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Full Length Article

Attenuation of Bleomycin-induced pulmonary fibrosis in rats by flavocoxid treatment

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ABSTRACT

Pulmonary fibrosis is a progressive fatal lung disorder with significantly high mortality rates. Bleomycin (BLM) is one of the most commonly used chemotherapeutic agents for treatment of several carcinomas. The most severe adverse effect of BLM is pulmonary toxicity; therefore, BLM has been repeatedly reported to be considered amongst the most widely used agents for induction of experimental pulmonary fibrosis. In the current study, flavocoxid has been investigated for its ability to ameliorate BLM-induced pulmonary fibrosis. BLM was instilled intratracheally and flavocoxid was administered orally (20 mg/kg) for 5 weeks; one week pre- and 5 weeks post BLM instillation. Flavocoxid significantly decreased lung/body weight index, BALF's lactate dehydrogenase activity, total protein content and total cell count, lymphocyte and neutrophil counts. Flavocoxid significantly decreased lung MDA content, increased lung GSH content, SOD activity, serum total antioxidant capacity and decreased lung NO content. Moreover, flavocoxid reduced lung content of IL-10. In addition, flavocoxid significantly ameliorated histological changes and prevented collagen deposition with paralleled decrease in lung hydroxyproline content. In conclusion; flavocoxid can be proposed to be a potential therapeutic agent for management of pulmonary fibrosis.

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1. Introduction

Pulmonary fibrosis is a progressive lethal lung disorder. It is the end stage of a wide range of lung inflammatory conditions. Loss of alveolar structure, accretion of myofibroblasts, remodeling of lung parenchyma and excessive extracellular matrix depositions are the main characteristic features of pulmonary fibrosis [1].

Pulmonary fibrosis is amongst the most common interstitial lung diseases affecting over 5 million individuals worldwide with a mean survival time of about 3 years [2]. In spite of extensive researches, there are no reports about any medication that can significantly ameliorate pulmonary fibrosis [3]. The only current effective approach is lung transplantation. Therefore, the search for novel drugs with significant efficacy and tolerability for the pulmonary fibrosis is inevitable [4].

Pulmonary fibrosis can developed as an adverse toxic effect of anti-neoplastic drugs such as bleomycin (BLM). Apart from this, cigarette smoking and inhaling mineral dusts/asbestos are added factors implicated in its pathogenesis [5].

Increased evidences suggest that pulmonary fibrosis mainly developed post alveolar epithelial injury and abnormal wound healing involving inflammation and T-helper type 2 cytokines, epithelial apoptosis and absence of appropriate re-epithelialization, fibroblast-myofibroblast migration and proliferation, epithelial mesenchymal transformation, and excessive extracellular matrix (ECM) deposition [6].

BLM is a chemotherapeutic antibiotic used for management of lymphomas, testicular cancer, and carcinomas amongst several types of tumors. It has been reported to induce marked functional and biochemical changes promoting pulmonary fibrosis [7]. Bronchial metaplasia, reactive macrophages recruitment, atypical alveolar epithelial cells, fibrinous edema, and interstitial fibrosis are amongst microscopical changes can be induced by BLM [8].

BLM-induced pulmonary fibrosis in rats and mice has been reported to be a useful tool to study mechanisms involved in the progression of human pulmonary fibrosis and the impact of various drugs on its progression. BLM induces reactive oxygen species (ROS) generation, which binds to DNA causing DNA damage, postu-

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lated to initiate inflammatory and fibro-proliferative responses. Furthermore, BLM is reported to promote the depletion of endogenous antioxidant defences, exacerbating oxidant mediated tissue injury [9].

Flavocoxid is a mixture of two flavonoids catechin and baicalin [10]. It is marketed as an FDA-regulated medical food, for management of osteoarthritis in the United States. Flavocoxid is the only currently marketed prescribed anti-inflammatory agent that modulates cycloxygenase (COX) enzymes via an anti-peroxidase activity. It also inhibits 5-lipoxygenase (5-LOX)-mediated leukotrines (LT) production. Flavocoxid has a wide range of strong antioxidant activities mainly via down-regulation of inducible inflammatory gene expression and neutralization of ROS, preventing the conversion of arachidonic acid to oxidized lipids [11].

The current study protocol focused on the evaluation the ability of flavocoxid to attenuate BLM-induced pulmonary fibrosis in a rat model and to draw a schematic conclusion about mechanisms involved.

2. Materials and methods

2.1. Experimental animals

Adult male Sprague Dawely rats (170–220 g) were purchased from Merck Research Center, Faculty of medicine, Mansoura University; they were kept under constant environmental and nutritional conditions throughout the experimental period. The research protocol complies with the ethical guidelines of experimental research; "Research Ethics Committee", Faculty of Pharmacy, Mansoura University, Egypt in accordance with "Guide for the Care and Use of Laboratory Animals", 1996.

2.1.1 Drugs and chemicals

Flavocoxid was purchased from Pimus pharmaceutical Inc. (Scottsdale, AZ, USA) and BLM was purchased from Nippon Kayaku Co. (Ltd., Tokyo, Japan). Ehrlich's reagent (*p*-dimethylaminobenzaldehyde), Chloramine-T, n-Propanol, Perchloric acid and thiopental sodium were purchased from Sigma Aldrich chemical Co. (St. Louis, MO, USA).

2.2. Experimental protocol

2.2.1. Induction of pulmonary fibrosis

Pulmonary fibrosis was induced by intratracheal instillation of BLM (5 mg/kg) as sulfate salt dissolved in 0.1 ml of normal saline [12]. Rats were anesthetized using thiopental sodium (20 mg/kg body weight, I.P.). A midline incision was made in the neck, the trachea was exposed and BLM was instilled. Rats were kept in vertical position and rotated several times to ensure uniform distribution of BLM within the lung tissues. The incision was surgically sutured sodium fusidate 2% was applied topically to the wound.

2.2.2. Animals grouping

Rats were randomly allocated to three experimental groups (12 rats/group) as follows: Normal control; 0.1 ml of normal saline was instilled to the trachea as previously described and rats received 0.2 ml of 0.5% carboxymethylcellulose (CMC) orally once daily for 5 weeks, BLM control: BLM was instilled intratracheally (5 mg/kg) and rats received 0.2 ml of 0.5% CMC orally once daily for 5 weeks, flavocoxid treated group: rats were treated with flavocoxid (20 mg/kg, oral) suspended in 0.5% CMC daily for one week prior to BLM instillation and for further 4 weeks post instillation for an overall period of five weeks of flavocoxid administration.

Four weeks post BLM instillation; rats were deeply anesthetized with thiopental sodium. Blood samples were collected via puncture of retro-orbital venous plexus, sera were separated and used immediately for biochemical assessments. Lungs were harvested, rinsed in ice-cold saline and weighed for calculation of lung/body weight index. The left lobes from all the lungs were isolated for preparation of lung homogenate and the right lobes were separated for histopathological examination.

2.2.2.1. Collection of bronchoalveolar lavage fluid (BALF). The thoracic cavity was opened and the tracheas were exposed, cannulated and 6 ml sterile 0.9% saline (3 times, 2 ml/time) were slowly infused into the lungs. 50–70% recovery was retrieved after compressing the chest gently several times. BALF was centrifuged at 2000 rpm, 4 °C for 10 min using cooling centrifuge. The sedimented cell pellets were pooled and re-suspended in 500 µl of sterile saline to quantify inflammatory cell contents; total and differential cell counts.

2.3. Assessment of BALF total protein content and lactate dehydrogenase (LDH) activity

Total protein content was assessed according to the method of Smith, Krohn [13] using commercial kit (Thermo Scientific, Rockford, USA) as instructed by manufacturer. Enzymatic LDH activity was assessed using commercial kit (Human diagnostics, Wiesbaden, Germany) according to Henry [14] as instructed by manufacturer.

2.4. Preparation of lung homogenate and biochemical assessment of nitric oxide (NO), malondialdehyde (MDA), reduced glutathione (GSH) contents and superoxide dismutase (SOD) activity

The isolated left pulmonary lobes were rinsed, weighed and homogenized in KCl (1.15%, pH 7.4) to yield 10% w/v tissue homogenate [15]. The homogenate was centrifuged at 2000 rpm, 4 °C for 15 min, and the supernatant was separated and used immediately for assessment oxidative/antioxidative stress biomarkers (MDA, GSH and SOD) as well as nitric oxide using commercially available Biodiagnostic assay kits (Giza, Egypt), as instructed by manufactures according to methods described by Ohkawa, Ohishi [16], Ellman [17], Marklund and Marklund [18] and Montgomery and Dymock [19] respectively.

2.5. Biochemical assessment of serum total antioxidant capacity (TAC)

Serum total antioxidant capacity was determined using commercially available kits (Bio-diagnostic, Giza, Egypt)) according to the supplied manufacturer's instructions as described by [20].

2.6. Assessment of lung interleukin-10 (IL-10) content

Lung content of IL-10 was quantilified using commercially available enzyme-linked immunosorbent assay (ELISA) kit (Uscn Life Science, INC. USA), according to the supplied manufacturer's instructions.

2.7. Quantification of lung hydroxyproline and collagen content

Lung hydroxyproline content was determined using colorimetric method described by Bergman and Loxley [21]. Lung collagen content calculated by multiplication of hydroxyproline content by 13.5 [22].

2.8. Histopathological examination of hematoxylin-eosin (H&E) and Masson's Trichrome stained lung specimen

The right upper pulmonary lobe was harvested, rinsed with ice-cold saline and fixed in 10% neutral-buffered formalin, embedded

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