Heme oxygenase attenuates allergen-induced airway inflammation and hyperreactivity in guinea pigs

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Heme oxygenase (HO), the heme-degrading enzyme, has shown anti-inflammatory effects in several models of pulmonary diseases. HO is induced in airways during asthma; however, its functional role is unclear. Therefore, we evaluated the role of HO on airway inflammation [evaluated by bronchoalveolar lavage (BAL) cellularity and BAL levels of eotaxin, PGE2, and proteins], mucus secretion (evaluated by bronchoalveolar lavage (BAL) cellularity and BAL levels of eotaxin, PGE2, and proteins), mucus secretion (evaluated by analysis of MUC5AC gene expression and periodic acid–Schiff staining), oxidative stress (evaluated by quantification of 4-hydroxy-2-nonenal adducts and carbonylated protein levels in lung homogenates), and airway responsiveness to histamine in ovalbumin (OVA)-sensitized and multiple aerosol OVA or saline-challenged guinea pigs (6 challenges, once daily, OVA group and control group, respectively). Airway inflammation, mucus secretion, oxidative stress, and responsiveness were significantly increased in the OVA group compared with the control group. HO upregulation by repeated administrations of hemin (50 mg/kg ip) significantly decreased airway responsiveness in control animals and airway inflammation, mucus secretion, oxidative stress, and responsiveness in OVA animals. These effects were reversed by the concomitant administration of the HO inhibitor tin protoporphyrin-IX (50 μmol/kg ip). Repeated administrations of tin protoporphyrin-IX alone significantly increased airway responsiveness in control animals but did not modify airway inflammation, mucus secretion, oxidative stress, and responsiveness in OVA animals. These results suggest that upregulation of the HO pathway has a significant protective effect against airway inflammation, mucus hypersecretion, oxidative stress, and hyperresponsiveness in a model of allergic asthma in guinea pigs.

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expression, mucus secretion, oxidative stress, and nonspecific airway hyperresponsiveness in models of immunized and multiple aerosol-challenged animals.

We have developed a model of OVA-immunized and multiple aerosol-challenged guinea pig. This model reproduces several of the characteristic features of asthma, including airway infiltration by inflammatory cells, particularly eosinophils, oxidative stress, and airway hyperresponsiveness to intravenously injected histamine (36). Furthermore, these animals have an increased pulmonary expression of HO-1 protein (38). Using this model, we have recently shown that HO modulates negatively the size of bronchial smooth muscle area (38). Therefore, the aim of the present study was to explore the effects of HO modulation on different parameters of lung inflammation, mucus secretion, oxidative stress, and airway hyperresponsiveness in these OVA-immunized and multiple aerosol-challenged guinea pigs.

MATERIALS AND METHODS

Guinea pig immunization and challenge. The protocol of OVA immunization and multiple aerosol challenge in guinea pigs was described previously (36). The experiments conducted in the present study were approved by our local institutional animal care and use committee and the protocol was in agreement with French legal recommendations related to animal studies. Two groups of animals were studied: a group of animals immunized and challenged with OVA (group OVA) and a group of animals immunized with OVA but challenged with saline. These animals were used as a control group (group C). In fact, we observed in separate experiments that the functional and biochemical parameters evaluated in the present study were not statistically different in group C animals compared with those that received saline intraperitoneally and had been subsequently exposed to aerosolized saline for 10 min on days 8, 9, 10, 13, 14, and 15.

Both group OVA and group C animals were randomly divided into four groups. One group received an inhibitor of HO activity, tin protoporphyrin IX (SnPP-IX; Porphyrin Products, London, UK) (42), given intraperitoneally at a dose of 50 μM/kg on days 8, 10, 13, and 15. A second group received the HO-1 inhibitor hemin (Porphyrin Products) given intraperitoneally at a dose of 50 mg/kg on days 7, 10, and 13. A third group received hemin and SnPP-IX at the same doses and on the same days as the first and second groups, respectively. A fourth group received the vehicle of SnPP-IX and hemin (1 M NaOH in PBS) on days 8, 10, 13, and 15. All compounds were administrated before the challenges.

A subset of animals of the different groups was killed 24 h after the last challenge for assessment of cytological and biochemical parameters in bronchoalveolar lavage (BAL) and lung homogenate, as described previously (36) (n = 6–12 animals per group). In another subset of animals (n = 4 per group), the lung was inflamed through a tracheal cannula at 25 cmH2O with 10% formol and fixed in paraﬁn to analyze mucus secretion. Finally, we evaluated airway responsiveness (n = 10–17 animals per group) in a third subset of animals at the same time point as the other measurements.

Cytological and biochemical parameters. BAL sampling, protein determination, and cell counting were performed as described previously (36). HO activity was evaluated by measuring bilirubin production by lung microsomes, as described previously (39).

Guinea pig eotaxin in BAL supernatant was determined, as previously reported (45), by ELISA using a murine monoclonal antibody as capture and a rabbit polyclonal antibody as detector (45). PGE2 levels in BAL supernatant were determined by an ELISA test (Amersham Biosciences, Uppsala, Sweden).

Gene expression of MUC5AC, a mucin whose expression increases in bronchial epithelium in asthma (2), was evaluated by RT-PCR as described previously (28). Quantification was performed by densitometric analysis and normalized by the expression of β-actin mRNA. Primers for specific detection of guinea pig MUC5AC were pairs of internal oligonucleotide sequences that would generate a 380-bp product: forward primer 5’-TCCCTACATGGAGTGGAAC-3’; reverse primer 5’-TGTTGACAGAAGTGGCTGAC-3’ (28). PCR amplification was performed for 30 cycles in 50 μl of volume using 5 μl of RT mix at the following conditions: 1.8 mM MgCl2, 40 s of denaturation at 95°C, 30 s of annealing at 66°C, and 1-min extension at 72°C (28). Mucus secretion was evaluated by staining paraffin sections with periodic acid-Schiff (PAS) reagent.

Levels of 4-hydroxy-2-nonenal (4-HNE) adducts were determined by Western blot analysis in lung homogenates using a specific polyclonal antibody (Calbiochem, San Diego, CA). Western blot analysis was performed as described previously, using a secondary antibody coupled with alkaline phosphatase (39).

The OxyBlot oxidized protein detection kit (Intergen, Strasbourg, France) was used to detect carbonyl groups formed as a result of protein side-chain oxidation, by Western blot analysis in lung homogenates, as described previously (39).

In all of the Western blot analyses, the molecular weights were calculated from the molecular weight of a standard ladder.

Copper- and zinc-containing superoxide dismutase (Cu/Zn SOD) and catalase activities were measured in lung homogenates with commercially available kits (RANDOX Laboratories) as described previously (7). Constitutive nitric oxide synthase activity was measured as described previously (36) by the conversion of l-[1-3H]arginine to l-[3H]citrulline.

Measurement of pulmonary inflation pressure. Pulmonary inflation pressure (Pip) was measured in anesthetized, mechanically ventilated guinea pigs at baseline and after intravenous injection of increasing concentrations of histamine, as described previously (36).

Statistical analysis. Values are given as means ± SE. Dose-response curves of histamine-induced bronchoconstriction in the different groups of animals were compared using two-way ANOVA for repeated measures. The other data were analyzed by one-way ANOVA. Differences between means were analyzed with Fisher’s protected least significant differences test. Signiﬁcance for all statistics was accepted at P < 0.05.

RESULTS

Effect of OVA challenge on HO activity. HO activity was augmented by 160% (P < 0.05) in group OVA compared with group C (Fig. 1).
Effect of OVA challenge on lung inflammation and oxidative stress. Different parameters were evaluated to assess lung inflammation. First, we analyzed BAL cellularity and eotaxin levels. OVA-challenged guinea pigs developed a 2.6-fold increase in the number of total inflammatory cells in the BAL fluid compared with saline-challenged animals (from $1.51 \pm 0.25 \times 10^5$ to $3.92 \pm 0.45 \times 10^5$ cells/ml, $P < 0.05$). Differential cell counting showed that the increased cellularity in OVA animals concerned the different types of inflammatory cells (macrophages, eosinophils, neutrophils, and lymphocytes; see Fig. 2, $P < 0.05$ for each type). Eosinophil and neutrophil numbers showed the most important proportional increase, being 10–12 times higher in group OVA compared with group C, whereas the other inflammatory cells were only 2–3 times higher in group OVA compared with group C. In group OVA, however, absolute neutrophil numbers were 10 times lower than eosinophil numbers (Fig. 2). Therefore, we then quantified in group OVA and group C animals BAL levels of the cysteine-cysteine chemokine eotaxin, a potent and selective eosinophil chemoattractant that is active on both guinea pig and human cells (20, 22). As previously reported (20, 22), eotaxin was constitutively expressed in guinea pigs and upregulated in allergen-challenged animals. The concentration of eotaxin increased by 35% in group OVA compared with group C (Fig. 3A, $P < 0.05$). Second, we measured PGE$_2$ levels in BAL supernatants, since eicosanoid products are important mediators of eosinophilic inflammation in human asthma and aeroallergen-induced animal models (11). PGE$_2$ levels were significantly higher in BAL supernatant in group OVA compared with group C (Fig. 3B, $P < 0.05$). Finally, we analyzed protein...
Mucus secretion was analyzed by quantifying gene expression of MUC5AC and by PAS staining. MUC5AC mRNA levels increased significantly in group OVA compared with group C (Fig. 4A, $P < 0.05$). This was associated with an increased number of PAS-positive bronchial epithelial cells (Fig. 4B).

Oxidative stress was evaluated by quantifying, with Western blot analysis in lung homogenates, levels of 4-HNE adducts, a specific and stable end product of lipid peroxidation (41), and carbonylated proteins, which are sensitive indexes of oxidative injury (27). The anti-4-HNE antibody detected two main protein bands in control lungs with molecular masses of 30 and 45 kDa (Fig. 5A). Repeated OVA challenge resulted in a significant increase in the intensity of these bands (Fig. 5B, $P < 0.05$ for the 2 bands). Measurement of carbonyl groups in lung homogenates indicated the presence of relatively few oxidized proteins in control lungs (apparent molecular masses of 97, 68, and 43 kDa; Fig. 6A). OVA challenge resulted in a significant increase in the intensity of these preexisting oxidized proteins (Fig. 6B, $P < 0.05$). Finally, we quantified the activity of the antioxidant enzymes Cu/Zn SOD and catalase in lung homogenates. These activities were similar in group C and group OVA animals (Table 1).

**Effect of OVA challenge on airway responsiveness.** Intravenous injection of increasing doses of histamine caused a dose-dependent bronchoconstriction in group C animals (Fig. 7A, $P < 0.005$). Histamine-induced bronchoconstriction was significantly potentiated in OVA animals, with respect to group C animals (Fig. 7B, $P < 0.05$).

*Effect of upregulation of HO pathway by hemin on airway inflammation and hyperresponsiveness induced by OVA challenge.* We first investigated whether upregulation of the HO pathway could protect against OVA-induced inflammation and bronchial hyperresponsiveness. In both group C and group OVA animals, repeated administration of hemin significantly and similarly increased lung HO activity ($P < 0.01$ vs. vehicle-treated animals, Fig. 1).

Hemin administration to group C animals did not significantly modify inflammation, mucus secretion, and oxidative stress markers (Figs. 2–6). By contrast, $P_{ip}$ was significantly lower in hemin-treated compared with vehicle-treated animals (Fig. 7A, $P < 0.05$ for comparison of whole dose-response curves).

In OVA animals, hemin significantly reduced inflammation, MUC5AC gene expression, mucus secretion, and oxidative stress markers except protein concentration in BAL, which was unaffected by the treatment (Figs. 2–6). Eotaxin, PGE$_2$ concentration, MUC5AC gene expression, and indexes of oxidative stress were decreased to a level similar to group C animals, whereas eosinophil numbers in BAL were decreased to an intermediate level between group OVA and group C animals ($P < 0.05$ hemin-treated OVA animals vs. vehicle-treated animals). $P_{ip}$ was also reduced by hemin administration (Fig. 7B, $P < 0.05$ for comparison of
histamine dose-response curves between group OVA H and group OVA. P_{ip} values were not statistically different between hemin-treated OVA animals and group C animals (Fig. 7B). The effects of hemin on inflammation, MUC5AC gene expression, and mucus secretion, oxidative stress markers, and P_{ip} were reversed by the concomitant administration of SnPP-IX (Figs. 2–7).

Effect of blockade of HO pathway by SnPP-IX on airway inflammation and hyperresponsiveness induced by OVA challenge. Having demonstrated that upregulation of the HO pathway protected against different markers of inflammation, oxidative stress and airway hyperresponsiveness in OVA animals, we explored the effects of HO inhibition on these parameters. In both group C and group OVA animals, repeated administration of SnPP-IX significantly reduced lung HO activity (P < 0.05 vs. vehicle-treated animals, Fig. 1). Because metalloporphyrins can modulate the activity of nitric oxide synthases (12), we measured the activity of the constitutive nitric oxide synthases [which include the activity of type I nitric oxide synthase, a main determinant of airway tone (8)] in SnPP-IX-treated and untreated group C and group OVA animals. We found no modification of this activity in SnPP-IX-treated animals; nitric oxide synthase activity (means ± SE, n = 8–10 per group) was 1.71 ± 0.88 and 1.41 ± 0.76 pmol·mg^{-1} minute^{-1} in group C and group OVA animals [not significant (NS)] and 1.22 ± 0.81 and 1.90 ± 1.01 pmol·mg^{-1} minute^{-1} in SnPP-IX-treated group C and group OVA animals (NS vs. the respective SnPP-IX-untreated animals).

Administration of SnPP-IX to group C animals did not modify inflammation and oxidative stress markers. Indeed, concentrations of protein, eotaxin, and PGE2 in BAL supernatant, BAL cellularity, and levels of 4-HNE and carbonylated proteins in lung homogenates were similar in SnPP-IX-treated and vehicle-treated group C animals (Figs. 2, 3, 5, and 6). Cu/Zn SOD and catalase activities were unmodified by administration of SnPP-IX (Table 1). By contrast, P_{ip} was significantly increased after the repeated injections of SnPP-IX (Fig. 7A). P_{ip} increased by ~35% for 10 μg/kg of histamine in SnPP-IX-treated vs. vehicle-treated group C animals (P < 0.05 for comparison of whole dose-response curves).

As with group C animals, repeated SnPP-IX administration to group OVA animals did not modify the levels of inflammatory and oxidative stress markers observed in these animals (Figs. 2–6), except for PGE2 levels, which were significantly increased in SnPP-IX-treated vs. vehicle-treated group OVA animals (Fig. 3B, P < 0.05). However, in contrast with group C animals, SnPP-IX administration did not enhance bronchial responsiveness: P_{ip} was not statistically different in SnPP-IX-treated vs. vehicle-treated group OVA animals (Fig. 7B).
DISCUSSION

In the present study, we examined the role of the HO pathway in the modulation of lung inflammation, oxidative stress, and airway hyperresponsiveness in OVA-immunized and multiple aerosol-challenged guinea pigs. We report that: 1) repeated OVA challenges induced an increase in HO activity in lung homogenates along with local inflammation and oxidative stress (increased BAL cellularity and BAL levels of eotaxin, PGE_2, and proteins, MUC5AC gene expression and mucus secretion, and increased levels of 4-HNE and carbonylated proteins in lung homogenates) and airway hyperresponsiveness; 2) in group C animals, neither hemin nor SnPP-IX modified inflammatory and oxidative stress markers, but they significantly attenuated and potentiated airway responsiveness, respectively; 3) in OVA animals, induction of HO activity with hemin significantly decreased the levels of both lung inflammatory and oxidative stress markers and attenuated airway hyperresponsiveness. By contrast, inhibition of lung HO activity by SnPP-IX had minimal effects on these parameters. Collectively, these results demonstrate, to the best of our knowledge and for the first time, that upregulation of the HO pathway in OVA-immunized and multiple aerosol-challenged guinea pigs has significant protective effects against airway inflammation, mucus hypersecretion, and hyperresponsiveness. This last effect could be related to the anti-inflammatory properties of HO as well as to a direct effect on airway smooth muscle contractility, as suggested by the modulation of airway responsiveness by hemin and SnPP-IX in group C animals.

Induction of HO-1 expression and activity has been demonstrated in experimental models of different pulmonary diseases, such as hyperoxia (33), influenza virus (13), or ozone-induced lung injury (17). In these cases, upregulation of HO-1 expression attenuated pathological alterations, demonstrating a clear protective role of HO. A similar induction and protective effect of HO has been shown in allergic lung disease models. Kitada and collaborators (25) demonstrated an enhanced HO-1 immunostaining in alveolar macrophages in a murine model of asthma, and Jia and coworkers (21) showed that HO induction with hemoglobin prevented bronchial plasma extravasation after intravenous OVA challenge in sensitized rats. The results of our study confirm and expand these latest data. Indeed, HO activity was increased in lung homogenates in the present guinea pig model of asthma, and HO upregulation with hemin prevented lung oxidative stress, MUC5AC gene expression and mucus hypersecretion, inflammation (with the exception of protein concentration in BAL), and airway hyperresponsiveness. The absence of effect of hemin on BAL proteins is in contrast with data from Jia and coworkers (21), which showed, in single OVA-challenged, sensitized rats, that HO-1 induction by SnPP-IX had minimal effects on these parameters. Collectively, these results demonstrate, to the best of our knowledge and for the first time, that upregulation of the HO pathway in OVA-immunized and multiple aerosol-challenged guinea pigs has significant protective effects against airway inflammation, mucus hypersecretion, and hyperresponsiveness. This last effect could be related to the anti-inflammatory properties of HO as well as to a direct effect on airway smooth muscle contractility, as suggested by the modulation of airway responsiveness by hemin and SnPP-IX in group C animals.

Fig. 7. Dose-response increase in pulmonary inflation pressure (P_{in}) expressed in cmH_2O, in response to intravenous injection of increasing doses (1–10 μg/kg) of histamine. Each point represents the means ± SE, n = 6–17 in each group. A: intravenous injection of histamine caused a dose-dependent increase in P_{in} in C animals (P < 0.005). This increase was significantly attenuated by H (P < 0.05 C vs. control H) and significantly potentiated by SnPP-IX (P < 0.05 C vs. SnPP). B: intravenous injection of histamine caused a dose-dependent increase in P_{in} in OVA animals (P < 0.005), which was significantly higher than in C animals (P < 0.05 C vs. OVA). H significantly attenuated the response to histamine in OVA animals (P < 0.05 OVA vs. OVA H).

Table 1. Cu/Zn SOD and catalase activities in the different groups of animals

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<th>Control</th>
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<td>Vehicle</td>
<td>Hemin</td>
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<tr>
<td>Cu/Zn SOD</td>
<td>4.37 ± 1.51</td>
<td>4.64 ± 1.42</td>
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<td>Catalase</td>
<td>13.82 ± 6.96</td>
<td>11.39 ± 3.45</td>
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<td>4.53 ± 0.91</td>
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<td>12.96 ± 3.21</td>
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Values are means ± SE, n = 6–10 in each group. OVA, ovalbumin-immunized and multiple aerosol-challenged animals; SnPP-IX, tin protoporphyrin-IX; Cu/Zn SOD, (copper- and zinc-containing superoxide dismutase) and catalase activities are expressed in units × 10^3/g protein. No significant differences were found between the different groups of animals.
with hemoglobin prevented plasma extravasation, quantified by the leakage of Evans blue dye in the airway wall. Methodological differences (animal species, single vs. multiple challenges with OVA and administrations of HO modulators, and different timing and techniques to measure plasma extravasation) could explain this discrepancy. Furthermore, we cannot exclude a proinflammatory effect of heme in our model, leading to an increased vascular permeability (43). The increased HO activity in our model reflected induction of HO-1 expression. Using immunohistochemical analysis, we have found an increased HO-1 protein expression in alveolar macrophages, bronchial epithelium, and bronchial smooth muscle in the same model of OVA-sensitized and challenged guinea pigs (38). Furthermore, lung HO-2 protein expression was not modified in this model (data not shown).

One can wonder which of the end products of heme catabolism could be responsible for the anti-inflammatory effect of HO activation. In fact, CO has shown a protective effect in a model of aeroallergen-induced inflammation in mice (6). In this case, exposure of OVA-sensitized mice to 250 ppm of CO (a dose considered to be equivalent to endogenous HO-related CO production) from 2 h before to 24 or 48 h after a single aerosolized OVA challenge significantly attenuated the increase in BAL levels of IL-5, a Th2-like cytokine involved in eosinophil differentiation, maturation, and mobilization from bone marrow to the blood, and the ensuing BAL fluid eosinophilia. It must be noted that levels of eotaxin, another Th2-like cytokine involved in selective eosinophil recruitment in lungs from the microvasculature (20, 22), were not modified in this mouse model. We also found that HO activation attenuated BAL eosinophilia as well as levels of eotaxin, which were increased in our guinea pig model, as reported previously (20, 22). Although we did not measure IL-5 levels, data from the literature show that this cytokine is also increased in allergic inflammation models in guinea pigs (20), suggesting that HO could modulate different Th2-like cytokines involved in recruitment of eosinophils from bone marrow to the lung. It must be noted, however, that although eotaxin levels in hemin-treated OVA animals were similar to controls, eosinophil numbers in these animals were intermediate between controls and OVA animals. This discrepancy could reflect the fact that other cytokines, such as IL-3 and granulocyte/macrophage colony-stimulating factor, can sustain tissue eosinophilia in the absence of eotaxin and IL-5 (35). HO-derived CO could also be involved in the decreased BAL PGE2 levels observed in the present study, since a similar finding was observed in the allergic mice model exposed to CO (6). This effect could be related to the properties of CO in stabilizing the membrane of the mastocytes (9, 31) since histamine, a product of mast cell degranulation, stimulates the release of PGE2 in isolated airway preparations (34).

In addition to CO, bilirubin could also be involved in the anti-inflammatory effect of HO activation in the present study. Although the effect of bilirubin on allergic inflammation is unknown, anti-inflammatory properties of bilirubin are increasingly recognized. Indeed, bilirubin decreases TGF-β expression in a rat model of pulmonary fibrosis (44) and stabilizes mast cell membrane in rats (14). Furthermore, in this last case, like CO, bilirubin also prevents venular leukocyte adhesion associated with mast cell degranulation. Moreover, bilirubin can also attenuate leukocyte venular adhesion via a downregulation of endothelial P-selectin translocation (14). The antioxidant properties of bilirubin (10) could also be postulated to explain its involvement in mediating the anti-inflammatory effects of HO. This hypothesis is supported by the fact that 1) a decrease in lung oxidative stress was found in the lung of OVA animals treated with hemin, as revealed by the decrease in 4-HNE and carbonylated protein levels, and 2) several lines of evidence strongly suggest a role of oxidative stress in airway inflammation and mucus hypersecretion in asthma (see Ref. 4 for review). For example, Henderson and coworkers (16) have recently shown that an inhibitor of the redox-sensitive transcription factors NF-κB and AP-1 prevents the increase in BAL eosinophilia and eotaxin levels in a mouse model of asthma. Moreover, oxidative stress has been shown to upregulate MUC5AC gene expression by transactivating the epidermal growth factor receptor (40).

In addition to inflammation, HO activation with hemin prevented the increase in airway responsiveness found in OVA animals. This effect could be related to the anti-inflammatory properties of HO. In fact, eosinophilic inflammation has been extensively postulated as an important mediator of airway hyperresponsiveness in human and animal studies (1, 3). However, recent studies challenged this notion (15, 26), and robust cause-effect relationships are still difficult to establish. Alternatively, a direct effect of HO on airway smooth muscle contractility could be postulated. Actually, CO is known to induce vascular and airway smooth muscle relaxation through activation of guanylyl cyclase and cGMP formation (24). Moreover, we have recently shown in guinea pig tracheal muscle that HO-formed bilirubin decreases airway smooth muscle contractility (37). This effect is mediated by bilirubin antioxidant properties via a reduction in intracellular ROS production and in the level of phosphorylation of the myosin light chain (37). Occurrence of this phenomenon, independent from inflammation, in the present study, is supported by the effect of hemin and SnPP-IX on airway responsiveness in control animals. However, the relative participation of the anti-inflammatory and antioxidant properties of HO in decreasing airway hyperresponsiveness in OVA animals cannot be discriminated from a direct effect on smooth muscle contractility, and both effects probably contribute to this phenomenon.

In contrast with the beneficial effect of HO activation with hemin, surprisingly, HO inhibition with SnPP-IX did not aggravate pathological changes induced by OVA challenge, except for PGE2 concentration in BAL. We are confident about the specificity of SnPP-IX since we verified that this molecule, at the dose used in the present study, effectively modulated HO activity, without any effect on the constitutive nitric oxide synthases, another pathway involved in the modulation of bronchial responsiveness (8, 36). Furthermore, SnPP-IX reversed the effects of hemin, thus confirming its ability to inhibit HO activity. The lack of aggravation of inflammatory and oxidative stress parameters and airway hyperresponsive-ness in SnPP-IX-treated OVA animals could be related to maximal OVA-induced alterations that cannot be further potentiated by HO inhibition and/or to a concomitant upregulation of antioxidant systems that could compensate for HO inhibition. We think that this last possibility is unlikely because both Cu/Zn SOD and catalase activities were not upregulated in group OVA compared with group C and were unaffected by SnPP-IX in our model. However, we cannot exclude the
existence of other redundant protective pathways that could explain the lack of effect of HO inhibition on the asthmatic phenotype in OVA animals. Clearly, this finding deserves further experiments.

In conclusion, this study provides evidence that HO activation decreases lung inflammation, mucus hypersecretion, oxidative stress, and airway hyperresponsiveness in a model of asthma in guinea pigs. The effect on airway responsiveness could result from the decrease in inflammation and/or a direct prorelaxant effect on airway smooth muscle. These results, and previous data showing that the HO-bilirubin pathway negatively modulates airway smooth muscle proliferation (38), suggest that induction of the HO pathway could be beneficial in asthma or other respiratory diseases leading to airway hyperresponsiveness, mucus hypersecretion, and remodeling.

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