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Research Article

Ameliorating effect of urokinase in bleomycin- induced pulmonary fibrosis in rats

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ABSTRACT
Pulmonary fibrosis (PF) is a chronic lung disorder that may be produced by infections, toxic agents, immune-mediated, or might be idiopathic. It frequently progressive with marked interstitial lung damage, ultimately fibrosis, and loss of the lung elasticity ended by respiratory failure.The objective of the study is to investigate the protective effects of urokinase administration during bleomycin treatment.A total of 32 male adult albino rats were equally distributed into four groups and treated as follow, Group1: rats received 0.5 ml normal saline / rat/ day ip., to serve as control. Group2: rats received bleomycin 15mg/kg bw ip., three times weekly for 8wks. Group3: rats received urokinase (uPA)10mg/ kg bw as a single dose daily for 8wks. Group4: rats received bleomycin and urokinase simultaneously at doses, route and periods similar to groups 2 and 3 mentioned above.At the end of the experiment, rats were anaesthetized with chloroform and venous blood samples were collected for estimation of serum SOD, GSHpx and HYP. After determination of relative lung weight, lung tissue samples were taken for histological examination. The current study showed that the lungs relative weight (mg/100g body weight) was significantly increased ($P<0.01$) in BLM treated group in comparison with control group. Urokinase alone did not induced significant changes in the lung relative weight($P<0.31$) in comparison with control. However, when it used with BLM, it significantly decreased ($P<0.01$) the lung relative weight in comparison to BLM group. On other hand, bleomycin significantly increased ($P<0.001$) seum HYP level, while, urokinase, in combination with BLM significantly ameliorated ($P<0.03$) HYPlevel. Histologically, the lungs of rat treated with bleomycin and urokinase showed reduced both, inflammatory cells infiltration and thickness of the interalveolar septa, and reduction of interalveolar deposition of collagen compared to bleomycin group. The current study revealed that the urokinase amelioratedthe pulmonary fibrosis induced by bleomycin in rats, and its use in combination with bleomycin significantly changed the biochemical parameters and reduced the histological lesions induced by bleomycin.

Keywords: Urokinase, bleomycin, pulmonary fibrosis, hydroxyprolin

INTRODUCTION

Pulmonary fibrosis (PF)is a chronic lung disorder that may be produced by infections, toxic agents,immune-mediated, or might be idiopathic.It frequently progressive with marked interstitial lung damage, ultimately fibrosis, and loss of the lung elasticity ended by respiratory failure (1).Bleomycin (BLM) is a common fibrogenic agent, provoking an initial adult respiratory distress syndrome-like injury with subsequent strong fibroproliferative response,severe abnormalities of the alveolar surfactant system accompanied by epithelial metaplasia (2).Furthermore, bleomycin is capable to cause cell damage independent from its influence on DNA, by inducing lipid peroxidation (3). Low doses of BLM can produce diffuse (PF) (4). The pathogenesis of PF is complex and the urokinase-type plasminogen activator (uPA)/plasminogen system participates in the repair process, where the balance between the activating enzyme uPA, and its inhibitor PAI-1, is a critical determinant of the amount of scar development that follows (5).During acute and chronic inflammatory lung diseases, the normal fibrinolytic activity in the alveolar space is inhibited by increased levels of plasminogen activator inhibitor 1 (PAI-1). Transgenic mice having increased fibrinolytic activity due to genetic deficiency of PAI-1 develop less fibrosis after bleomycin-induced lung inflammation (6). Therefore, using uPA may be a promising new therapeutic strategy in PF (7). In this study, a mouse model of bleomycin-induced pulmonary fibrosis was carried out to declare the prophylactic impacts of urokinase on BLM-induced PF in rats.

METHODS

Animals: A total of 32 male albino Wister rats (*Rattus* norvegicous), 175-225 gm, were used in this study. They were obtained from animal center of Thi-Qar university,College of Science. All animal were housed in conditioning room(22±3°C), in polypropylene cage andallowed to take food and received tap water *ad libitum*.

Drugs and kits: Bleomycin (BLM) was purchased from Sigma Chemicals company. Urokinas-plasnogen activator (uPA) from Boehringer Ingeheim, Germany and serum (HYP) kit from Cusabio Eliza, China.

Experimental design

Rats were equally distributed into four groups and treated as follow:

Group1:rats received 0.5 ml normal saline / rat/ day ip., to serve as control.

Group2:rats received bleomycin 15mg/kg bw ip., three times weekly for 8wks.

Group3:rats receivedurokinase(uPA)10mg/ kg bw as a single dose daily for 8wks.

Group4:ratsreceived bleomycin and urokinase simultaneously at doses, route and periods similar to groups 2 and 3 mentioned above.

The animals were starved over night for 12hrs and were anaesthetized with chloroform and venous blood samples were collected for estimation of serumHYP.

Histopthological examination: Lung tissue samples were fixed in 10% formalin, dehydrated, embedded in paraffin and 4µm thickness slices were stained with Hematoxylin and Eosin for examination.

Statistical analysis: The analysis of variance (ANOVA) was carried out to significant variations between groups.

RESULTS

As shown in table 1, the lungs relative weight (mg/100g body weight) was significantly increased ($P<0.01$) in BLM treated group in comparison with control group. Urokinase alone not induced no significant changes in the lung relative weight in comparison with control. While, when urokinase was used with BLM, it caused significant decrease($P<0.01$) in the lung relative weight in comparison to BLM (table 1).

BLM was significantly elevated (HYP) ($P<0.001$)in comparison with control group. Urokinase significantly decreased ($P< 0.05$) HYP level in healthy animals in comparison to (BLM) group, and it also significantly reduced the elevated level of (HYP) when used in combination with (BLM) in comparison to (BLM) alone groups (table 2).

Thehistopathological examination, revealed that sectionsof rat lung frombleomycin induced pulmonary fibrosis group showed extensive multiple infiltration areas of mononuclear inflammatory cells(Figure 1,B), multifocal areas of fibrosis and inflammatory exudates(Figure 1,C). Furthermore,in some sections of the lung of bleomycin –treated rat showed prominent multifocal areas of fibrosis with huge infiltration of mononuclear, inflammatory cells mostly macrophages(Figure 1,D) in comparison with normal lung histological appearance in control group (Figure 1, A). The lungs of rat treated with (bleomycin and urokinase) showed more patent alveolar space and reduced both, inflammatory cells infiltration and thickness of the interalveolar septa, and reduction of interalveolar deposition of collagencompared to bleomycin group (Figure 1, E &F).

DISCUSSION

Fibrosis is one of the major adverse effects of bleomycin in human cancer therapy. Different animal models of pulmonary fibrosis have been developed to investigate potential therapies in pulmonary fibrosis, but bleomycin is the most common model for induction of pulmonary fibrosisin rodents (mouse, rat and hamster) (8-11). Therefore, the current study was performed to evaluate the protective effect of urokinase in rat pulmonary fibrosis induced by bleomycin.

Many authors (10-11) mentioned that administration of bleomycin to rats produce pathological (included stimulation inflammatory mediators and cytokines) and histological alterations nearly similar to those found in human after bleomycin therapy.The current study showed that the lungs relative weight (mg/100g body weight) was significantly increased ($P<0.01$) in BLM treated group, it also significantly increased ($P<0.001$) seum HYP level. Histologically, it caused extensive multiple infiltration areas of mononuclear inflammatory cells multifocal areas of fibrosis and inflammatory exudates in the intraterminal bronchiolar. All these pathological and histological changes were significantly ameliorated by urokinase.The pathological changes induced by bleomycin could be attributed to stimulation of many pathological mediators. Among the several cytokines and chemokines that have been implicated in the pathogenesis of lung fibrosis, interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) through induction of the secretion of PDGF, were participated in pathogesesis of the disease. In both human and bleomycin induced pulmonary fibrosis, there was overexpression of IL-1β in lung epithelial cells which caused acute inflammation

and tissue destruction, followed by production of fibrogenic cytokines, such as TGF-β, and progressive interstitial fibrosis, it also exerted its profibrotic effects by inducing the expression in lung fibroblasts of osteopontin, a multifunctional matrix cellular protein upregulated. On the other hand, TNF-α triggered fibroblast proliferation and enhanced degradation of the extracellular matrix. Both mediators, IL-1 and TNF-α were induced by bleomycin(9, 12-15).Bleomycin induced marked increase in soluble collagen (bronchoalveolar lavage fluid) and hydroxyproline content, and typical histologic findings of pulmonary fibrosis in . Urokinase therapy resulted in normalization of compliance, suppression of soluble collagen and hydroxyproline and ameliorated the histological changes induced by bleomycin. Furthermore, pulmonary fibrosis was likewise increased in bleomycin-treated mice overexpressing PAI-1 and fibrosis was decreased in PAI-1 deficient mice (5-7). A number of experimental treatments showed that many drugs can ameliorate bleomycin induced lung fibrosis in rodents. These treatments possessed their effect via many mechanisms such as inhibition of macrophage activation and inactivation of oxidants produced during the inflammatory process, to the interference with blood cells (such as neutrophils, eosinophils and platelets) that augment lung inflammation, and decrease the concentration of known molecular mediators of fibrosis. Urokinase treatment of animals with established pulmonary fibrosis induced by bleomycin was found to diminish the collagen content of lungs to near control levels. The efficacy of urokinase could be attributed either to fibrinolytic activity or to other properties such as attenuation of fibrogenic cytokines which were the essential pathological mediators. (5-7, 16-17).

CONCLUSION

In conclusion, urokinase together with bleomycin significantly change the lung relative weight, hydroxyproline level and lessen the histological lesions induced by bleomycin. Accordingly, it represented a good protective remedy to avoid pulmonary fibrosis during bleomycin treatment.

Conflict of interests: The authors declared no competing interests.

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Ethical considerations: The study was approved by the ethical board of the South technical university-Iraq.

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Table 1: Relative lung weights of control and rats treated with BLM, Urokinase and a combination of BLM and Urokinase.

Treated groups	Mean ±SD mg/100g bw)	P-value
Control	0.711±0.074	
BLM	0.998±0.052	<0.01[a]
uPA	0.775±0.016	<0.31[b]
BLM+uPA	0.844±0.082	<0.01[c]

Data were calculated as relative weight of lung to 100 g animal body weight
Data are presented as mean ±(SD).
[a]BLM vs control group, [b] Urokinase group vs control group, [C]the combination vs BLM group

Table 2: Hydroxyproline (HYP µg / ml) concentration in control and rats treated with BLM, Urokinase and a combination of BLM and Urokinase.

Treated groups	Mean ± SD (µg/ml)	P-value
Control	1.229±0.099	
BLM	2.345±0.129	< 0.001[a]
uPA	1.116±0.153	< 0.05[b]
BLM±uPA	1.498 ±0.222	< 0.03[c]

[a]BLM vs control group, [b] Urokinase vs control group, [C] the combination vs BLM group.

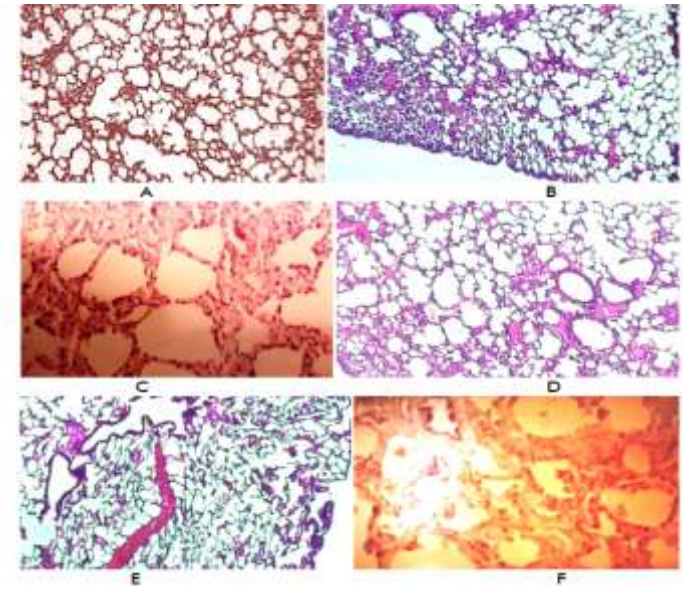

Fig.1: A. Section in normal lung tissue showed homogeneously distributed alveoli with normal interalveolar septa and capillary network around alveoli. B. Section in lung of (BLM) bleomycin group with higher magnification showed diffuse cellular infiltration. C. Section in rat lung from (PF) group showed extensive multiple infiltration areas of mononuclear inflammatory cells multifocal areas of fibrosis and inflammatory exudates in the intraterminal bronchiolar lumen. D. Section in the lung of bleomycin - treated rat showed prominent multifocal areas of fibrosis with huge infiltration of mononuclear, inflammatory cells mostly macrophages. E & F. Sections in the lungs of rat treated with (bleomycin and urokinase) showed more patent alveolar space and reduced both inflammatory cells infiltration and thickness of the interalveolar septa, low mononuclear inflammatory cells, and reduction of interalveolar deposition of collagen compared to bleomycin group.