Airway inflammation despite loss of bronchial hyper-responsiveness after multiple ozone exposures

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The effect of single and multiple exposures to ozone \((O_3)\) on airway responsiveness and inflammation was examined in guinea pigs. Airway responsiveness, measured as acetylcholine concentration needed to increase baseline airway resistance \((R_L)\) by 250% \((PC_{250})\), increased 1 h after exposure to ozone at 3 ppm for 3 h \((-\log PC_{250} = 3.88 \pm 0.17\) to \(4.78 \pm 0.18; P<0.05\), but returned to baseline at 8 h. An increase in neutrophil numbers was found at 8 h in bronchoalveolar lavage fluid (BALF). After \(O_3\) exposure on 4 successive days, baseline \(R_L\) increased and airway responsiveness decreased at 1, 8 and 72 h \((-\log PC_{250} = 2.88 \pm 0.17, 2.83 \pm 0.10\) and \(3.12 \pm 0.08\), respectively, compared to control value of \(3.48 \pm 0.05\). Repeated exposures to \(O_3\) also increased neutrophil numbers in bronchoalveolar lavage fluid and in bronchial submucosa. Thus, single exposure to \(O_3\) caused a rapid and transient increase in airway responsiveness, while multiple exposures induced a rapid but prolonged decrease in airway responsiveness associated with persistent bronchoconstriction. Both single and multiple exposures induced airway inflammation as evidenced by an increase in neutrophil influx. These studies demonstrated a dissociation between ozone-induced changes in airway responsiveness and neutrophil influx, and indicate that multiple exposures to \(O_3\) induce persistent bronchoconstriction with airway hyporesponsiveness.

Introduction

Ozone is an important component of the photochemical oxidation product of air pollution involving substrates emitted from automobile engines. Experimental exposure to ozone induces airways obstruction, airway hyperresponsiveness and airway inflammation in both humans and animals (1-6). In the guinea pig, ozone exposure below 1.5 ppm does not appear to induce any detectable increase in airway responsiveness (7,8), although at 3 ppm, airway hyper-responsiveness was observed in the guinea pig and rat (9-11). Although in the rat, ozone-induced bronchial hyper-responsiveness has not been associated with an increase in neutrophils in the tracheal submucosa or with airway oedema (11), in the guinea pig, an increase in inflammatory cells, particularly neutrophils, has been recorded following single exposure (1,12,13). As environmental exposure to ozone is likely to be a repeated occurrence, the effect of continued exposure is of greater importance. Attenuation of the effects of ozone on decrements in lung function has been observed in man following three exposures to ozone, with no significant effect on further exposure (14,15). Such functional adaptation has also been reported in rats, in which despite the occurrence of an attenuated response of pulmonary function to repeated exposures to ozone, biochemical and morphologic damage, such as epithelial damage and inflammation, and the levels of protein in lung lavage fluid continued to progress (16).

The present authors have investigated whether there was attenuation of bronchoconstriction and airway hyper-responsiveness after repeated exposures to ozone, and whether there was a
similar pattern of the pulmonary inflammatory response to repeated exposures as measured by the profile of cells recovered by bronchoalveolar lavage. Although adaptation of bronchoconstrictor responses has been reported after repeated exposures, it is not known whether airway hyper-responsiveness can be attenuated. The authors hypothesized that functional adaptation to ozone may occur but that inflammatory changes may continue to persist. In the present study, a concentration and duration of ozone exposure of 3 ppm and 3 h, respectively, were used as this level of exposure is known to increase bronchial responsiveness in the guinea pig.

**Methods**

Two separate series of experiments were performed on male Dunkin-Hartley guinea pigs (400–500 g) in order to study the effect of single or repeated exposures to ozone (O₃).

**PROTOCOL**

*Effect of Single Ozone Exposure*

Group 1 (Control group; n=9). Animals were exposed to laboratory air only. Lung resistance (Rₖ) and airway responsiveness to inhaled acetylcholine (ACh) were measured and bronchoalveolar lavage (BAL) fluid was collected.

Group 2 (O₃ × 1; 1 h group; n=6). Animals were exposed to O₃ for 3 h. One hour after exposure, Rₖ and airway responsiveness to inhaled ACh were measured and BAL fluid was collected.

Group 3 (O₃ × 1; 8 h group; n=6). Animals were exposed to O₃ for 3 h. Eight hours after exposure, Rₖ and airway responsiveness to inhaled ACh were measured and BAL fluid was collected.

Group 4 (O₃ × 1; 72 h group; n=7). Animals were exposed to O₃ for 3 h. Seventy-two hours after exposure, Rₖ and airway responsiveness to inhaled ACh were measured and BAL fluid was collected.

*Effect of Multiple Exposures to Ozone*

Similar groups were studied as for the single exposure protocol, except that these animals were exposed to ozone on four successive days: Group 1 (Control; n=7); Group 2 (O₃ × 4; 1 h; n=7); Group 3 (O₃ × 4; 8 h; n=6); and Group 4 (O₃ × 4; 72 h; n=7).

**OZONE EXPOSURE**

Ozone (0.5 l min⁻¹) was generated by passing laboratory air through a Sander Ozonizer (Model 500, Erwin Sander GmbH, Germany), mixed with compressed air (8 l min⁻¹) controlled by a gas flowmeter (Platon Flow Control, Basingstoke, U.K.) and fed into a purpose-designed perspex box (60 × 25 × 20 cm). The concentration of O₃ was determined by means of specific gas sampling tubes (Dragerwerk, AG, Germany) and maintained at 3 ppm by regular measurement at the output port of the box.

Conscious guinea pigs were placed in the box for 3 h and studied 1, 8 or 72 h later, as described above.

**MEASUREMENT OF Rₖ AND AIRWAY RESPONSIVENESS TO ACh**

Guinea pigs were anaesthetized intraperitoneally with urethane (8 mg kg⁻¹ of a 25% solution w/v in saline). Additional urethane was given as required to maintain adequate anaesthesia. A tracheal cannula (10 mm length and 2.7 mm internal diameter) was inserted into the lumen of the trachea through a tracheostomy, and tied snugly with suture material. A polyethylene catheter was inserted into the left carotid artery to monitor blood pressure and heart rate with a pressure transducer (Model PDCR 75, S/N 1506, Druck Ltd., U.K.). Animals were connected to a small animal respiratory (Harvard Apparatus Ltd., Edenbridge, Kent, U.K.) and ventilated with 10 ml kg⁻¹ air at a rate of 60 strokes min⁻¹. The ventilatory circuit had a total volume of 20 ml. Transpulmonary pressure was measured with a pressure transducer (Model FCO 40; ± 1000 mm H₂O, Furness Controls Ltd, Bexhill, Sussex, U.K.), with one side attached to a catheter inserted into the right pleural cavity and the other side attached to a catheter connected to a side port of the intratracheal cannula. Air flow was measured with a pneumotachograph (Model F1L; Mercury Electronics Ltd., Glasgow, U.K.) connected to a transducer (Model FCO 40; ± 20 mmH₂O:...
Furness Controls Ltd.). The signals from the transducers were digitalized with a 12-bit analogue-digital board (NB-MIO-16, National Instruments®, Austin, TX, U.S.A.) connected to a Macintosh II computer (Apple Computer Inc., Cupertino, CA, U.S.A.) and analysed with software (Labview®, National Instruments®) which was programmed to instantaneously calculate \( R_L \) by the method of von Neergaard and Wirz (17). Acetylcholine aerosols were generated with an ultrasonic nebulizer (Model 2511; PulmoSonic, DeVilbiss Co, PA, U.S.A.), and were administered to the airways through a separate ventilatory system that bypassed the pneumotachograph. The mean mass diameter of the aerosol was 3.8 \( \mu \)m, with a geometric standard deviation of 1.3, measured with a laser droplet and particle analyser (Model 2600C, Malvern Instruments, U.K.).

Animals were initially injected with propranolol (1 mg kg\(^{-1}\)) to inhibit adrenergic effects, and suxamethonium (1 mg kg\(^{-1}\)) to stop spontaneous breathing. At least 10 min after the animal was connected to the ventilator and administrated with propranolol and suxamethonium, the \( R_L \) was recorded before and after inhalation of 0.9% NaCl for 30 breaths, and the subsequent \( R_L \) value was used as baseline. Starting 3 min after saline exposure, increasing half-log_{10} concentrations of ACh were given by inhalation (30 breaths), with the initial concentration set at 10\(^{-5}\) M. Increasing concentrations were given when \( R_L \) returned to baseline (at least at 5–7-min intervals) with one hyperinflation of twice the tidal volume applied between each ACh concentration, performed by manually blocking the outflow of the ventilator. Airways secretions were suctioned as required to maintain clear airways. The challenge was stopped when an increase in lung resistance exceeding 400% over the initial baseline was obtained. The provocative concentrations to produce an increase in lung resistance of 250% over baseline (PC\(_{250}\)) was calculated by log-linear interpolation of concentration–response curves from individual animals.

BRONCHOALVEOLAR LAVAGE AND CELL COUNTING

After measurement of lung function and airway responsiveness, lungs were lavaged with 5 x 5 ml aliquots of 0.9% w/v sterile saline through a polythene tube introduced through the tracheostomy. Bronchoalveolar lavage fluid was collected. The samples were centrifuged at 5000 g for 10 min at 4°C. The supernatant was drained and phosphate-buffered saline (2 ml) was added. The suspension was shaken gently until cells were fully suspended. Kimura stain (90 μl) was added to a sample of the suspension (10 μl). The cells were counted in a Neubauer chamber and the suspension was further diluted to give a count of 1 million cells ml\(^{-1}\). Differential cell counts were made from cytospin preparations stained by May-Grunwald stain. Microscopy fields were chosen randomly and a total of 500 cells were counted for each slide. The total number of eosinophils, macrophages, neutrophils and lymphocytes in the BAL was calculated from the percentage found in the sample.

GRANULOCYTE INFLUX IN AIRWAY SUBMUCOSA (FOR MULTIPLE EXPOSURE STUDY)

At the end of the experiment, the guinea pigs were killed with an overdose of urethane. The lungs were inflated with 10% formaldehyde and the trachea was tied. Lung tissue was fixed in 10% formaldehyde, sectioned (3-μm sections) and stained with carbol-chromotrope. Eosinophils were distinguished by the bright red appearance of their granules, and neutrophils were distinguished by their distinct multilobar nuclei. The slides were coded and read. For quantification of the numbers of neutrophils or eosinophils in the airways, sections of main bronchus and right lower lung parenchyma were selected. Cells were counted within a given area using a graticule (area 175 × 175μm, at magnification × 400) positioned from the epithelium and extending into the subepithelial layers (for bronchi), or positioned at the edge of the lung next to pleural endothelial layer. For bronchi, cells within the graticule were further localized as being within epithelial, subepithelial and vascular areas. In total, four graticule sizes of each section were chosen and cells within the graticules were counted.
TABLE 1. Baseline lung resistance ($R_L$, cm $H_2O/ml$ s$^{-1}$) and acetylcholine provocative concentration (-log $PC_{250}$)

<table>
<thead>
<tr>
<th></th>
<th>Baseline $R_L$</th>
<th>$R_L$ after saline</th>
<th>-log $PC_{250}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single exposure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.08 ± 0.03</td>
<td>0.10 ± 0.03</td>
<td>3.88 ± 0.17</td>
</tr>
<tr>
<td>$O_3 \times 1$ h</td>
<td>0.13 ± 0.06</td>
<td>0.21 ± 0.08</td>
<td>4.78 ± 0.18*</td>
</tr>
<tr>
<td>$O_3 \times 1$ 8 h</td>
<td>0.16 ± 0.05</td>
<td>0.33 ± 0.09*</td>
<td>3.71 ± 0.15</td>
</tr>
<tr>
<td>$O_3 \times 1$ 3 day</td>
<td>0.31 ± 0.04</td>
<td>0.36 ± 0.06</td>
<td>3.67 ± 0.13</td>
</tr>
<tr>
<td><strong>Multiple exposures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.16 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>3.48 ± 0.05</td>
</tr>
<tr>
<td>$O_3 \times 4$ 1 h</td>
<td>1.15 ± 0.24†</td>
<td>1.55 ± 0.27†</td>
<td>2.88 ± 0.17†</td>
</tr>
<tr>
<td>$O_3 \times 4$ 8 h</td>
<td>1.00 ± 0.34†</td>
<td>1.23 ± 0.42†</td>
<td>2.83 ± 0.10†</td>
</tr>
<tr>
<td>$O_3 \times 4$ 3 day</td>
<td>0.34 ± 0.09*§</td>
<td>0.38 ± 0.11*‡</td>
<td>3.12 ± 0.08‡</td>
</tr>
</tbody>
</table>

Data expressed as mean ± sem. *$P<0.05$; †$P<0.01$ compared to control group; ‡$P<0.05$; §$P<0.01$ compared to $O_3 \times 4$ 1 h and $O_3 \times 4$ 8 h.

STATISTICAL ANALYSIS

Data are expressed as mean ± sem. $PC_{250}$ measurements were log$_{10}$-transformed. An increase in mean -log $PC_{250}$ represents an increase in airway responsiveness. Analysis of variance (ANOVA) was used to determine significant variance among the groups. If a significant variance was found, the unpaired $t$-test was used to determine any significant difference between two individual groups. Histological and BAL fluid data were analysed using Kruskal–Wallis and Mann–Whitney $U$-tests. Spearman rank correlation coefficient was used to assess the relationship between the cell counts in the tissues and bronchoalveolar fluid of all groups. A probability value of less than 0.05 was considered significant. Data were analysed with a Macintosh computer using standard statistical packages.

Results

EFFECT OF SINGLE OZONE EXPOSURE

Lung Resistance

There was no significant difference in the baseline $R_L$ either before or after saline challenge recorded among control or $O_3 \times 1$ h groups. Baseline $R_L$ after saline challenge in $O_3 \times 1$ 8 h group was significantly higher than that of the control group. Seventy-two hours after ozone exposure, $R_L$ had returned to baseline values (Table 1).

Airway Responsiveness

Inhaled $ACh$ caused a concentration-dependent increase in $R_L$. After inhalation of each concentration of $ACh$, peak $R_L$ was reached within 45 s, and recovered over 2–10 min. Mean -log $PC_{250}$ values for all the groups are shown in Table 1. One hour after single $O_3$ exposure, the mean -log $PC_{250}$ (4.78 ± 0.18) was significantly higher than that in control guinea pigs (3.88 ± 0.17; $P<0.05$), and returned to control level at 8 h [3.71 ± 0.15; Fig. 1(a)]. There was no significant difference in mean -log $PC_{250}$ between $O_3 \times 1$ 72 h group (3.67 ± 0.13) and control group [Fig. 1(a)].

Bronchoalveolar Lavage

There was no significant difference in numbers of total and specific cell types recovered in BAL fluid in $O_3 \times 1$ 1 h group when compared with control. There was a significant increase in the proportion of neutrophils in BAL fluid in $O_3 \times 1$ 1 h group (10 ± 1% vs. 7 ± 2%; $P<0.05$) and in $O_3 \times 1$ 8 h group (15 ± 1%; $P<0.05$). At 8 h after $O_3$ exposure, the numbers of neutrophils and lymphocytes (1.47 ± 0.21 $\times$ 10$^6$ ml$^{-1}$ and 0.27 ± 0.03 $\times$ 10$^6$ ml$^{-1}$, respectively) in BAL fluid increased as compared to control
Fig. 1. Mean $-\log PC_{250}$ ($\pm$ SEM) to acetylcholine in rats exposed to air or ozone (3 ppm for 3 h) measured at 1 h, 8 h or 3 days after exposure. (a) Effects of a single exposure, (b) effects of four exposures on four consecutive days. $PC_{250}$ is the concentration of acetylcholine needed to increase baseline lung resistance by 250%. *$P<0.05$ and †$P<0.01$ compared to control group, ‡$P<0.05$ compared to 1 h and 8 h groups.

Fig. 2. Mean ($\pm$ SEM) of total and specific (macrophage, eosinophil, lymphocyte and neutrophil) cell counts measured in bronchoalveolar lavage fluid in guinea pigs exposed to air or ozone (3 ppm for 3 h) measured at 1 h, 8 h or 3 days after exposure. (a) Effects of a single exposure, (b) effects of four exposures on four consecutive days. *$P<0.05$ and †$P<0.01$ compared to control; four $P<0.01$ compared to 1 h and 8 h groups. Solid bars, total counts; hatched bars, macrophage counts; stippled bars, eosinophil counts; cross-hatched bars, lymphocyte counts; open bars, neutrophil counts.

group $[0.70 \pm 0.22 \times 10^6 \text{ml}^{-1}$ and $0.12 \pm 0.04 \times 10^6 \text{ml}^{-1}; P<0.05$; Fig. 2(a)]. Three days after single $O_3$ exposure ($O_3 \times 1.72$ h group), the numbers of each inflammatory cell type in BAL fluid remained higher than those in the control group [$P<0.01$, Fig. 2(a)].

EFFECT OF MULTIPLE EXPOSURES TO $O_3$

Lung Resistance

There was a significant increase in the baseline $R_L$ both before and after saline challenge in
O₃ x 4 1 h, 8 h and 72 h groups when compared with the control group. Baseline $R_L$ before and after saline challenge in O₃ x 4 1 h and 8 h groups was significantly higher than that in O₃ x 4 72 h group (Table 1).

Airway Responsiveness

Multiple O₃ exposure caused a significant reduction in airway responsiveness to inhaled ACh. Mean $-\log PC_{250}$ in O₃ x 4 1 h, 8 h and 72 h groups (2.88 ± 0.17, 2.83 ± 0.10 and 3.12 ± 0.08, respectively) was significantly lower than that in the control group (3.48 ± 0.05; $P<0.01-0.05$). Mean $-\log PC_{250}$ in O₃ x 4 8 h group did not show any significant difference from that in O₃ x 4 1 h group, but it was significantly lower than that in the O₃ x 4 72 h group, suggesting partial recovery of airway responsiveness at 72 h [Fig. 1(b)].

Bronchoalveolar Lavage

Multiple O₃ exposure caused a significant increase in inflammatory cells in BAL fluid [Fig. 2(b)]. The increase in neutrophil counts was greater in O₃ x 4 1 h and 8 h groups (2.38 ± 0.38 x 10⁶ ml⁻¹ and 3.39 ± 0.54 x 10⁶ ml⁻¹, respectively) when compared to the O₃ x 4 72 h group (1.23 ± 0.21; $P<0.05$ and $P<0.01$, respectively). There was a significant increase in the proportion of neutrophils in BAL fluid in multiple O₃ exposure groups (15 ± 4%, 14 ± 2% and 10 ± 2%, respectively) as compared to the control group (3 ± 1%; $P<0.01-0.05$). The increase in neutrophils in BAL fluid was greater in O₃ x 4 1 h and 8 h groups than in O₃ x 4 72 h group ($P<0.05$, Fig. 2).

Granulocyte Counts in Bronchi and Lung Parenchyma

There was no significant difference in number of neutrophils and eosinophils counted in parenchymal tissues among all groups. Eosinophil counts in bronchial epithelium and subepithelium were also not different among these groups. A significant increase was found in neutrophil counts in the subepithelium in O₃ x 4 1 h and O₃ x 4 8 h groups (251-8 ± 40.2 cells mm⁻² and 104.5 ± 34.9 cells mm⁻², respectively) when compared with the control group (18.6 ± 14.0 cells mm⁻²; $P<0.01$ and $P<0.05$, respectively, Fig. 3). There was no significant difference in the number of neutrophils within the epithelium among all groups.

Relationship Between Cell Counts in BAL Fluid and in Tissues

There was a significant correlation between total cell recovery in BAL fluid and cell counts in bronchial tissue ($R_s=0.43; P<0.001$), and between neutrophil count in bronchial subepithelial layer and neutrophil proportion in BAL fluid ($R_s=0.06; P<0.05$).

Discussion

The present study has shown that following one or four exposures to O₃, there was a significant degree of bronchoconstriction which persisted for at least 8 h. While the effect after single exposure had worn off by 72 h, this still persisted after repeated exposures. By contrast, airway responsiveness to ACh was found to be reduced following repeated exposures for up to 72 h later, while it increased following a single O₃ exposure, an effect that occurred within 1 h after cessation of exposure and was significantly reduced by 8 h. Despite the attenuation of
airway responsiveness in the repeatedly exposed guinea pigs, there was a significant increase in neutrophils in BAL fluid and in bronchial airway tissue. By contrast, in the single-exposure guinea pigs, neutrophils in BAL fluid showed a small increase at 1 h when airway hyper-responsiveness was at its peak, but a greater degree of neutrophilia at 8 h, when airway hyper-responsiveness had disappeared. Interestingly, the degree of neutrophilia in BAL fluid nearly doubled at 8 h in the guinea pigs following multiple exposures as compared to a single exposure. Thus, there was a dissociation between the bronchoconstrictor response and bronchial hyper-responsiveness, and between the onset of bronchial hyper-responsiveness and neutrophil influx in the airways in the response to one and to four exposures to O_{3}. Both a reduction and an increase in bronchial responsiveness was observed in association with airway inflammation, as measured by the number of neutrophils in the airways. These observations indicate that repeated exposures to O_{3} may lead to chronic airway damage through persistent inflammatory processes.

The mechanisms underlying the decrease in airway responsiveness following multiple exposures to O_{3} are not clear. The decrease in airway responsiveness was associated with the presence of persistent bronchoconstriction, followed by a partial recovery of the reduced bronchial responsiveness when baseline lung resistance had partially recovered by 72 h. A decrease in bronchial responsiveness could have resulted from a decreased penetration of ACh aerosol into the airways, but bronchial responsiveness does not appear to change following bronchoconstriction (18). In guinea pigs exposed to O_{3} once, there was a significant increase in baseline lung resistance at 8 h after exposure without any changes in bronchial responsiveness. Following a single exposure to O_{3}, an increase in airway responsiveness in vivo is accompanied by airway smooth muscle responsiveness in vitro to ACh (19) and to electrical field stimulation in the dog (20), and to substance P in the guinea pig (21). Whether in vitro airway smooth muscle contractility is reduced following multiple exposures to O_{3} is not known.

The present authors' studies indicate that the degree of neutrophil influx into BAL fluid continues to increase with repeated O_{3} exposure, with no evidence of an attenuated response. Thus, the number of neutrophils recovered by BAL was nearly doubled in guinea pigs exposed on four consecutive days compared to guinea pigs only exposed once. Interestingly, in the multiple-exposure guinea pigs, the number of neutrophils fell at 3 days after exposure to non-significant levels at a time when there was a persistent increase in neutrophils in BAL fluid. A similar dissociation has been observed in guinea pigs exposed once to O_{3} (12). Neutrophils counted in the lung interstitium increased transiently over the 24 h after exposure, but the increase in neutrophils in BAL fluid was persistently elevated for up to 3 days (12). These observations suggest that there may be an enhanced transit of neutrophils from the airway and lung interstitial spaces into the airway lumen. Studies to assess the transit time of neutrophils in the lungs following O_{3} exposure are needed to confirm this hypothesis.

The increase in neutrophil influx with repeated O_{3} exposure is similar to what has been described in the rat with the persistence of epithelial damage and inflammation in the terminal bronchioles (16). It appears unlikely, from the present authors' data, that the neutrophil is playing a modulatory role in the changes in bronchial responsiveness induced by O_{3}. Observations from previous studies would also support such a conclusion. Thus, in the guinea pig, neutrophilic infiltration of the airways persisted at a time when bronchial hyper-responsiveness was in remission after exposure to O_{3} (1), and in rats, bronchial hyper-responsiveness induced by O_{3} developed in the absence of neutrophil influx into the trachea (11). Moreover, depletion of neutrophils by cyclophosphamide in the guinea pig did not attenuate O_{3}-induced bronchial hyper-responsiveness (22).

Bronchial hyper-responsiveness induced by a single exposure to O_{3} has been shown to be blocked by anti-oxidants, thus implying a role for reactive oxygen species (6,23). As endogenous anti-oxidants, particularly in the airway epithelium, could protect against damage induced by reactive oxygen species, it is possible that an increase in anti-oxidants caused by multiple exposures to O_{3} may activate protective mechanisms leading to bronchial hypo responsiveness.
Tracheal levels of the anti-oxidant, superoxide dismutase and lung glutathione levels were unchanged after continuous exposure to O₃ in the rat (16,24). However, levels of lung ascorbate remained persistently elevated after O₃ exposure (16) and, following one exposure to O₃, total lung superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase and catalase have been reported to be increased in the rat (25).

Studies of single exposure to O₃ have failed to implicate a role for the epithelial-derived relaxant factor in the dog in increased bronchial responsiveness (19). On the other hand, there is a potential role for increased release of tachykinins, perhaps through activation of local axon reflexes in the airways (26), concomitant with a reduction in levels of neutral endopeptidase (27). Increase in lavage concentrations of the prostaglandins PGF₂α and PGE₂ have been observed in rats exposed to O₃ concentrations of 4-0 ppm for 2–8 h (28). These prostaglandins appear to play an enhancing role since indomethacin prior to O₃ exposure decreased the injurious effects of O₃ in the rat (29). However, it is not known how repeated exposures to O₃ could affect the release of prostaglandins, and it is possible that repeated exposures may lead to a reduction or suppression of such a release.

Previous studies using repeated exposures to O₃ have been performed mostly in human subjects and have also demonstrated some degree of tolerance. Repeated daily exposures to concentrations of O₃ of 0.4–0.5 ppm usually caused a reduction in spirometric measurement on the first exposure day, maximal by the second day followed by attenuation of the initial response on later days (14,30,31). Repeated exposures to 0.12 ppm O₃ over 5 days led to an attenuation of symptom and spirometric measurements, but caused increased airway responsiveness to methacholine following each O₃ exposure, although some attenuation was observed on Days 4 and 5 in normal subjects (30). A more rapid and complete adaptation of bronchial hyper-responsiveness was observed in normal human subjects on the third day of exposure to O₃ at 0.4 ppm (15). The present findings are in line with the reports in humans that bronchial hyper-responsiveness is not observed following repeated O₃ exposures, but no studies in humans have reported bronchial hyporesponsiveness. It is possible that this may relate to the relatively higher levels of O₃ used in the guinea pigs, or that the guinea pig may release more broncho-protective agents on repeated exposures to O₃.

Acknowledgement

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References