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Anti-inflammatory effect of thalidomide in paraquat-induced pulmonary injury in mice

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ABSTRACT

Thalidomide has been used in inflammatory and autoimmune disorders due to its anti-inflammatory activity. Paraquat (PQ) poisoning causes severe lung injury. PQ-induced pulmonary inflammation and fibrosis are due to its ability to induce oxidative stress, inflammatory and fibrotic reactions. This study was designed to evaluate the anti-inflammatory and anti-fibrotic effect of thalidomide on PQ-induced lung damage in a mouse model. Mice were injected with a single dose of PQ (20 mg/kg, i.p.), and treated with thalidomide (25 and 50 mg/kg/day, i.p.) for six days. Lung tissues were dissected six days after PQ injection. The results showed that thalidomide ameliorated the biochemical and histological lung alterations induced by PQ. Thalidomide decreased production of inflammatory and fibrogenic cytokine tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and transforming growth factor (TGF)-β1. In addition thalidomide reduced myeloperoxidase (MPO), nitric oxide (NO), and hydroxyproline content in lung tissue. Taken together, the results of this study suggest that thalidomide might be a valuable therapeutic drug in preventing the progression of PQ-induced pulmonary injury.

1. Introduction

Paraquat (1,1′-dimethyl-4,4′-bipyridinium chloride) is a widely used herbicide that can cause severe lung injury in humans. Exposure to PQ leads to accumulation of PQ in the lungs resulting in pulmonary edema, alveolar destruction, proliferation of bronchial epithelial cells and eventually fibrosis. Pulmonary fibrosis is a major hallmark and a leading cause of death in PQ poisoning [1,2].

Paraquat redox cycling and subsequent generation of reactive oxygen species (ROS), hydroxyl free radical ($HO^{\bullet}$) and peroxynitrite ($ONOO^{-}$) is the primary mechanism for initiating lung damage by PQ. These mediators induce intracellular transcription factors and then many proinflammatory agents including inducible nitric oxide synthase (iNOS), inflammatory cytokines, and cyclooxygenase (COX) all of which exaggerate the inflammatory process [3,4]. From the molecular aspects, nitric oxide (NO) and proinflammatory cytokines, particularly TNF-α, IL-6, IL-1β, and TGF-β1 may be the core of the pathogenesis of PQ-induced lung injury and fibrosis [4–6]. Although there is no effective therapy for PQ poisoning, anti-inflammatory drugs have been used in the clinical treatment of PQ-poisoned patients [7,8]. Generally, corticosteroids and immunosuppressive drugs are the mainstay of treatment for PQ-induced lung injury.

As mentioned above, it may be hypothesized that an effective treatment against PQ-induced lung injury and fibrosis should have considerable anti-inflammatory and anti-fibrotic effects. It has been reported that thalidomide as an anti-inflammatory agent is effective in the prevention of pulmonary inflammation and fibrosis in the experimental models [9–11]. Thalidomide (α-N-phthalimido glutarimide) is a glutamic acid derivative that was initially introduced as a sedative drug but was withdrawn from the market for its teratogenic effects. Thalidomide has various pharmacological properties, including immunomodulation, anti-inflammation and anti-angiogenesis. Clinical and experimental studies have demonstrated the efficacy of thalidomide or its analogs in the treatment of a variety of disorders including erythema nodosum leprosum, multiple myeloma, rheumatoid arthritis, Crohn's disease, prostate cancer and lupus erythematosus [12]. It has also been shown that thalidomide exhibits anti-inflammatory and anti-fibrotic activity by suppressing the production of proinflammatory cytokines, growth factors and nitric oxide which play an important role in the tissue destruction and fibrosis in chronic inflammatory situations [11,13–15].

In the present study, we examined whether thalidomide had protective effects on PQ-induced lung injury in mice. Furthermore, we investigated the mechanism underlying the therapeutic effect of thalidomide on pulmonary inflammation and fibrosis.

2. Material and methods

2.1. Animal and chemicals

Male Swiss albino mice, weighing 25–30 g, were housed in a room with a 12-h light/dark cycle. Animals were allowed free access to tap water and ad libitum food. All animal procedures were performed in

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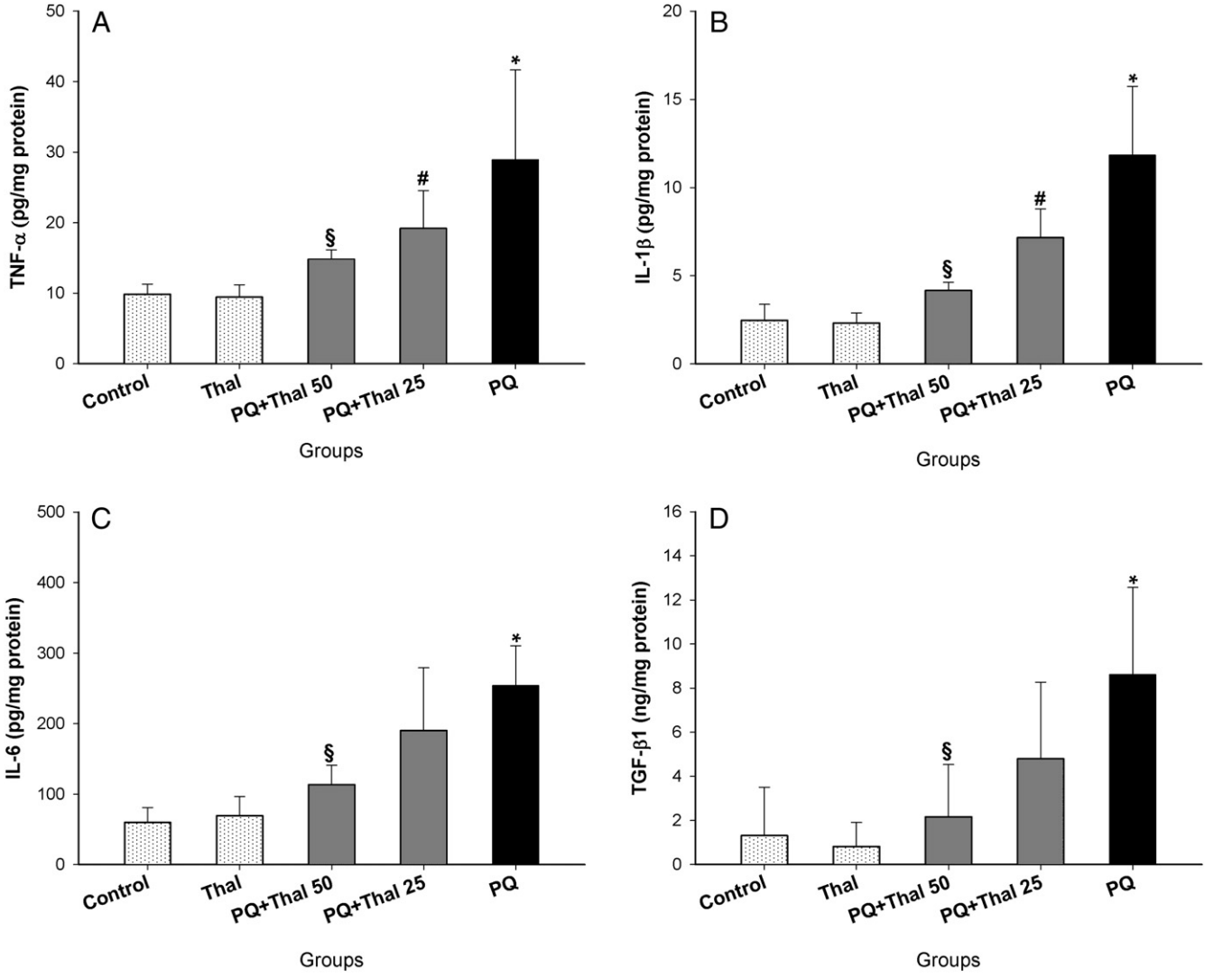

Fig. 1. Effect of thalidomide on PQ-induced production of TNF-α (A), IL-1β (B), IL-6 (C) and TGF-β1(D) in mouse lung tissues. Thalidomide treatment (50 mg/kg/day, i.p.) significantly reduced PQ-induced production of proinflammatory cytokines TNF-α, IL-1β, IL-6 and TGF-β1. Data are means ± SD (two replicates in each assay), n = 8. *$P < 0.001$ compared with normal group; §$P < 0.001$, #$P < 0.05$ compared with PQ group.

compliance with the "Guide for the Care and Use of Laboratory Animals" (National Academies Press, Washington, DC, USA, 1996). Thalidomide, tetramethylbenzidine, Tris–HCl buffer, dimethyl sulfoxide (DMSO), hydrogen peroxide (H_2O_2), hydroxyproline, chloramine T and p-dimethylaminobenzaldehyde were purchased from Sigma-Aldrich. Paraquat was purchased from Afrashimi Co. (Iran).

2.2. Experimental design

The animals were randomly divided into five groups (8 mice in each group), as follows: (1) Normal control group, mice received saline solution. (2) Thal group, mice received Thal (50 mg/kg/day, ip). (3) PQ group, mice received paraquat (20 mg/kg, i.p.). (4) PQ + Thal 25 group, mice received paraquat (20 mg/kg, i.p.) and thalidomide (25 mg/kg/day, i.p.). (5) PQ + Thal 50 group, mice received paraquat (20 mg/kg, i.p.) and thalidomide (50 mg/kg/day, i.p.). Paraquat was dissolved in saline solution (NaCl 0.9%) and injected intraperitoneally in a single toxic dose of 20 mg/kg of body weight. The dosage of PQ was based on our preliminary experiments showing induction of lung injury with lowest mortality. Thalidomide was dissolved in 0.5% carboxymethylcellulose and administered intraperitoneally for six consecutive days.

2.3. Tissue collection

At the end of the experiment, mice were anesthetized with ketamine and xylazine and their thoracic cavities were opened. One part of the right lung was fixed in formalin for histological examination, and the remaining lung tissues were immediately removed and washed in normal saline solution and frozen in liquid nitrogen.

2.4. Histological examination

Lung samples were fixed with 10% formalin embedded in paraffin and sections were stained with hematoxylin and eosin (H&E). The severity of lung damage was scored using the criteria as follows. Score 0 = no injury. Score 1 = alveolar inflammation without thickening of the alveolar septum. Score 2 = extensive alveolar inflammation and thickening of the alveolar septum. Score 3 = destruction of the alveolar spaces or extensive thickening of the airway/vessel wall [16].

2.5. Measurement of TNF-α, IL-1β, IL-6, and TGF-β1levels

The samples were homogenized in Tris–HCl buffer (pH = 7.4) containing protease inhibitors (trypsin and other serine and cysteine