Toxicology Letters 240 (2016) 161–171

Contents lists available at ScienceDirect

Toxicology Letters

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

Calcitriol inhibits bleomycin-induced early pulmonary inflammatory response and epithelial–mesenchymal transition in mice

Zhu-Xia Tan[a,1], Yuan-Hua Chen[b,c,1], Shen Xu[a], Hou-Ying Qin[a], Cheng Zhang[b], Hui Zhao[a,*], De-Xiang Xu[b,**]

[a] Second Affiliated Hospital, Anhui Medical University, Hefei 230022, China
[b] Department of Toxicology, Anhui Medical University, Hefei 230032, China
[c] Department of Histology and Embryology, Anhui Medical University, Hefei 230032, China

HIGHLIGHTS

- Calcitriol attenuates BLM-induced inflammatory cytokines in the lungs.
- Calcitriol inhibits BLM-activated NF-κB signaling in the lungs.
- Calcitriol inhibits BLM-activated PI3K/Akt and p38 MAPK in the lungs.
- Calcitriol inhibits BLM-induced EMT in the lungs.
- Calcitriol inhibits BLM-activated TGF-β-Smad signaling in the lungs.

ARTICLE INFO

Article history:
Received 27 August 2015
Received in revised form 18 October 2015
Accepted 25 October 2015
Available online 28 October 2015

Keywords:
Calcitriol
Bleomycin
Inflammation
Nuclear factor kappa B p65
Epithelial–mesenchymal transition
Lung
Mice

ABSTRACT

Early pulmonary inflammation and epithelial–mesenchymal transition (EMT) play important roles during lung fibrosis. Increasing evidence demonstrates that calcitriol, the active form of vitamin D3, has anti-inflammatory activities. The aim of this study was to investigate the effects of calcitriol on bleomycin (BLM)-induced early pulmonary inflammation and subsequent EMT. Mice were intratracheally injected with BLM (3.0 mg/kg). In three calcitriol + BLM groups, mice were intraperitoneal (i.p.) injected with different doses of calcitriol (0.2, 1.0 or 5.0 μg/kg) daily, beginning at 48 h before BLM injection. Twenty-four hours, seven and fourteen days after BLM injection, pulmonary inflammation and EMT were evaluated. As expected, BLM-induced infiltration of inflammatory cells in the lungs was attenuated by calcitriol. BLM-induced pulmonary inflammatory cytokines were repressed by calcitriol. Moreover, BLM-induced nuclear translocation of nuclear factor kappa B (NF-κB) p65 was blocked by calcitriol. In addition, BLM-induced phosphorylation of pulmonary p38 MAPK and protein kinase B (Akt) was inhibited by calcitriol. Further analysis showed that BLM-induced α-smooth muscle actin (α-SMA), a marker for EMT in the lungs, was significantly attenuated by calcitriol. BLM-induced transforming growth factor-beta 1 (TGF-β1) up-regulation and Smad phosphorylation were attenuated by calcitriol. In conclusion, calcitriol inhibits BLM-induced early pulmonary inflammation and subsequent EMT.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Idiopathic pulmonary fibrosis, characterized by fibroblast proliferation and extracellular matrix remodeling, is a chronic pulmonary disease of unknown origin ultimately leading to death (Borchers et al., 2011; King et al., 2011). Bleomycin (BLM), a widely used anti-neoplastic drug, causes a dose-dependent interstitial pulmonary fibrosis in humans and experimental animals (Adamson and Bowden, 1974; Lazo et al., 1990; Chen and Stubbe, 2005). BLM-induced pulmonary fibrosis, resembling human interstitial pulmonary fibrosis (Moore and Hogaboam, 2008), has been the most commonly used model for idiopathic pulmonary fibrosis (Moeller et al., 2008). Although the mechanisms of BLM-induced pulmonary fibrosis are not completely understood, alveolar cell

* Corresponding author.
** Corresponding author. Fax: +86 551 65161179.
E-mail addresses: zhaohuichenxi@126.com (H. Zhao), xudex@126.com (D.-X. Xu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.toxlet.2015.10.022
0378-4274/© 2015 Elsevier Ireland Ltd. All rights reserved.

damage followed by an infiltration of numerous inflammatory cells and a diffuse inflammatory response, epithelial–mesenchymal transition (EMT) and subsequent extracellular matrix deposition are involved in the pathogenesis of BLM-induced pulmonary fibrosis (Tanjore et al., 2009; Hashimoto et al., 2010).

Vitamin D, a secosteroid hormone, is known for its classical functions in calcium uptake and bone metabolism (Holick, 2006). Recently, vitamin D is recognized for its non-classical actions including the modulation of innate immune, antioxygenation and anti-inflammatory activity (Hewison, 2010; Xu et al., 2015; Chen et al., 2015a,b). Vitamin D itself is devoid of biological activity. The active form of vitamin D, calcitriol [1,25(OH)2D3], is produced by cytochrome p450 (CYP) 27B1 and inactivated by CYP24A1 (Schuster, 2011). The actions of vitamin D are mediated by vitamin D receptor (VDR) that binds calcitriol to induce both transcriptional and non-genomic responses (Dimitrov et al., 2014). Although VDR is highly expressed in the lungs (Menezes et al., 2008), its function remains unclear.

Vitamin D deficiency is common and increasingly recognized as a global public health problem (Chen et al., 2015c). Increasing evidence demonstrates that there is a cause association between vitamin D deficiency and childhood asthma (Paul et al., 2012; Litonjua, 2012; Poon et al., 2013). Moreover, vitamin D deficiency is linked with an increased risk of respiratory infections (Lowery et al., 2012; Chalmers et al., 2013; Jeon et al., 2013; Hong et al., 2014). According to a double-blind and randomized controlled trial, vitamin D3 supplementation alleviates eosinophilic airway inflammatory response in patients with nonatopic asthma with severe eosinophilic airway inflammation (de Groot et al., 2015). In addition, vitamin D3 supplementation protects against moderate or severe exacerbation in patients with chronic obstructive pulmonary disease (Martineau et al., 2015).

The aim of the present study was to investigate the effects of calcitriol on early pulmonary inflammatory response and subsequent EMT in the process of BLM-induced pulmonary injury. Our results showed that pretreatment with calcitriol alleviated early pulmonary inflammatory response through blocking activation of several inflammatory signaling in the lungs. We demonstrate that calcitriol attenuates BLM-induced EMT through suppressing pulmonary transforming growth factor-beta 1 (TGF-β1)-Smad signaling.

2. Materials and methods

2.1. Chemicals and reagents

BLM and calcitriol were purchased from Sigma Chemical Co. (St. Louis, MO). Phosphor-MAPK p38 (pp38), NF-κB p65, α-SMA, β-actin and Lamin A/C antibodies were from Santa Cruz Biotechnologies (Santa Cruz, CA). Phosphor-Akt (pAkt) and Akt antibodies were from Cell Signaling Technology (Beverley, MA, USA). Chemiluminescence (ECL) detection kit was from Pierce Biotechnology (Rockford, IL). TRI reagent was from Molecular Research Center, Inc. (Cincinnati, Ohio). RNase-free DNase was from Promega Corporation (Madison, WI). All the other reagents were from Sigma or as indicated in the specified methods.

2.2. Animals and treatments

Adult male C57BL/6J mice (8 week-old, 24–26 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. The animals were allowed free access to food and water at all times and maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment. The present study consisted of two independent experiments.

Experiment 1, to investigate the effects of pretreatment with different doses of calcitriol on BLM-induced early pulmonary pathological damage and inflammatory cytokines, thirty mice were divided into five groups. In BLM alone group, mice were intratracheally injected with 3.0 mg/kg of BLM (dissolved in 50 μL phosphate buffered saline (PBS). The doses of BLM used in the present study referred to others (Pilling et al., 2014). In three BLM + calcitriol groups, mice were intraperitoneally (i.p.) injected with different doses of calcitriol (0.2, 1.0 or 5.0 μg/kg) daily, beginning at 48 h before BLM injection, the second at 24 h before BLM injection, and the third at 1 h before BLM injection. The doses of calcitriol used in the present study referred to others with minor modification (Ito et al., 2013). Control mice were i.p. injected with normal saline (NS) daily, beginning at 48 h before PBS injection, the second at 24 h before PBS injection, and the third at 1 h before PBS injection. All mice were euthanized by exsanguination during pentobarbital anesthesia (75 mg/kg, i.p.) 24 h after an intratracheal BLM injection. Whole lung was weighed and left lungs were collected for measurements of inflammatory cytokines. After the lung vasculature was flushed, right lung were excised for histopathologic examination.

Experiment 2, to investigate the effects of calcitriol pretreatment on BLM-induced pulmonary pathological damage and inflammatory signaling at different time points, four–two mice were divided into three groups. In BLM alone group, eighteen mice were intratracheally injected with a single dose of BLM (3.0 mg/kg). In BLM + calcitriol group, eighteen mice were i.p. injected with calcitriol (1.0 μg/kg) daily, beginning 48 h before an intratracheal BLM injection. In Control group, six mice were i.p. injected with NS daily, beginning 48 h before an intratracheal PBS injection. In BLM alone and BLM + calcitriol groups, six mice each group were euthanized by exsanguination during pentobarbital anesthesia either 24 h or 7 d or 14 d after an intratracheal BLM injection. Whole lung was weighed and left lungs were collected for measurements of inflammatory cytokines. After the lung vasculature was flushed, the superior lobe of right lung was excised for histopathologic examination. The middle and lower lobes of right lung were excised for immunoblots.

This study was approved by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University (Permit Number: 13-0016). All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

Table 1
Oligonucleotide sequences and size of primers.

Genes	Sequences	Sizes (bp)
18S	Forward: 5′-GTAACCCGTTGAACCCCATT-3′ Reverse: 5′-CCATCCAATCGGTAGTAGCG-3′	151
tnf-α	Forward: 5′-CCCTCCTGGCCAACGGCATG-3′ Reverse: 5′-TCGGGGCAGCCTTGTCCCTT-3′	109
il-1β	Forward: 5′-GCCTCGTGCTGTCGGACCCATAT-3′ Reverse: 5′-TCCTTTGAGGCCCAAGGCCACA-3′	143
il-6	Forward: 5′-AGACAAAGCCAGAGTCCTTCAGAGA-3′ Reverse: 5′-GCCACTCCTTCTGTGACTCCAGC-3′	146
mcp-1	Forward: 5′-GGCTGGAGAGCTACAAGAGG-3′ Reverse: 5′-GGTCAGCACAGACCTCTCTC-3′	93
mip-1α	Forward: 5′-GCAACCAAGTCTTCTCAGCG-3′ Reverse: 5′-TGGAATCTTCCGGCTGTAGG-3′	77
mip-2	Forward: 5′-TTGCCTTGACCCTGAAGCCCCC-3′ Reverse: 5′-GGCACATCAGGTACGATCCAGGC-3′	175

Download English Version:

https://daneshyari.com/en/article/2598602

Download Persian Version:

https://daneshyari.com/article/2598602

Daneshyari.com