Biochemical and Biophysical Research Communications xxx (2015) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Chenodeoxycholic acid attenuates ovalbumin-induced airway inflammation in murine model of asthma by inhibiting the T_H2 cytokines

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ARTICLE INFO

Article history:
Received 18 May 2015
Accepted 29 May 2015
Available online xxx

Keywords:
Airway inflammation
Farnesoid X receptor
Nuclear receptor
T_H2 cytokines

ABSTRACT

Asthma is a complex highly prevalent airway disease that is a major public health problem for which current treatment options are inadequate. Recently, farnesoid X receptor (FXR) has been shown to exert anti-inflammatory actions in various disease conditions, but there have been no reported investigations of Chenodeoxycholic acid (CDCA), a natural FXR agonist, in allergic airway inflammation. To test the CDCA effectiveness in airway inflammation, ovalbumin (OVA)-induced acute murine asthma model was established. We found that lung tissue express FXR and CDCA administration reduced the severity of the murine allergic airway disease as assessed by pathological and molecular markers associated with the disease. CDCA treatment resulted in fewer infiltrations of cells into the airspace and peribronchial areas, and decreased goblet cell hyperplasia, mucus secretion and serum IgE levels which was increased in mice with OVA-induced allergic asthma. The CDCA treatment further blocked the secretion of T_H2 cytokines (IL-4, IL-5 and IL-13) and proinflammatory cytokine TNF-α indicate that the FXR and its agonists may have potential for treating allergic asthma.

1. Introduction

Asthma is a disease characterized by reversible airway obstruction, airway inflammation, that results in narrowing of airways leading to shortness of breath, chest tightness, coughing and wheezing. Prominent characteristics of allergic asthma is overproduction of antigen-specific IgE, extreme immunological responses, and consequent inflammation and clinical symptoms include excessive mucus secretion and bronchospasm [1]. Asthma may affect nearly 334 million people all-over the world according to the Global asthma report 2014, prevalence continuing to increase with tremendous socioeconomic impact in developing countries. Current medications targeting specific and crucial pathways have shown some beneficial effects, but the standard therapy is continuous use of potent anti-inflammatory glucocorticoids, but associated side effects limit enthusiasm for their use [2]. Furthermore, they fail to suppress significant aspects of asthma pathology and are ineffective in some patients [3,4], hence alternative, efficacious and safe anti-inflammatory strategies are required to combat this disease.

Nuclear receptors with anti-inflammatory properties have recently begun to attract attention to develop potential novel treatments, for better understanding and management of asthma [5,6]. Several nuclear hormone receptors, including peroxisome proliferator-activated receptor-γ (PPAR-γ) and farnesoid X receptor (FXR), have been shown to exert anti-inflammatory and anti-fibrotic activity [7,8]. The role of PPAR-γ in asthma and its potential as a therapeutic target is currently under active investigation, but there have been no reported investigations of FXR in this context. We accordingly propose to study the ability of FXR activation to reduce airway inflammation in a murine model of asthma generated by ovalbumin (OVA).

FXR is mainly expressed in liver, intestine, kidney and adrenal glands [9,10]. Recently, it is also found to be expressed in endothelial cells of lungs [11]. Chenodeoxycholic acid (CDCA), lithocholic acid, deoxycholic acid, and ursodeoxycholic acid (UDCA) are the natural FXR ligands [12–17]. FXR acts as a multipurpose

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http://dx.doi.org/10.1016/j.bbrc.2015.05.104
0006-291X/

nuclear receptor as it plays crucial roles in controlling bile acid homeostasis, lipoprotein and glucose metabolism. Thus its agonists proved useful in the treatment of various disorders including inflammation [10,18]. FXR has been proved to down-regulate the genes involved in inflammation. $FXR^{-/-}$ mice studies revealed that FXR deficiency as a significant risk factor in the development of various inflammatory diseases [19,20]. To assess whether CDCA, a natural agonist of FXR, might have therapeutic potential in asthma, we tested the effects of CDCA on inflammation and asthma-relevant pathophysiological markers in a murine model of allergic airway disease.

2. Materials and methods

2.1. Animals and experimental protocol

Female Balb/C mice at 6–7 weeks of age (22–24 g), free of murine specific pathogens, were obtained from National Institute of Nutrition, Hyderabad, India, and maintained on a 12-h light and 12-h dark cycle and fed with standard rodent chow and water *ad libitum*. All procedures were approved by the institutional animal ethics committee for the care and use of laboratory animals. Mice were randomly divided into three groups, namely control, ovalbumin (OVA) and OVA-CDCA group. To induce asthma mouse was sensitised and challenged as described previously with little modifications [21]. Briefly, mice of OVA and OVA-CDCA groups were sensitized by intraperitoneal injection of 100 μg of chicken egg white OVA (Grade V, sigma, USA) adsorbed to 1 mg of adjuvant aluminium hydroxide (alum) (IMject Alum; Thermoscientific, Rockford) in 100 μl of sterile PBS to each mouse on days 0, 10 and the control group mouse were injected with 200 μl PBS alone. The response was localised to airways by intranasally, on days 19–24, the anaesthetized mice of OVA group and OVA-CDCA group were challenged with OVA by intranasally with 1% OVA in 25 μl of sterile PBS and for control group with 25 μl of sterile PBS alone (Fig. 1A). After 24 h of final OVA challenge the mice were sacrificed for measurement of various markers associated with airway inflammation. To evaluate the therapeutic effects of CDCA, OVA-CDCA group received 100 mg/kg body weight by oral gavage [22] one day before intranasal OVA challenge and continued till the end of the study. CDCA (Sigma Aldrich–India) was dissolved in 0.5% carboxymethyl cellulose (Sigma) as a vehicle.

2.2. RNA isolation and RT-PCR

Total lung RNA was extracted by using TRIZOL reagent (Sigma) according to the manufacturer's instructions [23]. RNA was quantified by measuring absorption at 260 nm and stored at −80 °C unitl use. The total RNA was reverse-transcribed to cDNA using the first-strand cDNA synthesis system (Takara Clontech, India). Then these first strand cDNAs were used for PCR amplification of FXR and GAPDH. The primers used were as follows:

FXR-forward, TGGGCTCCGAATCCTCTTAGA,
FXR-reverse, CTCGTCCGTAGCCTGTAAATGGG and
GAPDH-forward, CTGAGTATGTCGTGGAGTCTAC,
GAPDH-reverse, GTTGGTGGTGCAGGATGCATTG.

2.3. Isolation of bronchoalveolar lavage (BAL) fluid cells and counting

Twenty-four hours after final intranasal challenge, mice were anesthetized and BAL fluid was collected as described previously [24]. Briefly, the trachea was cannulated with an 18-gauge needle, and the lungs were lavaged twice with 1 ml PBS. The lavage fluid was pooled and centrifuged at 1500 rpm for 10 min at 4 °C, and stained with trypan blue, and the cells were counted using a hemocytometer. For differential counts, cells were spun onto glass slides using a cytocentrifuge and stained with Diff-Quick (Fisher Scientific International).

2.4. Measurement of total and OVA-specific IgE

After 24 h following the final OVA challenge, the blood was collected from right ventricle and centrifuged at 2500 × *g* for 10 min. The serum was then separated and stored at −80 °C until use. Concentrations of total serum IgE (BD Biosciences) and OVA-specific IgE (Cayman chemicals) levels were determined according to the manufacturer's instructions. Concentrations were calculated using a standard curve generated with the kit's IgE standard.

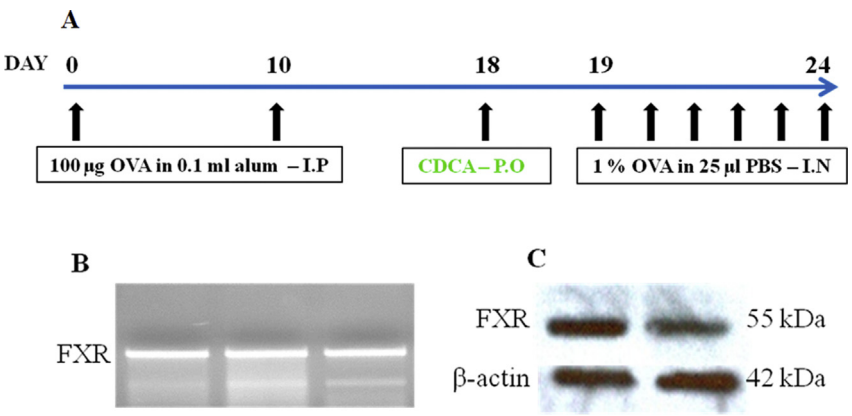

Fig. 1. A. Schematic representation of the acute ovalbumin (OVA)-induced asthma. Mice were immunized intraperitoneally (I.P) on days 0, and 10 with OVA. Intranasal (I.N) OVA challenges were administered on days 19–24. Control mice were sensitized and challenged with PBS alone. Mice were sacrificed 24 h after the final OVA challenge. Chenodeoxycholic acid (CDCA, 100 mg/kg body weight) was administered by oral gavage (P.O) starting on day 18, one day before the first I.N OVA challenge and then continued daily till the end of the study. B. Reverse transcriptase-PCR analysis of FXR mRNA of control mouse lung. C. Western blot analysis shows FXR is constitutively expressed in control mice lung at 45 kDa. Experiments were repeated three times and representative data was shown.

Please cite this article in press as: F.B. Shaik, et al., Chenodeoxycholic acid attenuates ovalbumin-induced airway inflammation in murine model of asthma by inhibiting the T_H2 cytokines, Biochemical and Biophysical Research Communications (2015), http://dx.doi.org/10.1016/j.bbrc.2015.05.104