Microbial Pathogenesis 125 (2018) 313–317

Contents lists available at ScienceDirect

Microbial Pathogenesis

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

Bacterial respiratory tract inflammation in neonatal rat model is attenuated by benzofuran through inhibition of GATA3

Aibibai Aierken[a], Peiru Xu[a,b,*]

[a] Department of Pediatrics, First Affiliated Hospital of Xinjiang Medical University, Urumqi, 830054, China
[b] The Xinjiang Uygur Autonomous Region Institute of Evidence-based Medicine, Urumqi, 830054, China

ARTICLE INFO

Keywords:
T-helper 2 cytokines
Transcription factor
Therapeutic
Methacholine

ABSTRACT

The current study was aimed to investigate the effect of benzofuran on asthma neonatal rat model. Twenty-five neonatal rats were assigned into five groups; Normal control, untreated, 1 mg/kg, 8 mg/kg and 10 mg/kg treatment groups. Methacholine was administered orally to the rats of untreated and treatment groups. Animals in the normal control group were given PBS as a vehicle. FlexiVent system employing a computer-controlled mouse ventilator along with respiratory mechanics was used for the analysis of airway resistance in the rats. Cytokine level and IFN-γ in the rat serum samples was performed by ELISA in accordance with the instructions of manufacturer. Methacholine administration into the rats caused a marked increase in lung airway resistance. However, treatment with 8 and 10 mg/kg doses of benzofuran led to marked decrease in the airway resistance. Benzofuran treatment prevented accumulation of macrophages and inflammatory cells in the lung airways. Inhibition of inflammation in methacholine administered rats by benzofuran was also confirmed by hematoxylin & eosin-staining. Examination of the rat serum showed significantly higher level of Th2 cytokines (IL-4, -5 and -13) in the untreated rats. However, treatment of methacholine administered rats with benzofuran significantly inhibited Th2 cytokine expression. The level of IFN-γ was increased by benzofuran treatment in methacholine administered rats. In methacholine administered rats the level of IgE was markedly higher however treatment of asthma rats with benzofuran inhibited up-regulation of IgE significantly. The expression of T-bet is decreased and that of GATA-3 is increased by methacholine administration in the rat lungs. Benzofuran treatment of methacholine administered rats prevented reduction in T-bet and up-regulation of GATA-3 expression in the rat lungs. The effect of benzofuran was significant at the doses of 8 and 10 mg/kg and non-significant at 1 mg/kg. These finding suggest that benzofuran inhibits expression of dominant T-helper 2 cytokines through targeting GATA-binding protein 3 transcription factor. Thus benzofuran can be of therapeutic importance for the treatment of asthma.

1. Introduction

Bacterial respiratory tract inflammation is a heterogeneous and chronic disease of respiratory tract involving inflammation of lung airways and problem in breathing. Moreover, asthma is characterised by the production of excessive airway mucus and higher level of serum immunoglobulin E (IgE). Globally 1–18% of the population is suffering from asthma and are being treated using corticosteroids [1]. Studies have revealed that Type 2 inflammation which plays important role in asthma pathogenesis is the result of Th (T helper)2, Th17 and regulatory T (Treg) cells [2]. Treg cells constitute a type of $CD4^+$ T cells and play an important role in the prevention of inflammation and immune tolerance [3]. The immune suppressive action of Treg cells is mediated by the expression of Forkhead box protein 3 (FOXP3) [4]. Treg cells inhibit the proinflammatory cytokine activation as well as suppress Th2 cell proliferation [5]. Thus Treg cells play a vital role in the asthma development by inhibiting Th2 cell activation, inflammatory cell infiltration and targeting IgE production [3].

Th2 cells express interleukin (IL)-4, −5 and −13 which are reported to be associated with development and progression of asthma. Th2 cells undergo differentiation in the presence of GATA-binding protein 3 (GATA-3) transcription factor which belongs to the zinc finger protein family [6]. Expression of GATA-3 is controlled by T cells through the involvement of T-bet. It is reported that T-bet inhibits production of IL-4 and promotes expression of interferon (IFN)-γ [7].

Natural as well as synthetic compounds containing benzofuran

* Corresponding author. The Xinjiang Uygur Autonomous Region Institute of evidence-based medicine, 137 South Road, Carp Mountain, Urumqi, the Xinjiang Uygur Autonomous Region, First Affiliated Hospital of Xinjiang Medical University, 830054, China.
E-mail address: xupeiru126@126.com (P. Xu).

https://doi.org/10.1016/j.micpath.2018.09.043
Received 1 July 2018; Received in revised form 28 September 2018; Accepted 28 September 2018
Available online 29 September 2018
0882-4010/

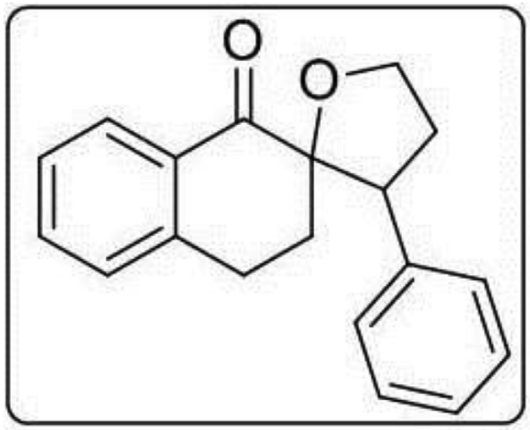

Fig. 1. Structure of benzofuran.

possess range of biological activities. They act as antimicrobial [8,9], anti-cancer [10], anti-inflammatory [11], antihyperglycemic and kinase inhibitor agents [12,13]. In addition, spirobenzofuran is the pharmacophore in several pharmaceutically significant compounds which exhibit broad spectrum of activities [14,15]. The present study was designed to discover effective treatment strategy and understand the mechanism of asthma fully. Herein, the effect of benzofuran (Fig. 1) on bacterial respiratory tract inflammation rat model was investigated. It was observed that benzofuran inhibits expression of dominant T-helper 2 cytokines through targeting GATA-binding protein 3 transcription factor. Thus benzofuran can be of therapeutic importance for the treatment of asthma.

2. Materials and methods

Experimental animals. Twenty five Sprague-Dawley neonatal rats 20–25 g in weight were obtained from the Center for Laboratory Animals of Academy of Military Medical Sciences (Beijing, China). The animals were maintained under standard laboratory atmosphere at a temperature 25 °C, with 60 ± 10% relative humidity and 12-h light/dark cycle. Standard pellet diet and sterilized water was provided to the animals. The rats were acclimated to the laboratory atmosphere for seven days before the pre-clinical studies. The experimental protocols for animals were approved by the ethics committee of the Capital Medical University (Beijing, China).

Experimental design and animal model preparation. Twenty-five neonatal rats were assigned into five groups (five animals in each group) after laboratory acclimation; Normal control, untreated, 1 mg/kg, 8 mg/kg and 10 mg/kg treatment groups. Methacholine (Sigma-Aldrich; St. Louis, MO, USA) 16 mg/ml was administered orally to the rats of untreated, 1 mg/kg, 8 mg/kg and 10 mg/kg treatment groups. Animals in the normal control group were given PBS as a vehicle. After methacholine administration rats in the three treatment groups received 1 mg/kg, 8 mg/kg and 10 mg/kg doses of benzofuran (Sigma-Aldrich; St. Louis, MO, USA).

Sensitization and treatment. Neonatal rats were subjected to sensitization using reported protocol [16] by intraperitoneal administration of two doses of egg albumin (40 μg) plus aluminum hydroxide (2.6 mg) in 200 μl PBS on 0 and 7th days. Methacholine (Sigma-Aldrich; St. Louis, MO, USA) 16 mg/ml was administered orally to the rats of untreated, 1 mg/kg, 8 mg/kg and 10 mg/kg treatment groups. Benzofuran at the doses of 1 mg/kg, 8 mg/kg and 10 mg/kg was given to the rats in three treatment groups daily from days 20–30. On day 31st the rats were sacrificed using ether anaesthesia (Sigma-Aldrich) by cardiac puncture. For evaluation of lung eosinophilia the rats were subjected to bronchoalveolar lavage.

Analysis of hyper response in lung airways. As reported in earlier studies [17] FlexiVent system (Synol High-Tech, Beijing, China) employing a computer-controlled mouse ventilator along with respiratory mechanics was used for the analysis of airway resistance in rats.

Broncho alveolar lavage fluid (BALF) collection. On day 31st the rats were sacrificed using ether anaesthesia to collect the broncho alveolar lavage fluid for analysis of cytokines and differential cell count. This was achieved by washing the rat lungs three times with PBS (0.5 ml) and EDTA (0.05 mM). After collection broncho alveolar lavage fluid was subjected to centrifugation for 5 min at 4 °C temperature at 4000 × g to obtain the cells. Supernatant was separated and kept at a temperature of −70 °C. Calculation of the cells suspended in PBS plus EDTA (0.05 mM) was performed using hemocytometer.

Measurement of cytokine level. Determination of the cytokine level (interleukin-5, interleukin-13 and interleukin-4) and IFN-γ in the rat serum samples was performed by ELISA (R&D Systems, Minneapolis, MN, USA) in accordance with the instructions of manufacturer.

Determination of serum immunoglobulin E. The wells of microtiter plate (Abcore, Ramona, CA, USA) were subjected to egg albumin coating at 4 °C for overnight. The plates were then washed and subsequently Tween 20 (0.5%) was used to block the non-specific sites. Into the wells coated with silver rat sera was put and the plates were incubated. After incubation biotinylated anti-mouse IgE (BD Pharmingen, San Diego, CA, USA) was used for the detection of immunoglobulin E.

Western blot analysis. On day 31st rats were sacrificed to collect lungs which were subsequently subjected to homogenization in aprotinin (1.5 μM), AEBSF (5 μM), leupeptin (0.01 μM), E−64 (10 μM) and phosphatase inhibitors [sodium orthovanadate (Na2VO4; 1 mM); sodium molybdate (1 mM) sodium tartrate dehydrate (4 mM); imidazole (2 mM) from Sigma-Aldrich]. The protein concentration in the lung homogenates was determined by bicinchoninic protein assay kit (Pierce, Rockford, IL, USA). The protein samples (30 μg) were separated by SDS-PAGE gel (10%) and subsequently transferred onto the polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA, USA) using electroblotting. Then the membranes were incubated in blocking solution for 1 h followed by PBS-Tween washing. The membranes were then incubated overnight with mouse polyclonal antibodies against GATA3 and T-bet antibodies (1:1000 dilution; Bio-Rad Laboratories, Inc.) and a monoclonal anti-β-actin as the internal loading control (Sigma-Aldrich) at 4 °C. The membranes were washed twice with PBS before incubation for 1 h at room temperature with horseradish peroxidase-conjugated polyclonal horse anti-rabbit (1:2000; Cell Signaling Technology, Inc., Danvers, Ma, USA). Enhanced chemiluminescence kit (Intron Biotechnology, Inc., Seongnam, Korea) was used for the detection of the blot.

Histological analysis. The lungs were extracted from the rats and left lung was subjected to fixing for 24 h in 10% formalin. Then the samples were subjected to paraffin embedding after dehydration. The paraffin embedded samples were sliced into thin 2-μm sections and subsequently subjected to hematoxylin & eosin staining. The histological examination of tissue samples was carried out using a light microscope (MCL-3000) connected with image-analysis system (Image-Pro Plus 4.0; Media Cybernetics, Minneapolis, MN, USA).

Statistical analysis. All the data presented are the means ± standard deviation (SD) obtained independently from three experiments. Analysis of the data was performed by one-way analysis of variance (ANOVA) and multiple comparisons were made by LSD method. The data were processed using GraphPad Prism 5 and SPSS 17.0. Differences were taken to be significant statistically at $P < 0.05$.

3. Results

Airway hyper responsiveness in asthma rat model is reduced by benzofuran. Methacholine administration into the rats caused a marked increase in the lung airway resistance compared to the control group. Treatment of methacholine administered rats with benzofuran