Steroids 76 (2011) 1305–1309

Contents lists available at ScienceDirect

Steroids

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

1α,25-Dihydroxyvitamin D_3 inhibits neutrophil recruitment in hamster model of acute lung injury

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ARTICLE INFO

Article history:
Received 1 May 2011
Received in revised form 23 June 2011
Accepted 24 June 2011
Available online 2 July 2011

Keywords:
Acute lung injury
Neutrophils
1α,25-Dihydroxyvitamin D_3
Hamsters

ABSTRACT

The chemokine interleukin-8 (IL-8) is involved in the pathogenesis of acute lung injury (ALI). Although several studies have reported that 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$) suppresses IL-8 production *in vitro* and *in vivo*, 1α,25$(OH)_2D_3$ has not been demonstrated to be effective in an animal model of ALI. Here, we determined its effects of 1α,25$(OH)_2D_3$ in a hamster model where ALI was induced by lipopolysaccharide (LPS) inhalation. 1α,25$(OH)_2D_3$ inhibited neutrophil recruitment in the lung by approximately 40% without increasing plasma calcium concentration, while it did not inhibit monocyte recruitment. Our findings show that vitamin D_3 analogues may be suitable as novel anti-inflammatory agents for ALI.

1. Introduction

The hormonally active form of vitamin D, 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$), has traditionally been associated with calcium and phosphorus homeostasis and maintenance of skeletal architecture in both young and adult mammals. Vitamin D achieves this prominent physiological role by promoting absorption of dietary calcium and phosphorus from the small intestine and by influencing the number and/or activity of osteoclasts and osteoblasts [1,2]. Recently, the 1α,25$(OH)_2D_3$-vitamin D receptor system was found to mediate immunomodulation to control cytokines such as interleukin (IL)-1α, IL-1β, IL-2, IL-6, IL-8, and tumor necrosis factor-alpha [3,4]. IL-8 is regarded as one of the most important endogenous chemotactic factors for neutrophils, and its infiltration has been observed in conditions such as acute lung injury (ALI), acute respiratory distress syndrome (ARDS), and chronic obstructive pulmonary disease (COPD) and has been considered associated with lung disorders [5]. However, whether or not 1α,25$(OH)_2D_3$ can inhibit neutrophil recruitment in ALI remains unclear. Here we report the inhibition of neutrophil recruitment in hamster ALI by 1α,25$(OH)_2D_3$.

2. Materials and methods

2.1. Reagents

The reagents used in this study and their sources were as follows: Lipopolysaccharide (LPS; *Escherichia coli*, 0111:B4) obtained from Difco Laboratories (Detroit, MI). Dexamethasone-21-acetate obtained was purchased from Sigma–Aldrich Co., (St. Louis, MO). Sodium carboxymethylcellulose was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). 1α,25$(OH)_2D_3$ was synthesized at the Teijin Institute for Bio-Medical Research. The compound was dissolved in 100% ethanol to determine concentration according to Beer's law and stored at −20 °C until use. The λ_{max} used was 264 nm. The extinction coefficient used was 18,200 M^{-1} cm^{-1}.

2.2. LPS-induced acute lung inflammation in hamsters

LPS-induced acute lung inflammation was induced as described by Mitsuhashi et al. [6]. Male Syrian golden hamsters (8–10 weeks old; 120–140 g; Charles River Laboratories, Wilmington, MA) were kept in an acrylic chamber (30 × 38 × 50 cm, 57 L) throughout the LPS exposure period. A solution containing 2 mg/ml LPS in saline was aerosolized with an ultrasonic nebulizer (NE-U12; Omron Co., Kyoto, Japan) and air pump coupled to a manometer (flow rate

Abbreviations: ARDS, acute respiratory distress syndrome; ALI, acute lung injury; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; IL, interleukin; LPS, lipopolysaccharide.

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doi:10.1016/j.steroids.2011.06.009

was monitored and adjusted to 4.8 L/min) for 30 min. All procedures involving animal handling and their care were performed in accordance with the Institute for Teijin Bio-Medical Research Guidelines for the care and use of laboratory animals.

2.3. Intra-tracheal and peroral administration of 1α,25(OH)$_2$D$_3$

1α,25(OH)$_2$D$_3$ was dissolved in vehicle (0.3% (v/v) ethanol-0.02% (v/v) Triton X-100 in saline). Where indicated, hamsters were anesthetized by Fluothane (Takeda Pharmaceutical Co., Osaka, Japan) with a small animal anesthetizer (Model TK-4, Bio Machinery, Tokyo, Japan) and administered 1α,25(OH)$_2$D$_3$ intratracheally (1 ml/kg) immediately after LPS-induced pulmonary inflammation.

Where indicated, hamsters were administered 1α,25(OH)$_2$D$_3$ (dissolved in vehicle (10% (v/v) ethanol-0.1% (v/v) Triton X-100 in saline)) perorally (5 ml/kg) 1 h before LPS-induced pulmonary inflammation.

2.4. Peroral administration of dexamethasone-21-acetate

Dexamethasone-21-acetate was dissolved in vehicle (0.5% (w/v) sodium carboxymethylcellulose in distilled water). Hamsters were administered 1α,25(OH)$_2$D$_3$ perorally (5 ml/kg) 1 h before LPS-induced pulmonary inflammation.

2.5. Measurement of neutrophil and monocyte in BAL fluids

Animals were anesthetized intraperitoneally with urethane (1 g/kg) 24 h after LPS inhalation. After semi-excision of the trachea, plastic cannulas were inserted and airspaces were washed with 2 mL of 0.9% NaCl to obtain bronchoalveolar lavage (BAL) samples. These operations were repeated three times using different syringes. Cells in the BAL fluids (BALFs) were attached to the glass slides by cytospin preparation (Auto Smear CF-120, Sakura Seiki, Tokyo, Japan), and neutrophils and monocytes were stained using the Differential Quick Stain kit (Dade Behring, Inc., Deerfield, IL) and counted by optical microscopy (OPTIPHOT, Nikon Co., Tokyo, Japan).

2.6. Measurement of concentrations of albumin in BALFs

The BALFs were centrifuged at 13,000*g* for 2 min at 4 °C, and the concentration of albumin in the supernatant was measured using the A/G B-Test WAKO with the Hitachi U-1080 Auto Sipper Photometer (Hitachi, Ltd., Tokyo, Japan).

2.7. Detection of calcium levels in the plasma

Blood was collected from eyeground vein of hamsters in heparinized capillary tubes at 24, 48 and 72 h after 1α,25(OH)$_2$D$_3$ or vehicle administration perorally and centrifuged at 1000*g* for 10 min at 4 °C to separate the plasma. The levels of calcium in plasma were determined with an autoanalyzer (model 7070, Hitachi, Tokyo, Japan) using Autosera Ca (Daiichi Pure Chemicals Co., Tokyo, Japan).

2.8. Statistical analysis

Statistical analysis was performed with StatView, version 4.11 (Abacus Concepts, Inc., Berkeley, CA). Data were expressed as mean ± SEM. Groups were compared by Dunnett's multiple comparison test, and group values were considered significant at $p < 0.05$.

3. Results

3.1. Time-course of LPS-induced acute lung inflammation in hamsters

Inhalation of LPS resulted in acute lung inflammation. The time-course of the number of cells in the BALFs is shown in Fig. 1A. From the differential cell count analysis, monocytes were observed; however, most of the cells in the BALFs were neutrophils. The number of neutrophils increased markedly and peaked at 24 h after LPS inhalation (1.8×10^3 cells/ml at $t = 0$ and 4.9×10^6 cells/ml at $t = 24$ h) and then decreased. On the other hand, the number of monocytes showed only a small increase (1.4×10^5 cells/ml at $t = 0$ and 7.3×10^5 cells/ml at $t = 24$ h) in response to LPS inhalation. Two peaks corresponding to neutrophil and monocyte cell number were simultaneously observed at 24 h after LPS inhalation, and the cellular proportion of neutrophils and monocytes in the BALFs was 85% and 15%, respectively. LPS inhalation also caused an increase in albumin concentration, a parameter of vascular permeability, in the fluid (Fig. 1B). The increase in albumin content also peaked at 24 h after LPS inhalation.

3.2. Efficiency of 1α,25(OH)$_2$D$_3$ in inhibiting neutrophil and monocyte recruitment in the lung

In order to investigate whether 1α,25(OH)$_2$D$_3$ could inhibit neutrophil and monocyte recruitment in the inflamed lung, we tested the effect of peroral administration of 1α,25(OH)$_2$D$_3$ at 0.7, 3.4, and 17 μg/kg, respectively. 1α,25(OH)$_2$D$_3$ dose-dependently inhibited neutrophil recruitment into the lung. On the other hand, no inhibition of monocyte recruitment was observed (Fig. 2A). To evaluate the efficiency of 1α,25(OH)$_2$D$_3$ in the lung more directly, 1α,25(OH)$_2$D$_3$ was intratracheally administrated at 0.0064, 0.032, 0.16, and 0.8 μg/kg, respectively. Inhibition of neutrophil recruitment by intratracheal administration of 1α,25(OH)$_2$D$_3$ was observed stronger than that by peroral administration, and no inhibition of monocyte recruitment was observed (Fig. 2B). The estimated effective dose of 40% inhibition (ED_{40}) by the treatment of 1α,25(OH)$_2$D$_3$ for neutrophil recruitment was calculated at 3.3 μg/kg for peroral administration and 0.3 μg/kg for intratracheal administration.

3.3. Effect of 1α,25(OH)$_2$D$_3$ on the level of plasma calcium

To examine the effect on plasma calcium level, we measured calcium levels in plasma with different doses (1, 10 and 100 μg/kg) of 1α,25(OH)$_2$D$_3$ after peroral administration (24, 48 and 72 h). A significant increase of plasma calcium was caused by 100 μg/kg of 1α,25(OH)$_2$D$_3$ at 72 h after administration (Fig. 3).

3.4. Efficiency of dexamethasone-21-acetate in inhibiting neutrophil and monocyte recruitment in the lung

Glucocorticoids exhibit a broad range of inhibitory inflammatory effects, including inhibition of cytokine transcription, cellular activation, and growth factor production [7,8]. To evaluate the effect of a corticosteroid in this model, we tested the efficiency of dexamethasone-21-acetate (1, 5, and 10 mg/kg, perorally administrated) for inhibiting neutrophil and monocyte recruitment activity. Dexamethasone-21-acetate dose-dependently inhibited both neutrophil and monocyte recruitment into the lung (Fig. 4). Estimated ED_{40} for neutrophil and monocyte recruitment was calculated as 8.6 and 3.8 mg/kg, respectively.

3.5. Administration timing of 1α,25(OH)$_2$D$_3$ for inhibiting neutrophil recruitment

We tested the administration timing of 1α,25(OH)$_2$D$_3$ to determine the time point when 1α,25(OH)$_2$D$_3$ can inhibit neutrophil recruitment under inflammation. At 1, 3, and 6 h after LPS inhalation, 1 μg/kg of 1α,25(OH)$_2$D$_3$ was immediately administered intratracheally, and BALFs were collected at 24 h after LPS inhalation. Inhibition of neutrophil recruitment was observed only at