

Nasal allergy-like symptoms aggravated by ozone exposure in a concentration-dependent manner in guinea pigs

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Abstract

Our previous study revealed that exposure to 0.4 ppm ozone (O₃) enhanced nasal allergy-like reactions in guinea pigs. In the present study, we investigated the concentration-dependency of the effects of exposure to O₃ on the aggravation of nasal allergy-like reactions induced by repeated nasal administration of antigen. Guinea pigs were exposed to filtered air or 0.1–0.6 ppm O₃ for 5 weeks. After each weekly administration of ovalbumin (OVA), sneezes and nasal secretions were measured. The number of eosinophils infiltrating the nasal septum and the titers of OVA-specific antibody were measured 24 h after the last administration. Ozone increased sneezing and nasal secretion induced by OVA, nasal responsiveness to physical stimuli, and the number of infiltrating eosinophils in a concentration-dependent manner. The titer of anti-OVA-IgG was increased in animals exposed to 0.6 ppm O₃. Thus, exposure to O₃ aggravated nasal allergy-like symptoms concentration dependently. The aggravation was caused by induction of nasal hyperresponsiveness, the infiltration of eosinophils, and the increase in the production of anti-OVA-IgG. The estimated *maximum* likelihood estimation concentrations (MLECs) and bench mark concentrations (BMCs) of O₃ for these indices were in the range of 0.09–0.18 and 0.02–0.06 ppm, respectively.

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Keywords: Nasal allergy-like symptoms; Ozone; Sneezes; Nasal secretion; Eosinophil; IgG; Threshold

1. Introduction

Epidemiological studies have shown that the prevalence rate of allergic rhinitis in air-polluted areas is higher than that in non-polluted areas (Kaneko et al.,

1980; Ishizaki et al., 1987; Corbo et al., 1993), thus suggesting that allergic rhinitis may be associated with the air pollutants. A previous study also showed that the rate of damage to the nasal mucosa in the presence of a high concentration of ozone (O₃) was higher than that in the presence of a low concentration of it (Boezen et al., 1999). O₃ exposure also increased bronchial responsiveness to allergens in subjects with airway allergy (Jorres et al., 2000). In animal studies, O₃ enhanced allergic asthmatic symptoms in mice and guinea pigs (Matsumura, 1970; Matsumura et al., 1972; Neuhaus-Steinmetz et al.,

Abbreviations: O₃, ozone; His, histamine; IL, interleukin; OVA, ovalbumin; NO, nitric oxide; PCA, passive cutaneous anaphylaxis

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2000; Sumitomo et al., 1990). Therefore, exposure to a high concentration of O_3 is associated with aggravation of allergy-related airway diseases such as rhinitis.

Our previous study (Iijima et al., 2001) showed that exposure of guinea pigs to 0.4 ppm O_3 aggravated nasal allergy-like reactions such as sneezing responses and nasal secretion induced by repeated ovalbumin (OVA) administration to non-sensitized animals. Exposure to O_3 enhanced nasal hyperresponsiveness and infiltration of eosinophils, which aggravated the nasal allergic reactions. Thus, it is important to clarify the concentration dependency of the effect of O_3 on nasal allergy-like reactions. The present study shows that exposure of guinea pigs to 0–0.6 ppm O_3 aggravated nasal allergy-like symptoms in a concentration-dependent manner. Increases in nasal hyperresponsiveness, infiltration of eosinophils, and the production of specific anti-OVA-IgG, all of which may aggravate the nasal allergic symptoms, were induced by O_3 concentration dependently.

2. Methods

2.1. Animals

This study proceeded after the approval of the Ethics Committee for Experimental Animals at the

National Institute for Environmental Studies. Male Hartley guinea pigs (about 250 g body weight and 4 weeks of age) were purchased from Japan SLC Inc. (Hamamatsu), fed the standard guinea pig chow (RC4 Oriental Yeast CO. LTD., Tokyo), and given water ad libitum. The animals were used for experiments at 5 weeks of age (about 350–450 g body weight).

2.2. Protocol

Guinea pigs were exposed to filtered air or to that containing 0.1, 0.2, 0.4 or 0.6 ppm O_3 . The protocol is shown in Fig. 1. The exposure period was 5 weeks (24 h per day, 6.5 days per week). The animals were taken outside of the chambers to assess the sneezes and nasal secretions (0.5 days per week). Once a week during the exposure period, 40 μ l/kg of 1% OVA in saline (eight animals per group) or just saline (seven animals per group) was administered into both anterior nares of the guinea pigs in both filtered air- and O_3 -exposed groups. Sneezes were counted for 20 min after OVA administration. Nasal secretion was also measured for 20 min after the OVA administration. The number of eosinophils that infiltrated the nasal epithelium and subepithelium and the titers of specific anti-OVA-IgG and anti-OVA-IgE were measured 24 h after the last administration of OVA.

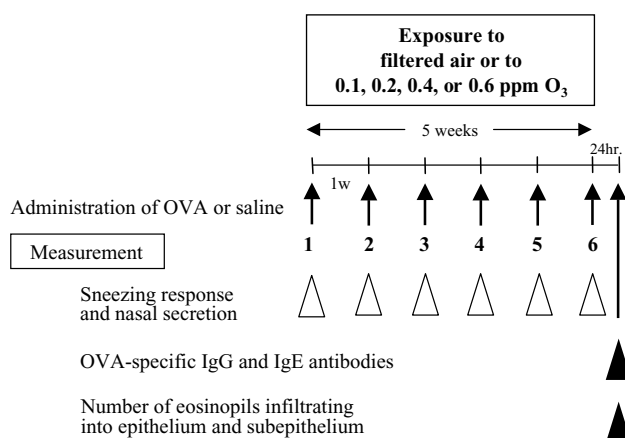


Fig. 1. Guinea pigs were administered 40 μ l/kg of 1% OVA–saline or just saline to both anterior nares once each week during exposure to filtered air or to 0.1, 0.2, 0.4 or 0.6 ppm O_3 for 5 weeks. The number of sneezes and nasal secretion were measured for 20 min after the administration. Titers of specific anti-OVA-IgG and anti-OVA-IgE and the number of eosinophils infiltrating the nasal epithelium and subepithelium were measured 24 h after the last administration.

2.3. Exposure to ozone

Guinea pigs were exposed to filtered air without or with 0.1, 0.2, 0.4 or 0.6 ppm O₃, for 5 weeks in identical stainless steel and glass chambers. For the former, filtered room air was passed through the chamber; and for the latter, filtered room air was first mixed with O₃ and then passed through the chamber. The chambers were maintained at 25 ± 1 °C, $55 \pm 5\%$ humidity and -5 mm H₂O relative to atmospheric pressure with an air flow of 110 m³/h. The concentration of O₃ was feedback-controlled by continuous monitoring with an O₃ analyzer (Monitor Labs model 8440, USA) operating on a chemiluminescence principle. The concentration of O₃ in chambers for exposure to 0.1, 0.2, 0.4 or 0.6 ppm O₃ was maintained at 0.10 ± 0.01 , 0.20 ± 0.01 , 0.40 ± 0.04 , and 0.60 ± 0.04 ppm, respectively.

2.4. Number of sneezes

Immediately after O₃ exposure, an intact, unanesthetized, spontaneously breathing guinea pig was placed in a two-chamber restrainer that kept the neck fixed and isolated from the body behind the neck. Altered airflow at the nose synchronized with the onset of sneezing was measured by using a pneumotachograph (A, Fleish Instruments, Lausanne, Switzerland) connected to a differential pressure transducer (model MP45-14, Validyne, Northridge, CA) (Kobayashi et al., 1998). Box pressure changes caused by abdominal movements synchronized with the onset of sneezing were determined by using a differential pressure transducer (model MP45-14) and a carrier demodulator (model CD72, Validyne, Northridge, CA). Airflow and box pressures were reported on a recorder (Linearrecorder F WR3701, Graphtec, Tokyo, Japan). Airflow and box pressure signals were also displayed simultaneously on an oscilloscope (SS-5802, Iwatsu Electric, Tokyo, Japan). A microphone (RP-3102E, National, Japan), amplified and reproduced by a loudspeaker (CFD-D77, Sony, Tokyo, Japan), picked up the sounds of sneezing, which were distinguishable from those of coughing. Sneezes were counted for 20 min after OVA administration.

2.5. Nasal secretion

Nasal secretion from the nostrils was measured as an index of the exocrine activity of the nasal mu-

cosa. The secretion was wiped off with a piece of pre-weighed paper (100 mm × 120 mm: Kimwipe, S-200, Jujo-Kimberly, Tokyo, Japan) at 20 min after nasal administration of OVA or saline. Then the paper was weighed again (Kobayashi et al., 1997).

2.6. Measurement of eosinophils infiltrating the nasal mucosa

Twenty-four hours after the last administration of OVA or saline, the guinea pigs were stunned and exsanguinated via the axillary artery and vein. After death by exsanguination, the lower jaw, skin, and musculature of the head were removed, and the nose was then immersed in an excess amount of 10% neutral phosphate-buffered formalin. After fixation, the noses were decalcified in decalcifying solution containing 0.5 M ethylenediaminetetraacetic acid disodium salt (pH 7.5) for 2 weeks and rinsed in tap water. A tissue block was removed from the anterior nasal cavity by making two transverse cuts perpendicular to the hard palate immediately posterior to the upper incisor teeth, as was described previously (Kobayashi, 2000). The tissue block was embedded in paraffin, and 5-mm thick sections were cut from the anterior surface. Sections were stained with hematoxylin–eosin to identify eosinophils. The number of eosinophils that infiltrated into the respiratory epithelium and subepithelium of the nasal septum were counted with a video micrometer (VM-30, Olympus, Tokyo, Japan), and the results were expressed as the number of eosinophils per 10³ μm².

2.7. Passive cutaneous anaphylaxis (PCA)

Twenty-four hours after the last administration of OVA, guinea pigs were stunned. Immediately after stunning, exsanguinated blood via the axillary artery and vein was collected. It has been reported that IgG and IgE persisted in the skin for 4 days (maximum at 3–6 h) and 3 weeks, respectively (Ovary, 1958; Ovary et al., 1976). Therefore, serum levels of specific anti-OVA-IgG and anti-OVA-IgE antibodies in guinea pigs were determined by homologous PCA tests with latency periods of 4 h and 7 days, respectively. Guinea pigs were passively sensitized by an intradermal injection of diluted sera given at 100 μl/animal. After 4 h or 7 days, these animals were injected

intravenously with saline (1 ml/kg) containing OVA (1%) and Evans blue (500 µg/ml). Dye that exuded in the cutaneous lesion was extracted with formamide and measured spectrophotometrically (620 nm). The initial increase in absorbance, which was estimated by extrapolation using dilution-absorbance curve, was used to determine the titer of specific anti-OVA-IgG or anti-OVA-IgE.

2.8. Calculation of threshold

Maximum likelihood estimation concentration (MLEC) and benchmark concentration (BMC) were calculated by use of the THC program (Clement Int. Corp., USA). This program fits a continuous polynomial regression model to animal toxicity data by the maximum likelihood estimation (Crump, 1984). Likelihood of the data can be written as

$$L = (2\pi)^{-g/2} \prod_{i=1}^g \frac{\sigma_i^{-1} \exp[-(N_i - 1)s_i^2 - N_i(\text{mean } x_i - m(c_i))^2]}{\sigma_i^2}$$

$$m(c_i) = \text{constant} + q_1(c - c_0) + \dots + q_k(c - c_0)^k, \\ \text{for } d > 0$$

$c_1 \dots c_g$: g concentration levels, N_i : number of animals in the i th concentration group, s_i^2 : sample variance, σ_i^2 : variance of the x_{ij} , x_{ij} : response of the j th animal in the i th concentration group ($j = 1, \dots, N_i, i = 1, \dots, g$). The mean function $m(c_i)$, which represents the average response at a concentration c_i , determines the concentration response (Crump, 1984).

THC calculates MLEC and statistical confidence limits for the concentration (BMC) corresponding to a given value of extra or additional risk. We calculated MLEC and BMC at 10% risk and the 95% lower limit corresponding to an extra risk. Threshold of sneezing responses and nasal secretion were calculated by using the values at the last administration for both.

2.9. Statistical analysis

Values were expressed as a percentage of the values for the filtered air group. Mann-Whitney's U -test was used to compare groups. A P -value of less than 0.05 was considered significant.

2.10. Chemicals

The following reagents were obtained: OVA from Seikagaku Corp. (Tokyo, Japan); sodium pento-barbital from Dainabot (Osaka, Japan); Evans blue, 10% neutral phosphate-buffered formalin, and decalcifying solution from Wako Pure Chemicals (Osaka, Japan); and formamide from Nacalai Tesque Inc. (Kyoto, Japan).

3. Results

3.1. Ozone augmented the number of sneezes induced by repeated nasal administration of OVA

The effect of 0.6 ppm O_3 exposure on the sneezing response induced by repeated nasal OVA administration is shown in Fig. 2a. The first nasal OVA administration to guinea pigs prior to exposure induced only a slight sneezing response. This may have been due to physical stimulation of the administration. O_3 at 0.6 ppm significantly augmented the sneezing response at the third and fourth administrations of OVA in comparison with the response for the filtered air group. At 0.4 ppm O_3 significantly augmented the sneezing response at the third, fourth, and fifth administrations of OVA (data not shown). O_3 at 0.2 or 0.1 ppm did not significantly augment the sneezing response (data not shown).

The concentration dependency of the effect of O_3 exposure on the sneezing response at the fifth administration of OVA is shown in Fig. 2b. Exposure to O_3 aggravated the sneezing response in a concentration-dependent manner.

3.2. Ozone augmented the nasal secretion induced by repeated nasal administration of OVA

The effect of exposure to 0.6 ppm O_3 on nasal secretion induced by repeated nasal OVA administration is shown in Fig. 3a. At 0.6 ppm O_3 significantly augmented the nasal secretion at the fifth and sixth administrations of OVA in comparison with the response for the filtered air group. O_3 at 0.4 ppm significantly augmented the nasal secretion at the sixth administration of OVA, but at 0.2 or 0.1 ppm, there was no effect (data not shown).

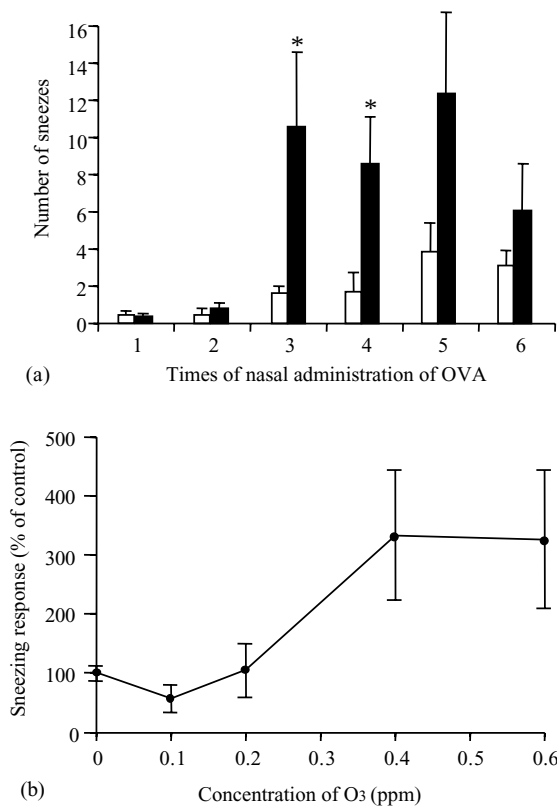


Fig. 2. (a) Effect of 0.6 ppm O₃ exposure on the sneezing responses induced by nasal OVA administration in guinea pigs. Sneezing responses were counted for 20 min after the administration of OVA. Open and solid columns represent air-exposed and 0.6 ppm O₃-exposed group, respectively: mean $\pm$ S.E.M.; * $P < 0.05$ vs. air-exposed group. (b) Concentration-dependency of the effect of O₃-exposure on nasal secretion induced by administration of OVA. Ratios of sneezes at the fifth administration of OVA to O₃-exposed animals vs. those in air-exposed animals are shown: $n = 8$ per group; mean $\pm$ S.E.M.

The concentration dependency of the effect of O₃ exposure on the nasal secretion at the fifth administration of OVA is shown in Fig. 3b. Exposure to O₃ aggravated the nasal secretion in a concentration-dependent manner.

3.3. Ozone augmented the nasal reaction induced by the physical stimulus of saline

The effects of exposure to 0.6 ppm O₃ on the sneezing response and nasal secretion induced by the physical stimulus of saline administration are shown in Figs. 4a and 5a. At 0.6 ppm O₃ significantly aug-

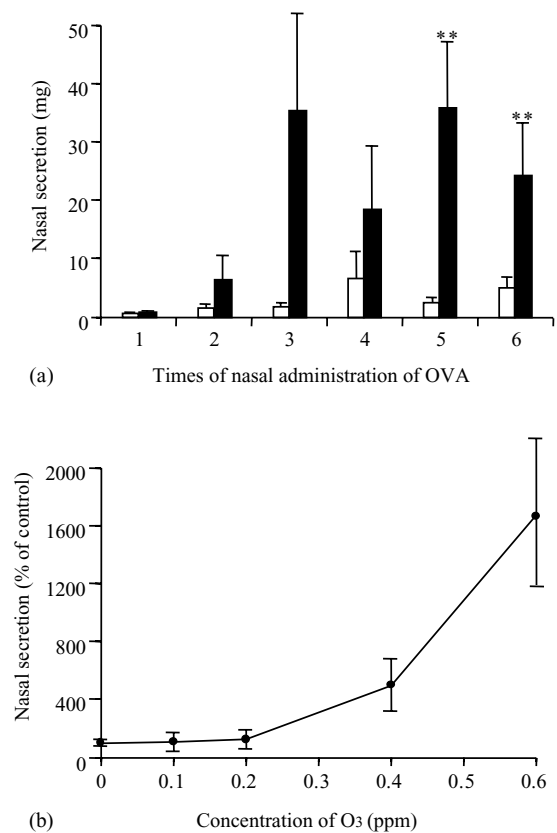


Fig. 3. (a) Effect of 0.6 ppm O₃ exposure on the nasal secretion induced by nasal OVA administration in guinea pigs. Nasal secretions were collected for 20 min after the administration of OVA. Open and solid columns represent air-exposed and 0.6 ppm O₃-exposed group, respectively: mean $\pm$ S.E.M.; ** $P < 0.01$, respectively, vs. air-exposed group. (b) Concentration-dependency of the effect of O₃-exposure on nasal secretion induced by administration of OVA. Ratios of nasal secretion at the fifth administration of OVA to O₃-exposed animals vs. that for air-exposed animals are shown: $n = 8$ per group; mean $\pm$ S.E.M.

mented the sneezing response at the fifth and sixth administrations of saline in comparison with the response for the filtered air group. At this concentration it also significantly augmented the nasal secretion at the fourth, fifth and sixth administrations of saline. O₃ at 0.4 ppm significantly augmented the sneezing response at the sixth administration of saline and the nasal secretion at the fifth administration of saline (data not shown). However, no significant enhancement of either sneezing response or nasal secretion was observed in animals exposed to 0.2 or 0.1 ppm O₃ (data not shown).

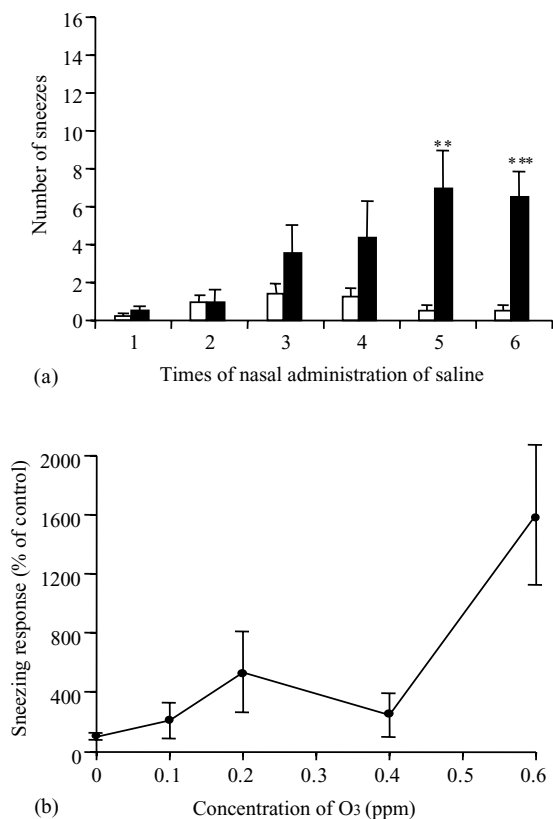


Fig. 4. (a) Effect of 0.6 ppm O₃ exposure on the sneezing responses induced by nasal saline administration in guinea pigs. Sneezing responses were counted for 20 min after the administration of saline. Open and solid columns represent air-exposed and 0.6 ppm O₃-exposed group, respectively: mean ± S.E.M.; ** $P < 0.01$ and *** $P < 0.001$, respectively, vs. air-exposed group. (b) Concentration dependency of the effect of O₃ exposure on the sneezing responses induced by administration of saline. Ratios of sneezes at the fifth administration of saline to O₃-exposed animals vs. those for air-exposed animals are shown: $n = 7$ per group; mean ± S.E.M.

The concentration dependency of the effect of O₃ exposure on the nasal hyperresponsiveness at the fifth administration of saline is shown in Figs. 4b and 5b. Exposure to O₃ aggravated both the sneezing response and nasal secretion induced by physical stimulus, almost concentration dependently.

3.4. Photomicrographs of epithelium and subepithelium of the nasal septum

The effect of exposure to O₃ on the epithelium and subepithelium of the nasal septum by

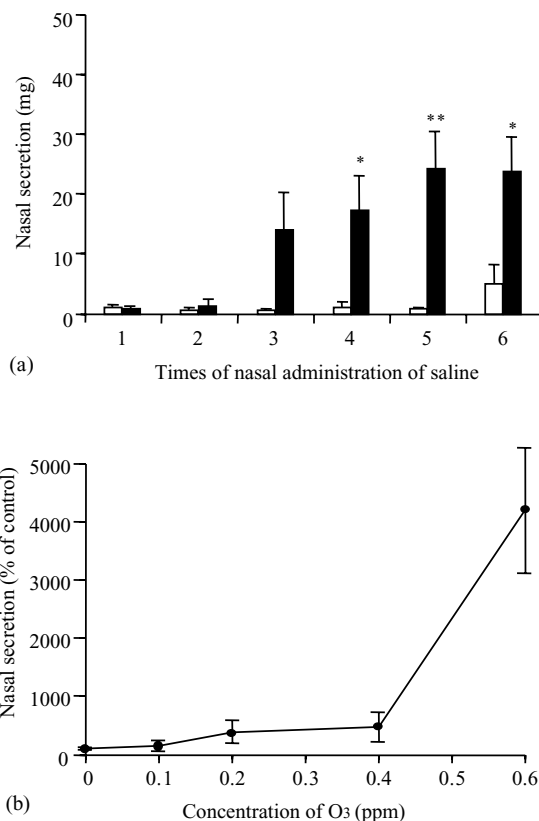


Fig. 5. (a) Effect of 0.6 ppm O₃ exposure on the nasal secretion induced by nasal saline administration in guinea pigs. Nasal secretions were collected for 20 min after the administration of saline. Open and solid columns represent air-exposed and 0.6 ppm O₃-exposed group, respectively: mean ± S.E.M.; * $P < 0.05$ and ** $P < 0.01$, respectively, vs. air-exposed group. (b) Concentration-dependency of the effect of O₃-exposure on nasal secretion induced by administration of saline. Ratios of nasal secretion at the fifth administration of saline to O₃-exposed animals vs. that for air-exposed animals are shown: $n = 7$ per group; mean ± S.E.M.

the OVA administration is shown in Fig. 6. In the filtered air-group, nasal epithelial cells were not damaged. Exposure to O₃ increased the number of goblet cells in the nasal epithelium. In the 0.4 ppm O₃-exposed group, the nasal epithelium was damaged, and at 0.6 ppm O₃, the nasal epithelium was damaged, and loss of cilia was observed. These results suggest that exposure to O₃ affected the nasal epithelium in a concentration-dependent manner.

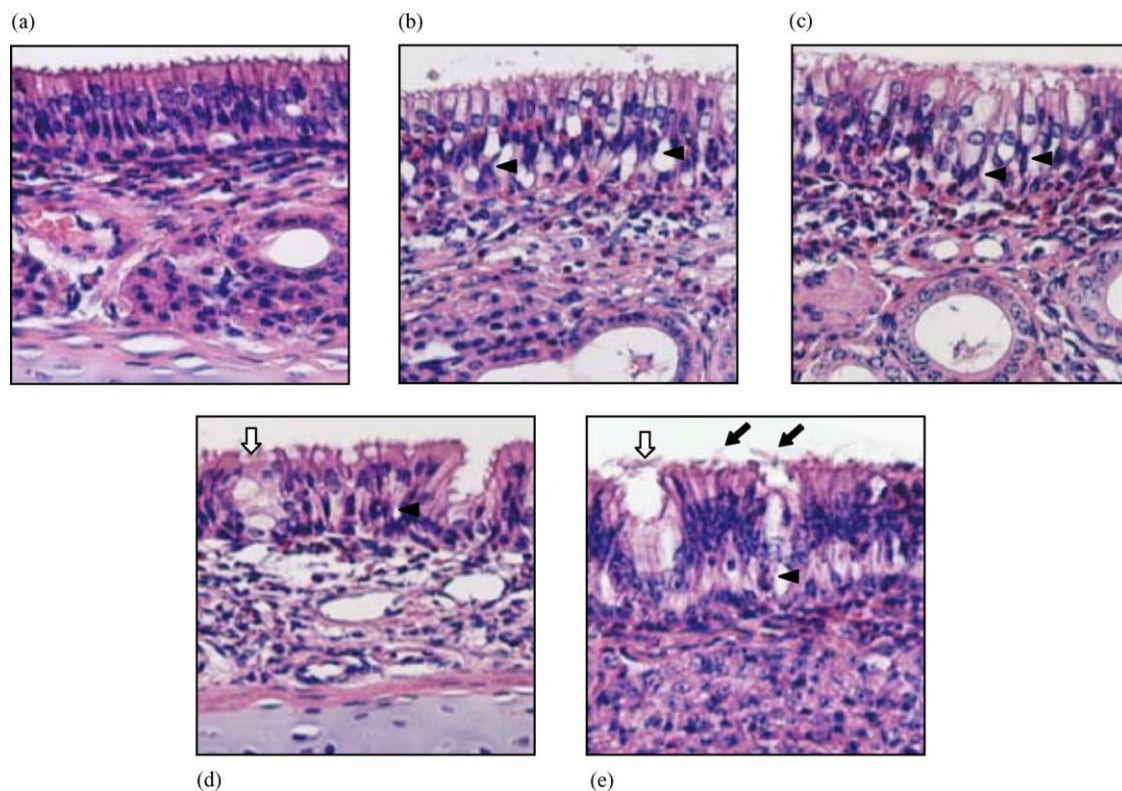


Fig. 6. Effect of exposure to O_3 on epithelium and subepithelium of the nasal septum by the administration of OVA from groups exposed to filtered air or O_3 . (a) Filtered air-exposed group; (b) 0.1 ppm; (c) 0.2 ppm; (d) 0.4 ppm; and (e) 0.6 ppm O_3 -exposed groups, respectively. Goblet cells are observed in the nasal epithelium (arrowheads in (b)–(e)). Nasal epithelium is damaged (open arrows in (d) and (e)), and detachment of the cilia is seen (solid arrows in (e)).

3.5. Infiltration of eosinophils into nasal epithelium and subepithelium

The effect of 5-week exposure to 0.6 ppm O_3 on the infiltration of eosinophils into the nasal epithelium and subepithelium induced by repeated nasal administration of OVA or saline is shown in Fig. 7a. In comparison with filtered air, 0.6 ppm O_3 significantly increased the number of eosinophils infiltrating the nasal epithelium and subepithelium. The concentration dependency of the effect of O_3 exposure on the eosinophils infiltrating is shown in Fig. 7b. At 0.4 ppm O_3 significantly increased the number of eosinophils infiltrating the nasal subepithelium in response to OVA (Fig. 7b). Exposure to 0.2 ppm O_3 tended to increase, but 0.1 ppm O_3 did not increase the number of eosinophils infiltrating after OVA

treatment (Fig. 7b). Exposure to O_3 aggravated the eosinophilic infiltration in concentration dependently. In comparison with the exposure to air, O_3 did not significantly increase the eosinophil influx induced by saline administration (data not shown).

3.6. Ozone enhanced the production of specific anti-OVA-IgG and anti-OVA-IgE

Fig. 8a shows the effect of exposure to 0.6 ppm O_3 on specific anti-OVA-IgG and anti-OVA-IgE titers in guinea pigs immunized by repeated intranasal administration of OVA. At 0.6 ppm O_3 significantly increased the anti-OVA-IgG titer. The concentration dependency of the effect of O_3 exposure on the anti-OVA-IgG and anti-OVA-IgE titers is shown in Fig. 8b. At either 0.4 or 0.2 ppm O_3 tended to increase,

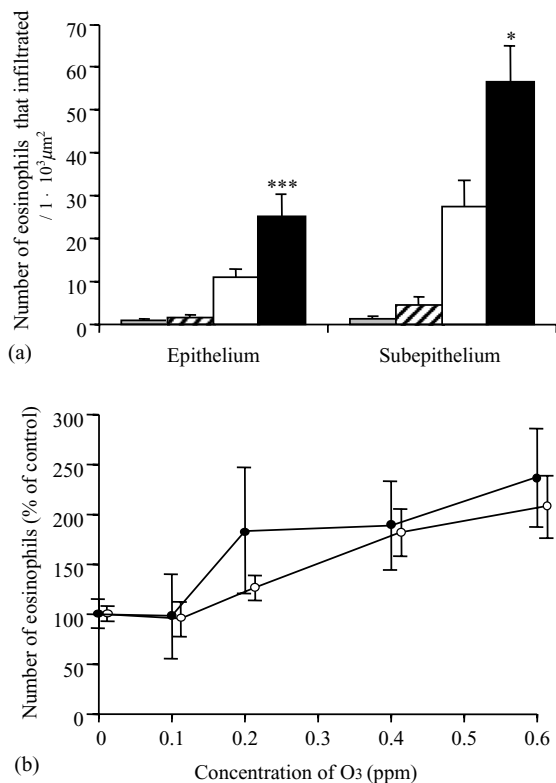


Fig. 7. (a) Effect of 0.6ppm O₃ exposure on the infiltration of eosinophils induced by nasal OVA or saline administration in guinea pigs. The eosinophils were counted at 24h after the last administration. Shaded, hatched, open, and solid bars represent air-saline, O₃-saline, air-OVA, and O₃-OVA groups, respectively: $n = 7$ or 8 per group; mean $\pm$ S.E.M.; * $P < 0.05$ and *** $P < 0.01$, respectively, vs. air-exposed group. (b) Concentration dependency of the effect of O₃ exposure on the number of eosinophils infiltrating the epithelium (closed circles) and subepithelium (open circles) induced by administration of OVA. Ratios of eosinophil numbers at the last administration of OVA to O₃-exposed animals vs. for those for air-exposed animals are shown: $n = 7$ – 8 per group; mean $\pm$ S.E.M.

but 0.1ppm O₃ did not increase, the anti-OVA-IgG titer (Fig. 8b). The anti-OVA-IgE titer was not increased by the exposure to O₃ (Fig. 8b).

3.7. Maximum likelihood estimate and benchmark concentration of O₃ for the effects on nasal allergic reaction

Maximum likelihood estimate concentration and benchmark concentration of O₃ for the effects on

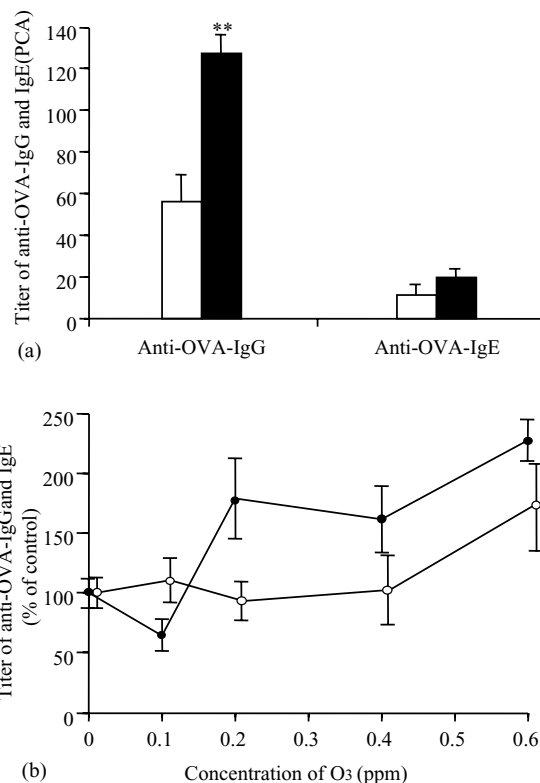


Fig. 8. (a) Effect of 0.6ppm O₃ exposure on the anti-OVA-IgG and anti-OVA-IgE in guinea pigs. The IgG and IgE were measured at 24h after the last administration. Open and solid bars represent air-exposed and 0.6ppm O₃-exposed groups, respectively: $n = 8$ per group; mean $\pm$ S.E.M.; ** $P < 0.05$, vs. air-exposed group. (b) Concentration dependency of the effect of O₃ exposure on anti-OVA-IgG (closed circles) and anti-OVA-IgE (open circles) titers. Ratios of IgG and IgE titers at the last administration of OVA to O₃-exposed animals vs. those for air-exposed animals are shown: $n = 8$ per group; mean $\pm$ S.E.M.

nasal allergic reactions are shown in Table 1. The MLEC was the lowest for infiltration of eosinophils into the epithelium (0.09ppm). MLECs for sneezing responses, nasal secretion, OVA-specific-antibody, and infiltration of eosinophils into the subepithelium ranged from 0.13 to 0.42ppm O₃. The BMC was the lowest for the number of eosinophils that infiltrated into the epithelium and subepithelium (0.02 and 0.04ppm, respectively). The BMC for sneezing, nasal secretion, and IgG were from 0.05 to 0.06ppm O₃. The BMC was the highest for IgE. A similar order was found for BMCs and MLECs.

Table 1
MLEC and BMC of O₃ effects on nasal allergic reactions

	MLEC (ppm)	BMC (ppm)
Allergic reaction		
Sneezing responses	0.18	0.06
Nasal secretion	0.14	0.05
OVA-specific-antibody		
IgG	0.13	0.06
IgE	0.42	0.11
Eosinophils		
Epithelium	0.09	0.02
Subepithelium	0.14	0.04

Threshold effects of O₃ on nasal allergic reactions were estimated as BMC and MLEC. Thresholds for sneezing responses and nasal secretion were calculated from data on sixth administration and fifth administration groups, respectively, which were the groups most affected by O₃.

4. Discussion

Our present results showed that O₃ enhanced nasal allergic reactions, such as the sneezing responses (Fig. 2b) and nasal secretion (Fig. 3b) both in a concentration-dependent manner.

The aggravation of nasal allergy-like reactions was caused by nasal hyperresponsiveness, the infiltration of eosinophils, and the increase in the production of anti-OVA-IgG in guinea pigs. The nasal responsiveness was measured by using indices of sneezing responses and nasal secretion induced by the physical stimuli of saline administration. Exposure to O₃ augmented the nasal responsiveness to this physical stimulus almost concentration dependently (Figs. 4b and 5b). Previous studies have already shown that O₃ enhanced nasal hyperresponsiveness to histamine (Kobayashi, 1996), and that exposure to O₃ enhanced damage to the nasal epithelium (Harkema et al., 1987; Hotchkiss et al., 1989). Fig. 6 showed that O₃ augmented damage to the nasal epithelium in a concentration-dependent fashion. It is possible that the infiltrating eosinophils also damaged the nasal epithelium. The damage may facilitate the transfer of irritants to sensory nerve endings, which irritation may enhance nasal responsiveness to physical stimuli. Damage to the epithelium may also increase nasal permeability. It was reported that short-term exposure to 0.4 ppm (Koren et al., 1990) or 0.8 ppm (Bhalla et al., 1986) O₃ increased the permeability of the nasal epithelium. This heightened permeability may also

enhance nasal responsiveness, for enhanced permeability facilitates the transfer of antigens through the epithelium to mast cells and antigen-presenting cells, consequentially leading to airway hyperresponsiveness (Hogg et al., 1979; Hulsmann et al., 1996) and increased antibody production. Fig. 7b showed that O₃ enhanced infiltration of eosinophils concentration dependently. Infiltrating eosinophils may release toxic granular proteins (Weller, 1991), which could damage nasal epithelial cells, as observed in asthma patients (Frigas and Gleigh, 1986). Ozone significantly increased allergen-induced release of eosinophil cationic protein in the nasal secretion (Peden et al., 1995), suggesting that O₃ may prime eosinophils for subsequent activation by allergen. Fig. 8b showed that 0.6 ppm O₃ increased the anti-OVA IgG titer and tended to increase the anti-OVA IgE titer. We previously reported that O₃ enhanced the antigen-presenting activity of broncho-alveolar lavage cells in rats (Koike et al., 2001). This enhanced antigen-presenting activity may have contributed to the increases in antibody production and infiltration of eosinophils. It is well known that the immediate allergic reaction in guinea pigs is mainly mediated by IgG. The increase in IgG titer may have aggravated the nasal allergy-like symptoms.

The MLEC of O₃ was the lowest for infiltration of eosinophils into the epithelium (0.09 ppm) among the effects of O₃ on our allergic rhinitis model. Considering the sensitive groups among the whole population, the BMC was calculated as threshold values. BMCs of O₃ for the increase in sneezing responses, nasal secretion, and anti-OVA-IgG titer were estimated to be 0.06, 0.05, and 0.06 ppm, respectively. BMCs of O₃ for eosinophilic infiltration into the epithelium and subepithelium were estimated to be 0.02 and 0.04 ppm, respectively. Therefore, BMC of O₃ was also the lowest for eosinophilic infiltration in the nasal allergic reactions.

Experiments on the nasopharyngeal removal of O₃ in laboratory animals suggested that the fraction of O₃ uptake depended inversely on flow rate, and uptake was greater for nose breathing than for mouth breathing (Yokoyama and Frank, 1972). Previous studies suggested that inspiratory flow and total respiratory tract O₃ uptake efficiency by nasal exposure in guinea pigs (Wiester et al., 1988a,b) were smaller than those in humans (Gerrity et al., 1988). Therefore, the dose of O₃ for the human nasal epithelium might be higher

than that for the guinea pig one during exposure to the same concentration of O₃.

In conclusion, O₃ aggravated nasal allergy-like symptoms in a concentration-dependent manner. Increases in nasal hyperresponsiveness, infiltration of eosinophils, and the production of specific anti-OVA-IgG, all of which may aggravate nasal allergic symptoms, were induced by O₃. The estimated MLEC and BMC of O₃ for these indices were in the range of 0.09–0.18 and 0.02–0.06 ppm, respectively. The differences in sensitivity and dosimetry of O₃ between guinea pigs and humans remain to be elucidated in order to extrapolate toxicological data of guinea pigs to humans.

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