International Immunopharmacology 26 (2015) 237–245

Contents lists available at ScienceDirect

International Immunopharmacology

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

Oxidative airway inflammation leads to systemic and vascular oxidative stress in a murine model of allergic asthma

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ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form 24 March 2015
Accepted 24 March 2015
Available online 3 April 2015

Keywords:
Airway inflammation
Blood
Oxidative stress
Antioxidants
Aorta
Allergens

ABSTRACT

Oxidant–antioxidant imbalance plays an important role in repeated cycles of airway inflammation observed in asthma. It is when reactive oxygen species (ROS) overwhelm antioxidant defenses that a severe inflammatory state becomes apparent and may impact vasculature. Several studies have shown an association between airway inflammation and cardiovascular complications; however so far none has investigated the link between airway oxidative stress and systemic/vascular oxidative stress in a murine model of asthma. Therefore, this study investigated the contribution of oxidative stress encountered in asthmatic airways in modulation of vascular/systemic oxidant–antioxidant balance. Rats were sensitized intraperitoneally with ovalbumin (OVA) in the presence of aluminum hydroxide followed by several intranasal (i.n.) challenges with OVA. Rats were then assessed for airway and vascular inflammation, oxidative stress (ROS, lipid peroxides) and antioxidants measured as total antioxidant capacity (TAC) and thiol content. Challenge with OVA led to increased airway inflammation and oxidative stress with a concomitant increase in vascular inflammation and oxidative stress. Oxidative stress in the vasculature was significantly inhibited by antioxidant treatment, N-acetyl cysteine; whereas hydrogen peroxide (H_2O_2) inhalation worsened it. Therefore, our study shows that oxidative airway inflammation is associated with vascular/systemic oxidative stress which might predispose these patients to increased cardiovascular risk.

1. Introduction

It has been well established now that oxidant–antioxidant imbalance plays an important role in repeated cycles of airway inflammation observed in asthma. Endogenous or exogenous environmental oxidants are critical to the inflammatory response through perpetuation and amplification of pro-inflammatory signaling pathways [1–4]. At the same time, endogenous antioxidant mechanisms are present to attenuate this ROS-mediated inflammatory response. It is when oxidative stress overwhelms antioxidant defenses that severe inflammatory state becomes apparent and may manifest in the form of mucus hypersecretion, increased vascular permeability, and airway remodeling [3–5].

Several studies have shown oxidant burden in different components of the lung and blood. Inflammatory cells such as eosinophils, neutrophils, and lymphocytes from the lung/blood, and also pulmonary resident cells such as bronchial epithelial/smooth muscle cells, have been shown to produce oxidants in response to various stimuli [1, 5–7]. For example, asthmatics have shown increased ROS generation such as superoxide and H_2O_2 from their airways as compared to controls [5,8–10]. Furthermore, ROS generation in asthmatic airways is associated with an increase in airway responsiveness and negatively correlated with FEV1 [7,10]. Oxidative stress in the blood of asthmatics has been shown to have a pattern similar to the lung by us and others [1, 11,12].

ROS-mediated airway responses and tissue injury depend on the nature of antioxidant defenses available in the lung/blood. ROS mediated depletion of antioxidants may result in amplification of inflammatory loop which results in insufficient protection of biomolecules [3,13]. Evidence for antioxidant imbalance in asthmatic airways has been shown in several studies which show alteration not only in the non-enzymatic but also in enzymatic antioxidants in different components such as BAL and sputum [14–16]. Similar derangements in antioxidants have been reported in different components of the blood of asthmatics as well [1, 11,17,18]. Of note, total antioxidant capacity (TAC) has been consistently shown to be positively correlated with lung function [1,18,19].

Abbreviations: BAL, bronchoalveolar lavage; H_2O_2, hydrogen peroxide; i.n., intranasal; NOX, NADPH oxidase; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.intimp.2015.03.032
1567-5769/

However, it needs further investigation whether oxidative stress observed in asthmatic airways also contributes to systemic/vascular oxidative stress.

Several studies in humans are beginning to show an association between chronic airway inflammation and cardiovascular complications [20–22]. Studies in animals and humans show that airway inflammation causes enhanced systemic inflammation and cardiovascular complications such as myocardial ischemia–reperfusion injury, endothelial dysfunction and inflammatory cell recruitment [10,23–26]. This might be a result of airway oxidants gaining entry into the systemic circulation due to ROS mediated dysfunction of pulmonary endothelial/epithelial barrier [5,27,28]. ROS produced in asthmatic inflammation such as hydrogen peroxide, hypochlorous acid and superoxide radical are known to increase airway epithelial and microvascular endothelial permeability in vitro and in vivo [29,30]. However, no study so far has attempted to establish a link between oxidative airway inflammation, and systemic/vascular oxidant–antioxidant balance in vivo. Therefore, this study was undertaken to investigate the effects of inhaled allergen/hydrogen peroxide on systemic and vascular oxidative stress in the context of allergic airway inflammation. The data from our study show that airway oxidative stress is concurrently associated with vascular and systemic oxidative stress in a murine model of asthma.

2. Materials and methods

2.1. Animals

Female Wistar rats (150–160 g), free of specific pathogens, were used in the experiments. The animals were obtained from Experimental Animal Care Center, College of Pharmacy, King Saud University. The animals were kept under standard laboratory conditions of 12-h light–dark cycle and 24–26 °C ambient temperature. All experimental animals used in this study were under a protocol approved by the Animal Care and Research Committee of College of Pharmacy, King Saud University.

2.2. Rat sensitization and challenge

Sensitization was performed according to the protocol described earlier by us with some modifications [31,32]. Rats were sensitized on days 1 and 8 with intraperitoneal (i.p.) injections of 200 μg ovalbumin (grade V) adsorbed to 4 mg alum. Non-sensitized control animals received only alum with the same volumes. Two weeks after the 1st sensitization, the mice were challenged intranasally (i.n.) under light anesthesia with 100 μg OVA once only on days 14, 17, 22, 25, 28 and 31. To study the role of oxidant and antioxidant on systemic/vascular oxidant–antioxidant balance in this model, rats were administered H_2O_2 by inhalation at 0.1% and NAC at 2 mmol/kg, i.p. respectively to the sensitized and challenged rats. We chose this concentration of H_2O_2 because it is 10 times lower than what has been published earlier to cause airway inflammation on its own [33] and it did not lead to significant changes in any of the parameters of this study when administered alone.

Rats were divided into the following groups: control group (CON): rats received only vehicles for sensitization and challenge; sensitized and challenged group (SEN + CHAL): rats were sensitized and challenged with OVA using the same protocol described above; sensitized and challenged group administered NAC (SEN + CHAL + NAC): rats were sensitized and challenged with OVA using the same protocol described above and NAC was administered i.p. on days 14, 17, 22, 25, 28 and 31 before each allergen challenge; and sensitized and challenged group administered H_2O_2 (SEN + CHAL + H_2O_2): rats were sensitized and challenged with OVA using the same protocol described above and H_2O_2 was administered by inhalation for 15 min on days 28 and 31 after allergen challenge.

2.3. Bronchoalveolar lavage (BAL)

The trachea was cannulated to perform BAL one day after the final allergen challenge; phosphate-buffered saline was introduced into the lungs via the tracheal cannula and the total cells were counted manually in a hemocytometer chamber followed by spinning of cells onto glass slides for differential count. A differential count of at least 300 cells was made according to standard morphologic criteria on cytocentrifuged Diff-Quik stained slides. The number of cells recovered per rats was calculated and expressed as mean ± SE per ml for each group.

2.3.1. Protein carbonyl assay

The content of protein bound carbonyls in the trachea/aorta/plasma, an indicator of protein oxidation, was measured at 380 nm using 2,4-dinitrophenylhydrazine (DNPH) by the method of Levine et al. [34] as described by us previously [1]. Briefly, after precipitation of proteins in sample supernatant/plasma by trichloroacetic acid, pellet was dissolved in DNPH followed by precipitation again after a waiting period of 1 h. The resulting pellet was dissolved in 6 M guanidine solution after several washes with ethanol:ethyl acetate solution. Absorbance of the sample was taken at 380 nm and carbonyl content was calculated using molar absorption coefficient of 22,000/M/cm. The final results were expressed as nmol/mg protein.

2.4. Thiol content assay

Plasma/tissue thiol content was measured at 412 nm using 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's reagent) by the method of Hu [35] as described earlier by us [1,36]. Total thiol content was calculated using molar absorptivity of 13.6 mM/cm for DTNB. The final results were expressed as mmol/l or μmol/mg protein.

2.5. Reactive oxygen species (ROS) assay

For reactive oxygen species generation, the harvested trachea/aorta were incubated with 100 μM 6-carboxy-2′,7′-dichlorofluorescein diacetate (DCFH-DA) for 30 min at 37 °C. DCFH-DA forms a fluorescent product, DCF (dichlorofluorescein) upon oxidation with ROS. Fluorescence caused by DCF in each well was measured and recorded for 30 min at 485 nm (excitation) and 530 nm (emission) by the method of Wang and Joseph [37] using a multi-mode fluorescent microplate reader (FLUOstar Omega, BMG LabTech, USA) with temperature maintained at 37 °C as described earlier by us [38]. The background fluorescence caused by buffer and DCF was subtracted from the total fluorescence in each well caused by the trachea/aorta in the presence of DCF. Fluorescence intensity was expressed as ROS generation (% control).

To assess the location of ROS generation in the aortas of allergen and oxidant challenged animals, the aorta was cut into equal pieces followed by mechanical removal of endothelium (this was done by placing a piece of thin wire in the lumen and rubbing the aortic ring over a wet blotting paper) in one half and leaving the other half intact as described previously by us [39]. This procedure leads to lack of relaxation in the endothelium denuded aorta in response to acetylcholine in an organ bath [39]. Afterwards, ROS generation in the endothelium intact and denuded aortas was measured as described above.

2.6. Lipid peroxide assay

Plasma/tissue lipid peroxides were measured as malondialdehyde (MDA)–thiobarbituric acid (TBA) adducts by the method of Jentzsch et al. [40] as described earlier by us [36]. Briefly, the sample was incubated with butylated hydroxyl toluene, ortho-phosphoric acid and TBA at 90 °C for 45 min, followed by ice-cooling and extraction of MDA–TBA adducts in n-butanol. Absorption was read at 535 and 572 nm for baseline correction in a multititer plate reader. MDA–TBA adducts