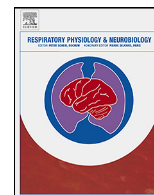
Respiratory Physiology & Neurobiology 201 (2014) 7–14

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol



Keratinocyte growth factor-2 is protective in lipopolysaccharide-induced acute lung injury in rats

Lin Tong[a,1], Jing Bi[a,1], Xiaodan Zhu[a], Guifang Wang[a], Jie Liu[a], Linyi Rong[a], Qin Wang[a], Nuo Xu[a], Ming Zhong[b], Duming Zhu[b], Yuanlin Song[a,c,d,**], Chunxue Bai[a,*]

[a] Department of Pulmonary Medicine, Zhongshan Hospital, Fudan University, Shanghai 200032, PR China
[b] Division of Critical Care Medicine, Zhongshan Hospital, Fudan University, Shanghai 200032, PR China
[c] Shanghai Public Health Clinical Center, Shanghai 201508, PR China
[d] Zhongshan Hospital, Qingpu Branch, Fudan University, Shanghai 201700, PR China

A R T I C L E I N F O

Article history:
Accepted 20 June 2014
Available online 26 June 2014

Keywords:
Acute lung injury
Keratinocyte growth factor-2
Lipopolysaccharide
Epithelial cells
Pulmonary surfactant proteins

A B S T R A C T

Keratinocyte growth factor-2 (KGF-2) plays a key role in lung development, but its role in acute lung injury has not been well characterized. Lipopolysaccharide instillation caused acute lung injury, which significantly elevated lung wet-to-dry weight ratio, protein and neutrophils in bronchoalveolar lavage fluid (BALF), inhibited surfactant protein A and C expression in lung tissue, and increased pathological injury. Pretreatment with KGF-2 improved the above lung injury parameters, partially restored surfactant protein A and C expression, and KGF-2 given 2–3 days before LPS challenge showed maximum lung injury improvement. Pretreatment with KGF-2 also markedly reduced the levels of TNF-α, MIP-2, IL-1β and IL-6 in BALF and the levels of IL-1β and IL-6 in lung tissue. Histological analysis showed there was increased proliferation of alveolar type II epithelial cells in lung parenchyma, which reached maximal 2 days after KGF-2 instillation. Intratracheal administration of KGF-2 attenuates lung injury induced by LPS, suggesting KGF-2 may be potent in the intervention of acute lung injury.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are common and potentially lethal respiratory diseases (Wheeler and Bernard, 2007). By new definition, ALI is the mild form of ARDS, which is also characterized by heterogeneous alveolar epithelium and endothelium damage with accumulation of plasma proteins and flooding in the alveoli causing refractory hypoxemic respiratory failure.

Keratinocyte growth factor-2 (KGF-2), namely fibroblast growth factor-10, has been shown to mediate epithelial–mesenchymal interactions, which are essential in lung development (Benjamin et al., 2007; Hyatt et al., 2004; Ware and Matthay, 2002). Recently KGF-2 showed preventive effects on lung injury from various stresses (Kim et al., 2009; Gupte et al., 2009; Upadhyay et al., 2004, 2005). With similarities to keratinocyte growth factor (KGF), KGF-2 is a heparin-binding protein predominantly expressed by mesenchymal cells. It binds with high affinity to a spliced variant of fibroblast growth factor receptor 2-IIIb which is expressed solely on epithelial cells. KGF-2 has a weaker affinity for fibroblast growth factor receptor 1-IIIb, which is expressed on epithelial/endothelial cells and is a functional transmembrane receptor for KGF-2 but not for KGF (Ware and Matthay, 2002; Beer et al., 2000; Igarashi et al., 1998; Yamasaki et al., 1996; Emoto et al., 1997). Thus, KGF acts specifically on epithelial cells, whereas KGF-2 seems to have broader cell type specificity (Kim et al., 2009).

The role of KGF in acute lung injury has been extensively studied since 1990s. But the concern about the carcinogenetic effect of KGF hindered its further translation to bedside (Chang et al., 2009; Mehta et al., 2010; Lin et al., 2011; Zang et al., 2009). However, KGF-2 had no *in vitro* or *in vivo* proliferative effects on human epithelial-like tumors (Alderson et al., 2002). This is critical to the safety profile of KGF-2 and provides its potential therapeutic feasibility.

The above studies suggest that KGF-2 might play an important role in ALI, but there are few studies directly assessed this possibility of exogenous KGF-2 *in vivo*. In this study, we used the ALI model

* Corresponding author at: 180 Fenglin Road, Shanghai 200032, PR China. Tel.: +86 21 64041990x3077; fax: +86 21 54961729.
** Corresponding author at: 180 Fenglin Road, Shanghai 20003, PR China. Tel.: +86 21 64041990x2422; fax: +86 21 54961729.
E-mail addresses: ylsong70@gmail.com (Y. Song), bai.chunxue@zs-hospital.sh.cn (C. Bai).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.resp.2014.06.011
1569-9048/© 2014 Elsevier B.V. All rights reserved.

induced by intratracheal instillation of lipopolysaccharide (LPS) to evaluate the effect of exogenous KGF-2.

2. Methods

2.1. Animals

Seventy male specific pathogen free Sprague-Dawley rats weighing 200–250 g were used in the experiments. Animals were maintained in the animal facility at Fudan University with clean, controlled temperature and independent ventilation environment. The animals had free access to food and water. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Zhongshan Hospital, Fudan University. All animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and efforts were made to minimize suffering and pain of the animals, and number in each group in our study.

2.2. Animal treatment

Rats were anesthetized with intraperitoneal injection of chloral hydrate (300 mg/kg). The instillate (recombinant human KGF-2 (rhKGF-2, recombinant product from *E. coli* after purification, MW 19.3 kDa, manufactured by Newsummit Pharmaceutical Company, Shanghai, China), phosphate buffered saline (PBS), or LPS) was then injected into the trachea using an 18G catheter attached to a 1-ml syringe as previously described (Su et al., 2004a). RhKGF-2 at a dose of 5 mg/kg was instilled through the catheter into the lungs of rats. The dose of KGF-2 used in the present study was based on previous experiments (She et al., 2012; Fang et al., 2014). Control animals received equal volume of PBS. After certain days (1, 2, 3, or 5 days), rats were intratracheally instilled with either 5 mg/kg LPS (*E. coli* O55:B5; Sigma, St. Louis, MO) dissolved in 0.3 ml PBS or vehicle (PBS). The rats were euthanized 24 h after LPS instillation with an intraperitoneal injection of urethane (1.5 g/kg) and exsanguinated through the cervical artery after sampling for arterial blood gas analysis. After wet weights were measured, the middle lobe of the right lung were placed in an oven at 60 °C for 72 h to allow determination of the wet-to-dry weight ratio.

2.3. Total cell count and differential cell count

The bronchoalveolar lavage was done in the left lung. Bronchoalveolar lavage fluid (BALF) samples were centrifuged, and the pellet was resuspended in PBS. The total number of nucleated cells in BALF was counted with a hemocytometer. Then the resuspended BALF was centrifuged onto slides and stained with Wright–Giemsa stain. The slides were used to quantify neutrophil number by counting a total of 200 cells per slide.

2.4. Protein concentration and cytokine levels in BALF

BALF protein concentration was measured using bicinchoninic acid protein assay (Thermo Fisher Scientific, MA, USA). Tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and macrophage inflammatory protein-2 (MIP-2) levels in BALF were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA) according to manufacture's guideline.

2.5. Lung morphometry analyses

The lungs were fixed in 10% formalin, and 5 μm sections were cut for hematoxylin and eosin staining. Lung injury was scored according to the following variables: alveolar and interstitial edema, neutrophil infiltration and hemorrhage. Each variable was graded according to a five-point scale: 1, no injury; 2, injury to 25% of the field; 3, injury to 50% of the field; 4, injury to 75% of the field; 5, diffuse injury (Su et al., 2004b). All samples were examined by 3 pathologists blinded to the experimental procedures and the mean score was used for comparison.

2.6. Immunohistochemistry analyses

Immunostaining of lung tissue was done using 5-μm paraffin sections. Labeling was obtained by incubation with rabbit anti-prosurfactant protein C (proSP-C) polyclonal antibody (Millipore, MA, USA), or mouse anti-Ki67 monoclonal antibody (Dako, Denmark). Sections were covered with DAB tetrahydroxychloride, and counterstained with hematoxylin.

2.7. Quantitative real-time polymerase chain reaction analyses

Total RNA was extracted using the Trizol reagent (Invitrogen, CA, USA). RNAs were reverse-transcribed, and real-time polymerase chain reaction was performed using primers for the examined transcripts (Table 1).

2.8. Western blot analyses

Lung tissues were homogenized and 20 μg of protein was electrophoresed. Membranes were exposed overnight at 4 °C to rabbit anti-proSP-C polyclonal antibody (Millipore, MA, USA), or rabbit anti-surfactant protein A (SP-A) polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, USA), or rabbit anti-β-actin polyclonal antibody (Abcam, UK) as loading control.

2.9. Statistical analyses

Each point corresponds to the mean ± SEM. Statistical differences were determined using the one-way analysis of variance, and $p < 0.05$ was considered significant. Individual groups were compared using the unpaired Student's *t* test.

3. Results

3.1. KGF-2 time-dependent protective effects on LPS-induced ALI

Intratracheal instillation of LPS induced ALI which is represented by a significant decrease in arterial PO_2, and a significant increase in BALF protein concentration, BALF total cell number, BALF neutrophil number, lung wet-to-dry weight ratio, and lung injury score 24 h after LPS administration compared to that in rats receiving PBS as control (Fig. 1).

In order to define the best response timing for rhKGF-2 on lung injury prevention, rhKGF-2 was instilled at different time points. Indeed, intratracheal instillation of rhKGF-2 1–5 days before LPS challenge showed different improvements in lung injury compared to animals without pretreatment (Fig. 1). Pretreatment with rhKGF-2 two or three days before LPS challenge resulted in a significant improvement in lung injury parameters compared with that in control rats receiving no pretreatment. Although there is no significant difference of above parameters between 2 and 3 days before LPS instillation, absolute number indicates pretreatment 3 days prior to insult might be slightly more effective than the other time points. So we decided to further investigate the protective effect of rhKGF-2 pretreatment 3 days before challenge in LPS-induced ALI.

Download English Version:

https://daneshyari.com/en/article/2846973

Download Persian Version:

https://daneshyari.com/article/2846973

Daneshyari.com