The effect of Lipopolysaccharide purified from Pseudomonas aeruginosa on lung tissue

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ABSTRACT

Samples of Pseudomonas aeruginosa were collected from different patients suffering from different injuries of burns and wounds then they identify by biochemical tests and molecular analysis by the PCR. Antimicrobial sensitivity test showed that the isolates were highly sensitive to Imipenem (87.5%) and have very low sensitive to Tobramycin (17.5%) and Tetracycline (22.5%). The lipopolysaccharide (LPS) was extracted by hot EDTA extraction method and partially purified by gel filtration chromatography using Sephadex- G200. Two doses of LPS (200 µg/ml and 500 µg/ml) were given intranasal for each experimental animal at one time point to evaluate the histopathological effect of the LPS in comparison to negative control, mice administrated distilled water intranasal. The result showed that 200 µg/ml dose increased the thickness of alveolar septae with formation of emphysema and after 72 h the effects almost disappeared, while the 500 µg/ml dose showed the same effect of previous dose but its effects continued after 72 h.

Keywords: Cytokines, Immune response, Proinflammatory, Pseudomonas aeruginosa.


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INTRODUCTION

The genus Pseudomonas is the most heterogeneous and ecologically significant group of known bacteria, and includes Gram-negative motile aerobic rods belonging to the bacterial family Pseudomonadaceae which includes other genera that are wide-spread throughout nature [1,2]. Pseudomonas species are measuring 0.5 to 0.8, µm by 1.5 to 3.0 µm. Species are distinguished by several tests like biochemical and DNA hybridization [3]. P. aeruginosa infection of the lung is associated with the highest mortalities [4] leads to entrance of the bacteria, apoptosis of the cell and release of few pro-inflammatory cytokines (interleukin- (1, 6, 8)) [5]. Most strains resistant for killing in serum alone, but the addition of polymorphonuclear leukocytes results in bacterial killing, it is most efficient in the presence of type-specific opsonizing antibodies, directed primarily at the antigenic determinants of Lipopolysaccharide (LPS) [2]. LPS are the main components of bacterial outer membranes.
where they perform as effective permeability barrier against antibiotics and the host cell defense system which released when the bacteria die or grow [6]. Consists of a lipid hydrophobic part (lipid A) and hydrophilic core oligosaccharide with an O-polysaccharide chain (O-antigen) attached, which defines the immunospecificity of the bacterium [7,8].

There are many techniques used to extract LPS include extraction with trichloroacetic acid (TCA), extraction with water at 80 °C, extraction with aqueous phenol and extraction with aqueous ether. The extraction with aqueous phenol has obtained widespread popularity not only because it can be used to many groups of bacteria and is a relatively simple technique, but also because it represents one of the few techniques to extract LPS from R-mutant bacteria [9]. There is EDTA-heat extraction method [10]. LPS able to eliciting an extensive variety of pathophysiological effect such as endotoxin shock, tissue injury and lethality in both human and animal [11]. LPS producing pulmonary pathological changes, pulmonary edema, lung injury scores, myeloperoxidase (MPO) activity, total cells, neutrophils, macrophages, TNF-α, IL-6 and IL-1β in bronchoalveolar lavage fluid (BALF) [12].

MATERIALS AND METHODS

Patients and Control

The samples were collected from patients suffering from burns and wounds during October 2014 to December 2015. These samples were cultured on differential MacConkey Agar. After identification the LPS was extracted by hot EDTA extraction method and partially purified by gel filtration chromatography.

Histology

The standard method of Zgair and Chhibber (2010) was followed to design the experiment, preparing the lung suction and read the yielded results [13].

RESULTS AND DISCUSSION

Fig 1 showed the section of normal lung that collected from mice installed intranasal with normal saline. Fig 2 showed histological results of lung section collected from mice post installed intranasal with LPS (200 µg/ml) at different time intervals. Mild thicken of alveolar septae with formation of emphysema was observed as early as 2 h post installation beside congestion in blood vessel (Fig 2a). At 24 h post installation (Fig 2b) blood congestion was observed in lung tissue besides increasing in fibroblast and mononuclear cells infiltration. Moreover, increase in thickness of walls of alveoli was observed. Infiltration of inflammatory cells was decreased at 48 h post installation (Fig 2c). Congestion in blood vessel, infiltration of inflammatory cells and thickness of alveolar was decrease to almost normal at time points 72 h (Fig 2d) with continuous appearance of emphysema.

In present study, the result showed that instillation with 500 µg/ml of LPS (group B) produced negative histological effect higher than instillation with 200 µg/ml of LPS (group A) on the epithelial tissue of the lung. The effect of LPS was continued in group B (congestion with inflammatory cells infiltration, also increase with alveolar septae thickness, in addition to present in emphysema phenomenon that may cause necrosis) for more time as compared with group A. Takashima [14] found that intranasal administration of LPS induced epithelial tissue injury and released cytokines. Whereas, Reutershan et al. (2005) found that significant numbers of leukocytes were found in lung after LPS administration, and their numbers increased moderately at four hours after LPS administration. TNF-α might damage vascular endothelial cells, increasing their permeability, and furthermore induces alveolar epithelial cells to produce other cellular factors and chemotactic factors [15]. Inhibiting the overproduction of pro-inflammatory cytokines such as TNF-α and IL-1 showed the lessening of pulmonary injury in models administrated with intranasal LPS [16,17].

Other studies depicted that intratracheal instillation of LPS resulted an increase in airway epithelium barrier
permeability, as demonstrated by a significant increase in albumin concentration in lung tissues. However, after 4 h of LPS exposure, there was no change in vascular permeability. Other study also found that *P. aeruginosa-*derived LPS increased airway epithelial permeability in rabbits in a time dependent manner without changes in

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**Fig 2** Sections of mice lung installed intranal with 200 µg/ml of LPS. A, Section of lung at time point 2 h post installation; B, Section of lung at time point 24 h post installation; C, Section of lung at time point 48 h post installation; D, Section of lung at time point 72 h post installation (X 200).

**Fig 3** Lung sections show histopathological variation of mouse lung administrated intranasal with 500 µg/ml of LPS. A, Section of lung at time point 2 h post installation; B, Section of lung at time point 24 h post installation; C, Section of lung at time point 48 h post installation; D, Section of lung at time point 72 h post installation (X 200).
vascular permeability at this early stage [18,19]. Inflammatory cells involved in lung injury include macrophages and neutrophils, which released inflammatory mediators, including oxygen radicals, proteases and cytokines. However, the major factor in alveolar capillary wall permeability is the epithelial rather than the endothelial barrier [20]. Accordingly, it has been demonstrated that the migration of neutrophils across the alveolar capillary wall does not necessarily induce severe damage to this barrier. However, if the injury does not occur during the migration process, lesions seem to be caused by the toxic products released by neutrophils in the alveolar spaces after their migration [18,21,22]. Al-Ajeely, (2006) found that some concentration of P. aeruginosa LPS may result in epithelial tissue inflammation, however, other concentration may not result in the inflammation because the large quantity immune response that released in tissue [23]. The partial purified LPS have a good ability to promote the pro-inflammatory immune response in the lung of mice and LPS resulted in inflammation response in mucosal tissue of lung that leads to changes in epithelial cells.

Conflict of interest
The authors declare that they have no conflict of interests.

REFERENCE