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Research Article

Lipopolysaccharide-induced acute lung injury in experimental male albino rats.

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Abstract

Acute lung injury (ALI) is a life-threatening lung disorder marked by rapid development of hypoxemia, severe dyspnea, tachypnea, widespread pulmonary interstitial edema, and alveolar edema, ultimately leading to respiratory failure and death. ALI can be induced both directly and indirectly. Lipopolysaccride (LPS) is the main cause of the toxicity linked to gram-negative bacteria and is frequently used to establish ALI models. **Purpose of the study;** this study was assigned to investigate the short-term LPS-induced lung injury. **Basic procedures;** rats were randomly assigned into 2 equal groups: Control group: where rats were allowed to run about in their cages with unrestricted access to food and water, Lipopolysaccharide (LPS) treated group: in which rats were received single intra-tracheal dose of LPS 3mg/kg on the 8th day after the beginning of the experimental study then sacrificed 24 hours after intra-tracheal LPS injection. **Main findings;** Intra-tracheal administration of LPS produced a significant elevation of plasma level of Ferritin as well as resulted in elevation of the level of pulmonary malondialdehyde (MDA) and tumor necrosis factor alpha (TNF-α) as compared to control group. **Principle conclusion:** LPS was proved to produce extensive lung tissue damage as proved in the present study by inflammatory and oxidative markers. So, LPS can be considered as one model of lung injury.

Key words: lipopolysaccharide, Acute lung injury, Ferritin, Tumor necrosis factor alpha, malondialdehyde

Introduction

Severe cases of pneumonia and other forms of severe illness caused by bacteria, viruses, and other pathogens may lead to a condition known as acute respiratory distress syndrome (ARDS). Diffuse alveolar destruction, pulmonary edema, hyaline membrane development, systemic inflammation, coagulopathy, and bilateral opacities on chest imaging are all hallmarks of acute respiratory distress syndrome (ARDS), a potentially fatal type of lung failure [1]. Covid-19 sickness is a potentially fatal condition caused by infection of the respiratory system with a novel strain of coronavirus termed SARS-COV-2 [2].

Alveolar bleeding, bilateral widespread alveolar injury, pulmonary oedema, hyaline membrane development, and pulmonary fibrosis are all possible findings on a chest x-ray. Many people with severe illness may eventually have multi-organ failure and die as a result [3].

Gram-negative bacteria have an outer membrane containing lipopolysaccharide (LPS), which may be inhaled (a direct, pulmonary insult) or injected intravenously (an extra-pulmonary insult)[4]. Inflammatory responses in the lungs are triggered by LPS, leading to an increase in pulmonary capillary permeability and the infiltration of polymorphonuclear leukocytes (PMNLs)[5]. The purpose of the present work is to study LPS effects on lung tissue.

Patients and Methods

Chemicals:

Lipopolysacchide (LPS) from Escherichia Coli was obtained in powder form. It was dissolved in saline and given by gavage (Med-chemexpress, USA).

Experimental animals:

Twenty adult male albino rats (Sprague-Dawley strain) weighing 230±20 gm was used in this study. Rats were acclimated to their new environment over the course of two weeks by being housed in groups of four in stainless steel cages (40 cm x 40 cm x 25 cm) that provided adequate space for free movement and wandering. Cages were kept at room temperature with natural dark/light cycles, and rats were given free access to water and commercial rat's diet (Nile Company, Egypt). The origin of the rats, the caging, the comfort, the health state, and the entire experimental design and methods were all authorized by the Ethics Committee "FMREC" Faculty of Medicine, Minia University, Minia, Egypt. Approval No. 294:3/2022.

Experimental design:

Rats were assigned evenly between two groups of 10 rats each :

(1) Control group: This allowed rats unrestricted access to food and water as they roamed freely in their cages. On the 8th day after the beginning of the experimental study, control rats were given single intra-tracheal dose of saline 3 µL/g body weight then sacrificed 24 hours after intra-tracheal saline injection .

(2) Lipopolysaccharide (LPS) treated group: in which rats were received single intra-tracheal dose of LPS 3mg/kg on the 8th day after the beginning of the experimental study then sacrificed 24 hours after intra-tracheal LPS injection [6]

I- Animal sacrifice and sample collection:

Five hours before decapitation, the tail veins of all rats were progressively injected with Charcoal macromolecules (0.5 mg) suspended in normal. The phagocytic cells were activated by charcoal, which served as a foreign antigen[i]. After an overnight fast, the animals were slain through decapitation, and blood samples were taken from the jugular vein and stored at room temperature in a tube with 0.5 percent heparin as the anticoagulant before being spun in a cooling (Hettich) centrifuge at 3000 rpm for 15 minutes. The collected plasma was frozen at -80 °C until it was time to assay ferritin (using a rat ferritin (FE) ELISA kit from Elab science in the United States, according to the Sandwich-ELISA principle.

II- Analysis of lung homogenates :

After being removed and rinsed in ice-cold saline, the right lung was submerged in liquid nitrogen and frozen at 80°C for later biochemical investigation. Cold potassium phosphate buffer was used to homogenize lung tissue (0.05 M, pH 7.4). The homogenates were centrifuged for 10 minutes at 4°C and 5000 rpm (revolutions/rotor). The resultant supernatant was analyzed for both TNF-α and MDA levels using specific ELISA kits (a rat Tumor Necrosis Factor-Alpha (TNF-α) ELISA kit from Elab science in the United States, and a Malondialdehyde (MDA) ELISA kit from Bio-diagnostics in Egypt, respectively.(

Statistical analysis of data

IBM SPSS 20.0 statistical program was used for the data analysis (IBM; Armonk, New York, USA). For quantitative measurements, data were presented as mean SE, minimum and maximum of range; for classified data, data were presented as number and %. For parametric data, we utilized ANOVA to compare among groups, and then performed the Tukey post hoc test to determine if there were significant differences between groups. The cutoff for significance was set at a p value of 0.05.

Results

- Effect of LPS on pulmonary MDA level:

When comparing the LPS group to the control group, the LPS group showed a significant rise in lung MDA. (Table 1).

- Effect of LPS on pulmonary TNF-α level:

The result of the current study demonstrated that the pulmonary TNF-α concentration was the highest mean value in the LPS treated group (Table 2).

- Effect of LPS on plasma ferritin level :

When compared to the control group, the plasma level of Ferritin increased significantly after LPS administration. (Table 3)

Table (1); show the effect of LPS induced lung injury on MDA level

	Control	LPS	p-value
MDA **Mean ± S.E.M**	**4.58 ± 0. 04**	**14.08 ± 0.68**	**<0.0001***
P-value between each two group **Control** **LPS**		**<0.0001***	

Data are expressed by mean ± S.E.M of 10 rats in each group. Control group (C), Lipopolysaccharide (LPS), Malondialdehyde (MDA). * Significant level of p- value is < 0.05. * p value of means was calculated by One-Way ANOVA test followed by Tukey post-hoc test.

Table (2): show the effect of LPS induced lung injury on TNF-α level

	Control	LPS	p-value
TNF-α Mean ± S.E.M	2.65 ± 0.08	6.39 ± 0.12	<0.0001*
P-value between each two group **Control** **LPS**		<0.0001*	

Data are expressed by mean ± S.E.M of 10 rats in each group. Control group (C), Lipopolysaccharide (LPS), Tumor Necrosis Factor-Alpha (TNF-α). * Significant level of p- value is < 0.05. * p value of means was calculated by One-Way ANOVA test followed by Tukey post-hoc test.

Table (3); show the effect of LPS induced lung injury on Ferritin level

	Control	LPS	p-value
Ferritin Mean ± S.E.M	31.36 ± 0.98	66.50 ± 0.34	<0.0001*
P-value between each two group **Control** **LPS**		<0.0001*	

Data are expressed by mean ± S.E.M of 10 rats in each group. Control group (C), Lipopolysaccharide (LPS). * Significant level of p- value is < 0.05. *p value of means was calculated by One-Way ANOVA test followed by Tukey post-hoc test.

Discussion

Acute lung injury (ALI) is a serious medical emergency. Acute respiratory distress syndrome (ARDS) is the most severe type of ALI and is associated with very high death and morbidity rates. Direct and indirect lung damage are both contributors to ALI.[8]. Inflammatory cell infiltration increased oxidative stress, and an overproduction of pro-inflammatory mediators are the pathological hallmarks of acute lung injury/acute respiratory distress syndrome (ALI/ARDS). These factors lead to damage to the alveolar epithelial and capillary endothelial cells, pulmonary edema, impaired gas exchange, and respiratory failure [9]

The development and symptoms of ALI are significantly influenced by oxidative stress and the consequent production of oxidative stress radicals. Damage to proteins, lipids, DNA, and carbohydrates from oxidative stress radicals disturbs the regular molecular structure and physiological functioning of cells[10]. As oxidetive stress levels rise, antioxidant defenses are decreased, the cellular autophagy /mitophagy processes are affected and the cell survival regulatory mechanisms are also affected, which enhances cell and tissue damage [11]

In the current ALI model, lung tissue damage was discovered along with significant oxidative stress biomarker changes. Malondialdehyde (MDA) levels were observed to be significantly higher in lung tissue after LPS administration. These findings are consistent with those of other researchers who found that LPS induced severe lung damage, accelerated the inflammatory response, and elevated oxidative stress in the lung [12].

Free radicals start the lipid peroxidation process within an organism. MDA is a result of polyunsaturated fatty acid peroxidation in cells. Free radical production has increased, which has led to an excess of MDA. Because of this, MDA levels are often used to assess the antioxidant state and oxidative stress in cancer patients [13].

One example of a cytokine with widespread effects is tumor necrosis factor alpha (TNF-α). It has a role in the pathophysiology of several inflammatory illnesses and is well recognized as a critical regulator of inflammatory responses. Macrophages, T lymphocytes, and natural killer cells that have been activated are the primary sources of TNF-α. Its primary function is to stimulate the production of more inflammatory chemicals, such as cytokines and chemokines [14].

The result of the current investigation shown that intratracheal injection of LPS significantly elevates lung tissue TNF-α. Increased production of proinflammatory cytokines like tumor necrosis factor alpha (TNF-α) is a result of LPS binding to toll-like receptor 4 (TLR4) and activating the nuclear factor kappa B (NF-KB) pathway [15]. Macrophages may be switched to the M1 phenotype when exposed to lipopolysaccharide. Through the production of a wide variety of cytokines and growth factors, macrophages play a crucial role in the start, maintenance, and resolution of inflammation. The inflammatory cytokines, such as TNF-α, are secreted by activated M1 macrophages [16].

Ferritin is a protein that has been characterized as a cytosolic protein. It is also found in the nucleus, mitochondria, and lysosomes in addition to a small amount of ferritin being present in the serum. Ferritin plays an important role in iron storage and metabolism beside this function ferritin regulates cellular iron concentrations, securing iron from invasive pathogens, and protecting cells from oxidative stress. In the presence of an infection or cancer, ferritin levels dramatically rise as endotoxins cause the gene that codes for ferritin to upregulate, which raises ferritin levels. Moreover, Ferritin is an acute phase- reactant so it has a role as a marker of inflammation [17].

The current study shows that following intratracheal delivery of LPS, plasma ferritin levels rise considerably. This is consistent with [18]'s finding that the LPS-induced model of ALI involves the NF-KB signaling pathway. The synthesis of ferritin and other inflammatory cytokines follows LPS delivery into the trachea, which causes phosphorylation of the NF-kappa B signaling pathway. Lung damage triggers the release of inflammatory cytokines, which in turn drive immune cells like macrophages to increase ferritin production [19].

Conclusion

the present study concluded that LPS produced

extensive lung tissue damage as proved in the present study by inflammatory and oxidative markers. So, LPS can be considered as one model of lung injury .

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Declaration of interest

The authors report no conflicts of interest.

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