International Immunopharmacology 39 (2016) 57–62

Contents lists available at ScienceDirect

International Immunopharmacology

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

Increased glutathione levels contribute to the beneficial effects of hydrogen sulfide and inducible nitric oxide inhibition in allergic lung inflammation

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ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form 15 June 2016
Accepted 8 July 2016
Available online 15 July 2016

Keywords:
Reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio
Aconitase
Ovalbumin
Oxidative stress
1400 W
Sodium hydrosulfide

ABSTRACT

Objective: The interaction between nitric oxide (NO) and hydrogen sulfide (H_2S) in the airways could have significant implications for the pathogenesis and therapeutic effects of both on lung diseases. In this study we investigated whether the beneficial effects of H_2S on asthma could be comparable to that inhibition of inducible NO synthase (iNOS).
Methods: Female BALB/C mice sensitized with ovalbumin (OVA) received either the H_2S donor sodium hydrosulfide (NaHS, 14 μmol/kg) or the iNOS inhibitor 1400 W (1 mg/kg), 30 min before each OVA challenge during six days. On the first, second and sixth days, the leucocyte infiltration in lung parenchyma and bronchoalveolar lavage was evaluated. The aconitase activity (a sensor of $O_2^{\bullet-}$ formation) and lipid peroxidation, as well as levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined in the lung tissues.
Results: OVA-challenge caused a significant and time-dependent increase in the eosinophil number in the airways, which was accompanied by a significant decrease of aconitase activity and GSH/GSSG ratio along with enhanced lipid peroxidation in the lungs. Treatment with NaHS or 1400 W significantly attenuated the airways eosinophilia that was paralleled by an increase in aconitase activity and decrease of lipid peroxidation. NaHS or 1400 W treatments also reversed the decreased GSH/GSSG ratio seen after OVA-challenge.
Conclusions: The present study shows for the first time that the increased GSH/GSSG ratio caused by either H_2S supplementation or iNOS-inhibition is a potential mechanism protecting airways against oxidative stress and inflammatory lung diseases.

1. Introduction

Asthma is an airway chronic inflammation characterized by a large quantity of eosinophils, mast cells, and activated T helper lymphocytes absorbed into lung tissue, bronchoconstriction, mucus secretion and remodelling [1].

Oxidative stress plays an important role in the pathogenesis of asthma due to excessive production of reactive oxygen (ROS) and nitrogen (RNS) species, such as superoxide anion ($O_2^{\bullet-}$) and nitric oxide (NO•). The imbalance between ROS/RNS production and antioxidant defence are determinants for the oxidative stress in airways. Antioxidants enzymes such as superoxide dismutase (SOD) and catalase and reduced glutathione (GSH) contribute to the decrease of chronic inflammation process that characterizes allergic asthma [2]. Glutathione constitutes the first line of the cellular defence mechanism against oxidative injury. Evidence suggests that the intracellular redox status, namely the balance between intracellular GSH and oxidized (GSSG) glutathione, regulates various aspects of cellular function, and that glutathione is an important immune modulator [3].

Nitric oxide (NO) and hydrogen sulfide (H_2S) are two endogenous gaseous transmitters whose regulatory functions are highly associated with the development of respiratory diseases, such asthma. NO is produced by NO synthase isoforms (NOS), and high NO levels produced by inducible NOS (iNOS) have been detected in the lungs of asthmatics [4]. H_2S is generated from L-cysteine by enzymes cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST). The H_2S-generating enzyme CSE is mainly distributed in airway and vascular smooth muscle cells in rat peripheral

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http://dx.doi.org/10.1016/j.intimp.2016.07.009
1567-5769/

lung tissues [5]. However, as opposed to NO, a decrease in H_2S levels has been demonstrated in serum and lung tissue of OVA-induced asthma in rats [6]. Studies demonstrated that H_2S is a high reactive molecule that could react with both ROS and RNS produced in inflammatory conditions [7]. Thus endogenous H_2S might be a new antioxidant in the organism.

Our previous results demonstrated that inhibition of iNOS isoform significantly reduces the eosinophil migration into the lung of ovalbumin (OVA)-challenged mice [8]. In addition, using the same animal model, we reported that exogenous H_2S has beneficial effects on pulmonary allergic inflammation by inhibiting the neutrophil and eosinophil recruitment to the lungs, as well as by increasing the endogenous antioxidant defences such as SOD, catalase, glutathione reductase (GR) and glutathione peroxidase (GPx) activities [9].

Therefore, the interaction between NO and H_2S in the airways could have a significant implication for the pathogenesis and therapeutic function. Considering that H_2S-donor treatment inhibits iNOS expression and activity [9,10], in this study we investigated whether the beneficial effects of H_2S on experimental allergic lung inflammation could be comparable to that shown by inhibition of iNOS-derived NO.

2. Material and methods

2.1. Drugs

Ovalbumin (OVA grade V), sodium hydrosulfide (NaHS), N-3-aminomethyl-benzyl-acetamidine-dihydrochloride (1400 W), protease inhibitor cocktail, protein assay kit, reduced and oxidized L-glutathione, and reagents used for the enzyme activity assays were purchased from Sigma Chemical (St. Louis, MO, USA).

2.2. Ethics and animal experimentation

All animal care and experimental procedures were in accordance with the Brazilian and American Guidelines for the Care and Use of Laboratory Animals, and were approved by the local Animal Ethics Committee (San Francisco University, Brazil; licence number 0021108). A total of 50 female BALB/c mice (5 to 8 week old) were obtained from the Multiinstitutional Center for Biological Investigation (CEMIB, UNICAMP, Brazil). The mice were maintained in polypropylene cages (five per cage) under standard controlled conditions (22 °C, 12 h light/dark cycle) with food and water *ad libitum*.

2.3. Sensitization, airway challenge and treatments

Mice were sensitized at days 0 and 7 by the subcutaneous (s.c.) injection of 400 μl of a suspension containing 100 mg of grade V ovalbumin (OVA) bound to 4 mg of aluminum hydroxide in sterile saline. Seven days after the second sensitization, the animals were briefly anesthetized by inhaled halothane (5% in oxygen) and intranasally (i.n.) challenged twice daily, over a period of 6 days, with OVA (10 μg in 50 μl of sterile saline solution; OVA group) or the same volume of sterile saline solution (Saline group). Thirty minutes prior to OVA-challenge, mice were treated *via* i.p. with sodium hydrosulfide (NaHS; 14 μmol/kg dissolved in 300 μl sterile saline solution), the iNOS inhibitor N-3-aminomethyl-benzyl-acetamidine-dihydrochloride (1400 W; 1 mg/kg dissolved in 300 μl sterile saline solution) or the same volume of sterile saline solution (untreated OVA-challenged group). Mice were euthanized, by exsanguination by cutting the cervical vessels under anaesthesia (halothane), on the first, second and sixth day following OVA-challenge. Thus, we totalized four experimental groups in each time-point, namely, OVA-sensitized non-challenged (Saline; $n = 8$);

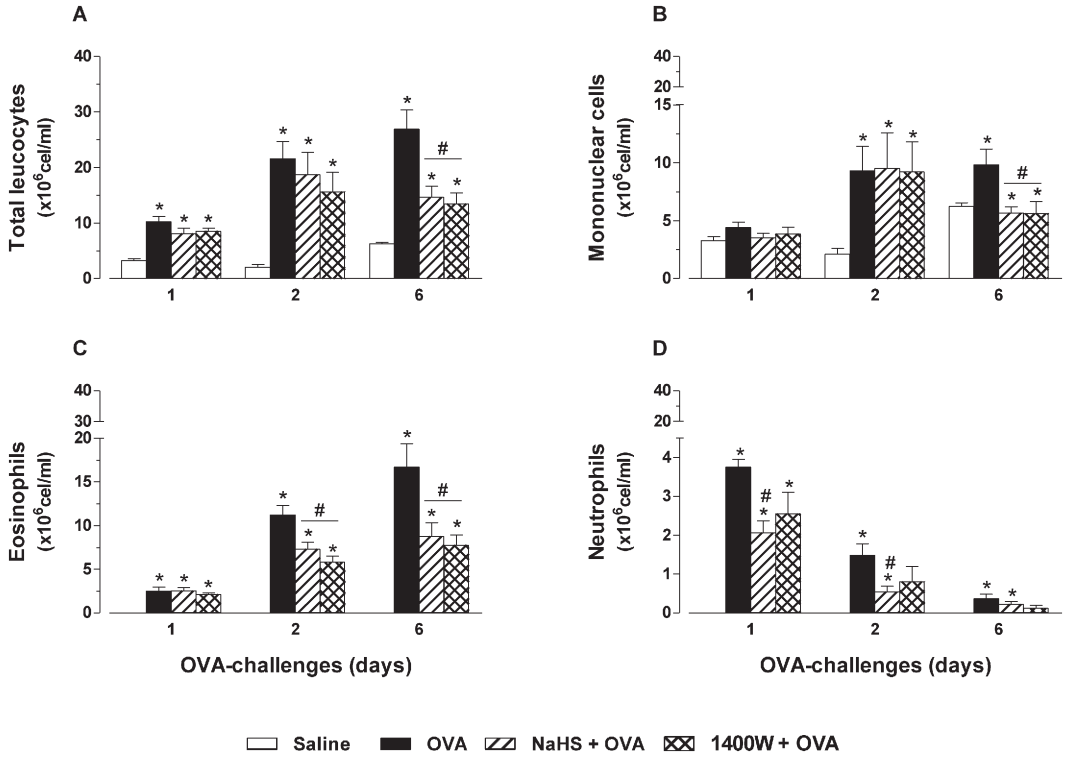

Fig. 1. Time-response curves of the comparative effect of a H_2S donor (NaHS) and a selective iNOS inhibitor (1400 W) on total and differential cells in BAL. Animals were treated (−30 min, i.p.) with NaHS or 1400 W prior each OVA challenge. Bars in panels A, B, C and D represent the mean ± S.E.M. of $n = 6$–8 animals. Values of $^{*}P < 0.05$ compared to saline. $^{\#}P < 0.05$ compared to OVA on the same day.