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Hydrogen sulfide inhibits oxidative stress in lungs from allergic mice in vivo

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ABSTRACT

Recent studies show that endogenous hydrogen sulfide (H_2S) plays an anti-inflammatory role in the pathogenesis of airway inflammation. This study investigated whether exogenous H_2S may counteract oxidative stress-mediated lung damage in allergic mice. Female BALB/c mice previously sensitized with ovalbumin (OVA) were treated with sodium hydrosulfide (NaHS) 30 min before OVA challenge. Forty eight hours after antigen-challenge, the mice were killed and leukocyte counting as well as nitrite plus nitrate concentrations were determined in the bronchoalveolar lavage fluid, and lung tissue was analysed for nitric oxide synthase (NOS) activity, iNOS expression, superoxide dismutase (SOD), catalase, glutathione reductase (GR) and glutathione peroxidase (GPx) activities, thiobarbituric acid reactive species and 3-nitrotyrosine containing proteins (3-NT). Pre-treatment of OVA-sensitized mice with NaHS resulted in significant reduction of both eosinophil and neutrophil migration to the lungs, and prevented the elevation of iNOS expression and activity observed in the lungs from the untreated allergic mice, although it did not affect 3-NT . NaHS treatment also abolished the increased lipid peroxidation present in the allergic mouse lungs and increased SOD, GPx and GR enzyme activities. These results show, for the first time, that the beneficial in vivo effects of the H_2S-donor NaHS on allergic airway inflammation involve its inhibitory action on leukocyte recruitment and the prevention of lung damage by increasing endogenous antioxidant defenses. Thus, exogenous administration of H_2S donors may be beneficial in reducing the deleterius impact of allergic pulmonary disease, and might represent an additional class of pharmacological agents for treatment of chronic pulmonary diseases.

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1. Introduction

In the bronchopulmonary airways, oxidative stress can affect a variety of endogenous molecular targets (phospholipids, proteins, nucleic acids) and is mediated by the unbalanced production of the so-called reactive oxygen species, including superoxide anion—$O_2^{-\cdot}$, hydrogen peroxide—H_2O_2, hydroxyl radical—OH●, singlet oxygen, as well as reactive nitrogen species, mainly nitric oxide (NO) and the derived species dinitrogen trioxide (N_2O_3), peroxynitrite anion ($ONOO^-$), nitrogen dioxide (NO_2), nitrosoperoxycarbonate anion ($ONOOCO_2^-$). These species are usually involved and mediate cellular dysfunction and inflammation in humans and other mammals (Comhair and Erzurum, 2010). Particularly, reactive oxygen species and/or derived

biomarkers for their production have been found in the lung of individuals with respiratory diseases, including chronic obstructive pulmonary disease (COPD) and asthma (Kirkham et al., 2006). In this way, the development of defensive biological mechanisms is crucial to lessen the potential damage secondary to oxidative stress, as an imbalance between reactive oxygen/nitrogen species production and antioxidant enzyme activities, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase, contributes to the chronic inflammation process that characterizes asthma (Dworski, 2000).

A growing number of observations suggest that, similarly to NO, hydrogen sulfide (H_2S) might be of biological relevance as an endogenous gasotransmitter in the pathogenesis of airway diseases, such as COPD and asthma (Chen and Wang, 2012). Chen et al. (2009) observed that in ovalbumin (OVA)-sensitized rats, exogenously supplied H_2S alleviated airway inflammation, characterized by a diminished influx of eosinophils and neutrophils into the lungs and abnormal metabolism and function of H_2S, in addition to significantly attenuated pulmonary iNOS activation.

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In patients with COPD, it was found that serum total sulfide concentrations were negatively correlated with the number of neutrophils in sputum, but positively correlated with the proportion of lymphocytes (Chen et al., 2005).

Although the underlying mechanisms of action of H_2S are incompletely understood to date, it has been shown that this mediator can induce cell hyperpolarization by activation of ATP-dependent K^+ (K_{ATP}) channels, a mechanism that can account for H_2S effects as a vasodilator and inhibitor of leukocyte adherence to mesenteric venule endothelium (Zanardo et al., 2006).

H_2S is endogenously produced in mammalian lung and airways tissues by mutiple transsulfuration reactions catalyzed by the enzymes cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE); however, the dominant reactions comprise H_2S synthesis from cysteine (by both CBS and CSE) and homocysteine (by CBS; Kabil et al., 2010).

As previously stated, H_2S may react with reactive oxygen/nitrogen species produced under inflammatory conditions (Lowicka and Beltowski, 2007), and this has led us to hypothesize on the possibility that exogenously supplied H_2S may counteract the oxidative stress-mediated lung damage that occurs in allergic mice.

In the present study, we show some results related to the effects of treatment of allergic mice with sodium hydrosulfide (NaHS), an H_2S donor, on oxidative stress in lung inflammation.

2. Material and methods

2.1. Drugs

Protein assay kit, acrylamide, bisacrylamide, sodium dodecyl sulfate (SDS) and nitrocellulose membrane were purchased from Bio-Rad Laboratories (CA, USA). Antibodies (anti-iNOS, anti-actin and goat anti-rabbit IgG coupled to horseradish peroxidase) were from Upstate Biotechnology (NY, USA). Chemiluminescence substrate (SuperSignal-West Pico) was purchased from Thermo Scientific (IL, USA). Other reagents were purchased from Sigma Chemical (St. Louis, MO, USA).

2.2. Animals

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, 5–8 week old, were obtained from the Multi-institutional 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. Ovalbumin sensitization protocol

The study design comprised 3 experimental groups: OVA-sensitized non-challenged ($n=6$), untreated OVA-sensitized and challenged (control; $n=8$) and NaHS-treated OVA-sensitized and challenged ($n=8$) mice. Mice were sensitized at days 0 and 7 by the subcutaneous (s.c) injection of 400 μl of a suspension containing 100 mg grade V ovalbumin (OVA) bound to 4 mg of aluminum hydroxide in sterile phosphate buffered saline (PBS) solution. Seven days after the second sensitization, groups of animals were briefly anesthetized with halothane and intranasally challenged with OVA (10 μg in 50 μl of sterile saline solution), or received the same volume of sterile saline solution alone. These OVA or saline

exposures were performed twice a day during 2 consecutive days. A set of animals from the challenged group ($n=8$) received intraperitoneal (i.p.) injections of freshly prepared sodium hydrosulfide solution (NaHS; 14 μmol/kg) twice a day, 30 min before the OVA challenge; the untreated challenged animals received the same volume of sterile saline alone ($n=8$). All the mice were killed 48 h after the first challenge.

2.4. Cell collection and sample processing

To obtain the bronchoalveolar lavages, mice were anesthetized with halothane and lungs were washed three times with 500 μl of saline. The samples were immediately centrifuged (20 °C, 300g, 10 min); the cell pellets were resuspended in PBS containing 2 mM ethylenediaminetetraacetic acid (PBS/EDTA) and the supernatants were collected and frozen at -80 °C for further analysis.

Total leukocyte number in the bronchoalveolar lavage samples was determined using standard hematological procedures. Differential leukocyte count was carried out on a minimum of 400 cells using cytospin preparations and the cells were classified as neutrophils, eosinophils or monoclear cells based on standard morphological criteria, as previously described (Ferreira et al., 1998). The lungs were then homogenized with cold Tris–HCl buffer (50 mM, pH 7.4) containing 1% protease inhibitor cocktail and 0.5 mM PMSF, and the homogenates were centrifuged at 800g for 10 min at 4 °C. The supernatants was aliquoted, quickly frozen in liquid nitrogen and kept at -80 °C until analysed.

2.5. Western blot for inducible nitric oxide synthase (iNOS) expression

The presence of iNOS in the lung homogenates was detected by Western blotting. Briefly, after sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE with 7% total polyacrilamide) of 25 μg of total proteins, the bands were electro-transferred to nitrocellulose membranes (Bio-Rad, USA), and following blockade of non-specific sites with 1% BSA, the membranes were incubated overnight at 4 °C with a polyclonal rabbit IgG anti-iNOS antibody (2.5 μg/ml). A horseradish peroxidase-conjugated goat anti-rabbit IgG was used as a secondary antibody and the immunoreactive bands were visualized using a chemiluminiscence detection system (SuperSignal-West Pico; Pierce Biotechnology, Rockford, IL, USA) by exposure to a photographic film (Kodak-Medical X-ray Film, NY, USA). The developed films were scanned and the band intensities were estimated by densitometry using the UN-SCAN-IT gel software (Silk Scientific Inc., UT, USA).

2.6. Measurement of nitric oxide synthase (NOS) activity

Ca^{2+}-dependent and -independent NOS activity present in the lung homogenates were determined based on the [^{3}H]-L-arginine to [^{3}H]-L-citrulline conversion, as previously described (Teixeira et al., 2002).

2.7. Measurement of total nitrite+nitrate concentration

Total concentrations of nitrite plus nitrate (NO_x) anions in the bronchoalveolar lavage samples were determined by the Griess reaction for nitrite anion after the nitrate reductase-catalyzed reduction of nitrate to nitrite, as previously described (Grisham et al., 1996).

2.8. Slot blotting analysis of nitrotyrosine-containing proteins

The presence of proteins containing 3-nitrotyrosine (NT) residues was analyzed by slot blotting, as previously described

(Sultana et al., 2008). Immunoreactivity of the slots was detected using a chemiluminescence system and their intensities were estimated by densitometric analysis (ChemImager 5500 system, Alpha Innotech Corp., USA). Results were normalized by the intensity values obtained after staining of the slots with a Ponceau S dye solution.

2.9. Measurement of antioxidant enzyme activities

Total superoxide dismutase (SOD) activity was estimated by the rate of inhibition of cytochrome c oxidation at 550 nm, as previously described (McCord and Fridovich, 1968). Catalase activity was measured by the decrease in absorption of H_2O_2 at 240 nm, as previously described (Nelson and Kiesow, 1972). Glutathione peroxidase (GPx) activity was determined by measuring the rate of formation of oxidized glutathione from reduced GSH in the presence of H_2O_2 (detected by the change in absorbance at 340 nm due to NADPH oxidation), as previously described (Lawrence and Burk, 1976). Glutathione reductase (GR) activity was measured by monitoring the rate of decrease of NADPH absorbance at 340 nm, considering that one unit of glutathione reductase catalyzes the oxidation of 1 μmol of NADPH/min, as previously described (Carlberg and Mannervik, 1975). The enzyme activity values are expressed as relative to the total protein contents, which was determined by the method of Bradford (1976) using bovine serum albumin as standard.

2.10. Analysis of thiobarbituric acid reactive species

Lipid peroxidation was estimated by the quantification of thiobarbituric acid reactive species present in the lung homogenates, as previously described (Ohkawa et al., 1979).

2.11. Data analysis and statistical procedures

Except for the iNOS expression experiments, all data are expressed as mean $\pm$ S.E.M. of n experiments and were analyzed by one-way ANOVA followed by the Student–Newman–Keuls test for multiple comparisons. The densitometric values for iNOS band expression are expressed as medians and were analyzed by non-parametric statistics, using the Kruskal–Wallis test followed by the Mann–Whitney test. Statistical significance was established at $P < 0.05$.

3. Results

3.1. Leukocyte lung infiltration

As shown in Fig. 1, leukocytes present in the bronchoalveolar lavage samples collected from non-challenged animals are exclusively mononuclear cells (Fig. 1). However, 48 h after OVA challenge, a significant increase in total leucocytes (associated with eosinophils, neutrophils and mononuclear cells) was observed in the bronchoalveolar lavages samples, and pre-treatment with NaHS resulted in significant reduction of both eosinophil and neutrophil infiltration into the lungs (Fig. 1B and C).

3.2. Lung NOS and 3-NT residues contents

After 48 h, OVA challenge did not affect lung Ca^{+2}-dependent NOS activity, but resulted in significant elevation of iNOS expression and activity (Ca^{+2}-independent NOS), which was avoided by NaHS pre-treatment. In addition, in the OVA challenged mice, bronchoalveolar lavages NO_x (nitrite plus nitrate) concentrations and lung 3-NT-containing protein content were significantly augmented in relation to the non-challenged group, but unaffected by NaHS pre-treatment (Fig. 2, panels C and D, respectively).
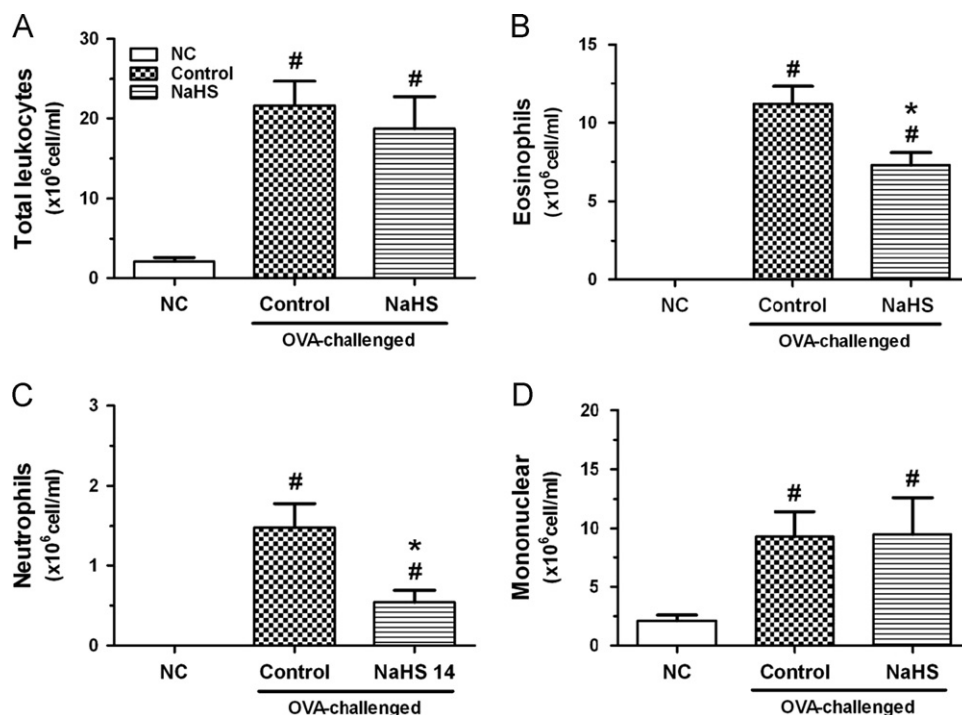

Fig. 1. Effect of NaHS on leukocyte migration to the lungs of OVA-sensitized and challenged mice. Bronchoalveolar lavages were obtained from non-challenged mice (NC; $n=6$), untreated controls ($n=8$) or NaHS-pre-treated mice ($n=8$) 48 h after the first OVA-challenge. NaHS was administered intraperitoneally 30 min before each OVA administration. Each column represents the mean $\pm$ S.E.M. of n mice. $^{\#}P < 0.05$ vs. NC and $^{*}P < 0.05$ vs. Control.
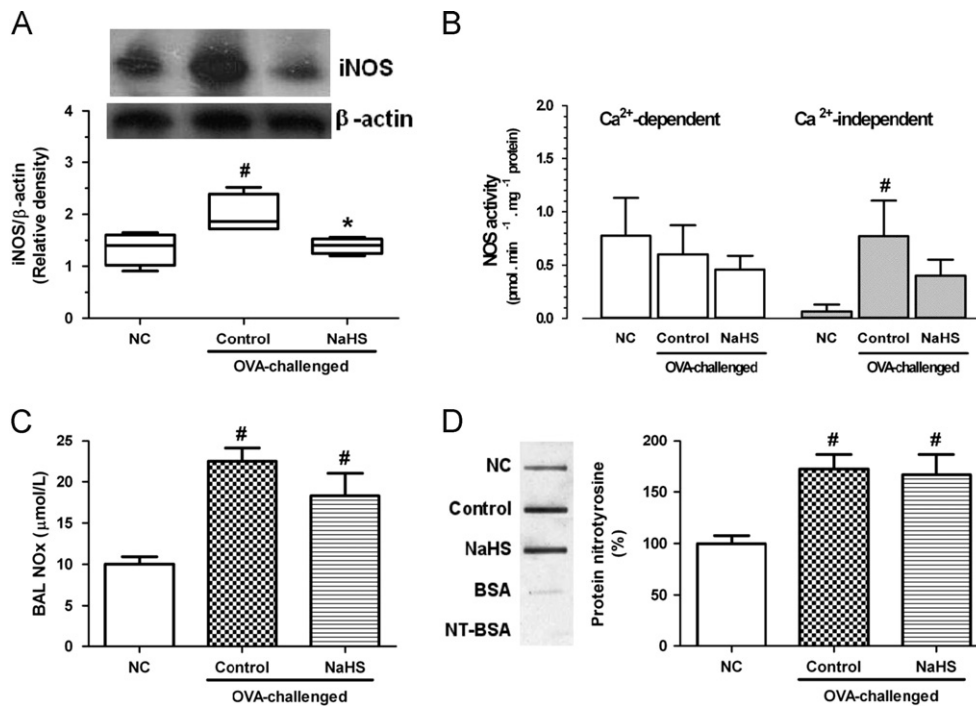
Fig. 2. Effect of NaHS on lung iNOS expression, NOS activity, 3-NT–containing proteins and bronchoalveolar lavage NO_x concentrations in OVA-sensitized and challenged mice. Panel A shows a representative Western blot for iNOS and the densitometric analysis of its expression in all the experimental groups ($n=4$). Panel B: lung NOS activities (in pmol/min/mg protein) in terms of their dependence on Ca^{2+} ($n=5$). Panel C: Bronchoalveolar lavage NO_x (nitrite plus nitrate) concentrations ($n=8$) in µmol/l. Panel D: Representative slot blots of 3-NT containing proteins in lungs from the experimental groups and densitometric analysis (relative to the control NC group; $n=7$). $^{\sharp}P < 0.05$ vs. NC; $^{*}P < 0.05$ vs. Control.
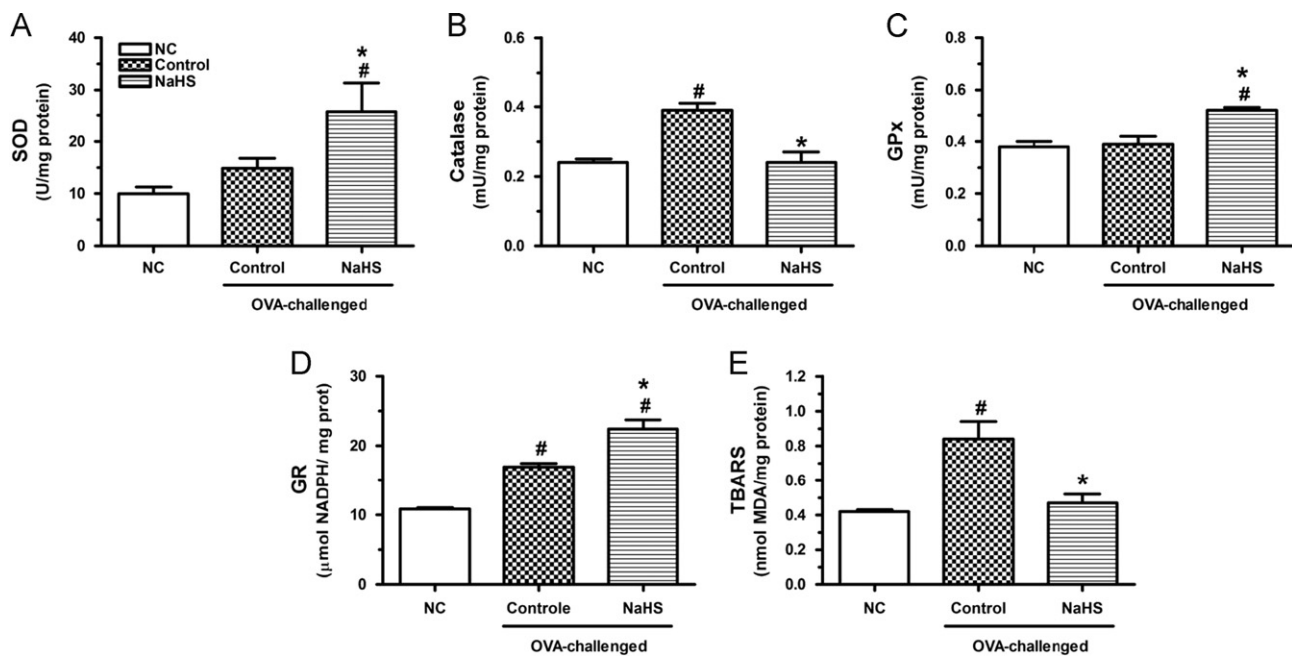

Fig. 3. Effect of NaHS on SOD, catalase, GPx and GR activities and thiobarbituric acid reactive species contents in lungs obtained from OVA-sensitized and challenged mice. Panels A–C shows SOD, Catalase and GPx activity, respectively (in U/mg protein). Panel D: GR activity (in µmol NADPH/mg protein). Panel E: lung thiobarbituric acid reactive species contents (in nmols MDA/mg protein). The lungs were obtained from non-challenged (NC; $n=6$), control ($n=8$) or NaHS-treated mice ($n=6$) 48 h after the first OVA-challenge. All the bar graphs represent the results as mean $\pm$ S.E.M. $^{\sharp}P < 0.05$ compared to NC; $^{*}P < 0.05$ compared to Control.

3.3. Lung SOD, catalase, GPx, GR and thiobarbituric acid reactive species

As shown in Fig. 3, OVA-challenge did not affect pulmonary SOD activity, although NaHS pre-treatment resulted in significant increase of this activity (panel A). Both lung catalase activity and thiobarbituric acid reactive species content were significantly higher in the OVA-challenged group in comparison with the non-challenged animals, and these increases were prevented by NaHS-pre-treatment (panels B and E). GR activity was also

significantly elevated in the OVA-challenged group, and was potentiated by NaHS pre-treatment (panel D). In contrast, OVA challenge did not affect lung GPx activity, but NaHS pre-treatment resulted in significant increase of this enzyme activity (panel C).

4. Discussion

Compelling evidence suggests a pathophysiological relevance for the new gasotransmitter, hydrogen sulphide (H_2S), in both acute and chronic inflammatory processes (see reviews: Wallace et al., 2012; Vandiver and Snyder, 2012). Additionally, while recent findings indicate an inverse correlation between total circulating sulfide concentrations and the severity of chronic inflammatory respiratory diseases (e.g., asthma; Wang et al., 2012; Chen and Wang, 2012), little is known about the effects and mechanisms of H_2S donors in allergic inflammation in the lung.

In the present study we provide the first in vivo evidence that the H_2S donor-mediated protective effect in ovalbumin-induced allergic airway inflammation in mice (assessed by the increased eosinophils and neutrophils) is related to both iNOS inhibition and upregulation of antioxidant defenses (e.g., SOD, GPx and GR). These results are in tune whith the fact of bronchial obstruction during asthma being associated with increased production of oxygen-derived free radicals (Mak et al., 2006).

In both animal and humans with allergic lung inflammation, eosinophils and lymphocytes are the main inflammatory cells found in the bronchoalveolar lavage or sputum, respectively, thus making of these cells useful markers for evaluation of asthma severity (Ferreira et al., 1998, 2004; Hamid and Tulic, 2009; Spahn, 2012). Although macrophages can also play an important role in asthma due the release of inflammatory mediators, the number of resident pulmonary macrophages is actually decreased in relation to the non-allergic conditions; in additon, and more importantly, H_2S does not affect this situation (Chen et al., 2009). Regarding neutrophils, despite bronchoalveolar neutrophilia has been considered an additional feature of allergic lung diseases, its significance is not yet completely understood (Barnes, 2011; Nakagome et al., 2012). Therefore, and since eosinophils peak at 48 h after OVA-challenge, we decided to study the cell migration response at this time-point .

Activated eosinophils and macrophages produce $O_2\bullet^-$, via the membrane associated NADPH-dependent complex, and the subsequent dismutation of $O_2\bullet$ gives rise to H_2O_2. Both neutrophil myeloperoxidase and eosinophil peroxidase can catalyse the formation of the powerful oxidant $OH\bullet$, as well as nitrating intermediates from the nitrite anion (an NO end product) and H_2O_2 (Ricciardolo et al., 2006). We have previously shown that treatment with the iNOS inhibitor 1400 W does not affect neutrophil migration, but significantly inhibits eosinophil migration to the lungs of allergic mice (Pelaquini et al., 2011), a fact that led us to postulate whether NO could be involved in the observed H_2S effects. Here, it is shown that in lungs from allergic mice pre-treated with the H_2S donor NaHS there is a marked reduction of iNOS activity and upregulation of SOD, GPx and GR activities. Corroborating these findings, Szabó and co-workers (2011) demonstrated that, in addition to K_{ATP} channel activation, the cardioprotective actions of H_2S also involves increased gene expression of antioxidant enzymes secondary to activation of the cytoprotective Nrf-2 gene.

The report from Abe et al. (2006) give support to our results on the signifcant elevation of NO_x concentrations in bronchoalveolar lavage samples from control (non-treated) allergic mice, in parallel with increased Ca^{2+}-independent iNOS activity and expression in the lungs. As shown in this study, the inhibitory effect of exogenous H_2S on iNOS activity and expression has been also previously observed in the carrageenan-induced knee joint synovitis in rats, an inflammatory conditions unrelated to allergic inflammation (Ekundi-Valentim et al., 2010). Curiously, and in contrast to our findings, Chen et al. (2005) show that NaHS treatment does not significantly affect increased iNOS expression in the lung of OVA-chellenged rats. These discrepancies might be related to either the experimental protocol (i.e., exposure of rats to OVA and/or NaHS-treatment during 2 weeks) and/or the animal species (i.e., rats) used by these authors.

It is noteworthy that although NaHS treatment inhibited lung iNOS activity, it failed to affect the increased NO_x—concentrations in the bronchoalveolar lavage. In fact, it must be considered that Ca^{2+}-dependent NOS activity was not significantly altered by NaHS. In addition, NO_x—values just represent $NO_2^- + NO_3^-$ anion concentrations, but not real NO which could easily undergo alternative reaction pathways (e.g., reaction with thiols, amines, etc.).

In line with our previous report showing that the H_2S donor Lawesson's reagent was devoid of effects on the 3-NT-containing proteins present in the knee joint synovial membrane of rats with carrageenan-induced synovitis, despite the beneficial effects of this H_2S donor on other inflammatory markers (Ekundi-Valentim et al., 2010). In these study, we also show that the increased amount of lung 3-NT-containing proteins observed at 48 h after OVA-challenge was unaffected by NaHS-pre-treatment . Nitration of protein tyrosine residues has been observed in diverse acute and chronic inflammatory diseases, including asthma (Ulrich et al., 2008). The presence of protein 3-NT residues has been proposed as a marker for the highly reactive peroxynitrite anion ($ONOO^-$), which can be formed by the reaction between $O_2\bullet^-$ and $\bullet NO$, mainly in situations where high amounts of iNOS-derived NO are present (Ischiropoulos et al., 1992). However, protein nitration can also occur in an iNOS-independent manner, and as a result of the presence of peroxidases (van der Vliet et al., 1997) or abundant heme proteins (such as myoglobin; Kilinc et al., 2001). Interestingly, Duguet et al. (2001) have shown that 3-NT formation secondary to allergen challenge in mice is dependent on eosinophil peroxidase activity and independent of increased NO production. However, all the above mentioned tyrosine nitration mechanisms fail to support the observed lack of effects of the NaHS treatment on lung protein nitration, considering the reported inhibitory properties of this H_2S donor on leukocyte (neutrophil+eosinophil) migration and on iNOS activity/expression. Consequently, alternative compensatory mechanisms (e.g., lung denitrase enzymes) could be involved (Kamisaki et al., 1998).

In addition to NO (and other NO-derived species), reactive oxygen species also contribute to the oxidative stress status present in asthma. In this study we show that lipid peroxidation is increased in the lungs of OVA-sensitized mice (as demonstrated by the higher thiobarbituric acid reactive species content) and that NaHS treatment prevents this increase. Lipid peroxidation is a chain reaction usually initiated by the electrophilic attack of unsaturated lipids by free radicals such as hydroxyl—$OH\bullet$, or hydroperoxyl—$HO_2\bullet$. In biological systems, $OH\bullet$ usually originates from hydrogen peroxide—H_2O_2 in the presence of trace amounts of Cu^+ or Fe^{2+} (as described by Fenton reaction) or, to a lesser extent, from $H_2O_2, +O_2^-\bullet$ in the presence of trace amounts of Fe^{3+} (according to the Haber–Weiss reaction). According to Beckman et al. (1990), $OH\bullet$ can also result from the homolytic decomposition of protonated peroxynitrite (ONOOH) in aqueous solutions.

The observed inhibitory effects of the H_2S-donor treatment on lipoperoxidation thus shows that H_2S (or the resultant sulfide

ions in aqueous medium at physiological pH) can either directly scavenge OH● radicals or interfere with the mechanisms of OH● production. Sulfide anion (either as soluble or insoluble metal salts) not only fails to inhibit H_2O_2-derived OH● formation, but also increases OH● formation, as detected by electronic spin resonance in a cell-organelle free system (Shi et al., 1994), liver microsomes in vitro (Fontecave et al., 1990) or by thiobarbituric acid reactive species measurement in *E. coli* cultures (Berglin et al., 1985). Therefore, the decrease in lipid peroxidation observed in the NaHS-treated rats may be secondary to diminished OH● formation.

According to Fig. 3, lung SOD, GPx and GR activities were increased in response to NaHS treatment. Taking into account the reactions catalysed by each of these three enzymes, we should expect a net fall in $O_2^- ●$ and H_2O_2 (due to the increased SOD and GPx activities, respectively) that would limit OH● production through substrate availability. Moreover, and strengthening these observations, GR activity was also increased by the H_2S-donor . Thus, the increased availability of reduced glutathione (i.e., the GPx substrate) does not limit H_2O_2 consumption by GPx, and consequently lowers the availability of H_2O_2 to undergo decomposition to OH● through the Fenton or the Haber–Weiss reactions.

Corroborating our results, H_2S-induced upregulation of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase or thioredoxin, has been shown in rats subjected to intestinal ischemia-reperfusion (Liu et al., 2009), and in brain endothelial cells under methionine-induced oxidative stress in vitro (Tyagi et al., 2009). Furthermore, H_2S protects osteoblasts exposed to H_2O_2 in vitro by diminishing NADPH oxidase activity (Xu et al., 2011), thus showing another mechanism by which H_2S can lower OH● production and consequent lipid peroxidation.

Lung catalase activity was augmented in the OVA-sensitized rats and treatment with NaHS resulted in a reversal of this increase to control values, as shown Fig. 3. It is feasible to speculate that NaHS-induced catalase decrease is due to an adaptation of the system to the low H_2O_2 concentrations that emerge from the GPx up-regulation . However, direct effects of H_2S on the enzyme cannot be ruled out.

The inhibitory effects of sulfide ion on catalase activity were recognized early by Stern (1932), and later confirmed by the spectroscopic studies performed by Nicolls (1961), and involve the formation of a complex of this anion with the iron of the heme group of catalase. In fact, catalase inhibition by non-lethal concentrations of Na_2S potentiated the mutagenic and lethal effects of H_2O_2 on Salmonella typhimurium cultures (Carlsson et al., 1988). In contrast, it has also been reported that H_2S can increase catalase activity, as part of the beneficial antioxidant effects that this mediator shows in some disease models such as, lung ischemia-reperfusion (Fu et al., 2008), heroin-induced hippocampal damage (Jiang at al., 2011) and isoproterenol-induced myocardial infarction (Sojitra et al., 2012).

To the best of our knowledge, the present study is the first to address the relationship between lung catalase activity and H_2S in allergic mice, thus it is difficult to compare our results with other data obtained from different experimental disease models. However, and independently of the exact mechanisms involved, based on the lipid peroxidation results discussed above, it is clear that the fall in catalase activity secondary to NaHS treatment, did not compromise the general antioxidant effects of this treatment. Furthermore, shifting the H_2O_2 consuming system from catalase to glutathione is quite beneficial in terms of antioxidant activity, considering the large differences in the K_m values for H_2O_2 that exist between these enzymes (low micromolar concentrations for peroxidase vs. millimolar for catalase (Flohé et al., 1972; Jones et al., 1968).

5. Conclusion

The present study shows the beneficial effects of the H_2S-donor, NaHS, on the pathogenesis of allergic airway inflammation in terms of inhibition of both eosinophil and neutrophil recruitment to the lung and increase of the endogenous antioxidant enzymes SOD, GPx and GR. Thus, H_2S donors may emerge as an additional class of pharmacological agents for the treatment of allergic pulmonary diseases.

Acknowledgements

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