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Inhibition of airway inflammation and remodeling by sitagliptin in murine chronic asthma

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ABSTRACT

In this study the role of sitagliptin, dipeptidyl peptidase inhibitor, DPP-4, and dexamethasone in ameliorating inflammation and remodeling of chronic asthma in a mouse model were investigated. Mice sensitized to ovalbumin were chronically challenged with aerosolized antigen for 3 days a week continued for 8 weeks. During this period animals were treated with sitagliptin or dexamethasone daily. Assessment of inflammatory cell, oxidative markers, total nitrate/nitrite (NOx), interleukin (IL)-13, transforming growth factor-beta1 (TGF-β1) in bronchoalveolar lavage (BAL) and/or lung tissue were done. Also histopathological and immuno-histochemical analysis for lung was carried out. Compared with vehicle alone, treatment with sitagliptin or dexamethasone significantly reduced accumulation of eosinophils and chronic inflammatory cells, subepithelial collagenization, and thickening of the airway epithelium. Also both drug reduced goblet cell hyperplasia, oxidative stress, TGF-β1, IL-13 and epithelial cytoplasmic immunoreactivity for nuclear factor κ-B (NFκ-B). These data indicate that sitagliptin like dexamethasone may play a beneficial role reducing airway inflammation and remodeling in chronic murine model of asthma.

1. Introduction

Asthma is a chronic inflammation of the airways with a variety of structural changes such as mucous cell hyperplasia/metaplasia, subepithelial fibrosis, and increased smooth muscle mass, which are collectively named as airway remodeling [1]. Changes of remodeling correlate with the development of airway hyperreactivity (AHR) [2] and with persistent airflow limitation in chronic asthmatics [3]. Airway remodeling includes airway wall thickening, subepithelial fibrosis, and hyperplasia of mucus glands, myofibroblasts, smooth muscle, and vasculature [4]. Airway remodeling is thought to occur as a result of an imbalance in the mechanism of regeneration and repair.

Some studies have addressed the importance of oxidative stress in asthma physiopathology. Eosinophils, alveolar macrophages, and neutrophils from asthmatic patients produce more ROS than do those from normal subjects. The overproduction of ROS or depression of protective mechanisms results in bronchial hyperreactivity, which is characteristic of asthma [5]. Evidence for increase of oxidative stress in asthma is further provided by the finding of defective endogenous antioxidant capacity in asthmatic patients [6].

Airway mucosal inflammation is characterized by an influx of activated eosinophils and T lymphocytes [7], and numerous investigations have shown that Th2 cytokines (particularly IL-4, IL-5, and IL-13) play critical roles in orchestrating the allergic inflammatory response leading to AHR [8]. Among various transcription factors, NF-κB is a transcriptional factor that regulates and co-ordinates the expression of various inflammatory genes and inflammatory mediators, including cytokines, chemokines, adhesion molecules, immunoreceptors and growth factors. Therefore, it is often termed as a "central mediator of human immune response". NF-κB is the master switch for pro-inflammatory genes and has been involved in asthma both in experimental models and in humans [9,10]. Transforming growth factor (TGF)-β1, has emerged as a key mediator of pulmonary fibrosis, which accelerates fibrotic changes through the accumulation of extracellular matrix, may play a key role in this airway remodeling process. TGF-β1 expression correlates with basement membrane thickness and fibroblast number [4]. Furthermore, although TGF-β1 is reported to be an important factor in the regulation of acute pulmonary inflammation, as in pneumonia [11] its role in asthma remains to be defined.

Sitagliptin is a selective dipeptidylpeptidase-4 (DPP-4/CD26) inhibitor indicated for the treatment of type II diabetes mellitus. DPP-4 inhibitors protect endogenous GLP-1 and GIP from N-terminal degradation thereby prolonging their bioactivity. DPP-4 occurs in all organs including the small intestine, biliary tract, exocrine pancreas, spleen, and brain in both rodents and humans. This widespread organ distribution indicates that DPP-4 has pleiotropic biological activities [12]. DPP-4/CD26 has been reported to be expressed in the serosal submucosal glands of the human bronchus and in human BAL fluid [13]. Also, DPP-4/CD26 is expressed on a fraction of resting T cells and it is strongly

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up-regulated following T cell activation. It truncates several chemokines as well as neuropeptides and influences immune responses via modulation of cell adhesion and T cell activation, suggesting an involvement of DPP-4/CD26 in asthmatic and airway inflammation [14]. Moreover, sitagliptin inhibited immediate-type hypersensitivity allergic reaction through inhibition of histamine release and proinflammatory cytokine production in rat peritoneal mast cells [15]. It has been demonstrated in an acute murine model of bronchial asthma that sitagliptin can enhance antioxidant status and downregulate some inflammatory cytokines expression through exerting antiinflammatory/immunomodulatory activities [16]. In this study, using a chronic model of allergic airway inflammation with subepithelial fibrosis, the effect of sitagliptin on underlying mechanisms potentially involved in airway inflammation and remodeling was investigated.

2. Material & methods

2.1. Drugs and chemicals

Ovalbumin (OVA; chicken egg, Grade V), aluminum hydroxide hydrate and urethane were purchased from Sigma, St. Louis, MO, USA. Dexamethasone (gifted from Amoun Pharmaceutical Co., Egypt). The highly water soluble sitagliptin phosphate monohydrate was obtained as tablets from the Medical Center hospital pharmacy of Mansoura University.

2.2. Animals

Female BALB/c mice were purchased from Urology & Nephrology center, Mansoura University, Egypt and initiated into experiments at 7–9 weeks of age (20 ± 2 g). Mice were housed under specific pathogen-free conditions following a 12-h light–dark cycle. Cages, food, and bedding were autoclaved. The experimental protocol conducted in the study complies with the ethical guidelines and the principals of care, use and handling of experimental animals adopted by "The research Ethics Committee", Faculty of Pharmacy, Mansoura University, Egypt which is in accordance with "Principles of Laboratory Animals Care" (NIH publication No. 85-23, revised 1985).

2.3. Sensitization and antigen challenge

The mice were housed in environmentally controlled pathogen free conditions for 1 week prior to the study and during the experiments. They were sensitized on days 0 and 14 by intraperitoneal injection of 10 μg OVA emulsified in 0.5 mg of aluminum hydroxide in a total volume of 200 μl saline. Seven days after the last sensitization, mice were exposed to OVA aerosol (1% *w/v* diluted in sterile physiological saline) for up to 30 min three times per week for 8 weeks. Aerosolization was performed for 30 min by placing the mice in custom-built cylindrical chamber (20 × 30 × 35 cm) connected to the ultrasonic air nebulizer (Omron, Vernon Hills). The outlet of the chamber was connected to a vacuum pump, and a constant flow rate of 15 ml/min and particle size 2–6 μm was ensured by a flow meter (Hoffer Flow Controls, USA). Sixty female mice were randomly divided into six groups with 10 mice in each group, and treated as follows; (i) Normal group; mice were treated with saline, (ii) Control group; saline challenged group: Mice were sensitized by I.P. injection of 0.5 mg Alum in 0.2 ml saline, challenged by nebulization of saline solution and received 0.5% CMC (10 ml/kg, orally) 1 h before saline challenge, (iii) Sita-group; mice were sensitized by I.P. injection of 0.5 mg Alum in 0.2 ml saline, challenged by nebulization of saline solution and received by oral gavage 10 mg/kg sitagliptin, (iv) OVA-group; OVA-sensitized/challenged group mice were sensitized and challenged with OVA. (v) Dexamethasone-treated group; (Dexa–OVA) comprised mice that were sensitized and challenged as in the asthmatic group described above, and treated with 2 mg/kg dexamethasone by ip injection 1 h before challenge and (vi) Sita–OVA treated group: mice were sensitized and challenged as in asthmatic mice, and were given 10 mg/kg sitagliptin orally 1 h before challenge.

At the end of the experimental study, the animals were anesthetized using urethane (2.5 mg/kg) 24 h after the last challenge. The chest was opened and the trachea with the heart–lung package was excised from the thorax then the left main bronchi were clamped. A cannula was inserted into the trachea in situ, and the right lung was lavaged three times with portions of 1 ml PBS solution and BAL fluid was collected for subsequent analysis. Thereafter, the left lung and the trachea were collected for histopathological examination. The inferior lobe was dissected and homogenized for subsequent analysis.

2.4. BAL preparation and measurement of inflammatory cell counts

The BAL samples were centrifuged for 5 min at 1000 g and 4 °C to collect whole cells in the pellet. The supernatant was carefully removed and stored at −80 °C for subsequent analysis. The pellet of cells that remains after centrifugation of BAL samples was resuspended in an equal volume of fresh PBS (100 μl). Total inflammatory cell number was assessed by counting of cells in at least five squares of a hemacytometer. Smears of BAL cells prepared with Cytospin II (Shandon, Runcorn, UK) were stained with Diff-Quik solution (B4132-1A; Dade Behring Inc., Deerfield, IL) for differential cell counting. Approximately 200 cells were counted in each of four different random locations at 40 magnifications. Differential cell count was performed through quantification of the slides for eosinophils, lymphocytes, neutrophils and macrophages.

2.4.1. Measurement of nitric oxide in BAL fluid

The concentration of total nitrate/nitrite (NOx) was measured in BAL fluid as an indicator of NO production using NO assay kit according to the supplier's specifications (R&D Systems, Minneapolis, USA). The amount of NOx in the test samples were determined by interpolation of the result into the standard curve of nitrite prepared from serial dilutions of a 2000 μM stock solution. All assays were performed in duplicate and expressed as μM/g tissue.

2.4.2. Measurement of IL-13 & TGF-β1 levels

The amount of IL-13 and TGF-β1 in BAL fluid was measured using enzyme linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, USA) according to the manufacturer's directions.

2.5. Preparation of lung homogenate

The tissue samples of lung were weighed and homogenized (1:10, *w/v*) in 0.1 M phosphate buffer (pH 7.4) in an ice bath. The homogenate was centrifuged at 3000 g for 20 min at 4 °C. The supernatant was used for measurement of MDA, SOD and GSH.

2.5.1. Measurement of oxidative stress in lung tissue

2.5.1.1. Measurement of malondialdehyde (MDA). The MDA concentration was determined as an indicator of lipid peroxidation in lung tissue. The MDA content was measured using a commercial kit purchased from Bio-diagnostic Company (Giza, Egypt). MDA was expressed as nmol/g tissue.

2.5.1.2. Measurement of superoxide dismutase (SOD). The SOD activity was estimated using a commercial kit purchased from Bio-diagnostic Company (Giza, Egypt). The SOD activity was expressed as units/g tissue.

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