SMA, collagen Iα and IV) and ROS production (NOX1, NOX4, iNOS, GPX1) was determined in the lung tissues.

2. Materials and methods

2.1. Chemicals

Pirfenidone was obtained from Intermune Company (United States). Paraquat, hydroxyproline, 4-dimethylaminobenzaldehyde, chloramine T and malondialdehyde (MDA), and all the reagent for histological staining were purchased from Sigma–Aldrich Chemical Co. (USA). TRIzol® RNA isolation reagent and HyperScript™ first strand cDNA synthesis kit were purchased from Invitrogen (Germany). SYBR Green Master Mix was obtained from Takara (Japan). TGF-β1 kit was purchased from Bioassay Technology Laboratory (China).

2.2. Animals

Male NMRI mice, weighing 18–25 g, were obtained from Neuroscience Research Center, Kerman University of Medical Sciences and housed in normal laboratory conditions at 21 ± 2 °C under a 12 h/12 h light–dark cycle. All the mice had free access to water and standard laboratory food and were kept in standard cages. All the animals were treated humanely according to the guideline approved by the Animal Experimentation Ethics Committee of Kerman Neuroscience Research Center (EC/KNRC/90).

2.3. Paraquat-induced pulmonary fibrosis

Mice were randomly divided into five groups (except for BALF and lung wet/dry weight ratio experiments) eight mice in each: PQ group (PQ 20 mg/kg), PQ + water group (10 ml/kg as vehicle), PF100 group (PQ + pirfenidone 100 mg/kg), PF200 group (PQ + pirfenidone 200 mg/kg), and control group (normal animals without any treatment). For BALF analysis and lung wet/dry weight ratio experiments, four groups of 14 mice in each were given no treatment (as control), PQ (PQ + water), PQ + PF100, and PQ + PF200. Pulmonary fibrosis was induced by intraperitoneal (i.p.) injection of PQ at the dose of 20 mg/kg body weight. The dosage of PQ was based on our preliminary experiments showing induction of lung injury with no mortality

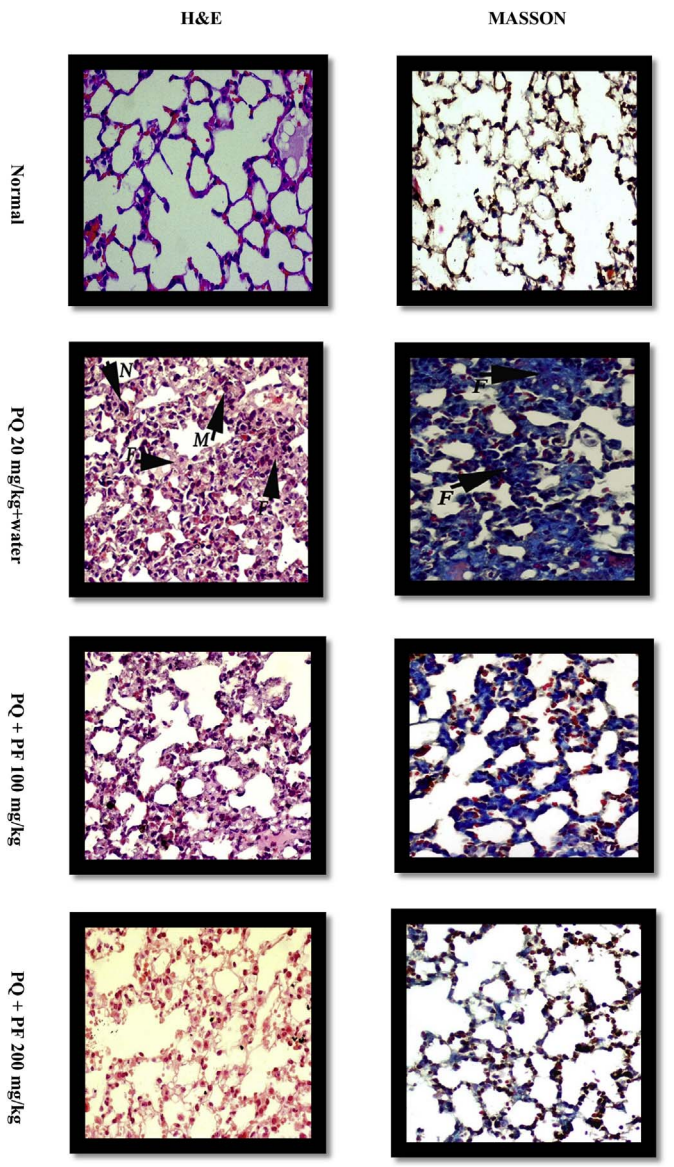

Fig. 1. Histopathological changes in the lungs of the mice in PQ-induced lung fibrosis. Lung fibrosis was induced in the lungs of the mice by injection of PQ (20 mg/kg, i.p.). They were then treated with PF 100 or 200 mg/kg/day for 28 days by oral gavage. The morphopathological changes (400×) in the lung tissues were analyzed by hematoxylin and eosin (H&E), and Masson's trichrome staining.
PQ: paraquat; PF: pirfenidone, M: Macrophage; N: Neutrophil; F: Fibrosis.

Table 1
Histopathological lesions of the lung tissue subsequent to therapeutic effects of PF.

Histological features	Control	PQ + water	PQ + PF100	PQ + PF200
Alveolar hemorrhage	absent	focal (mild)	mild	absent
Alveolar macrophages	present	increase	present	present
Interstitial inflammation	absent	moderate	mild	absent
Peribronchial fibrosis	absent	present	absent	absent
Interstitial fibrosis	absent	severe	moderate	mild

Mice treated with 100 and 200 mg/kg PF for 28 days after i.p. injection of single dose of PQ.
PQ: paraquat 20 mg/kg; PF: pirfenidone.