Ameliorating effect of Alteplase in bleomycin-induced pulmonary fibrosis in adult rats

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Abstract

Fibrosis is the formation of excess fibrous tissue in tissue in a reparative process. Pulmonary fibrosis (PF) characterized by interstitial lung damage with tissue fibrosis, and loosing of lung elasticity. The using of bleomycin as anticancer drug associated with high incidence of PF. The current research was designed to investigate the ameliorating effect of alteplase in bleomycin-induced pulmonary fibrosis in adult rats. Alteplase 0.9mg/ kg i.p. as a single dose daily for 3weeks, significantly ameliorated the decreased serum levels of SOD and GSHpx, and significantly decrease the elevated level of hydroxyproline in bleomycin (BLM) treated rats. Sections of lung of BLM treated rats showed extensive damage of the lung tissue with loss of the normal alveolar architecture and the majority of the alveolar walls were occupied by collagenous fibers with diffuse cellular infiltration, as well as bands of collagen fibers, with an increased macrophages and fibroblast cells (figure 3). The sections of the lung of rats treated by alteplase in combination with (BLM), revealed that fibrotic lesions appeared less, with mild inflammatory cells, minimum blood vessels congestion and most parenchyma tissue appeared similar to normal structure. These results clearly confirmed the protective role of alteplase in pulomary fibrosis associated with (BLM) therapy. So, the findings may be offer one of therapeutic strategy to prevent the lung fibrosis associated with anticancer therapy.

Keywords: Alteplase, Bleomycin, Pulmonary fibrosis, rats, GSH,SOD, HYP

Introduction

Fibrosis is the formation of excess fibrous tissue in tissue in a reparative process (1). Pulmonary fibrosis (PF) characterized by interstitial lung damage with tissue fibrosis, and loosing of lung elasticity. PF may be secondary to a wide range of diseases or may be idiopathic with no known underlying cause. Furthermore, there were three types of lung tissue fibrosis: Replacement fibrosis secondary to lung damage e.g. infarction, tuberculosis and pneumonia, focal fibrosis in response to irritant e.g. coal dust and silica and diffuse parenchymal lung disease (DPLD), which occurs in fibrosing alveolitis (IPF) and extensive allergic alveolitis (2). There are numerous different methods for modeling (PF) and/or to induce lung tissue damage in animal models to get a realistic picture of human disease, these are pathologic similarities between fibrotic reactions in human and rodent lungs, and as such, animal models present an excellent tool to investigate pathologic changes in vivo. (3-4). However, different animal models of (PF) have been developed to investigate potential therapies for (PF). Among them chemicals induction by bleomycin (BLM), represents the most common model in rodents (mouse, rats and hamster) (3). Most of pulmonary fibrosis inducing models were stimulated lung-injury in response to oxidative stress.

Bleomycin, the anticancer drug, caused lung cell damage and fibrosis by inducing lipid peroxidation, independent from its effect on DNA. Therefore, one of the serious side effects of BLM in humans has been observed in the lungs, which may progress to fatal diffuse (PF) (5-8).

This research was designed to investigate the ameliorating effect of alteplase in bleomycin-induced pulmonary fibrosis in adult rats

Materials and methods

Animals

Male Wister Albino Wister rats (*Rattus norvegicous*) weighing 200-250 g were obtained from animal center of Thi-Qar University/College of Science . All animal were given food and tap water *ad libitum*, temperature (22±3) °C with an alternating cycle of 12-h light and dark.

Experimental design

Animals were equally divided into four groups (10 rats each). Rats in the group 1 (Control group) received sterile saline solution (the vehicle) 0.5ml/animal/daily i.p. Group 2 received bleomycin 15mg/ kg/ i.p. three times weekly for 3 weeks. Group 3 received alteplase 0.9mg/ kg i.p. as a single dose daily for 3weeks (Benoitet al., 2010). Group 4 received bleomycin and alteplase simultaneously at doses, periods and administration similar to that mentioned in the groups 2 and 3. All the treatments were dissolved in normal saline and were administered for 3 weeks (9). In the end of the experiment, the animals were starved for 12hrs and the rats were anaesthetized with chloroform, venous blood samples were collected by direct heart puncture into sterilized tubes. Sera were stored in deep freezer.

Estimation of GSHpx, SOD and HYP

The serum glutathione peroxidase (GSHpx), superoxide dismutase (SOD) and hydroxyproline (HYP) were measured on the basis of the instructions of kits (Cusabio Eliza kit, catalo no. CSB-E 121144r.)

Histopthological examination

Lung tissue samples were obtained from sagittle slices of the lungs, fixed in 10% formalin for 24 and then washed, dehydrated, cleared and embedded in paraffin, sections about 4μm were stained with Hematoxylin -Eosin for microscopic examination.

Statistical analysis

Differences among groups were determined using one-way ANOVA. Differences were considered statistically significant at $P<0.05$.

Results

The serum level of SOD in the control was 9.42±1.08 Pg/ml, administration of alteplase didn't induce significant changes in the serum SOD (9.270±0.591 Pg/ml), however, SOD was significantly decreased in bleomycin group (7.77±0.644 Pg/ml) ($P<0.01$), while administration of alteplase with bleomycin significantly restored the serum SOD level to the normal. The level of seum GSHpx was also decreased significantly in bleomycin treated group [17.04±1.52 milli international unit (MIU/ml)] compared with control (33.18±3.444 MIU/ml) ($P<0.001$), while alteplase didn't affected the level of GSHpx when administered alone (30.62±3.92 MIU/ml) but it restored the level of GSHpx to normal limit when administered with bleomycin (30.07±2.00 MIU/ml). On the other hand, the serum level of hydroxyproline was 1.44±0.20 μg/ml in the control group, it was didn't change by alteplase (1.67±0.19 MIU/ml), but it was significantly increased by bleomycin (2.86±0.12 MIU/ml) ($P<0.0001$), while alteplase significantly ameliorated the effect of bleomycin on hydroxyproline (1.54±0.34 MIU/ml) (table 1).

The microscopic observations of the rat lung sections of control group revealed normal architecture, spongy appearance of the lung with thin alveolar septa, clear alveolar cavities and normal alveolar

ducts. The lung tissue appeared normal and alveoli were homogeneously distributed. The interalveolar septa were formed from the epithelial tissue lining of the alveoli and loose connective tissue contained extensive capillary network around the alveoli. The alveoli ducts have numerous alveoli opening along their length and the alveolar sacs occurred at the termination of the alveolar ducts (Figure 1). The histological examination on lung sections of rats treated with alteplase, also showed normal histological features similar to the sections of control group (figure 2).

Sections of lung of BLM treated rats showed extensive damage of the lung tissue with loss of the normal alveolar architecture and the majority of the alveolar walls were occupied by collagenous fibers with diffuse cellular infiltration, as well as bands of collagen fibers, with an increased macrophages and fibroblast cells (figure 3). The sections of the lung of rats treated by alteplase in combination with (BLM), revealed that fibrotic lesions appeared less, with mild inflammatory cells, minimum blood vessels congestion and most parenchyma tissue appeared similar to normal structure (figure 4 & 5).

Table 1: Effect of alteplase on superoxide (SOD), glutathione peroxidase (GSHpx) and hydroxyproline (HYP) in blood serum of rats with pulmonary fibrosis induced by bleomycin.

Groups	SOD Pg/ml	GSHpx MIU/ml	HYP µg/ml
Control	9.42±1.08[a]	33.18±3.44[a]	1.44±0.20[a]
Bleomycin	7.77±0.644[b]	17.04±1.52[b]	2.86±0.12[b]
Alteplase	9.27±0.591[a]	30.62±3.92[a]	1.67±0.19[a]
BLM+Alteplase	9.08±1.09[a]	30.07±2.00[a]	1.54±0.34[a]

Vertically similar letter means not significant

Discussion

Pulmonary fibrosis is a common side effect caused by antineoplastic drugs**.** This study was designed to evaluate the protective effect of alteplase. Different animal models of pulmonary fibrosis have been developed to investigate potential therapies for pulmonary fibrosis (PF). Bleomycin-induced lung fibrosis possesses classical inflammatory pattern and is the most common model for PF In rodents (mouse, rat and hamster). Usuki and Fukua (10) reported that administration of bleomycin to rodents produce histological alterations nearly similar to those found in human pulmonary fibrosis.

As in the current results, the previous studies also showed that rat serum hydroxyproline was significantly increased, in bleomycin induced pulmonary fibrosis in rats (11-15). Furthermore, bleomycin induced significant deleterious changes in antioxidant status. The decreased antioxidant status in this study was also recorded by many workers (12, 16). They found that the balance between oxidants and antioxidants was one of the important conditions to maintain the lung normal homeostasis and protect the lung from many pathological conditions and promote normal repair.

The histopathological lesions appeared with bleomycin in this study were similar to that recorded by many authors (17-18). The histopathological lesions induced by bleomycin confirmed that injurious effects of bleomycin. Bleomycin–treated animals showed prominent parenchymatous disruption manifested by the appearance of over expanded alveoli with thinning, destruction of the interalveolar septa and connection of many alveoli together. These finding, were similar to the picture of emphysematous lung previously described (19). These could be attributed to proteolytic parenchyma destruction. Furthermore, it was reported that damage of the interstitial tissue could be followed by alveolar and interstitial edema, inflammation and breakdown of connective tissue (20-21), all these findings matched with the current histological results. In current study, the treatment with alteplase has been proven beneficial as it attenuated lung pathology induced by bleomycin. Where, it reduced pulmonary inflammation, reduced extensive areas of collagen deposition whether interalveolar, around alveoli or peribronchioleoli, around blood vessels and subpleura zone. It also decreased inflammatory cells infiltration, re-epithelization of cellular lining alveoli. The effect of alteplase could be attributed to its fibrin dissolving characteristics and its antioxidant effects in BLM-induced pulmonary damage, where ROS led to depletion of glutathione and oxidant/antioxidant imbalance, subsequently. Alteplase blocked this process and eventually improved the case of the lung.

Conclusion

According to the current results, alteplase decreased hydoxyproline which elevated in pulmonary fibrosis induced by bleomycin. It also increase the antioxidant status which decreased by bleomycin. Furthermore it ameliorated the histological alterations in the lung. Accordingly, the results confirmed the protective effects of fibrinolytic drugs in pulmonary fibrosis.

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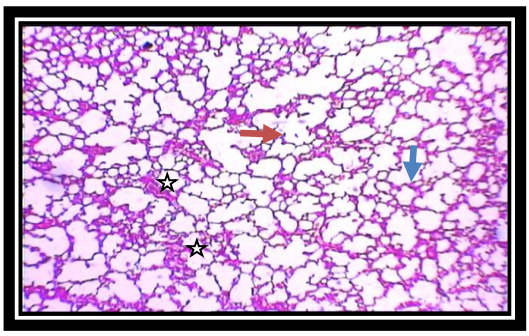

Figure1: Section in normal lung tissue showing that alveoli were homogeneously distributed ( ) with normal interalveolar septa ( ) and capillary network around alveoli ( ) **(H&E 40X)**.

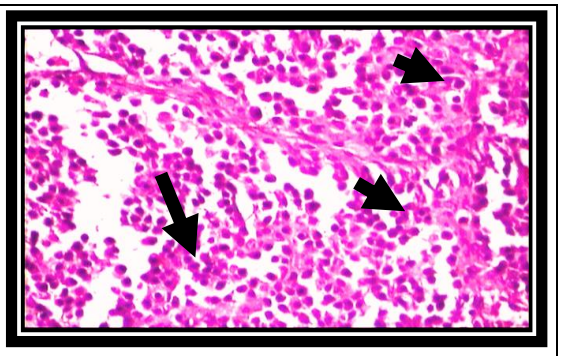

Figure 2: Section of the lung of rats treated with alteplase showed heavily invasion of lung tissue with mononuclear cells prominently macrophages ( ) , lymphocytes with fewer other inflammatory cells, also fewer fibroblast infiltrate interalveolar septa **(H&E. 400X).**

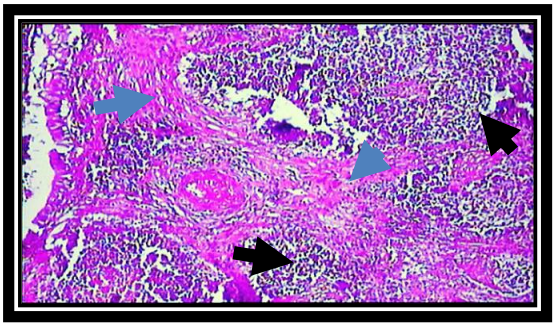

Figure 3: Section of the lung of bleomycin-treated rat, revealed an increase in cellularity of alveolar septum, look-like lymphatic nodule ( ) and intra-alveolar fibrosis with collagenous bands accompanying great septal thickness ( ) **(H&E. 100X).**

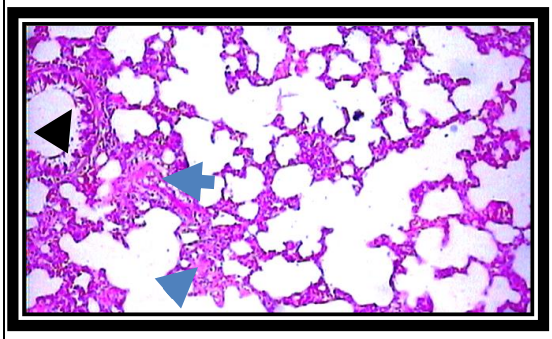

Figure 4: Section in the lung of rat treated with (bleomycin and alteplase) showed mild inflammatory cells, less than other groups, limited areas of fibrosis ( ) and collapse, with clear lumen of bronchioles lining with normal folded epithelium ( ) **(H&E. 100X).**

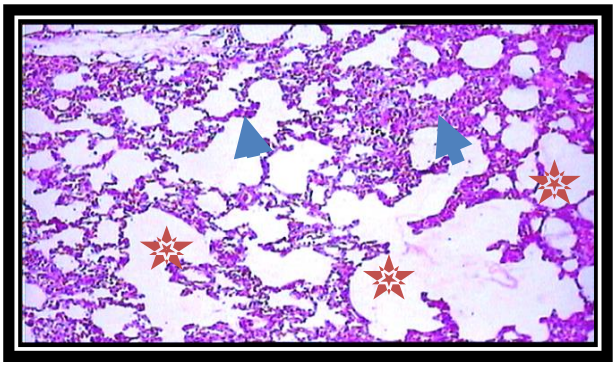

Figure 5: Section in the lung of rat treated with (bleomycin and alteplase) showed more patent alveolar space ( ) and reduced both inflammatory cells infiltration and thickness of the interalveolar septa (**H&E. 40 X).**