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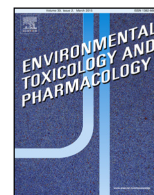

Perfluorocarbon attenuates inflammatory cytokines, oxidative stress and histopathologic changes in paraquat-induced acute lung injury in rats

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ABSTRACT

The effects of perfluorocarbon (PFC) on paraquat (PQ) induced acute lung injury (ALI) was evaluated among rats.

Twenty four Wistar rats were divided into 4 groups: control group injected by saline physiologic 0.9%, PFC group injected by Perfluorocarbon, PQ group injected by PQ and PQ + PFC group injected by PFC one hour after receiving paraquat. Bronchoalveular fluid content, inflammatory cytokines, oxidative and histopathologic changes were measured after 72 h.

The levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and transforming growth factor-β1(TGF-β1) in the PQ group were increased compared to either control or PFC groups, but their levels decreased in PQ + PFC group significantly ($p < 0.05$). Also, histopathologic evaluation revealed an increase in malondialdehyde (MDA) and hydroxyproline (HP) in the PQ group but a decrease in PQ + PFC group significantly ($p < 0.01$).

PFC emulsion by its anti-inflammatory, anti-oxidative and anti-fibrotic properties can reduce the inflammatory and fibrotic alterations, pulmonary oedema, and pulmonary histopathologic changes created by PQ.

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1. Introduction

Acute lung injury (ALI), induced by several inner or outer pulmonary factors, has been reported by longitudinal studies (Inoue and Takano, 2010). Evidences indicate that acute inflammatory responses and increased pulmonary microvascular permeability are among the most important pathological changes in ALI (Gao et al., 2012). Poisoning with pesticides applied for agricultural purposes is one of the most important health problems in the society, particularly among developing countries (Chen et al., 2015). More than 20,000 deaths and 2 million hospitalizations happen annually, due to poisoning with pesticides and chemical substances used during plantations. Environmental pollutants and food contaminants produced by industry; for example pesticides, flame retardants, plasticizers or dyes, may have adverse effects on human health, as revealed by numerous epidemiological studies (Vrzal et al., 2015). In many countries, notably within the European Union, the use of paraquat is restricted due to its potentials to contaminate the environment and particularly due to its high aqueous solubility which increases the risk of contamination of rivers and groundwater. Lung is the main organ affected and its treatment is difficult (Grillo et al., 2014).

PQ (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride) is one of the most widely used herbicides which held the largest share of the global herbicide until recently. However, this role is now overtaken by glyphosate. PQ is applied on large and small farms, plantations and estates and in non-agricultural weed control. It is a quick acting, non-selective herbicide which destroys green plant tissue on contact and translocation within the plant and is a classical toxic substance for lungs (Wen-Tien, 2013). A key aspect of PQ could be

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its toxicity for humans, so that its mortality rate is about 70% among poisoned patients (Hsin-Ling et al., 2008). About 350,000 patients were poisoned by different gardening or agricultural chemical substances during 1985–1990 in the USA and PQ has been responsible for only 0.34% of these poisonings. However, despite this low prevalence, its mortality rate is 13% among poisoned patients compared to other toxic agents (Klein-Schwartz and Smith, 1997). Rather than its application for agricultural purposes, PQ is also used as a suicide tool in some societies (Papiris et al., 1995). PQ can enter the human body through ingestion, inhalation, or skin contact, and rapidly spreads in most body tissues, gets easily absorbed into the pulmonary epithelial cells despite its high rate of renal excretion (Chan et al., 1998) and eventually causes death via ALI or acute respiratory distress syndrome (ARDS).

Although the lung injury mechanisms induced by PQ are still not clearly defined, several studies have shown that inflammatory mediators induced by PQ, cause pulmonary inflammation and tissue damage. Respiratory distress as a result of destruction of alveolar epithelial cells, as well as hemorrhage, infiltration of inflammatory cells into the alveolar and interstitial space, edema, proliferation of fibroblasts, progressive fibrosis and collagen deposition, are among the mechanisms discussed in the context of PQ-induced pulmonary toxicity (Göcgeldi et al., 2008). TNF-α and IL-1β are among the inflammatory markers, which are induced by PQ. Also, PQ can induce reactive oxygen species (ROS) which can leads to increase in TNF-α and IL-1β (He et al., 2012). Despite its considerable mortality and morbidity, specific and effective detoxification materials for PQ toxicity are still unknown (Morán et al., 2010). PFC is a molecule composed of carbon and fluorine with some chemical or physical characteristics such as high levels of gas dissolvability in water, rapid release, low surface pressure, high volume quality, average volatility, good histocompatibility, and no absorption or metabolism in vivo (Nakashima-Kamimura et al., 2009). Application of PFC into the alveolar space during the partial or total ventilation improves gas exchange, breathing mechanisms, and lung structure in many models of lung damage (Wang et al., 2014). Also, due to the small size of PFC, it can be easily injected intravenously and can also improve oxygen delivery in allogenic transfusion and tissue oxygenation (Spahn, 1999). Previous studies have shown antioxidant and anti-inflammatory properties of PFCs in different models (Nakata et al., 2007). We hypothesized that the toxicity of PQ leads to ALI through increased inflammatory cytokines and lipid peroxidation, histological changes, and impaired gas exchange and that PFCs may demonstrate a protective role against PQ toxicity. This study aimed to assess anti-inflammatory and protective effects of PFCs on oxidative stress and histological changes in the PQ-induced ALI models in rats.

2. Materials and methods

2.1. Materials

PQ (1,10-dimethyl-4, 40-bipyridinium dichloride) and PFCs (60% w/v perflubron-based formulation, AF0144) solutions were purchased from Sigma (St. Louis, MO, USA) and Double Crane Pharmaceuticals, Beijing, China, respectively.

2.2. Animals

The experimental protocols used in this study were approved by the Institutional Animal Care Committee of the Ilam University of Medical Science, Iran. Animals were prepared from vivarium section of Physiology Department, Ilam University of Medical Sciences, Iran. Experiments were conducted on 24 male Wistar rats (4–5 months old and weighting 200 ± 10 g) in this study. Animals were located individually in alternate cycles of 12-h light and darkness, at a room temperature of 22–23 °C and given free access to food and water ad libitum.

2.3. Experimental design

In this study, 24 Wistar rats were randomly divided into 4 groups including a control group ($n = 6$) injected by 10 mg/kg of 0.9% physiologic saline (NaCl 0.9%) intraperitoneally (I.P), PFC group ($n = 6$) injected by 6 mg/kg PFC via femoral vein, PQ or ALI group ($n = 6$) injected by a single dose of 20 mg/kg PQ (I.P) that was dissolved in NaCl 0.9% (Amirshahrokhi and Bohlooli, 2013) and PQ + PFC group ($n = 6$) injected by 6 mg/kg PFC emulsion via femoral vein, one hour after receiving PFC by the same dosage as mentioned above. Different studies have used dosages ranged from 2 to 30 mg/kg for PFC in different conditions. The selected dosage applied in the current study was 6 mg/kg based on the study performed by Shike et al. (2014) who reported the beneficial effects of this dosage on molecular and cellular pathogenesis of ALI/ARDS.

2.4. Bronchoalveolar lavage fluid (BALF) evaluation

BALF was collected according to a previously reported method (Xiaoyu et al., 2012). Briefly, after cannulation of the left trachea, fluid lavage was collected from the left lung with 4 mL phosphate-balanced saline solution in 2.5 mL aliquots. The collected BALF was centrifuged at $1000 \times g$ for 10 min and the supernatant was collected and stored at −70 °C. For total cell and PMN counts, first the cell pellet was eluted twice by phosphate buffer saline (PBS) and the total cell count was then performed via haemocytometer apparatus. In the next step, Wright–Giemsa stain was performed on the fluid sample for differentiation of cells. The number of PMN was measured by dividing the number of PMN by the total cell counts. Total protein was measured via the micro bicinchoninic acid (BCA, Pierce, Rockford, IL, USA) method. The level of lactate dehydrogenase (LDH) was measured according to the manufacturer's kit instructions (Roche Molecular Biochemicals, Mannheim, Germany) and was expressed as U/L.

2.5. Wet-to-dry weight (W/Dc) ratio assay

The rats were killed and the left lung was isolated. Then lungs were excised en bloc and dissected away from the heart and thymus. After blotting off blood and other contaminants, the wet weight of lung tissue was measured. The lungs were then dried in a 70 μC oven for 72 h and the ratio of wet/dry weight was used to quantify lung's water content.

2.6. Hydroxyproline (HP) assay

Hydroxyproline was assessed using calorimetric assay by the method reported by Murrell et al. (2008). According to this method, 10 mg of lung tissue was homogenized in 100 mL of water and transferred into a pressure-tight vial with PTFE-lined cap or 2.0 mL polypropylene tube. A total of 100 mL of concentrated hydrochloric acid (HCl, ~12 M) was then added cap tightly, and hydrolyzed at 120 °C for 3 h. A volume of 10–50 mL of the supernatant was then transferred to a 96 well plate. Chloramines T/Oxidation Buffer Mixture—100 mL was required for each reaction well. For each well, 6 mL of Chloramines T Concentrate was added to 94 mL of Oxidation Buffer and mixed well. Totally, 100 mL of the Chloramines T/Oxidation Buffer Mixture was added to each sample and standard well and incubated at room temperature for 5 minutes and the absorbance rate was measured at 560 nm (A560).

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