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Research Article

Effect of mast cell stabilizer on lipopolysaccharides induced acute lung injury in rats

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Abstract

Acute lung injury (ALI) describes a form of parenchymal lung disease representing a wide range of severity from short-lived dyspnea to terminal failure of the respiratory system or acute respiratory distress syndrome (ARDS). No sufficient data is addressing the role of oxidative stress and the effect of mast cell stabilizer on lipopolysaccharides (LPS) induced ALI model. This study aimed to investigate the effect of ketotifen on LPS-induced ALI in rats with an explanation of possible underlying mechanisms. Rats were allocated into 4 groups: (1): control group, (2): ketotifen group (received ketotifen 1 mg/kg/day orally for 2 weeks), (3): LPS group (received LPS 5mg/kg intratracheal single dose at 5th day) and (4): ketotifen+LPS group: received both ketotifen plus LPS in the same previous doses. Rats were sacrificed after 2 weeks. The lung oxidative damage effect of LPS was evaluated by measuring the lung tissue level of malonaldehyde (MDA) and histopathological assessment. ALI induced by LPS revealed statistically significant improvement in rats treated with ketotifen compared with LPS alone group. These results suggest that ketotifen can protect against LPS-induced ALI. Ketotifen effects rely, at least partially, on its antioxidant effect.

Keywords: Lipopolysaccharides; Acute lung injury; Ketotifen.

Introduction

Acute lung injury (ALI) is a life-threatening state characterized by diffuse pulmonary interstitial and alveolar edema that causes respiratory failure and death. ALI may trigger acute respiratory distress syndrome (ARDS), the most severe form of ALI, and result in numerous organ failures with high mortality (approximately 30%-50%) [1]. However, the pathogenesis of these diseases has not been fully elucidated, and no specific and effective pharmacological intervention for ALI/ARDS is currently available [2].

Lipopolysaccharide (LPS) is a main component of the outer membrane of Gram-negative bacteria. It is commonly used to establish ALI models and intratracheal administration of LPS has been used to construct animal models of ALI. Mechanisms of ALI may include activation of multiple signal pathways which stimulate the release of inflammatory mediators, reactive oxygen species (ROS) and growth factors [3].

Ketotifen is a mast cells stabilizer. It has been widely used for the treatment of allergic diseases, such as bronchial asthma, allergic rhinitis, atopic dermatitis, and chronic urticaria[4]. Previous studies showed that ketotifen significantly protected against inflammatory and apoptotic damage in both animal and human studies [5] [6].

The mechanisms of ketotifen action include its ability to inhibit the release of mediators from mast cells involved in hypersensitivity reactions and ameliorate the status of inflammation and oxidative stress

functioning in systemic homeostasis[7]. Furthermore, ketotifen was proven to down regulate the activation of the Toll-like receptor 4/nuclear transcription factor kappa B (TLR4/ NF-κB) mediated signaling pathways with subsequent suppression of inflammatory cytokines production [3].In addition, it is a second-generation histamine H1 receptor antagonist which can effectively inhibit the release of histamine by bronchial submucosal mast cells to avoid the occurrence of respiratory tract non-specific inflammation [8].

The antioxidant and anti-inflammatory effects of ketotifen have been evaluated in different previous studies [5] [6] but still no clear data addressing the protective role of ketotifen in ALI model so the current study aimed to explore the effect of ketotifen in ALI model through possible antioxidant and anti-inflammatory activities.

Materials and methods

Animals

Thirty–two albino rats (250-300g), aged 10 weeks. Rats were obtained from the National Research Center, Giza, Egypt. Rats were fed a standard diet of commercial rat chow and tap water and left to acclimatize to the environment for 2 weeks before the start of the experiments. The research was performed in accordance with the guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Protocols were applied by the Committee for the Care and Use of Laboratory. Animals of the Faculty of Medicine, Minia University; (Approval No: 680:2020).

Chemicals

Ketotifen powder was obtained from (Multiapex Pharma Co, Egypt). LPS of Escherichia -coli was obtained from (Sigma, USA).

Experimental design:

Grouping

Rats were weighed and randomly divided into four groups (n=5-8). (1): Control group: received normal saline as a vehicle orally for 2 weeks and a single intratracheal saline injection (100 µL) on the 5th day of the experiment; (2): ketotifen group: received orally ketotifen (1mg/kg/day), dissolved in saline [9] for 2 weeks and a single intratracheal saline injection (100 µL) on the 5th day of the experiment. (3): LPS group: received oral normal saline for 2 weeks and a single intratracheal administration of E-coli LPS (5 mg/kg) dissolved in saline and injected in a volume of 100 µL [10] on the 5th day of the experiment; (4) LPS+ ketotifen group; rats received ketotifen dose as mentioned previously and a single intratracheal LPS injection on the 5th day of the experiment.

Induction of acute lung injury:

Rats were anesthetized with ketamine/xylazine (100/10 mg/kg) [11]. Then placed in a supine position with the extremities pulled caudally to aid in the exposure of the trachea. This procedure was used to induce ALI using LPS as described by **An and his team study** [10]. The trachea had been visible through an anterior neck incision, and a direct puncture was made at two to four tracheal rings below the larynx using a 24-gauge needle and a 1 ml tuberculin syringe. Then tuberculin syringe was removed after injecting saline or LPS into the lung. Finally, the neck was repaired with sutures. After 2 weeks, animals were anesthetized, sacrificed and the lungs of each animal were surgically removed.

Sample collection

At the end of the experiment, the animals were anesthetized, weighted then sacrificed. Each rat's lung was weighted to determine the lung weight ratio. The lung was washed by cold saline and divided into parts and one part was put in 10% formalin for histopathological examination and the other parts were kept for homogenization. Homogenization was done using homogenizer (Tri-R Stir-R homogenizer, Germany) and ice-cold phosphate buffer (0.01 M, pH 7.4; 20% w/v). The homogenate was centrifuged for 15 min at 5000 rpm using (Jantezki, T30, Germany) centrifuge and the supernatant was stored at –80°C till used to measure the biochemical parameter.

Assessment of lung tissue oxidative stress biomarker

This method measures the lung MDA which is the breakdown product of lipid peroxides. The lung content of lipid peroxides was determined using the thiobarbituric acid method described by **Buege and Aust** [12] which measures the thiobarbituric acid reactive substances concentration, sometimes referred to as MDA concentration.

Histological examination of lung tissue

Lung tissue sections from all groups were fixed in 10% formalin, embedded in paraffin and dehydrated in ascending concentrations of ethyl alcohol (70%-100%). 5 µm tissue sections were stained with hematoxylin and eosin (H&E) [13]. The histological changes of the lung among the different experimental groups were done. The

structural changes of tissue were assessed according to the degree of the thickened interalveolar septum; vascular congestion; and inflammatory cell infiltration.

Statistical analysis of the data:

Results were expressed as means and standard error of mean (S.E.M) and analyzed by One-way analysis of variance followed by Tukey's test. *P* values <0.05 were considered significant. Graph Pad Prism software was used (version 5.01 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com

Results

Effect of ketotifen on lung oxidative stress biomarker.

The lung tissue MDA level was significantly increased in the LPS group in comparison to the control group. In contrary, ketotifen pretreatment succeeded in significantly reducing lung tissue MDA level in comparison to LPS group **(Table 1).**

Histopathological assessment

Effect of ketotifen on lung histopathological pattern in LPS- induced ALI.

The control group and ketotifen group revealed normal lung architecture. The alveoli and alveolar sacs were patent and lined with pneumocytes type I (PI) and II (PII). PI was flat and more numerous, and PII-rounded cells that might appear protruded into the alveolar lumen. The interalveolar septa were delicate containing blood capillaries and macrophages **(Fig: 1a &1b)**. LPS group showed distorted histological architecture of the lung tissue with collapsed and compensatory dilated alveoli with thick inter-alveolar septa studded with extravasated RBCs and inflammatory cells infiltration. Numerous hemosiderin-laden alveolar and interstitial macrophages were obviously noticed. Areas of consolidation and interstitial edema were noticed **(Fig: 1c&1d)**. As regards ketotifen+LPS group, the lung tissue apparently returned back to normal **(Fig: 1e &1f).**

Table 1: Effect of ketotifen on malondialdehyde (MDA) in lipopolysaccharides (LPS) induced acute lung injury (ALI).

Groups	MDA (nmol/g tissue)
Control	41.9 ± 0.60
K	43.63 ± 0.69
LPS	93.99 ± 3.31[a]
K+LPS	71.50 ± 3.24 [b]

Values represent the mean ± SEM (n=5-8). Results are considered significantly different when $p < 0.05$, [a] significantly different from control group, [b] significantly different from LPS group, K: ketotifen; MDA: malondialdehyde; LPS: lipopolysaccharides.

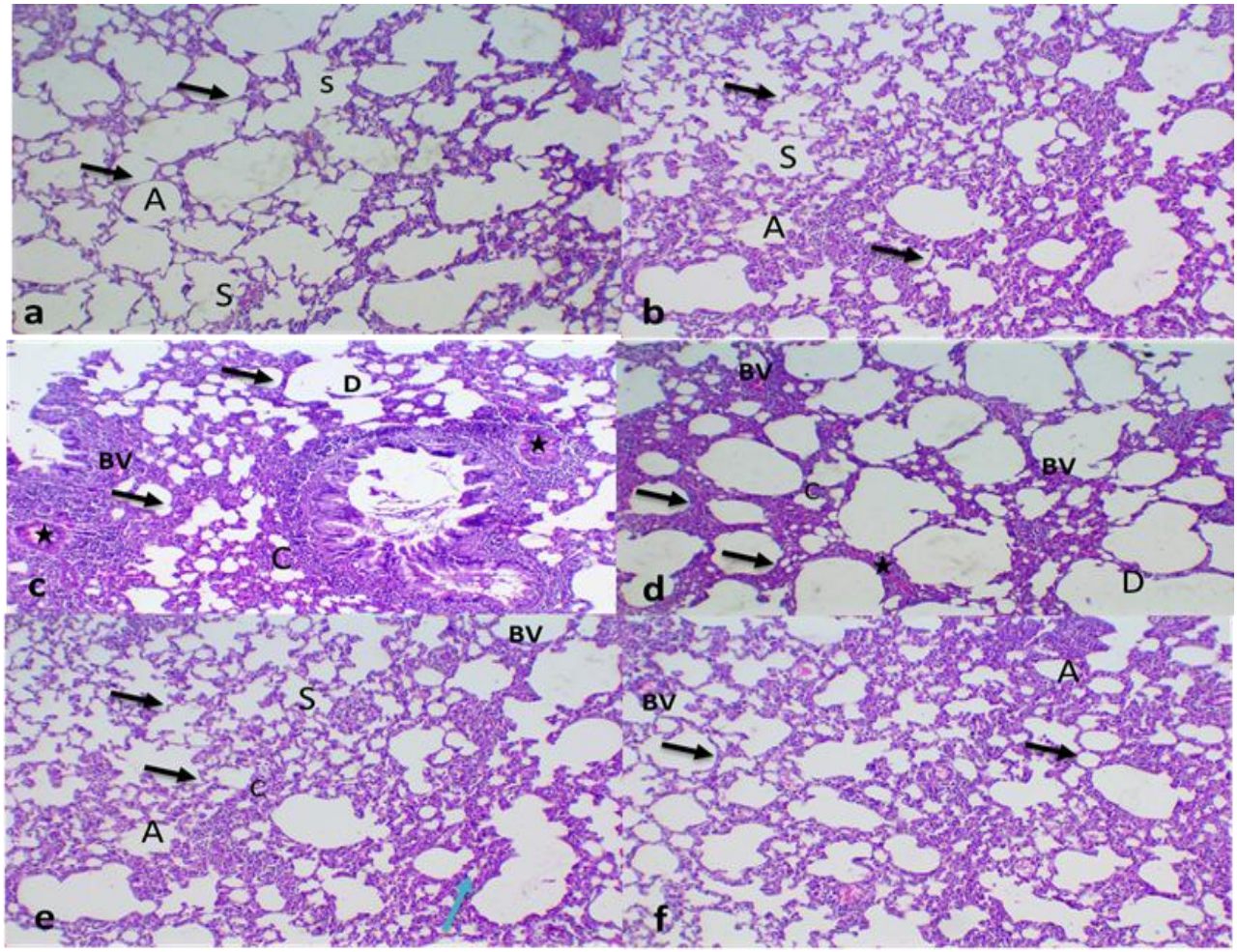

Figure 1: Effect of ketotifen on histopathological pattern stained by haematoxylin and eosin (H&E) in lipopolysaccharides (LPS) induced acute lung injury (ALI).
Representative photomicrographs of lung of adult male albino rats of different experimental groups in; control, ketotifen groups (a, b) respectively; showing alveoli (A), alveolar sacs (S), and blood vessels (B.V). Notice thin inter-alveolar septa (arrows). LPS group (c & d); showing marked thickening of the inter-alveolar septa (arrows). Some alveoli are collapsed (c) and other are dilated (D). The congestion of the blood vessels (B.V) is obvious. Notice dilated congested blood vessels (star). Ketotifen with LPS group (e & f); showing apparently normal alveoli (A), alveolar sac (s), blood vessels (BV) and thin interalveolar septum (black arrows). Notice presence of some collapsed alveoli (C) and thick inter alveolar septum (blue arrow). (H&E X 100; inset X 400 , scale bar=200 μm).

Discussion

ALI is a severe clinical condition with high morbidity and mortality that usually results in the development of multiple organ dysfunction [14]. The pathophysiological process of ALI/ARDS basis on alveolar-capillary damage [15], increased vascular permeability leading to diffuse pulmonary interstitial and alveolar edema, over-activation of macrophages and neutrophils, excessive release of inflammation-associated proteases, ROS and pulmonary hemorrhage [16]. Inflammation, oxidative stress, and accumulation of inflammatory cells in the airways are thought to play key roles in the pathophysiological process of ALI [17].

Ketotifen is a mast cell stabilizer and histamine H1 antagonist with antioxidant and anti-inflammatory activities[8]. However, the potential role and the underlying mechanisms of ketotifen in ALI are still undiscovered. For the above goal, we utilized a well-established rat model of LPS- induced ALI and evaluated the effects of ketotifen on this model. The design of

this study offers the advantage that it allowed us to record the evidence of lung oxidative stress in the form of elevation of MDA, in addition to record the evidence of inflammatory lung damage in the form of histopathological changes and inflammatory cell infiltration.

In the present study, ALI was induced after intratracheal administration of LPS which obviously appeared as significant histopathological changes in the form of distorted histological architecture of the lung tissue compared to the control group with marked thickening of the inter-alveolar septa and inflammatory cell infiltration. Some alveoli were collapsed and others were dilated, to some extent similar findings were reported by other investigators [18, 19] who revealed distorted histological pictures in ALI induced by LPS. On the other hand, the group pretreated with ketotifen revealed a significant improvement in the histopathological images and decrease inflammatory cell infiltration in which lung tissue apparently returned back to normal whereas revealed well-formed alveoli and alveolar sacs. Thus, our findings confirmed the ameliorating effect of ketotifen in ALI. This is in line with a previous study done by Zhang and his coworkers [20] who conducted the effect of ketotifen in improvement of histopathological changes in ALI after orthotopic autologous liver transplantation in rats.

Oxidative stress and production of oxidative stress radicals have been demonstrated to play an important role in the development and manifestations of ALI. Oxidative stress radicals can induce lipid peroxidation to cellular and subcellular organelle membranes, causing serious damage to cellular structure and function [21]. Oxidative stress and the release of inflammatory mediators are considered to be the causative factors of LPS-associated damage in the lung tissue [18].

MDA is a degradation product of oxygen-derived free radicals and lipid oxidation, which reflects the damage caused by ROS [22]. Excessive accumulation of ROS and MDA holds an important position in the pathogenesis of lung injury and cell death in LPS-induced ALI [23]. This is confirmed in the present study by the significant increase in MDA level of the lung tissue after intratracheal injection of LPS compared to control group indicating lipid peroxidation. This coincides with the previous study of Dong and Yuan [24] which reported accelerated inflammation and oxidative stress induced by LPS in acute lung injury.

Ketotifen successed to restore the oxidant-antioxidant balance manifested by reduction of MDA level compared to LPS challenged group. The current study revealed that treatment by ketotifen caused significant fall in MDA level compared to LPS group which indicates attenuation of lipid peroxidation and oxidative stress induced by LPS through inhibiting degranulation of mast cells, histamine release and inflammatory mediators. Our data are in good agreement with the previously reported study of Huang and his study team [25] which showed the ameliorating effect of cromolyn sodium, ketotifen and protamine on oxidative stress in the form of reducing MDA level in ALI model induced by small intestinal ischemia reperfusion. These results demonstrate that the prevention of ALI injury by ketotifen is partially attributed to its antioxidant property which was previously reported in different models and different tissues [9, 26].

Conclusion

The present study concluded that ROS may largely participate in the mechanism of pathogenesis of ALI. Ketotifen has ameliorative activity against ALI at least in part by its antioxidant effect.

Acknowledgements Declaration of interest

The authors report no conflicts of interest

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Authors' contributions

MK, SA, HK, KA suggested the point of research suggestion, performed the experiment, statistically analyzed the data, write, revised and proofread the final manuscript. **MG** carried out the histological part of the experiment and wrote the corresponding parts of the manuscript.

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