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Original article

Keratinocyte growth factor-2 inhibits bacterial infection with *Pseudomonas aeruginosa* pneumonia in a mouse model

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ABSTRACT

To determine protective effects of concurrent administration of Keratinocyte growth factor-2 (KGF-2) with *Pseudomonas aeruginosa (P. aeruginosa)* inoculation on the induced pneumonia.

KGF-2 (5 mg/kg) was concurrently administered into the left lobe of 55 mice with *P. aeruginosa* PAO1 (5×10^6 CFU, half-lethal dose); 55 mice in the control group were concurrently administered PBS with the PAO1. We detected and analyzed: body temperature; amount of *P. aeruginosa* in homogenates; count of total number of nucleated cells and of mononuclear macrophages; protein concentration in bronchoalveolar lavage fluid (BALF); lung wet-to-dry weight ratio; cytokines in BALF and blood; and lung morphology. To study survival rate, concurrent administration of KGF-2 (experimental group) versus PBS (control) with a lethal dose of PAO1 (1×10^7 CFU was performed, and survivorship was documented for 7 days post-inoculation.

The bacterial CFU in lung homogenates was significantly decreased in the KGF-2 group compared to the control group. There were significantly more mononuclear macrophages in the BALF from the KGF-2 group than from the control group ($p < 0.05$). KGF-2 increased the surfactant protein and GM-CSF mRNA in lung at 6 h and 72 h after inoculation. Significant reduction of lung injury scores, protein concentrations, lung wet-to-dry weight ratio, and IL-6 and TNF-α levels was noted in the KGF-2 treated rats at 72 h after inoculation ($p < 0.05$). The 7-day survival rate of the KGF-2 group was significantly higher than that of the control group ($p < 0.05$).

Concurrent administration of KGF-2 facilitates the clearance of *P. aeruginosa* from the lungs, attenuates *P. aeruginosa*-induced lung injury, and extends the 7-day survival rate in mice model with *P. aeruginosa* pneumonia.

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*), an opportunistic pathogen, frequently causes lower airway infection in patients with impaired immunity [1]. Antibiotic-resistant strains of *P. aeruginosa* are emerging and spreading in many countries. Infection with multidrug-resistant (MDR) and extensively drug-resistant (EDR) isolates that are insensitive to all standard antipseudomonal antibiotics (including carbapenems, fluoroquinolone and aminoglycosides) except colistin represents a special challenge, as physicians may have exhausted all effective antibiotics in treating *P. aeruginosa* infection [2,3]. New therapeutics, that can potently inhibit *P. aeruginosa* infection but do not frequently generate drug resistance, are urgently needed. Keratinocyte growth factor-2 (KGF-2), a member of the fibroblast growth factor family, is a 208 amino acid polypeptide, also known as fibroblast growth factor-10 (FGF-10) [4]. Similar to KGF, KGF-2 is mainly expressed and secreted by stromal cells; once released, it binds with high affinity to FGF receptors 2IIIb (FGFR2IIIb) and 1IIIb (FGFR1IIIb) that are expressed exclusively on epithelial and endothelial cells [5]. KGF-2 is also similar to KGF in

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structure and biological function. Thus, KGF-2 acts as a paracrine mediator, facilitating epithelial cell proliferation/differentiation, angiogenesis and barrier function of capillary monolayers [6–8]. KGF-2 was found to accelerate healing of corneal epithelial wounds [9] and incisional wounds *in vivo* [10] and to reduce inflammatory bowel disease in a preclinical model [11]. These known biological functions of KGF-2 deem it a potential therapeutic. Recently, KGF-2 has been in phase II trials for topical treatment of chronic venous ulcers and systemic treatment of ulcerative colitis [12]. KGF-2 also prevented mucositis in patients who received chemotherapy after bone marrow transplantation [13].

Prophylactic application of KGF-2 requires further investigation. Our recent studies demonstrated that pretreatment with KGF-2 attenuated severe pulmonary edema [14] and lung injuries induced by ischemia/reperfusion [15], ventilator [16] and lipopolysaccharide [17] in rats. Viget et al. [18] showed that KGF pretreatment had antibacterial effects at the acute stage of *P. aeruginosa* pneumonia in rats. Wu et al. [19] also found KGF pretreatment could facilitate clearing Gram-negative pathogens from the lung within 24 h post-infection. Lee et al. [20] showed that KGF secreted by mesenchymal stem cells could kill *E. coli* perfused from human lungs under *ex vivo* culture conditions. Those results suggest that KGF-2 had great potential as a prophylactic and therapeutic, but definite effects of KGF-2 in inhibiting *P. aeruginosa* infection in the lungs remains to be established. The aim of this study was to determine whether concurrent administration of KGF-2 with *P. aeruginosa* could inhibit bacterial infection and mitigate *P. aeruginosa* pneumonia in early (6-h) and late (24-h and 72-h) pneumonia after pathogen instillation in a mouse model.

2. Materials and methods

2.1. Animals

A total of 180 female BALB/c mice, 6–8 weeks old and certified as specific pathogen-free (SLAC Laboratory Animal, China), were housed in Zhongshan Hospital Animal Care Facility. All animals had access to food and water ad libitum. The experimental protocol was approved by the Animal Care and Use Committee, Fudan University, Shanghai.

2.2. Bacteria

This study used the *P. aeruginosa* PAO1 strain, for which the lethal dose was previously titrated 1×10^7 CFU (unpublished data). *P. aeruginosa* was incubated in tryptic soy broth (Oxide Microbiology Products, England) at 37 °C; then the culture was washed twice and resuspended in sterile PBS.

2.3. Experiment grouping

2.3.1. Half-lethal dose of P. aeruginosa and KGF-2

One hundred and ten mice were randomly divided into an experimental group (intratracheal administration of *P. aeruginosa* and KGF-2) and a control group (intratracheal administration of *P. aeruginosa* and PBS). In the experimental group, recombinant human KGF-2 (rhKGF-2, 5 mg/kg in 0.05 ml; New Summit Biopharma, Shanghai, China) was administered to mice concurrently with inoculation with *P. aeruginosa* (5×10^6 CFU, half-lethal dose) by the intratracheal route.

2.3.2. Lethal dose of P. aeruginosa and KGF-2

To study the effect of KGF-2 on survival of *P. aeruginosa*-infected mice, the remaining 40 mice were randomly divided into a experimental group (intratracheal instillation of 1×10^7 CFU *P. aeruginosa* and rhKGF-2, 5 mg/kg in 0.05 ml; n = 20) and a control group (intratracheal instillation of 1×10^7 CFU *P. aeruginosa* and 0.05 ml PBS; n = 20). The survival of mice in all groups was documented for 7 days after inoculation.

2.3.3. KGF-2 and PBS

To study the effect of KGF-2 on the total cell number and the macrophages counts in BALF, 10 mice were randomly divided into a KGF-2+PBS group (rhKGF-2, 5 mg/kg in 0.05 ml) and PBS group (PBS,0.05 ml).

2.4. Intratracheal instillation

Mice were inoculated by instilling the bacteria with KGF-2 or PBS into the left lung as previously described [21]. Briefly, following anesthesia with avertin, mice were placed on a board with their heads elevated at 45°. Then 50 μl of bacterial working solution was instilled into the left lung through the trachea using a 22G gavage needle. Mice were recovered for 15 min prior to replacement into their cages. All animals were active and appeared normal in 30 min post-inoculation.

2.5. Monitor of rectal temperature

Rectal temperature was hourly monitored for initial 12 h post bacterial instillation, then daily for 7 days (5 mice for each group).

2.6. Lung and blood bacterial enumeration

At 6, 24, and 72 h after inoculation, 5 mice per group in the half-lethal dose experiment were euthanized with a high dosage of avertin. Blood and lungs were collected under sterile conditions. The left lung was removed and placed in 1 ml of sterile PBS and homogenized for quantification of bacteria. Serial ten-fold dilutions of lung homogenates and whole blood in sterile PBS were carried out. Then different dilutions were spread onto pseudomonas centrimide agar plates (Oxide Microbiology Products, England) for overnight incubation at 37 °C prior to enumeration.

2.7. Lung wet to dry weight ratio (W/D)

Mice were euthanized with averting, the left lung was isolated and the wet weight was recorded. The lungs were then placed in a 60 °C incubator for 3 days, during which the dry weight was recorded to determine the wet-to-dry weight ratio.

2.8. Bronchoalveolar lavage fluid

Upon euthanasia of 5 mice per group in the half-lethal dose experiment at each time point described above, bronchoalveolar lavage fluid (BALF) was also collected prior to lung removal from the left lung by cannulating the trachea and gently flushing the left lung three times with 0.5 ml PBS. BALF was immediately centrifuged at 1500 × g for 10 min at 4 °C, then the supernatant was stored at −80 °C for further analysis, while the cell pellet was used for cell cytospin preparation.

2.9. Total cell count and differential cell count

The total number of nucleated cells in BALF was counted with hemocytometer. The resuspended BALF cells were centrifuged, transferred to slides and stained with Wright's–Giemsa (Jiancheng, Nanjing, China). Finally, leukocytes and macrophages on all slides were quantified by counting a total of 200 cells/slide at 40× magnification.