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Tamoxifen citrate: a glimmer of hope for silicosis

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ABSTRACT

Background: Inhalation of crystalline silica nanoparticles causes pulmonary damage resulting in progressive lung fibrosis. Currently, there is no effective treatment for silicosis. Tamoxifen citrate is a selective estrogen receptor modulator, which is one of the adjuvant treatment choices for breast cancer. It is also known with its inhibitory effect on the production of transforming growth factor-beta (TGF-β) and studied for the anti-fibrotic effect in some fibrotic diseases. The aim of the study was to determine the effect of tamoxifen citrate on the prevention of pulmonary fibrosis and the treatment of silicosis.

Methods: A total of 100 adult female Wistar Albino rats (200–250 g) were used in this study. The rats were divided into five groups including 20 rats in each. Rats were exposed to silica for 84 d in all groups. In group 1, rats were sacrificed on the day 84 without receiving treatment. In group 2, rats received 1 mg/kg tamoxifen (tmx1 + 1), from the first day of the study for the whole 114 d of the study. In group 3, (tmx10 + 10) rats were given 10 mg/kg tamoxifen from the first day of the study for the whole 114 d of the study. In group 4 (tmx1), rats were started 1 mg/kg of tamoxifen on day 84 and were given until day 114. In group 5 (tmx10), rats were fed with 10 mg/kg tamoxifen starting from day 84 to day 114. All rats except group 1 were sacrificed on 114 day of the study. Lung inflammation and fibrosis scores, serum TGF β levels, lung smooth muscle antigen and tissue transforming growth factor β (t-TGF-β) antibody staining levels, and number of silicotic rats were compared between groups.

Results: Silicosis was caused successfully in all rats in group 1. There were six silicotic rats in group 3 and it was the lowest number of all groups. Plasma TGF-ß levels and fibrosis

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score were significantly lower in all groups when compared with the control group. Tamoxifen could have preventive or treating effects in silicosis and found that lung fibrosis score was significantly lower in rats treated with tamoxifen.

Conclusions: Tamoxifen treatment after and/or before induction of silicosis decreased lung fibrosis score with blood TGF-β levels. We hope that this study may introduce a new indication as prophylactic use of tamoxifen in high-risk groups for silicosis and for treatment of silicosis.

1. Introduction

Inhalation of crystalline silica nanoparticles causes pulmonary damage resulting in progressive lung fibrosis. Currently, there is no effective treatment for silicosis. Silica has a wide range of industrial applications: quarries, sand blasting, chemical mechanical polishing cosmetics, printer toners, varnishes, and food preparation [1,2]. Tamoxifen citrate has been used for the treatment of breast cancer for its selective estrogen receptor modulator action. It also inhibits the production of transforming growth factor-beta (TGF-β) and insulin-like growth factor [2,3]. TGF-β has important roles in the formation of fibrosis such as the modulation of inflammation, wound repair, and immunity by decreasing lymphocyte proliferation and encouraging growth of fibroblasts [4]. These effects have been studied in postoperative adhesions [5], keloid fibroblast proliferation, and reducing collagen production [6,7]. In this study, we aimed to investigate possible effects of tamoxifen on progression, prevention, and treatment of the fibrotic pulmonary silicosis disease considering the anti-fibrotic effect shown in aforementioned studies.

2. Material and methods

A total of 100 adult female Wistar Albino rats (200–250 g) were housed in wire cages under stable temperature (21 ± 2°C) with a 12-h light–dark cycle for 114 d. The animals were allowed free access to water and standard rat chow. The rats were divided into five groups, 20 rats in each. All the rats were exposed to silica for the first 84 d. The exposure characteristics of the rats in all five groups are given in Table 1. All the rats fed with tamoxifen with oral gavage.

Crystalline silica (approximately 98%, between 40 and 100 nm) was purchased from MMR Refrakter Co (Konya, Turkey). Rats were exposed to silica via dry air compressor released to the cage with a volume of 10 m^3 and were inhalated 6 h a day and 5 d a week for 12 wk and the rats were followed up for 84 d. On 1st, 42th, and 84th d, nonspecific respirable dust concentrations and respirable crystalline silica concentrations were measured with gravimetric method [8]. Exposure concentration was 23.375 mg/m^3, particle composition >98.5% crystalline silica, particle size 40 and 100 nm, and mean respirable crystalline silica concentration level was 0.268 mg/m^3–2.39%.

All animals were anesthetized by intramuscular injection of 30 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, Istanbul) and 5 mg/kg xylazine (Rompun, Bayer, Istanbul). After the rats were anesthetized, the thorax was shaved and povidone iodine (Repithel Mundipharma GmbH -Limburg, Germany) was applied.

Under sterile conditions, thoracotomy was performed. Lung samples were obtained from the lower lobe of the right lung together with the hilar lymphoid structures. Blood samples were centrifuged at 30,000 rpm for 5 min at 4°C. Then the serum was aspirated and stored at −80°C until analyzed.

The tissue samples from the right lung were evaluated histopathologically for fibrosis and also for the presence of inflammation. The histopathologic analysis was carried out in the Pathology Department of Kecioren Research and Training Hospital. Histopathologic examination was performed by using a light microscopic analysis. The samples obtained from the lower lobe of the right lung were fixed in 10% neutral buffered formalin solution for 2 d. Tissues were washed in running water and were dehydrated with increasing concentrations of ethanol (50%, 75%, 96%, and 100%). After dehydration, specimens were placed into xylene to obtain transparency and embedded in paraffin. Embedded tissues were cut into 5-μm-thick sections and were stained with hematoxylin and eosin and trichrome. Histopathologic examinations were performed by a pathologist blinded to exposure status and scored for fibrosis and inflammation using a ×4 objective. Each field was assessed for severity of fibrosis and inflammation, and then graded (Fig. 1). If normal tissue predominated in the field without the fibrosis, it was classified as A. Mild–moderate fibrous thickening of bronchial and alveolar walls was placed in the category of B. Increased fibrosis with formation of fibrous bands was classified as C. The observer graded the inflammation into no, mild, and severe categories according to the extent and the severity of the pulmonary inflammation. The lung tissue without inflammation classified as D; mild peribronchial and alveolar inflammation as E, and severe and large inflammatory areas classified as F.

Tissue sections were taken on poly-L-lysine coated slides. After deparaffinization, they were stained with SMA (smooth muscle actin) and TGF-beta antibody staining levels by immunohistochemistry. Histopathologic examination was performed by using a light microscopic analysis. After checking the staining intensity, photographs were taken.

2.1. *Immunohistochemistry procedure*

Deparaffinization of the tissue was provided via incubating the slides for 1 h at 70 C in the oven. Then tissues were kept for half hour in heated xylene and then removed from the oven for cooling process at room temperature for 10 min. Samples were kept in 90% alcohol for 1 min, in 70% alcohol for 1 min, in 50% alcohol for 1 min, and in distilled water for 1 min.