

Airway function, oedema, cell infiltration and nitric oxide generation in conscious ozone-exposed guinea-pigs: effects of dexamethasone and rolipram

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1 The effects of ozone inhalation (90 min, 2.15 ± 0.05 p.p.m.) and their modification by dexamethasone (20 mg kg^{-1}) or the phosphodiesterase-4 inhibitor, rolipram (1 mg kg^{-1}), administered (i.p.) 24 and 0.5 h before and 24 h after ozone exposure were examined in conscious guinea-pigs.

2 Ozone caused an early-phase bronchoconstriction (EPB) as a fall in specific airways conductance (sG_{aw}) measured by whole body plethysmography, followed at 5 h by a late-phase bronchoconstriction (LPB) and increased respiratory rate. Rolipram did not alter this profile but dexamethasone inhibited the EPB.

3 Airway hyperreactivity to inhaled histamine (1 mM, 20 s) occurred at 0.5, 2, 12, 24 and 48 h after ozone inhalation, the 2 h change being abolished by rolipram and dexamethasone.

4 Bronchoalveolar lavage fluid (BALF) macrophages, eosinophils and neutrophils were significantly ($P < 0.05$) elevated at 12, 24 and 48 h after ozone exposure, the 48 h influx being significantly attenuated ($P < 0.05$) by rolipram and dexamethasone.

5 BALF nitric oxide (NO) metabolites decreased 0.5 h after ozone exposure by 52%, recovered at 2 h and significantly increased at 12 (101%) and 24 h (127%). The elevated NO was unaffected by rolipram or dexamethasone.

6 Lung oedema, measured from wet/dry weight differences, was significant 12, 24 and 48 h after ozone exposure, the latter being significantly attenuated ($P < 0.05$) by rolipram and dexamethasone.

7 Ozone exposure of guinea-pigs produced features common to COPD. Although rolipram and dexamethasone did not affect the airway function changes, they inhibited the inflammation, airway hyperreactivity and oedema.

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Abbreviations: AHR, airway hyperreactivity; BALF, bronchoalveolar lavage fluid; breaths min^{-1} , respiratory rate; cAMP, adenosine 3', 5' cyclic monophosphate; COPD, chronic obstructive pulmonary disease; DMSO, dimethylsulphoxide; EPB, early-phase bronchoconstriction; FAD, flavin adenine dinucleotide; FEV₁, Forced expiratory volume in 1 s; HEPES, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid]; I- κ B, inhibitory nuclear transcription factor- κ B; iCOX₂, inducible cyclo-oxygenase₂; iNOS, inducible nitric oxide synthase; L-NAME, N^ω-nitro-L-arginine methyl ester; LPB, late-phase bronchoconstriction; LPS, lipopolysaccharide; NADPH, nicotinamide adenine dinucleotide phosphate; NANC, nonadrenergic noncholinergic; NED naphthylethylenediamine; NEP, neutral endopeptidase; NF- κ B, nuclear transcription factor- κ B; NO, nitric oxide; NOS, nitric oxide synthase; PDE4, phosphodiesterase isoenzyme 4; ROS, reactive oxygen species; sG_{aw} , specific airways conductance; TNF- α , tumour necrosis factor- α

Introduction

Oxidative stress can be defined as an increased exposure to oxidants (e.g. reactive oxygen species: ROS) and/or decreased anti-oxidant capacity, and is a widely recognized central feature of both asthma (Barnes *et al.*, 1999) and chronic obstructive pulmonary disease (COPD) (Repine *et al.*, 1997). Nearly 90% of all COPD patients are smokers (Repine *et al.*, 1997). Considerable evidence now links the rich source of oxidants in cigarette smoke with pulmonary oxidative stress in COPD (Stockley, 1995; Repine *et al.*, 1997). A release of

ROS, by activated inflammatory cells in the airways, is a shared feature of both asthma and COPD. Both COPD and asthma are also associated with an exaggerated constrictor response of the airways to spasmogens (airway hyperreactivity: AHR), reduced air flow, dyspnoea (shortness of breath) and oedema (Vrugt & Aalbers, 1993). In COPD, the airway obstruction is characterized by a progressive and irreversible decline in FEV₁ and an airways inflammation driven by neutrophils and macrophages (Postma & Kerstjens, 1998). In contrast, in asthma, the episodic obstruction, typically characterized by early- (EPB) and late- (LPB) phase bronchoconstrictions, is reversible by β -adrenoceptor agonists

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and the airways inflammation is driven by eosinophils (Bousquet *et al.*, 2000).

Exposure of asthmatic subjects to antigen (Alving *et al.*, 1993) and exacerbations in individuals with COPD (Bhowmik *et al.*, 1998; Corradi *et al.*, 1999) are associated with increases in levels of exhaled nitric oxide (NO). Derived from the nitric oxide synthase (NOS) conversion of L-arginine to L-citrulline, a basal level of the free radical, NO, maintains bronchodilatory airway tone, suppresses leukocyte activation and inhibits microvascular leakage (Barnes *et al.*, 1999; Colasanti & Suzuki, 2000). However, excess airways NO, although having beneficial anti-bacterial and anti-viral activity, increases microvascular permeability to cause pulmonary oedema and promotes leukocyte influx and adhesion in the airways (Barnes *et al.*, 1999). Under conditions of inflammatory-derived oxidative stress, excess NO also rapidly reacts with ROS (e.g. superoxide), to yield powerful nitrogen oxidants (e.g. peroxynitrite), which promote airway epithelial damage and AHR (Nijkamp & Folkerts, 1995; Giaid *et al.*, 1998). Naturally occurring anti-oxidants and scavenging enzymes like superoxide dismutase, catalase and glutathione protect from such inflammatory cell-derived ROS and oxidative stress (Repine *et al.*, 1997). In asthmatics and patients with COPD, exhaled NO also correlates with the degree of AHR, leukocyte influx and peroxynitrite formation, and negatively correlates with a decline in lung function (FEV₁) (Saleh *et al.*, 1998; Agusti *et al.*, 1999; Ichinose *et al.*, 2000).

Anti-inflammatory steroids are the mainstay of severe asthma treatment (Saleh *et al.*, 1998; Bousquet *et al.*, 2000). Although recent studies suggest that there is little clinical benefit from administering steroids in COPD, other than improving the patient's perceived quality of life and the incidence of disease exacerbation (Burge, 1999; Calverley, 2000). Recent studies suggest that inhibiting the adenosine 3', 5' monophosphate (cAMP)-selective phosphodiesterase (PDE)-4 isozyme, is a potential therapeutic target for improving COPD and severe asthma (Torphy *et al.*, 1999). PDE4 inhibitors elevate cAMP levels, by inhibiting its degradation *via* PDE4, and induce airway smooth muscle relaxation (bronchodilation), inhibit allergen- and lipopolysaccharide (LPS)-induced AHR and suppress excess NO generation and immunocompetent cell activation and migration (Danahay & Broadley, 1997; Torphy *et al.*, 1999; Toward & Broadley, 2001a).

Ozone (O₃) is an unstable allotropic species of oxygen that spontaneously decomposes into molecular (O₂) and ionized (O⁻) oxygen. Anti-oxidant and scavenger enzymes normally sequester increased levels of ROS after ozone exposure, however, oxidative stress occurs if the level of ozone exceeds the capacity of the anti-oxidative mechanisms (Cardile *et al.*, 1995). In animals and humans, inhaled ozone (O₃) induces AHR to various spasmogens (Schultheis, *et al.*, 1994; Seltzer *et al.*, 1986; Arakida *et al.*, 2000), decreases expiratory flow and volume (Hazucha, 1987; Joad *et al.*, 1994) and bronchial biopsies and lavage exhibit evidence of leukocyte influx, oedema and epithelial damage (Murlas & Roum, 1985; Aris *et al.*, 1993). Thus, oxidant stress arising after ozone inhalation shares many features associated with COPD and asthma. Previous studies from this laboratory have examined the effects of ozone inhalation on AHR, leukocyte influx into the airways and oedema in guinea-pigs (Johnson *et al.*, 1998).

However, as with many previous studies investigating the airways response to ozone (Murlas *et al.*, 1993; Schultheis *et al.*, 1994; Holbrook *et al.*, 1996; Inoue *et al.*, 2000; Nakano *et al.*, 2000), anaesthesia was utilized to determine AHR, which may influence vagal tone or sensory reflexes. In the current study, we assessed both AHR and airway function changes in conscious guinea-pigs following ozone exposure. This technique has the advantage of preserved neural reflexes and the ability to monitor the time-course of airway function (changes in airway calibre and respiratory rate) over several days, with paired analysis before and after ozone exposure in the same animal. Thus, the first aim of this study was to examine the hypothesis that changes in respiratory function over time correlate with airways hyperreactivity, leukocyte infiltration, oedema and NO generation. NO metabolites in bronchoalveolar lavage fluid (BALF) have previously been shown to be elevated at 5 h after ozone exposure in guinea-pigs (Inoue *et al.*, 2000), but in the present study we monitored changes at intervals up to 48 h. To validate the ozone model further as a suitable paradigm of COPD and for evaluating potential drugs for its treatment, we also determined the profile of activity of two well-studied standard classes of anti-inflammatory agent – the corticosteroid, dexamethasone, and the PDE4 inhibitor, rolipram. Preliminary findings from this study have been presented to the British Pharmacology Society (Toward & Broadley, 2000a).

Methods

Animals

Groups of six male Dunkin-Hartley guinea-pigs, weighing 300–400 g were used throughout. Animals received food and water *ad libitum*, and room temperature (22 ± 2°C) and lighting (maintained on a 12 h cycle) was regulated. This work complied with the guidelines for care and use of laboratory animals according to the Animals (Scientific Procedures) Act 1986 and GlaxoSmithKline policy.

Measurement of respiratory function (*sG_{aw}* and *breaths min⁻¹*)

Whole-body plethysmography was used to monitor respiratory function in the conscious guinea-pig. Changes in airway calibre (bronchoconstriction and bronchodilation) were recorded as specific airways conductance (*sG_{aw}*) and respiratory rate was measured as breaths per minute (breaths min⁻¹). The method was as described by Griffiths-Johnson *et al.*, 1988, although a computerized data acquisition system replaced the original oscilloscope and angle resolver (Toward & Broadley, 2000b). The guinea-pig was placed in a restrainer and a face mask was fitted over the snout of the animal. The restrained guinea-pig was then sealed inside the plethysmography chamber. As the animal breathed, a computerized Biopac data acquisition system (Biopac systems Inc., Santa Barbara, CA, U.S.A.) acquired and stored data (Acknowledge software) referring to air flow across a pneumotachograph (Mercury FIL). The resulting change in box volume (pressure) was simultaneously measured. Two UP pressure transducers (Pioden Controls Ltd, Canterbury, Kent, U.K.) measured changes in air flow (UP1) and box pressure (UP2).

The resultant waveforms could then be rapidly analysed by comparing the gradients of the flow and the box pressure waves at a point where flow tended towards zero, i.e. in the first 30 ms of expiration. A function of these parameters, correcting for ambient pressure and the weight of the animal, determined a value for sG_{aw} . The breathing rate over the 5 s reading was multiplied by 20 to determine an integer value for respiratory rate (breaths min^{-1}). At least five breaths were analysed for each animal at each time point. Before each experiment the animals were handled and familiarized with the equipment to reduce stress.

Ozone exposure

Groups of six guinea-pigs were box-exposed for 90 min to ozone generated *via* medi-air (0.35 $\text{l}\cdot\text{min}^{-1}$, 18.8–23.5% oxygen in nitrogen) passed through an ozonizer (model 100; Sander, Frankfurt, Germany). The level of ozone was regulated by a variable voltage supply. The ozone level within the exposure chamber (620 × 300 × 420 mm) was monitored every 15 min using a colourmetric indicator tube (ozone 0.05/b) and pump (Accuro) system (Dragger Sicherheitstechnik GmbH, Germany) and regulated accordingly to maintain a constant level of ozone (2.15 ± 0.05 p.p.m.). Murlas & Roum (1985) have previously shown that this level of ozone exposure was effective at causing AHR and mild-inflammation in guinea-pig airway, without fatality. Control animals were exposed for 90 min to medi-air. The average of two baseline determinations of respiratory function (sG_{aw} and breaths min^{-1}) was obtained prior to exposure to ozone or medi-air and then at regular intervals (0, 15, 30 min and hourly) after the exposure.

Spasmogen exposures

Airway reactivity to nebulized histamine (1 mM, 20 s), delivered as a nose-only exposure, was assessed 24 h before and at 0.5, 2, 24, 48 or 72 h after box-exposure. This dose of histamine caused a small threshold bronchoconstriction in naïve animals, compared with 3 mM which was required to produce significant bronchoconstriction ($-22.6 \pm 6.7\%$ decrease from baseline sG_{aw} value). Histamine *via* inhalation has been shown to be at least a 100-fold more efficacious on functional changes in the guinea-pig lung compared to the nasal cavity (Finney & Forsberg, 1994). A nasal component of the changes in sG_{aw} was considered negligible compared to those in the lung. Measurements of sG_{aw} were taken before and at 0, 5 and 10 min after exposure to the spasmogen.

Administration of anti-inflammatory compounds

In other animals, the phosphodiesterase-4 (PDE4) inhibitor, rolipram (1 $\text{mg}\cdot\text{kg}^{-1}$), the corticosteroid, dexamethasone (20 $\text{mg}\cdot\text{kg}^{-1}$), or vehicle were administered (i.p.) 24 and 0.5 h before exposure to ozone and 24 h afterwards. In these animals, AHR was assessed at 2 h after the completion of ozone exposure and BALF was removed at 48 h. These doses were selected based upon data from other studies using models of inflammation and that were without adverse effects (Toward & Broadley, 2001a,b). No animal appeared to be in respiratory distress, or exhibit other signs of discomfort during the exposure regimes or during any of the other procedures described.

Bronchoalveolar lavage

Within 20 min of assessing airway reactivity to histamine, 48 h after medi-air exposure or 48 h after ozone exposure with or without rolipram or dexamethasone treatment, the guinea-pigs were overdosed with pentobarbitone sodium (400 $\text{mg}\cdot\text{kg}^{-1}$, i.p., Euthatal®). The thoracic cavity was opened to allow access to the lungs, and the trachea was exposed. A ligature was placed around the right large-bronchi, to prevent fluid from entering the right side of the lung. The trachea was then cannulated and the animals underwent a bronchoalveolar lavage of the left lung lobes. Saline (0.9% NaCl: 0.5 $\text{ml}\cdot 100\text{ g}^{-1}$ body weight) was flushed through the cannula into the lungs, and recovered 3 min later. This was repeated and a total cell count (cells. ml^{-1}) of the pooled bronchoalveolar fluid (BALF) sample was determined using a haemocytometer (Neubauer). A Cytospin smear (Shandon Centrifuge: 1000 r.p.m., 7 min) of the sample was differentially stained (Leishman's: 1.5% in methanol, 6 min), and a minimum of 500 cells (macrophages, eosinophils and neutrophils) were counted. Lymphocytes were not identified or counted. In all the groups studied there was no significant difference in the volumes of reclaimed BALF (typically, 70–80% recovery).

Measurement of nitric oxide (NO) production

The remaining BALF sample was then centrifuged (1200 r.p.m., 6 min) and the supernatant was removed and frozen (-20°C). A spectrophotometric assay was used to determine the stable decomposition products of NO (nitrite and nitrate) in BALF. The assay was based on the Griess reaction (Grisham *et al.*, 1996) as previously described (Toward & Broadley, 2000b). Briefly, 100 μl samples of the BALF were incubated (37°C) for 30 min with HEPES buffer (50 mM, pH 7.4), FAD (5 μM), NADPH (0.1 mM), distilled water (290 μl) and nitrate reductase (0.2 $\text{U}\cdot\text{ml}^{-1}$) for the conversion of nitrate to nitrite. In an identical set of tubes, nitrate reductase was omitted for determining nitrite only. Any unreacted NADPH in the solution (500 μl) was then oxidised by incubating (25°C , 10 min) with potassium ferricyanide (1 mM). The samples were then incubated (25°C) with 1 ml of the Griess reagent (NED: 0.2% ($\text{w}\cdot\text{v}^{-1}$), sulfanilamide: 2% ($\text{w}\cdot\text{v}^{-1}$), solubilized in double distilled water (95%) and phosphoric acid (5%) ($\text{v}\cdot\text{v}^{-1}$)) for 10 min and the absorbance measured at 543 nm. Levels of nitrite (NO metabolite) in the BALF samples were calibrated against Griess reagent absorbance from a sodium nitrite (0–150 μM) standard curve. To maintain standard conditions, all the samples to be compared were assayed at the same time. The maximum linear limit of detection for the assay was 1 mM.

Measurement of wet/dry lung weight

Changes in lung oedematous fluid after exposure to medi-air, ozone or ozone and rolipram or dexamethasone treatment, were quantified as the difference in the wet/dry lung weights. An increase in lung wet weight after ozone exposure, compared to naïve lung, was considered to be the result of oedema (Hwang *et al.*, 2001). After removing BALF from the left side of the lung, the right lung lobes were removed below

the ligature, by dissecting through the right large-bronchi. The lung lobes were blotted dry, weighted in a pre-weighted tube and placed in an oven (80°C) with silica crystals. They remained in the oven until there was no further reduction in lung weight (without charring), normally a period of 24–48 h. The difference between the wet and oven-dried lung weight was a measure of fluid in the lung. The dry lung weight was not significantly different in any of the groups studied.

Drugs and solutions

Dexamethasone-21-phosphate disodium salt, dimethylsulphoxide (DMSO), flavin adenine dinucleotide (FAD) disodium salt, histamine diphosphate salt, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid] (HEPES) free acid, nicotinamide adenine dinucleotide phosphate (NADPH: reduced form), naphthylethylenediamine (NED), sodium nitrite, phosphoric acid, potassium ferricyanide, rolipram and sulfanilamide were purchased from Sigma (Poole, Dorset, U.K.), Euthatal[®] from Rhone Merieux (Harlow, Essex, U.K.) and Aspergillus nitrate reductase (NADPH: nitrate oxidoreductase, EC 1.6.6.2) from Boehringer Mannheim (Indianapolis, U.S.A.). Rolipram and dexamethasone were dissolved in 50% DMSO, 50% saline (0.9% NaCl: Baxter Healthcare, Thetford, Norfolk, U.K.) and introduced as 1.0 ml volumes made up with sterile saline. The final volume of DMSO was less than 5% in the vehicle and the treatment. This vehicle has previously been shown to have no effect in naïve animals, or on lung function and the inflammatory response in other inflammatory models (Toward & Broadley, 2001a, b). Unless otherwise indicated, all other solutions were made up in sterile saline.

Data analysis

To reduce inter-subject variability, changes in sG_{aw} from the baseline sG_{aw} values taken before a procedure, are presented as a percentage of the mean baseline value preceding ozone, medi-air or histamine challenge. Absolute values of baseline sG_{aw} are stated in the figure legends. Changes in respiratory rate are presented as the number of breaths per minute (breaths min^{-1}). The BALF cell counts, the combined NO metabolites and the wet/dry lung weights were compared using analysis of variance, followed by Scheffe's *post hoc* analysis. Changes in airway function (sG_{aw} and breaths min^{-1}) were compared using analysis of variance followed by the appropriate paired or unpaired Student's *t*-test. Differences were considered statistically significant when $P < 0.05$ (Motulsky, 1995).

Results

Lung function

Exposure of guinea-pigs to ozone caused an immediate fall in sG_{aw} , indicating bronchoconstriction (-52.2 ± 6.5 peak per cent decrease from baseline sG_{aw} , $P < 0.001$), compared with medi-air ($-13.4 \pm 3.3\%$) (Figure 1). This early-phase bronchoconstriction (EPB) recovered 2 h later. However, at 5 h after ozone exposure, a late-phase bronchoconstriction (LPB) commenced (5 h: $-19.0 \pm 3.1\%$ decrease from baseline sG_{aw} , $P < 0.01$). During the EPB, the respiratory rate was unaltered from baseline values (94 ± 13 breaths min^{-1}) (Figure 2). However, at 5 h after exposure to ozone, an increase in respiratory rate (120 ± 18 breaths min^{-1} , $P > 0.05$) com-

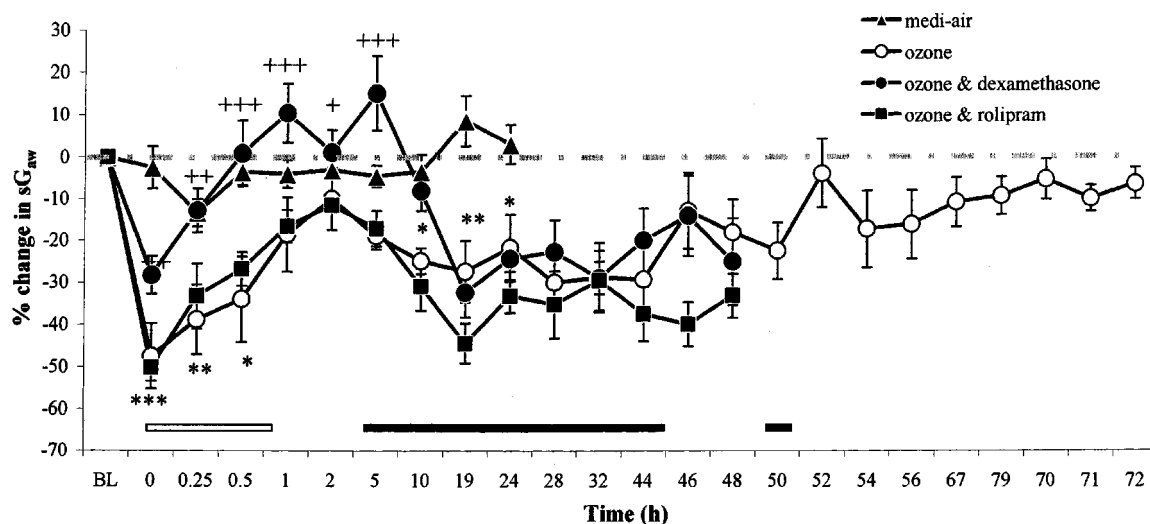


Figure 1 The effect of exposure (90 min) to medi-air (0.35 l min^{-1} , 18.8–25% oxygen in nitrogen), or medi-air *via* an ozone generator (2.15 ± 0.05 p.p.m.) with and without dexamethasone (20 mg kg^{-1}) or rolipram (1 mg kg^{-1}) treatment on lung function, in conscious guinea-pigs. Treatment was administered 24 and 0.5 h before exposure to ozone and 24 h afterwards. Each point represents the mean \pm s.e. mean ($n = 6$) change in sG_{aw} expressed as a percentage of the baseline sG_{aw} values (sG_{aw} ($\text{sec}^{-1} \text{ cmH}_2\text{O}^{-1}$): medi-air = 0.29 ± 0.01 ; ozone = 0.34 ± 0.01). Negative values represent bronchoconstriction. The EPB is depicted from 0–1 h (white bar) and the LPB is shown from 5–50 h (black bar) after exposure to ozone. Significance of differences in changes of sG_{aw} after ozone exposure: from baseline values (black and white bars: $P < 0.05$); or between medi-air exposure ($*P < 0.05$, $**P < 0.01$ and $***P < 0.001$); or with and without dexamethasone treatment ($+P < 0.05$, $++P < 0.01$ and $+++P < 0.001$), were determined by analysis of variance (single factor), followed by a Student's *t*-test (paired, unpaired and unpaired, respectively). There was no significant ($P > 0.05$) difference between the lung function in ozone exposed guinea-pigs with and without vehicle treatment (data not shown).

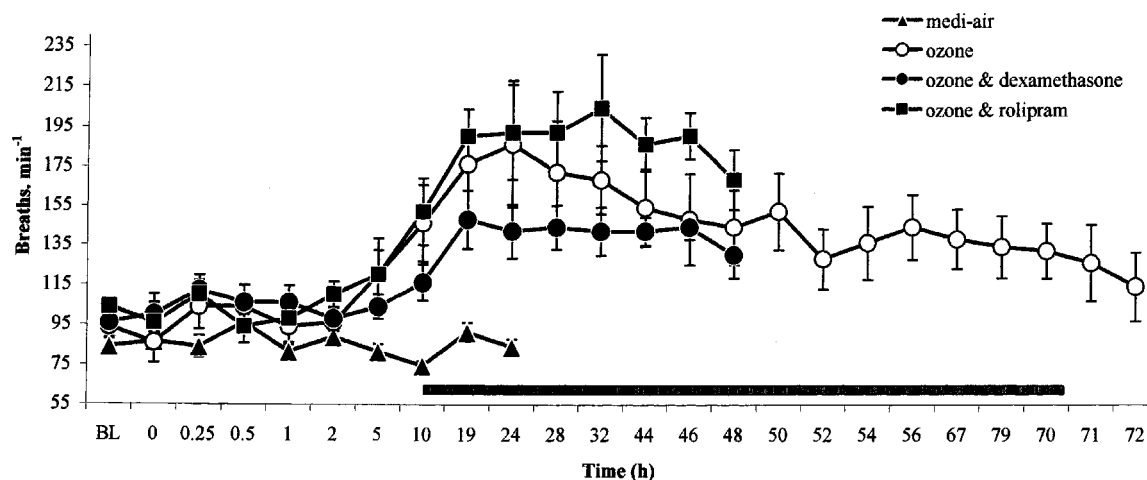


Figure 2 The effect of exposure (90 min) to medi-air (0.35 l min^{-1}), or medi-air *via* an ozone generator ($2.15 \pm 0.05 \text{ p.p.m.}$) with and without dexamethasone (20 mg kg^{-1}) or rolipram (1 mg kg^{-1}) treatment on respiratory rate, in conscious guinea-pigs. Treatment was administered 24 and 0.5 h before exposure to ozone and 24 h afterwards. Each point represents the mean \pm s.e. mean ($n=6$) change in respiratory rate (breaths min^{-1}). Black bar denotes the significance of differences ($P < 0.05$) between respiratory rate before and after exposure to ozone as determined by analysis of variance (single factor), followed by Student's unpaired *t*-test. There was no significant ($P > 0.05$) difference between the respiratory rate in ozone exposed guinea-pigs with and without rolipram, dexamethasone or vehicle (data not shown) treatment.

menced that coincided with the development of a LPB. Both the increase in respiratory rate ($186 \pm 32 \text{ breaths min}^{-1}$, $P < 0.01$) and LPB (-29.9 ± 5.7 peak per cent decrease from baseline sG_{aw} , $P < 0.01$) peaked at 24 h after ozone exposure and recovered to baseline values at 72 h (Figures 1, 2). Interestingly, cyanosis was observed in the ears, paws and nose of the guinea-pigs, especially during the LBP, that also recovered at 72 h. Exposure to medi-air failed to significantly ($P > 0.05$) alter lung function from baseline values of sG_{aw} or respiratory rate (Figures 1, 2).

In ozone-exposed animals treated with vehicle there was a similar profile of EPB followed by a LPB accompanied by increased respiratory rate (data not shown). Rolipram treatment did not affect this profile (Figures 1, 2). However, dexamethasone treatment attenuated ($P < 0.01$) the EPB after ozone exposure, but failed to significantly alter the LPB (Figure 1). Animals treated with dexamethasone also displayed a slight ($P > 0.05$) reduction in the increased respiratory rate associated with the LPB (24 h: $142 \pm 14 \text{ breaths min}^{-1}$), compared with ozone exposed animals (24 h: $186 \pm 32 \text{ breaths min}^{-1}$) (Figure 2). In vehicle, rolipram or dexamethasone treated animals, the LPB and associated increase in respiratory rate recovered to baseline levels at 72 h after ozone exposure (Figures 1, 2). There was no statistically significant difference between starting baseline values of sG_{aw} or respiratory rate for any of the groups studied.

Airway responsiveness to inhaled histamine

Inhalation of histamine (1 mM, 20 s) before ozone exposure failed to produce a significant bronchoconstriction (Figure 3a,b). However, at 0.5 h after a single exposure to ozone, there was a significant ($P < 0.05$) bronchoconstriction to inhaled histamine, compared to before exposure, indicating AHR (Figure 3a,b). There was also AHR to inhaled histamine at 2 h ($P < 0.01$), 12 h ($P < 0.05$), 24 h ($P < 0.05$)

and 48 h ($P < 0.05$) after ozone exposure, a lack of bronchoconstriction before ozone exposure being converted to a significant bronchoconstriction afterwards (Figure 3b). At 72 h after exposure to ozone, the increased reactivity towards histamine (i.e. AHR) subsided (Figure 3b). Exposure to medi-air failed to alter airway responsiveness to histamine at 2 h after exposure (Figure 3b).

Compared with before ozone (-2.2 ± 8.4 peak per cent decrease from baseline sG_{aw}), the peak increase in airway responsiveness to histamine ($-34.3 \pm 9.6\%$, $P < 0.01$) occurred at 2 h after exposure to ozone (Figure 3b). The airway responsiveness to histamine was not significantly ($P > 0.05$) increased at 2 h after ozone exposure in guinea-pigs treated with rolipram ($-4.7 \pm 5.3\%$) or dexamethasone ($-7.4 \pm 10.9\%$), compared with before exposure ($-5.2 \pm 5.7\%$ and $-8.4 \pm 4.9\%$, respectively), indicating an inhibition of the acute ozone-induced AHR (Figure 3b). Treating animals with the rolipram and dexamethasone vehicle failed ($P > 0.05$) to alter the airway responsiveness to histamine (Figure 3b). Treating control animals with dexamethasone or rolipram fails to affect the responsiveness to histamine as shown in our previous studies (Toward & Broadley, 2001a).

Leukocyte infiltration

In animals exposed to ozone, there was no significant ($P > 0.05$) increase in the population of leukocytes in BALF removed at 0.5 and 2 h later, compared with naïve animals (Figure 4). However, there was a marked increase in the number of macrophages ($P < 0.05$), eosinophils ($P < 0.05$) and neutrophils ($P < 0.01$) retrieved from the BALF at 12 h after ozone exposure, compared to that removed from naïve animals. Macrophages and eosinophils further increased at 24 h and remained elevated at 48 h, which coinciding with the peak in LPB and peak increase in respiratory rate. However, the neutrophil influx that peaked at 12 h began to

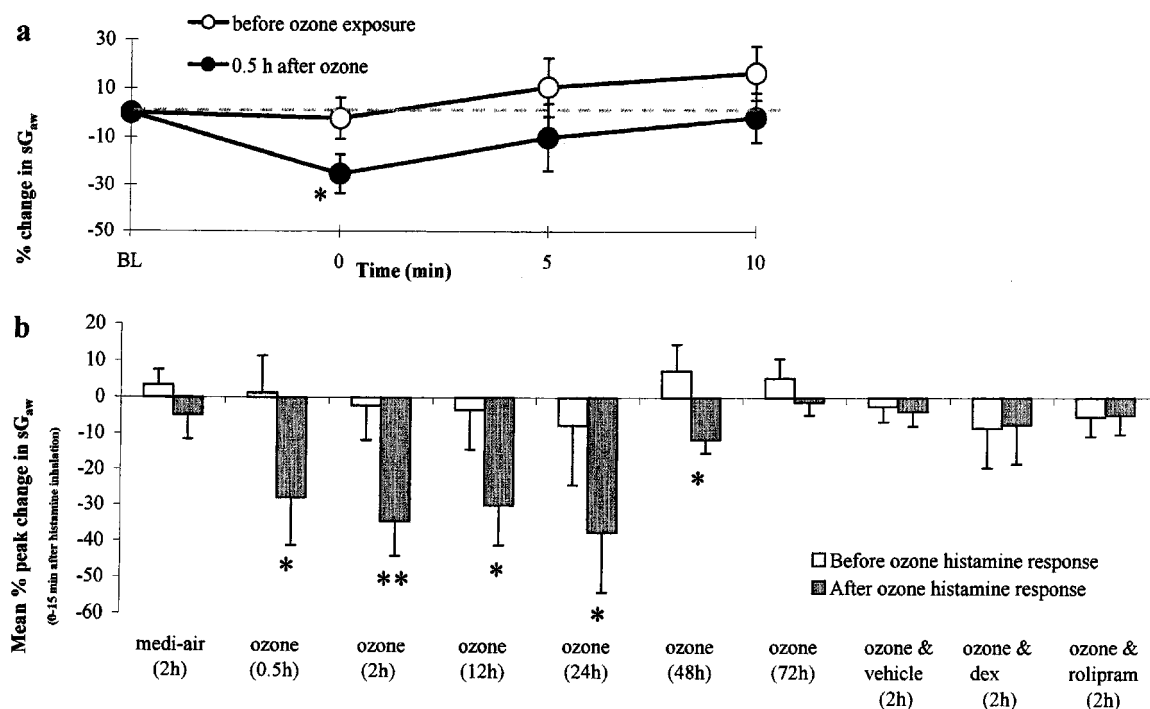


Figure 3 The airways responsiveness of a nose-only exposure (20 s) to histamine (1 mM) before and (a) 0.5 h after exposure (90 min) to ozone (2.15 ± 0.05 p.p.m.); (b) 0.5, 2, 12, 24, 48 and 72 h after exposure (90 min) to ozone, 2 h after exposure (90 min) to medi-air, and 2 h after ozone with vehicle, dexamethasone (dex, 20 mg kg^{-1}), or rolipram (1 mg kg^{-1}) treatment, in conscious guinea-pigs. Treatment was administered (i.p.) 24 and 0.5 h before exposure to ozone. Each point ($n=6$) represents the (a) mean \pm s.e. mean change in sG_{aw} expressed as a percentage of the baseline sG_{aw} values (sG_{aw} ($\text{sec}^{-1} \text{ cmH}_2\text{O}^{-1}$) before = 0.37 ± 0.02 and after = 0.40 ± 0.02); (b) mean \pm s.e. mean of individual peak change in sG_{aw} from baseline sG_{aw} values, 0–10 min following inhalation of histamine expressed as a percentage of the baseline, 24 h before and: 2 h after medi-air (before = 0.27 ± 0.01 and after = 0.28 ± 0.01); 0.5 h (before = 0.37 ± 0.02 and after = 0.40 ± 0.02), 2 h (before = 0.31 ± 0.01 and after = 0.33 ± 0.02), 12 h (before = 0.33 ± 0.02 and after = 0.31 ± 0.03), 24 h (before = 0.34 ± 0.03 and after = 0.35 ± 0.02), 48 h (before = 0.32 ± 0.01 and after = 0.28 ± 0.01), 72 h (before = 0.31 ± 0.01 and after = 0.30 ± 0.01) after ozone, and 2 h after ozone and vehicle (before = 0.33 ± 0.01 and after = 0.32 ± 0.01), dex (before = 0.34 ± 0.02 and after = 0.33 ± 0.03), or rolipram (before = 0.33 ± 0.02 and after = 0.27 ± 0.02) treatment. Negative values represent bronchoconstriction. Differences between before and after exposure to histamine were determined by analysis of variance (single factor), followed by a Student's (paired) t -test and denoted as * $P < 0.05$ and ** $P < 0.01$.

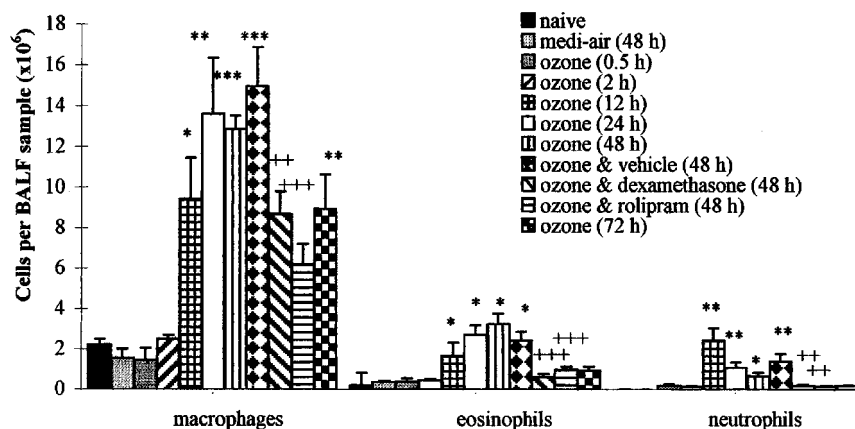


Figure 4 Differential cell (macrophage, eosinophil and neutrophil) counts of bronchoalveolar lavage fluid (BALF) removed before (naïve) and after exposure (90 min) to medi-air (48 h), ozone (2.15 ± 0.05 p.p.m.) (0.5, 2, 12, 24, 48 and 72 h), or ozone with vehicle, dexamethasone (20 mg kg^{-1}), or rolipram (1 mg kg^{-1}) treatment (48 h). Treatment was administered (i.p.) at 24 and 0.5 h before exposure to ozone and at 24 h afterwards. Each point represents the mean \pm s.e. mean ($n=6$) of the differential cells per BALF sample ($\times 10^6$) removed from the left lung lobes, *via* the left bronchi. The significance of differences in airway cell influx compared with naïve guinea-pigs (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$), and ozone exposure and vehicle *vs* dexamethasone or rolipram treatment (++++ $P < 0.01$ and +++++ $P < 0.001$), as determined by analysis of variance (single factor), followed by Scheffe's *post hoc* analysis.

wane at 24 h. At 48 h, the eosinophil and macrophage influx peaked. At 72 h, the eosinophils began to subside and along with the neutrophils were not significantly different from naïve levels. Macrophages were also significantly reduced, but remained significantly ($P < 0.01$) elevated compared with naïve animals. At 48 h after exposure to media-air, the levels of leukocytes in BALF were not significantly ($P > 0.05$) different from those observed in naïve guinea-pigs (Figure 4).

In ozone-exposed guinea-pigs treated with the vehicle there was no significant ($P > 0.05$) difference in the peak increase in BALF macrophages ($14.9 \pm 1.9 \times 10^6$ cells sample⁻¹), eosinophils ($2.4 \pm 0.4 \times 10^6$) and neutrophils ($1.4 \pm 0.4 \times 10^6$) at 24 h, from that observed in ozone-only exposed animals (Figure 4). Treatment with rolipram or dexamethasone significantly attenuated the 48 h peak airway influx of macrophages, eosinophils and neutrophils by 59, 59 and 42% and 42, 75 and 56% respectively, compared with ozone-exposed animals treated with vehicle (Figure 4). Treatment of control guinea-pigs with dexamethasone and rolipram enhance the levels of macrophages and eosinophils to a small extent, as shown in our previous studies (Toward & Broadley, 2001a).

Nitric oxide

The variation in concentrations of the individual NO metabolites (nitrate and nitrite) in BALF were synchronous. However, lower levels of nitrate were analysed, due to the rate limiting nitrate reductase conversion of nitrate to measurable nitrite (data not shown). Therefore, to describe changes in the NO metabolites assayed, the combined nitrate and nitrite values are shown (Figure 5) and referred to.

There was a basal level of combined NO metabolites in BALF removed from naïve animals (Figure 5). At 0.5 h after ozone exposure, the concentration of combined NO

metabolites in the BALF were significantly ($P < 0.05$) decreased, by 52%, compared to that found in naïve animals (Figure 5). At 2 h after ozone, the NO-deficiency recovered and was increased by 75% ($P > 0.05$), compared to that observed in naïve animals. At 12 h ($P < 0.05$) and 24 h ($P < 0.01$), the levels of combined NO metabolites in BALF were further increased (101 and 127% increase from naïve levels, respectively). This also coincided with the LPB, increase in respiratory rate and cell influx. At 48 h after ozone exposure, the NO levels remained significantly (131%, $P < 0.01$) elevated compared with naïve animals, but like the LPB, increase in respiratory rate and leukocyte influx, began to subside at 72 h. Exposure to medi-air failed to affect the basal production of NO at 48 h.

The significantly ($P < 0.01$) increased NO metabolites at 48 h after ozone exposure, were not affected by treatment with vehicle ($96.7 \pm 11.2 \mu\text{M}$ 100 μl BALF⁻¹, $P < 0.01$) (Figure 5). Treating the animals with either rolipram ($93.8 \pm 7.7 \mu\text{M}$, $P < 0.01$) or dexamethasone ($96.6 \pm 11.5 \mu\text{M}$, $P < 0.01$) failed to alter the significantly elevated levels of BALF NO metabolites, compared with vehicle treated animals at 48 h after ozone exposure. Dexamethasone or rolipram have also been shown in our previous studies to not affect BALF NO metabolites in control animals not exposed to ozone (Toward & Broadley, 2001a).

Lung wet/dry weight

At 0.5 and 2 h after exposure to ozone there was no significant ($P > 0.05$) increase in the lung wet/dry weight difference, compared with naïve animals (Figure 6). However, at 12 and 24 h there was a significant increase ($P < 0.05$) in the lung wet/dry weight difference. At 48 h, the lung wet/dry weight difference further significantly ($P < 0.001$) increased,

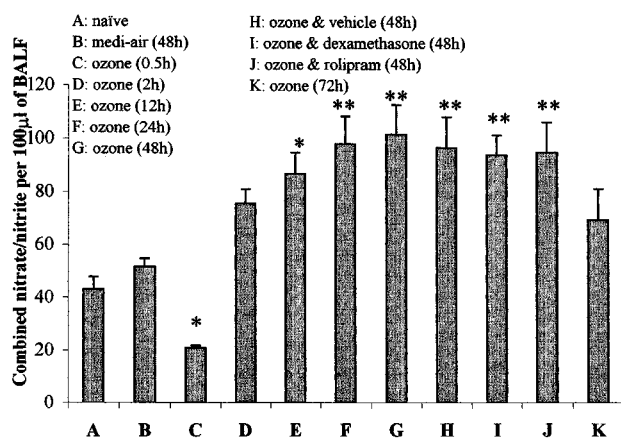


Figure 5 Airways levels of combined nitric oxide (NO) metabolites (nitrate and nitrite) recovered before (naïve) and after exposure (90 min) to medi-air (48 h), ozone (2.15 ± 0.05 p.p.m.) (0.5, 2, 12, 24, 48 and 72 h), or ozone with vehicle, dexamethasone (20 mg kg^{-1}), or rolipram (1 mg kg^{-1}) treatment (48 h). Treatment was administered (i.p.) at 24 and 0.5 h before exposure to ozone and at 24 h afterwards. Each point represents the mean \pm s.e.mean ($n = 6$) of the concentration of combined NO metabolites (μM) per 100 μl of BALF removed from the left lung lobes, *via* the left bronchi. * $P < 0.05$, and ** $P < 0.01$ denotes the significance of differences in airway NO levels compared with naïve guinea-pigs, as determined by analysis of variance (single factor), followed by Scheffe's *post hoc* analysis.

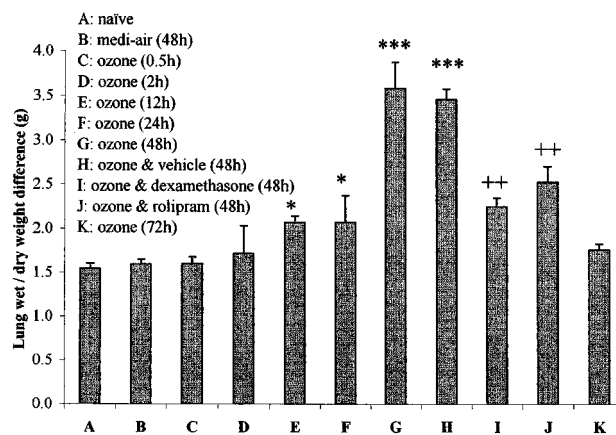


Figure 6 Difference in wet and oven-dried lung weights before (naïve) and after exposure (90 min) to medi-air (48 h), ozone (2.15 ± 0.05 p.p.m.) (0.5, 2, 12, 24, 48 and 72 h), or ozone with vehicle, dexamethasone (20 mg kg^{-1}), or rolipram (1 mg kg^{-1}) treatment (48 h). Treatment was administered (i.p.) at 24 and 0.5 h before exposure to ozone and at 24 h afterwards. Each point represents the mean \pm s.e.mean ($n = 6$) of the difference in right lung lobe weights (grams), removed below the right bronchi, before (wet) and after oven drying. The significance of differences in wet/dry lung weights compared with naïve guinea-pigs (* $P < 0.05$ and *** $P < 0.001$), and ozone exposure and vehicle versus dexamethasone or rolipram treatment (++) $P < 0.01$), was determined by analysis of variance (single factor), followed by Scheffe's *post hoc* analysis.

but at 72 h, it decreased and was not significantly ($P > 0.05$) different from that observed in naïve animals.

In guinea-pigs treated with vehicle there was no significant ($P > 0.05$) difference in the peak increase in oedematous fluid (3.5 ± 0.3 g) at 48 h after ozone exposure, to that observed in ozone-only exposed animals (Figure 6). Treatment with rolipram or dexamethasone significantly attenuated the peak increase in lung wet/dry weight difference by 27 and 35% respectively, compared with ozone-exposed animals treated with vehicle at 48 h (Figure 6).

Discussion

In COPD and severe asthma, chronic inflammatory-derived ROS in the airways are thought to contribute to pulmonary oxidative stress (Repine *et al.*, 1997; Barnes *et al.*, 1999). The aim of this study was to induce oxidative stress in conscious guinea-pig airways by exposure to ozone and to establish a model that displays the features of COPD. To test the validity of this model, the modification of these features by two standard anti-inflammatory agents, dexamethasone and rolipram were examined.

Exposure of guinea-pigs to ozone caused an immediate EPB that recovered at 2 h. This was, followed at 5 h, by a LPB that peaked at 24 h and recovered at 72 h. These two distinct phases of bronchoconstriction have not been reported before following ozone exposure, probably because a prolonged time-course, as used here, has not previously been examined in conscious guinea-pigs. Early- and late-phase bronchoconstrictions are commonly associated features of the asthmatic response to inhaled allergen and are thought to reflect two distinct pathological events (Bousquet *et al.*, 2000). In asthmatics and atopic animals, allergen exposure causes an immediate mast cell-derived release of histamine and other bronchoconstrictor mediators into the airways that contribute to the EPB (Bousquet *et al.*, 2000). The allergen-induced LPB, however, is often associated with an eosinophilic infiltration of activated leukocytes and a further increase in airways pro-inflammatory mediators that contribute to bronchoconstriction, AHR and oedema (Barnes *et al.*, 1999; Bousquet *et al.*, 2000).

In this study, the EPB after ozone exposure was not associated with a change in respiratory rate, oedema, or an increase in BALF leukocyte levels, although an increase in peripheral airway leukocytes cannot be ruled out. There was, however, a deficiency in airway NO and AHR to histamine at 0.5 h after ozone. In human macrophages, Cardile *et al.* (1995) have also shown that NO synthesis was attenuated after exposure to ozone. The authors suggested that oxidative damage to NOS was an unlikely cause of the NO-deficiency, but that enzymatic synthesis of NO was antagonized by ozone-derived ROS. An increase in mucosal mast cells and increased histamine in guinea-pig airways has been reported immediately after ozone exposure (Murlas & Roum 1985; Igarashi *et al.*, 1998). Other bronchoconstrictor metabolites may also occur from the generation of ROS-induced lipid peroxidation of arachidonic acid to form isoprostanes. The most prevalent of which, 8-epi-prostaglandin F_2 , causes potent constriction of human airways (Barnes *et al.*, 1999). In human airway smooth muscle, NO is the sole inhibitory nonadrenergic noncholinergic (NANC) bronchodilator (Nij-

kamp & Folkerts, 1995). Thus, in this study, an absence of opposing bronchodilatory NO coinciding with a mast cell-derived release of histamine or ROS-induced peroxidation of arachidonic acid to yield isoprostanes, may contribute to the development of the ozone-induced EPB. Another explanation for the bronchoconstriction is that ozone inhibits cholinesterase activity in guinea-pig lungs (Gordon *et al.*, 1981) and also causes a loss of pre-junctional inhibitory M_2 muscarinic receptor function either directly or *via* inflammatory cell-derived mediators (Schultheis *et al.*, 1994). Both of these effects would increase the release and longevity of acetylcholine in the synapse, and subsequently enhance post-synaptic vagal nerve stimulation and explain the bronchoconstrictor activity.

In this study, AHR to histamine occurred as early as 0.5 h after ozone exposure. Previously, we have shown in LPS-exposed guinea-pigs (Toward & Broadley, 2001b) that AHR to inhaled histamine coincided with NO-deficiency, at 1 h after exposure. Furthermore, we demonstrated that NO might aid recovery from LPS-induced AHR, as inhibiting NO generation with the non-selective NOS inhibitor, L-NAME, prolonged the duration of AHR (Toward & Broadley, 2000b). The AHR to inhaled histamine at 0.5 h after ozone, might also be a consequence of NO-deficiency failing to oppose an inhaled histamine-induced bronchoconstriction or to the reduced airway calibre due to the EPB. At 2 h after ozone exposure, however, there was no residual bronchoconstriction or NO-deficiency, but there was a maximal increase in airways responsiveness to histamine. One explanation for the AHR at 2 h is that ozone inactivates neutral endopeptidase (NEP) in the airway (Holbrook *et al.*, 1996), which is a peptidase involved in the degradation of pro-inflammatory peptides, such as tachykinins released from sensory nerve endings in guinea-pig lung (Spina *et al.*, 1998; Barnes *et al.*, 1999). Tachykinins cause hypersensitive vagal stimulation and bronchoconstriction (Spina *et al.*, 1998; Barnes *et al.*, 1999) and an ozone-induced inactivation of NEP would elevate their levels and cause neuronal hypersensitivity and an increased bronchoconstrictor response to inhaled histamine (i.e. AHR).

In contrast to the EPB, the LBP was associated with an increase in leukocytes. Initially, neutrophils transiently infiltrated the airways and peaked at 12 h. As neutrophils subsided, macrophage and eosinophil levels peaked between 24 and 48 h, and at 72 h, the levels of BALF granulocytes recovered to those in naïve animals. However, macrophage levels remained significantly elevated. Ozone and other ROS activate nuclear transcription factor- κ B (NF- κ B), which orchestrates the expression of multiple inflammatory genes that are up-regulated in both COPD and asthma (Stockley, 1995; Barnes & Adcock, 1997; Barnes *et al.*, 1999). Ozone-induced activation of NF- κ B would cause resident macrophages, lymphocytes and epithelial cells in the airway to express the neutrophil chemotactic factors, interleukin (IL)-8 and tumour necrosis factor (TNF)- α , which may explain the initial neutrophilia in this study (Barnes & Adcock, 1997). NF- κ B and TNF- α also induce a complex cascade of pro-inflammatory mediators responsible for macrophage and eosinophil recruitment (macrophage inflammatory protein-1 α , monocyte chemotactic protein-3 and eotaxin), adhesion molecule expression and promoting leukocyte survival (granulocyte-macrophage colony stimulating factor) (Barnes

& Adcock, 1997; Barnes *et al.*, 1999). As in asthma and COPD, in this study during the LBP, AHR to histamine correlated with a decline in airway function and the development of underlying inflammation (Postma & Kerstjens, 1998). Furthermore, the recovery in AHR also coincided with a resolution in leukocyte influx and the LPB. This may suggest that during the ozone-induced LBP, leukocyte influx and airway function are related to AHR.

The inducible inflammatory enzymes, iNOS and cyclooxygenase-2 (iCOX₂) are also up-regulated by NF- κ B and TNF- α . In this study, the elevated levels of NO during the LBP could be due to NF- κ B-mediated iNOS induction. Haddad *et al.* (1995) demonstrated an increase in iNOS mRNA expression at 2 and 8 h after ozone exposure of Brown Norway rats. Interestingly, Colasanti & Suzuki (2000) suggest that basal levels of NO suppress NF- κ B dissociation from the inhibitory nuclear transcription factor (I- κ B) complex and also prevent NF- κ B binding to the promoter region of the iNOS gene. Thus, in the present study, the initial NO-deficiency during the ozone-induced EPB, could facilitate activation of the machinery to up-regulate iNOS expression during the LBP. The high levels of bronchodilatory NO at 24 and 48 h, may be a rebound response to attenuate the ozone-induced LBP (Barnes *et al.*, 1999). However, excessive NO also causes leukocyte adhesion and microvascular leakage that promotes leukocyte influx and oedema (Barnes *et al.*, 1999). These effects might be further compounded due to underlying oxidative stress from exposure to ozone and ROS derived from activated inflammatory cells, that combine with NO to form oxidative nitrogen species (e.g. peroxynitrite). These oxidative nitrogen species would exacerbate airway damage and oedema and also cause the AHR associated with the LBP in this study (Nijkamp & Folkerts, 1995).

In the present study, the peak leukocyte influx and generation of NO (metabolites) was also associated with a doubling in lung wet/dry weight difference at 48 h after ozone. The increase in wet lung weight is likely to arise from oedema (Hwang *et al.*, 2001). An increase in lung oedematous fluid may be a result of an increased capillary blood pressure or an increase in permeability of the capillary wall. Ozone and pro-inflammatory-derived arachidonic acid metabolites, synthesised by iCOX₂ (e.g. thromboxane) or 5-lipoxygenase (e.g. leukotriene: C₄, D₄, E₄), induce vasoconstriction to elevate trans-capillary blood pressure. Tachykinins, ROS, mast cell-derived histamine, excess NO and ROS-activated proteolytic enzymes may cause increased permeability of the capillary wall (Barnes *et al.*, 1999). Oedematous extravasation from the capillaries into the airway and oedematous airway wall swelling, would reduce airway calibre and contribute to the LBP (Pare & Hogg, 1989). However, as ozone and ROS are potent mucus secretagogues, the secretion of mucus from goblet cells and submucosa glands could also increase the lung wet weight and reduce airway calibre, thus causing bronchoconstriction (Barnes *et al.*, 1999; Bousquet *et al.*, 2000). A reduced airway calibre is also a likely cause of the AHR during the LBP, since it parallels the increase in lung wet weight (Hwang *et al.*, 2001), and a reduced geometric airway calibre would potentiate spasmogenic contractility to inhaled histamine (Pare & Hogg, 1989). However, tachykinin-induced neuronal hypersensitivity, peroxynitrite, or epithelial damage exposing airway sensory nerves are also likely

contributors of the AHR associated with the LBP in this study (Nijkamp & Folkerts, 1995; Spina *et al.*, 1998).

The profile of LBP, unlike the EPB, was also paralleled with an increase in respiratory rate. As far as the authors are aware, this is the first report of distinct phases of bronchoconstriction and an increase in respiratory rate associated with only the LPB, in conscious animals exposed to ozone. One explanation for the increased respiratory rate is that ROS compete with oxygen, for haemoglobin in erythrocytes (Repine *et al.*, 1997; Barnes *et al.*, 1999). This would increase carbon dioxide tension in the blood, causing acidosis. Chemoreceptors located on the ventrolateral medulla region of the brainstem and peripherally in the carotid bodies, in response to acidosis, would vagally stimulate an increase in respiratory rate (Staub, 1993). Levels of acidosis in the blood and vagal activity were not examined. However, cyanosis in the animals after ozone exposure, would indicate a reduced haemoglobin oxygen capacity, a possible trigger of increased respiratory rate.

Treatment of the ozone-exposed animals with dexamethasone or rolipram, inhibited the AHR at 2 h and attenuated the leukocyte influx and oedema at 48 h. Interestingly, dexamethasone but not rolipram also attenuated the EPB. However, neither treatment significantly affected the LPB or the associated increase in respiratory rate and excess NO generation at 48 h. The inhibitory effects of dexamethasone can be attributed to inhibition of NF- κ B activation and subsequent expression of pro-inflammatory mediators responsible for leukocyte influx into the airways (e.g. iCOX₂ and iNOS), through an increased transcription of I- κ B (Barnes & Adcock, 1997). Rolipram is a PDE4-inhibitor and elevates intracellular levels of cAMP that suppress cell activation and inflammatory processes (Torphy *et al.*, 1999), which would also explain the attenuated leukocyte influx at 48 h after ozone. That both treatments attenuated the oedema was probably a consequence of a reduced leukocyte influx into the airways, inflammatory cell activity and a subsequent release of pro-inflammatory mediators capable of inducing oedema (e.g. eicosanoids, leukotrienes and proteolytic enzymes) (Barnes *et al.*, 1999; Torphy *et al.*, 1999). However, neither dexamethasone nor rolipram treatment attenuated the LPB, nor the excess generation of airways NO. This is contrary to our previous findings in parainfluenza 3-infected guinea-pigs (Toward & Broadley, 2001b) and LPS-exposed guinea-pigs (Toward & Broadley, 2001a), whereby both excess airways NO and leukocyte influx were inhibited using this dosing regime. Similarly, Haddad *et al.* (1995) showed that dexamethasone inhibited iNOS mRNA expression in ozone-exposed rats. It was unlikely that ozone itself artificially generated the free radical NO, since passing ozone through BALF removed from naïve animals did not affect the level of NO metabolites (unpublished observations). Furthermore, the increase in NO occurred 48 h after ozone exposure.

The AHR at 2 h after ozone exposure was sensitive to both dexamethasone and rolipram. In previous studies, we have demonstrated using this dosing regime that neither dexamethasone nor rolipram caused an anti-histamine or bronchodilator effect that would functionally antagonise a histamine-induced bronchoconstriction (Toward & Broadley, 2001a). Thus, the inhibition of AHR by these compounds may be related to their anti-inflammatory activity. It is

plausible that ozone or ROS derived from inflammatory cells inactivate NEP and thus elevate airway tachykinin levels that cause neuronal hypersensitivity and contribute to the AHR at 2 h after ozone. Murlas *et al.* (1993) have reported in ozone-exposed guinea-pigs a reduction in tachykinin levels after dexamethasone treatment. Furthermore, Jacoby *et al.* (2001) have shown that dexamethasone reduced stimulation of tachykinin release from sensory nerves by activated leukocytes in rats exposed to ozone. Rolipram has also been reported to prevent tachykinin release *via* inhibiting vagal stimulation of sensory nerves (Holbrook *et al.*, 1996). Thus, dexamethasone or rolipram treatment may inhibit AHR at 2 h after ozone by prevention of tachykinin-dependent AHR.

In summary, this study demonstrates, that oxidative stress induced by ozone exposure of conscious guinea-pigs causes an EPB and LPB. The EPB was associated with a deficiency in NO metabolites, whereas the LPB, as in COPD, was associated with dyspnoea, elevated NO levels, leukocyte influx and an increase in lung oedema. In this model, as

with COPD and asthma, AHR also correlated with the airways leukocyte influx, oedema, excess NO generation and dyspnoea associated with the LPB. Both dexamethasone and rolipram inhibited the ozone-induced AHR, leukocyte influx and oedema. Although dexamethasone attenuated the EPB, neither dexamethasone nor rolipram affected the excess NO, dyspnoea or associated LPB. Thus, this model provides many of the features of COPD and the results support the development of PDE4 inhibitors for the treatment of oxidant stress-induced inflammation and AHR in COPD or severe asthma.

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