SHORT COMMUNICATION

Concentration- and Time-Dependent Increase in Specific Airway Resistance after Induction of Airway Hyperresponsiveness by Subchronic Exposure of Guinea Pigs to Nitrogen Dioxide


In the present study utilizing male Hartley guinea pigs, we investigated (1) the concentration and time dependency of the effects of subchronic exposure to nitrogen dioxide (NO₂) on airway responsiveness and (2) the concentration and time dependency of the effects on specific airway resistance under room air (SRaw₀). Animals were exposed to filtered air, 0.06, 0.5, 1.0, 2.0, or 4.0 ppm NO₂ for 6 and 12 weeks (24 hr/day). Airway responsiveness and SRaw₀ were determined 1 week before the beginning of exposure at 10 weeks of age and on the day of termination of the exposure. Our results revealed that (1) subchronic exposure to NO₂ induces airway hyperresponsiveness, both concentration and time dependently, (2) such exposure also induces an increase in SRaw₀, both concentration and time dependently, and (3) subsequent to the airway hyperresponsiveness induced by NO₂, an increase in the SRaw₀. Therefore, NO₂ could be a potent risk factor for alteration of pulmonary function and airway responsiveness.

Nitrogen dioxide (NO₂), which is produced by a variety of combustion processes, is a major component of air pollution in urban environments. In large metropolitan areas in Tokyo, Osaka, and other prefectures, the daily average at most monitoring stations exceeds 0.06 ppm, the upper limit of the Japanese environmental quality standard. Epidemiological studies have shown that there is a possible relationship between NO₂ and alteration of pulmonary function and airway responsiveness (Monstardi et al., 1981). Epidemiological studies have also revealed that airway hyperresponsiveness is a high risk factor in the pathogenesis of chronic obstructive pulmonary disease (Rijken et al., 1987; Pham et al., 1984). Therefore, if NO₂ induces airway hyperresponsiveness, then NO₂ could be one of the important factors in the etiology of chronic obstructive pulmonary diseases. Several studies revealed that airway hyperresponsiveness is induced in healthy and asthmatic subjects by short-time exposure to NO₂ (Kleinman et al., 1983; Orehek et al., 1976). However, conflicting results have also been presented (Linn et al., 1985; Hazucha et al., 1983). In studies on experimental animals, short-term exposure to high concentrations of NO₂ induces airway hyperresponsiveness (Abraham et al., 1980; Silbaugh et al., 1981; Kobayashi and Shinozaki, 1992). However, no report about the effect of subchronic exposure to NO₂ on airway responsiveness is available. It has been shown that short-term exposure to a high concentration of NO₂ causes obstructive changes in airways (Abraham et al., 1980; Murphy et al., 1964; Niehouser and Kleinerman, 1973). In the case of subchronic exposure to NO₂, however, conflicting results about such obstructive changes have been reported (Davidson et al., 1967; Freeman et al., 1968; Wagner et al., 1965). Differences in species, exposure time, and concentration of NO₂ might be the cause of these conflicting results. Therefore, at present, concentration and time dependency of the effect of subchronic exposure to NO₂ on airway responsiveness and airway resistance remain to be elucidated. In addition, no time course study has been reported on the relationship between the effects of NO₂ on airway responsiveness and airway resistance. Therefore, in the current study we investigated (1) the concentration and time dependency of the effects of subchronic exposure to NO₂ on airway responsiveness and (2) the concentration and time dependency of the effects on specific airway resistance (SRaw₀).

METHODS

Experimental animals. This study was performed with the approval of the National Institute for Environmental Studies Ethics Committee for Experimental Animals. Forty-eight and 60 male Hartley-strain guinea pigs (about 250 g body wt and 4 weeks of age) were used in first and second experiments, respectively. To examine more animals within the limitation of the exposure chamber, we chose small animals. Among them, we chose

Abbreviations used: NO₂, nitrogen dioxide; SRaw, specific airway resistance; Pb, barometric pressure; Pw, water vapor pressure at body temperature; g, geometric means; SRaw₃₇₉, SRaw after inhalation of 0.9% NaCl aerosol; EC₉₀Hist, effective molar concentration of histamine that produced a doubling of SRaw₃₇₉; SRaw₀, specific airway resistance under room air conditions.
Nitrogen dioxide exposure. Animal exposure techniques for nitrogen dioxide have previously been described (Kobayashi et al., 1983). Briefly, the guinea pigs were exposed to filtered air or NO₂ in one of four identical chambers with a volume of 1.3 m³ in all experiments. The chambers were operated under dynamic conditions at 23 ± 1°C, 55 ± 5% humidity, and 55 ± 5% air change/hour. The concentrations of NO₂ and NO were feedback-controlled by continuous monitoring with an NO₂ analyzer (Monitor Labs Model 8440, USA).}

Measurement of specific airway resistance. The methods for measurement of specific airway resistance (SRaw) have previously been reported (Agrawal, 1981; Johanson and Pierce, 1971). Briefly, SRaw was measured in a constant-volume chamber (about 12,000 cm³, 17 × 43 × 16.5 cm) body plethysmograph based on the design of Agrawal (1981). An intact, unanesthetized, spontaneously breathing guinea pig was placed in a two-chamber restrainer that kept the head fixed and isolated from the body behind the neck. Airflow and volume changes at the nose were measured with a pneumotachograph (no.0, Fleish Instruments, Lausanne, Switzerland) connected to a differential pressure transducer (Model MP45-14, Validyne, Northridge, CA), a carrier demodulator (Model CD72, Validyne), and an integrator (AR-601G, Nihonkohden, Tokyo, Japan). Box pressure changes were determined with a differential pressure transducer (Model MP45-14) and a carrier demodulator (Model CD72). Airflow and box pressure were recorded on a recorder (RJC-4124, Nihonkohden). Airflow and box pressure signals were also displayed simultaneously on an X-Y digital storage oscilloscope (SS-5802, Iwatsu Electric, Tokyo, Japan). The signal was stored ever 0.5 m sec. Loops, formed from the signals, were recorded using a three-way stop-cock connected to the inlet of the anterior chamber. At this airflow, each nebulizer was adjusted to deliver a 1.55 mg/min wet weight of the 0.9% NaCl aerosol. At this airflow, aerodynamic mass median diameter determined by a Marple personal cascade impactor (Series 290, Sierra Instruments, Carmel Valley, CA) was 1.2 μm (σg = 1.25). To protect the rotameter, we connected a filter (2500QAT-UP, Pallflex Products Corp., Putnam, CT) between the outlet of the anterior chamber and the air pump. At first, SRaw was determined before inhalation of the aerosols (SRawo). After inhalation of each dose for 30 sec, SRaw was determined within 1 min. A challenge dose was given every 5 min until SRaw increased to twice the value of SRawNacI (SRaw after inhalation of 0.9% NaCl aerosol). Concentration-response curves were constructed by plotting on semilogarithmic paper. The effective molar concentration of histamine (EC₂₀₀ His) in the solution that produced a doubling of SRawNacI was determined by interpolation of such concentration-response curves.

Statistical analysis. EC₂₀₀ His and SRawo were expressed as means ± SE. Statistical significance was assessed by using Student’s t test for paired data of the preexposure and postexposure of NO₂ or filtered air after checking the uniformity of variances by Bartlett’s test. The difference between groups was considered to be significant at p < 0.05.

Drugs. Histamine dihydrochloride was purchased from Wako (Osaka, Japan).

RESULTS

Effects of NO₂ Exposure on Airway Responsiveness

Prior to and post 6 and 12 weeks exposure to NO₂, no significant differences were observed in body weight between filtered air group and NO₂ group. Effects of 0.06, 0.5, and 4 ppm NO₂ or filtered air exposure for 6 and 12 weeks on airway responsiveness in guinea pigs are shown in Fig. 1a. Prior to exposure to filtered air or 0.06, 0.5, or 4 ppm NO₂, EC₂₀₀ His concentrations of inhaled histamine necessary to double SRawNacI were 1.46 ± 0.40, 1.70 ± 0.22, 1.72 ± 0.34, and 1.70 ± 0.27 mm, respectively, and corresponding 6-weeks exposure values were 1.51 ± 0.51, 1.63 ± 0.30, 1.65 ± 0.40, and 0.96 ± 0.20 mm. The results show that exposure to 4 ppm NO₂ for 6 weeks caused a significant increase in airway responsiveness to the inhaled histamine aerosol (p < 0.01). EC₂₀₀ His values after 12 weeks of exposure to filtered air, 0.06, and 0.5 ppm NO₂ were 1.31 ± 0.29, 1.80 ± 0.34, and 1.63 ± 0.38 mm, respectively. Thus, exposure to filtered air alone, 0.06, or 0.5 ppm NO₂ had no significant effect on the airway responsiveness to subsequently inhaled histamine aerosol. In the case of exposure to 4 ppm NO₂, SRawo and SRawNacI were significantly increased after 12-weeks exposure in more than 90% of the guinea pigs. Therefore, the extrapolation of EC₂₀₀ His for the longer exposure to 4 ppm NO₂ was not made.
Effects of exposure to filtered air, 1.0, 2.0, or 4.0 ppm NO₂ for 6 and 12 weeks on airway responsiveness to inhaled histamine aerosol are shown in Fig. 1b. EC₂₀₀₃₅₇ values prior to exposure were 1.20 ± 0.16, 1.15 ± 0.18, 1.15 ± 0.10, and 1.10 ± 0.15 mm, respectively. Corresponding 6 weeks exposure values were 1.28 ± 0.12, 1.24 ± 0.13, 0.73 ± 0.09 (p < 0.05), and 0.60 ± 0.08 mm (p < 0.05). Six weeks of exposure to 2 and 4 ppm NO₂ induced airway hyperresponsiveness, whereas exposure to filtered air or 1 ppm NO₂ for 6 weeks had no significant effect on airway responsiveness to inhaled histamine aerosol. EC₂₀₀₃₅₇ values after 12 weeks exposure to filtered air, 1, and 2 ppm NO₂ were 1.34 ± 0.21, 0.78 ± 0.10 (p < 0.05), and 0.50 ± 0.10 mm (p < 0.01), respectively. In the case of exposure to 4 ppm NO₂, since SRaw₀ and SRaw₉ were significantly increased after exposure, the extrapolation of EC₂₀₀₃₅₇ at 12 weeks exposure to 4 ppm NO₂ was not shown. In the case of exposure to 2 and 1 ppm NO₂, SRaw₀ and SRaw₉ increased to over twice those prior to exposure in 6 and 2 of 15 animals, respectively. These animals were excluded from the calculation of EC₂₀₀₃₅₇.

Relative Frequency of Occurrence of Animals Having SRaw₀ over Twice That prior to Exposure

Relative frequency of occurrence of animals having an SRaw₀ value over twice that found SRaw₀ prior to exposure was calculated. Results show that no such animals in this category after 6 weeks of exposure to filtered air, 0.06, 0.5, 1.0, or 2.0 ppm NO₂. In the case of exposure to 4 ppm, 13–16% of the animals had an SRaw₀ over twice that prior to exposure. After 12 weeks of exposure to filtered air, 0.06, or 0.5 ppm NO₂, no such animals were found, whereas with exposure to 1.0, 2.0, or 4.0 ppm NO₂, 13, 40, and 93–100% of the animals showed the elevated baseline.

DISCUSSION

The results of the present study showed that subchronic exposure of guinea pigs to NO₂ induces airway hyperresponsiveness to inhaled histamine aerosol (Fig. 1). It has been reported that airway hyperresponsiveness is induced by acute exposure to a high concentration of various air pollutants such as O₃ (Lee et al., 1977), SO₂ (Abraham et
data on the effect of subchronic exposure to air pollutants on airway responsiveness in experimental animals is limited. Figure 1 showed that airway hyperresponsiveness induced by subchronic exposure progressed to a higher degree concentration dependently. Figure 1 also showed that with exposure to NO₂ at 1 or 2 ppm airway hyperresponsiveness developed to a higher degree with the passage of exposure time. Therefore, our results suggested that a higher concentration of NO₂ induces airway hyperresponsiveness faster than does a lower concentration of NO₂. Airway obstruction, as indicated by an increase in airway resistance, is developed by chronic airway irritation due to exposure to various air pollutants such as SO₂ (Scanlon et al., 1987; Seltzer et al., 1984) and NO₂ (Davidson et al., 1967). Figure 2 showed that SRaw₀ after 12 weeks of exposure increased concentration dependently. Figure 2 also showed that SRaw₀ increased to a higher degree with the passage of exposure time. Comparison of the results of rabbits exposed to 8–13 ppm NO₂ for 3 months (Davidson et al., 1967) with our results showed that even a lower concentration of NO₂ (2 ppm) can induce the increase in SRaw₀. Tepper et al. (1993) reported that although not significantly increased, expiratory resistance was elevated in rats exposed to simulated urban profile of NO₂ exposure-time dependently. Figures 1b and 2b also showed that subsequent exposure to 2 and 4 ppm NO₂ after induction of airway hyperresponsiveness (6 weeks exposure) increased SRaw₀ significantly at 12 weeks. In the case of 1 ppm NO₂, 12 weeks exposure induced only airway hyperresponsiveness. These results indicate that the induction of airway hyperresponsiveness precedes the increase in SRaw₀. There is no information about time course study on the relationship between airway hyperresponsiveness and airway obstruction in experimental animals. On the contrary, Pham et al. (1984) showed that subjects having higher airway responsiveness to inhaled acetylcholine aerosol are at a higher risk for chronic obstructive lung disease. This epidemiological observation supports our present results.

Although the exposure to 4 ppm NO₂ for 6 weeks and 1 ppm NO₂ for 12 weeks did not cause a significant increase in SRaw₀ (Fig. 2), 13–16 and 13% of the animals among the respective 4 and 1 ppm NO₂-exposed guinea pigs had SRaw₀ over twice that found prior to exposure. Although exposure to 2 ppm for 12 weeks caused a significant increase in SRaw₀, 60% of these animals did not have an SRaw₀ over twice that prior to exposure. These results suggest that facility of induction of the increase in SRaw₀ by NO₂ exposure differs individually. Therefore, populations in which the increase in SRaw₀ is easily induced by air pollutants such as NO₂ appear to be at high risk in air-polluted areas. Ambient air quality standard in Japan was determined in 1978. At the present time, no information about the effects of subchronic and chronic exposure to NO₂ in airway responsiveness is available. To assess a risk of NO₂, our present results could be useful. Overall, our results indicate the following: (1) Airway hyperresponsiveness was induced by subchronic exposure to NO₂, both concentration and time dependently. (2) Subchronic exposure to NO₂ also induced an increase in SRaw₀, both concentration and time dependently. (3) Subsequent to the hyperresponsiveness induced by NO₂ is an increase in the SRaw₀. Therefore, NO₂ could be a potent risk factor of alteration of pulmonary function and airway responsiveness.

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REFERENCES


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Takahiro Kobayashi
Takashi Miura

Department of Basic Medical Sciences
National Institute for Environmental Studies
Tsukuba 305, Japan

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