Role of nitrous acid in the association between nitrogen dioxide and asthma symptoms: effect of nitrous acid exposure on specific airway resistance in guinea pigs

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Abstract

Epidemiological evidence regarding the effects of nitrogen dioxide (NO_2) on asthma and chronic obstructive pulmonary disease (COPD) is inconclusive because NO_2 assays measure nitrous acid (HONO) as NO_2. Epidemiological study using separate measurements of indoor HONO and NO_2 suggests that indoor HONO levels are associated with decrease in lung function, and HONO exposure experiment in rats suggests that HONO adversely affects respiratory function more than NO_2. Effects of NO_2 on respiratory tract resistance have not been observed in rats but have been observed in guinea pigs, including baseline specific airway resistance (sRaw). The present study aimed to investigate the effects of HONO exposure on baseline sRaw in guinea pigs. Eighteen male Hartley guinea pigs were divided into Groups C, M, and H ($n = 6$ per group) and exposed to HONO at three concentrations, respectively, 24 h/d for 7 weeks. Double-flow plethysmography was used to measure the sRaw once a week and grade respiratory waveforms, indicating increased airway resistance. The experiment was performed twice. Mean HONO concentrations of two experiments in Groups C, M, and H were 0.02, 0.66, and 3.43 parts per million (ppm). The sRaw increased significantly in Groups H and M compared with sRaw in Group C, and in a concentration-dependent manner after the 4th week of HONO exposure. Increased airway resistance was observed in 50% of animals in Group M and 100% of animals in Group H in some weeks. However, in some animals from Group M, increased airway resistance was observed earlier than the airway resistance increases observed in all animals in Group H. These sRaw results suggest that HONO affects asthma symptoms or COPD, at a lowest observed adverse effect level (LOAEL) of < 0.66 ppm. HONO may affect baseline sRaw after shorter exposure periods and at lower concentrations compared with the reported effects of NO_2. The present results suggest that HONO is the most important nitrogen oxide affecting asthma and COPD indoors, and that HONO may be the causative agent in the association between NO_2 and asthma symptoms in epidemiological studies.

Keywords: Nitrous acid, Specific airway resistance, Asthma, Chronic obstructive pulmonary disease, Nitrogen dioxide, Guinea pig

Background

Numerous epidemiological studies have shown a relationship between NO_2 and alterations in respiratory function or asthma symptoms [1–3]. However, conventional nitrogen dioxide (NO_2) assays measure both nitrous acid (HONO) and NO_2 as NO_2 [4]. HONO contamination in NO_2 measurements causes uncertainty in

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epidemiological studies regarding whether NO_2 exposure has an independent effect on asthma development. According to the US Environmental Protection Agency (EPA) [5], this uncertainty has been partly reduced by experimental animal studies by Kobayashi & Miura characterizing the potential mode of action of NO_2 in asthma development [5]. Kobayashi & Miura (1995) conducted an NO_2 exposure experiment in guinea pigs using baseline specific airway resistance (sRaw) as an index of asthma and chronic obstructive pulmonary disease (COPD); exposure of guinea pigs to NO_2 at 2 and 4 parts per million (ppm) for 12 weeks induced a concentration-dependent increase in sRaw compared with a control group [6]. However, no increase in sRaw was observed at 12 weeks of exposure to NO_2 at 0.06, 0.5, and 1.0 ppm, or at 6 weeks of NO_2 exposure at up to 4 ppm [6].

We previously reported that HONO exposure at 5.8 ppm with secondary products of 0.7 ppm NO_2 and 2.3 ppm NO in rats significantly increased the baseline pulmonary resistance (RLung) and Muc5ac mRNA levels in the lung but had no effects on dynamic lung compliance (Cdyn) and pulmonary fibrosis [7]. RLung is used as an indicator of asthma symptoms and COPD in rats. Until the publication of our HONO exposure experiment, sulfur dioxide (SO_2) was the only known air pollutant that increased RLung in rats. 250 ppm SO_2 causes not only increased RLung, but also pulmonary fibrosis and reduced Cdyn in rats [8]. However, pulmonary fibrosis is not always observed in asthmatics. The HONO exposure experiment in rats suggested that HONO has more adverse effects on respiratory function than NO_2 or SO_2 [7].

The World Health Organization (WHO) [9] reported that the extent of health effects that can be attributed to NO_2 itself, or rather to primary and secondary combustion-related products (i.e., organic carbon and HONO) is unclear in epidemiological studies [9]. For instance, Jarvis et al. conducted an epidemiological study using separate measurements of indoor HONO and NO_2 and found that indoor HONO levels, but not indoor NO_2 levels, are associated with a decrease in lung function [10]. Additionally, in an epidemiological pilot study, Ohyama et al. found that indoor HONO and nitric oxide (NO), which are mainly produced by the combustion of certain substances, were significantly associated with asthma attacks, as revealed via statistical analysis using the Mann–Whitney *U*-test [11]. However, WHO revised the annual air quality guidelines (AQG) for NO_2 from 40 μg/m^3 (approximately 0.020 ppm) in 2005 to 10 μg/m^3 (approximately 0.005 ppm) in 2021 based on the effects of long-term NO_2 on all-cause mortality and respiratory mortality according to epidemiological studies without HONO measurements [12]. One of the reports that

contributed to this revision in the AQG demonstrated that the evidence for NO_2 effects on COPD mortality was the most reliable [13].

Although atmospheric NO_2 levels has been decreasing in recent years [2], numerous epidemiological studies have demonstrated an association between indoor NO_2 and asthma [14–18]. Hansel et al. [14] suggested that interventions that lower NO_2 concentrations in homes will reduce asthma morbidity in preschool children [14]. According to Belanger et al. (2013), asthmatic children exposed to indoor NO_2 levels below the US EPA outdoor standard (53 ppb) are at risk for increased asthma morbidity [16]. However, these reports did not consider the involvement of HONO. Lee et al. reported correlations between indoor HONO and indoor NO_2, with a correlation coefficient of 0.51, and between indoor HONO and outdoor NO_2, with a correlation coefficient of 0.35, but not between outdoor HONO and indoor and outdoor NO_2 [19]. The involvement of HONO in indoor NO_2 effects should be considered even more than the outdoor effects, as the ratio of HONO to NO_2 is about 1:3–4 indoors [10] and 1:20–60 outdoors [20].

Further animal studies are needed to determine whether HONO or NO_2 has stronger effects on asthma and COPD. The present study aimed to investigate the effects of HONO exposure on baseline sRaw in guinea pigs over time. This experiment will help us compared the effects of HONO versus NO_2 on asthma and COPD.

Methods
Animals
Eighteen male Hartley guinea pigs (5 weeks of age, specific-pathogen free) were purchased from SLC (Shizuoka, Japan). Guinea pigs were divided randomly into three groups of 6 animals each (control group, Group C; middle concentration exposure group, Group M; high concentration exposure group, Group H). The animals in each group were housed preliminarily in flat-floored hanging 3 cages for 4 weeks in each exposure chamber, as described previously [7, 21, 22]. Briefly, the exposure chambers were custom-made out of acrylic, and had volume of 0.2 m^3 and flow air volume of 15 L/min. Room air was filtered using about 5 kg of charcoal-activated granules and 15 sheets of American air filters for vinyl isolators (Clea Japan, Inc., Tokyo, Japan), air compressors (0.4LE-8S; Hitachi Industrial Equipment Systems Co., Ltd., Tokyo, Japan), dehumidifiers (RAX3F; Orion Machinery Co., Ltd., Nagano, Japan), high-pressure regulator valves (with the largest supply pressure of about 0.078 MPa; model No. 44–2263-241; Kojima Instruments Inc., Kyoto, Japan), and mass flow controllers to control air flow (model 8350MC-0-1-1; Kojima Instruments Inc., Kyoto, Japan). The internal pressure in the chambers was

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adjusted to about $+1$ mm H_2O relative to atmospheric pressure. Food and water were available freely during all experimental periods. The animal room was maintained under a dynamic temperature of 24 ± 3 °C to stabilize the chamber temperature and humidity. The room lighting was turned on/off by staff at 9:00 and 17:30.

HONO exposure

A HONO generation system (as described previously) was used with the exposure chambers described above [23, 24]. Briefly, the HONO generation system sprayed a mixture of aqueous sodium nitrite ($>98.5\%$ pure sodium nitrite; Wako Pure Chemical Industries, Ltd. Osaka, Japan) with aqueous acid (85–92% lactic acid; Wako Pure Chemical Industries, Ltd. Osaka, Japan) solution in a porous polytetrafluoroethylene (PTFE) tube (TB-1008, approximately 15 cm length; Sumitomo Electric Fine Polymer, INC., Osaka, Japan) with filtrated room air through an atomizer-nozzle (BN90s-IS[V], SUS316L, 1/8PT, M14; Atomax Co., Shizuoka, Japan). Air outside the porous PTFE tube, including that containing HONO, was continuously supplied to the exposure chamber with secondary products of NO_2 and NO for 7 weeks. The concentration of aqueous sodium nitrite was 200 and 80 mmol/L in Groups H and M, respectively. The concentration of lactic acid was 250 and 100 mmol/L in Groups H and M, respectively. The flow velocity of each solution was maintained at approximately 0.4 mL/min, and the flow velocity of clean air was maintained at 15 L/min. HONO exposure was stopped for approximately 1 h every week during exchange of cages and cleaning of the chambers.

Measurement of nitrogen oxides in chamber air

Within the same sampling period, we used a non-continuous method to measure HONO levels and a continuous method for measuring NO_2 and NO with a NOx analyzer (Model 42 i, Thermo Fisher Scientific., Yokohama, Japan). In measurement of HONO, we used a Harvard EPA Annular Denuder System consisting of two annular denuders (URG-2000-30 $\times$ 150-3CSS; URG Corporation, NC) coated with sodium carbonate ($>99.5\%$ pure, Wako Pure Chemical Industries, Ltd. Osaka, Japan) and glycerol ($>99\%$ pure, Wako Pure Chemical Industries, Ltd. Osaka, Japan) [25, 26]. Chamber air was aerated to the denuder by a NOx analyzer equipped with a device that switched six paths hourly. The amount of HONO in the denuder extracts was measured by ion chromatography (700 series; Metrohm Japan LTD., Tokyo, Japan). The concentrations of HONO were calculated by the aeration volume data of the NOx analyzer and the ion chromatography data. The concentrations of NO_2 and NO were measured in the air passing through the denuder. Only

stable data collected from 20 min after the switching of sampling routes were used.

Measurement of sRaw and observation of respiratory waveform

The method for measurement of sRaw by double-flow plethysmography has been reported previously [27–29]. sRaw was assessed weekly in nonanesthetized spontaneously breathing guinea pigs using the PULMOS-I system (MIPS Co. Ltd., Osaka, Japan) from the stage of preliminary housing to the end of HONO exposure. The object animal range of the PULMOS-I system was fixed at the range for "guinea pig". The data of 100 respirations following the 10th respiration after the start of measurement were used for analysis. sRaw values were calculated as the mean of these data. Any data obtained while the guinea pigs struggled were excluded. Raw is used as an indicator of asthma symptoms or COPD in guinea pig.

Respiratory waveforms can be observed while airway resistance is being measured using the PULMOS-I system. Under normal conditions in guinea pigs, expiratory flow in the first half of expiration is greater than the expiratory flow in the latter half of expiration. However, when airway resistance is increased, expiratory flow in the latter half of expiration is greater than expiratory flow in the first half of expiration. We classified the respiratory waveforms of each measurement based on these characteristics into three grades: normal, larger flow in the first half of expiration than in the latter half of expiration; affected, larger flow in the latter half of expiration than in the first half of expiration; and strongly affected, clear and large flow in the latter half of expiration than in the first half of expiration.

Measurement of tidal volume (TV) and peak expiratory flow (PEF)

TV and PEF were measured by the PULMOS-I system in 100 respirations, as for sRaw.

Experiment to confirm reproducibility

A same above mentioned experiment, as described above, was conducted again to confirm reproducibility. Therefore, the total animal number of animals in each group was 12.
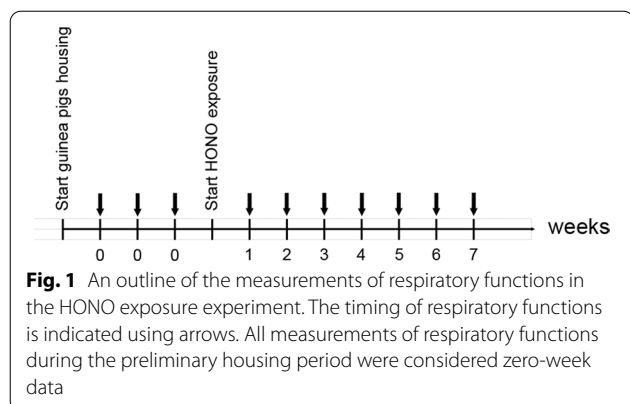
Statistical analysis

The mean $\pm$ standard deviation values of sRaw, TV, and PEF were calculated for 12 animals in each group. Statistical analyses were performed for twice experiments data using analysis of variance followed by Dunnett's multiple comparison tests. $p < 0.05$ was considered significant. All data analyses were performed using SPSS statistical software, version 26 (IBM Corp, Armonk, NY, USA).

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Table 1 Concentrations of nitrogen oxides in each camber

Group	HONO (ppm)	NO$_2$ (ppm)	NO (ppm)
Experiment 1			
Group C	0.005	0.044 ± 0.063	0.027 ± 0.062
Group M	0.775	0.174 ± 0.291	1.032 ± 0.495
Group H	4.179	1.374 ± 1.175	3.997 ± 1.626
Experiment 2			
Group C	0.030	− 0.011 ± 0.0 35[a]	0.094 ± 0.131
Group M	0.542	0.000 ± 0.042	0.721 ± 0.219
Group H	2.670	0.385 ± 0.212	2.639 ± 0.569
Average[b]			
Group C	0.018 ± 0.013	0.017 ± 0.028	0.061 ± 0.0335
Group M	0.659 ± 0.117	0.087 ± 0.087	0.877 ± 0.156
Group H	3.425 ± 0.755	0.880 ± 0.495	3.318 ± 0.679

[a] Negative value due to inadequate of calibration of the NOx analyzer

[b] Data are presented as the mean value ± SD based on the average value of 2 experiments

Fig. 1 An outline of the measurements of respiratory functions in the HONO exposure experiment. The timing of respiratory functions is indicated using arrows. All measurements of respiratory functions during the preliminary housing period were considered zero-week data

Results
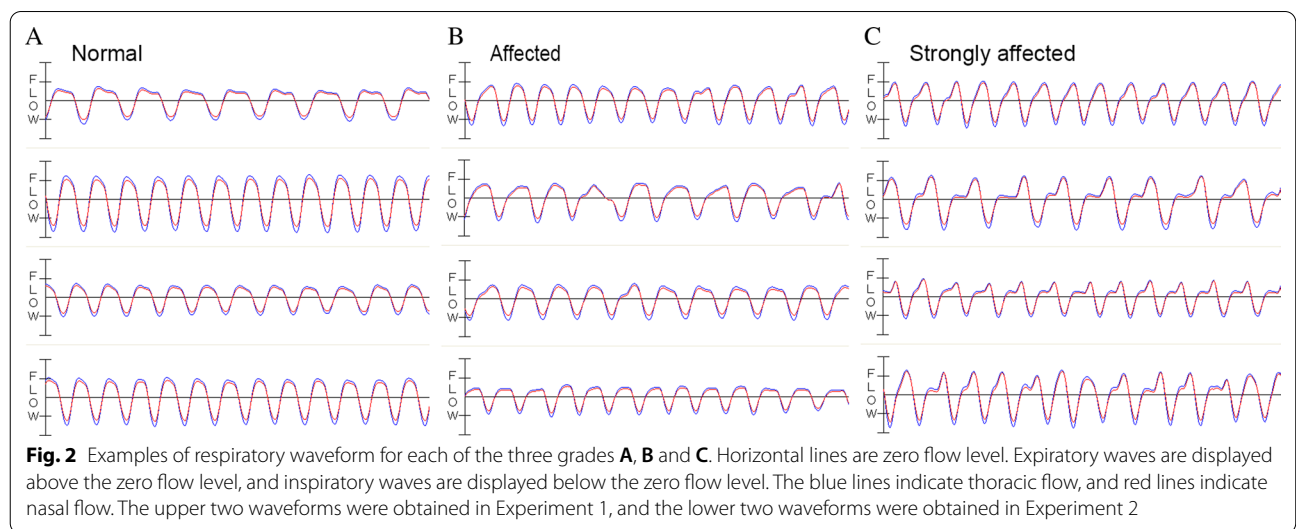
Concentrations of nitrogen oxides in chamber air
Table 1 shows the concentrations of HONO, NO$_2$, and NO, in chamber air for each group. Although the nitrogen oxide concentrations were lower in Groups H and M in Experiment 2 than in Experiment 1, the concentration ratios of Groups H/M were similar for HONO and NO in Experiment 1 and 2 (Groups H/M for HONO and NO: 5.4 and 3.9 in Experiment 1, and 4.9 and 3.7 in Experiment 2, respectively). The concentration level of NO$_2$ was lower those of HONO and NO in both Experiment 1 and 2. Although a slight drift of NOx analyzer was observed in the measurement of Experiment 2, the NO$_2$ concentration in Group M was similar to that in Group C.

Respiratory function
Figure 1 schematically depicts the plan for measuring respiratory functions during the HONO exposure experiment. Arrows indicate the measurement of respiratory functions. The average values of three measurements of respiratory functions obtained during the preliminary housing period were considered zero-week data.

Respiratory waveforms of the three grades
Figure 2 (A, B, and C) shows examples of respiratory waveforms for the normal, affected, and strongly affected respiratory waveform grades. In each subfigure, the upper two waveforms were obtained in Experiment 1, and the lower two waveforms were obtained in Experiment 2. All examples of the normal grade were respiratory waveforms of Group C, and the examples of the affected and strongly affected grades were respiratory waveforms of Groups M and H in each experiment.

Fig. 2 Examples of respiratory waveform for each of the three grades **A**, **B** and **C**. Horizontal lines are zero flow level. Expiratory waves are displayed above the zero flow level, and inspiratory waves are displayed below the zero flow level. The blue lines indicate thoracic flow, and red lines indicate nasal flow. The upper two waveforms were obtained in Experiment 1, and the lower two waveforms were obtained in Experiment 2

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Although breathing speed varied among the animals, no effects of breathing speed on the respiratory waveform grades were observed. Respiratory waveforms like those in Fig. 2A were classified as normal. The expiratory flows in the first half of expiration were slightly greater than the expiratory flows in the latter half of expiration in most respiratory waveforms. Respiratory waveforms like those in Fig. 2B were classified as affected. The expiratory flows in the latter half of expiration were slightly greater than the expiratory flows in the first half of expiration in some respiratory waveforms, and some respiratory waveforms corresponded to normal. Respiratory waveforms like those in Fig. 2C were classified as strongly affected. The expiratory flows in the latter half of expiration were clearly greater than the expiratory flows in the first half of expiration in most respiratory waveforms. In each group, similar waveforms were observed in the two experiments.

Effects on sRaw and the respiratory waveform grades

Figure 3 shows changes in sRaw and the respiratory waveform grade for each animal over time. Although the sRaw for each animal was not very stable over time, all respiratory waveforms in Group C were graded as normal. In the respiratory waveforms of Groups M and H, waveforms were often graded as affected or strongly affected when sRaws were greater than 2 cm $H_2O \times$ mL/(mL/s), and waveforms were sometimes graded as affected when sRaws were less than 2 cm $H_2O \times$ mL/(mL/s). Animals with sRaw above 3 cm H_2O/(mL/s) in some week tended to have similar sRaw in other weekly measurements. In both Experiments 1 and 2, the strongly affected or affected grades were observed in either week six animals in Group H and three animals in Group M. Although the HONO concentration of Group M in Experiment 2 was lower than the concentration in Experiment 1, the strongly affected weeks earlier in Experiment 2 than in Experiment 1. Moreover, strongly affected or affected grades were observed in five animals in Group M and one animal in Group H within 3 weeks of exposure.

Table 2 shows the results of the statistical analysis of changes in sRaw in each group for combined Experiments 1 and 2. Significant increases in sRaw were observed after the 4th week of HONO exposure in Groups H and M and in the 1st week of HONO exposure in Group M compared with the sRaw in Group C, and in a concentration-dependent manner after the 4th week of HONO exposure. Changes in sRaw for Groups M and H were similar after the 2nd week of HONO exposure.

Effects of HONO exposure on TV and PEF

Figure 4 shows changes in the average TV over time for each group for the combined Experiments 1 and 2. The TV for each group ranged from approximately 3–4 mL. No significant changes over time were observed, and TVs in Groups H and M were not significantly different from TV in Group C.

Figure 5 shows changes in the average PEF over time for each group for the combined Experiments 1 and 2. The PEF for each group ranged from approximately 14–19 mL/s. No significant changes over time were observed, and PEFs in Groups H and M were not significantly different from PEF in Group C.

Discussion

Most NO_2 epidemiological studies discuss the effects of NO_2 without measuring HONO, even though conventional NO_2 assays measure HONO as NO_2 [4]. Moreover, HONO is in equilibrium with NO, NO_2, and H_2O [30]: $2HONO \rightleftarrows NO_2 + NO + H_2O$. Therefore, some NO_2 may have changed to HONO in animal experiments focused on NO_2 exposure. Even in the present HONO exposure experiment, the secondary products of NO_2 and NO could not be eliminated. However, since HONO and NO_2 were both measured, we can discuss whether the effects were due to HONO or NO_2.

The NO_2 concentration in Group M from Experiment 1 was 0.17 ppm, and the NO_2 concentration in Group M from Experiment 2 was similar to the NO_2 concentration in Group C from Experiment 2. However, the effects on respiratory waveforms in Group M were similar between Experiments 1 and 2. This result suggests that 0.17 ppm NO_2 levels in HONO exposure is not important in respiratory resistance. Although the highest concentration of NO_2 in this experiment was approximately 1.4 ppm in Group H of Experiment 1, Kobayashi & Miura reported no effect on sRaw even at 4 ppm NO_2 6 weeks exposure [6]. Moreover, the sRaws of Groups M and H in this experiment were similar. In addition, we observed the pulmonary emphysema-like alterations in guinea pigs exposed to 1.7 ppm HONO with secondary products of 0.0 ppm NO_2 and 0.2 ppm NO [22]. Based on these results and knowledges, the increases in sRaw in Groups M and H are not likely to be due to the secondary product NO_2. Although the concentrations of NO are similar to the concentrations of HONO in each group, NO is not very toxic and is used for treating respiratory insufficiency in certain conditions [31]. Overall, our results suggest that the significant increase in sRaw was due to HONO and the LOAEL was less than 0.66 ppm.

Although nitrogen oxide concentrations in Groups H and M were lower in Experiment 2 than in Experiment 1, the experimental conditions, such as reagent concentrations, remained the same, and the reason for this finding is unknown. According to the results of Experiment 1 and 2, there was no air leakage based on the identical
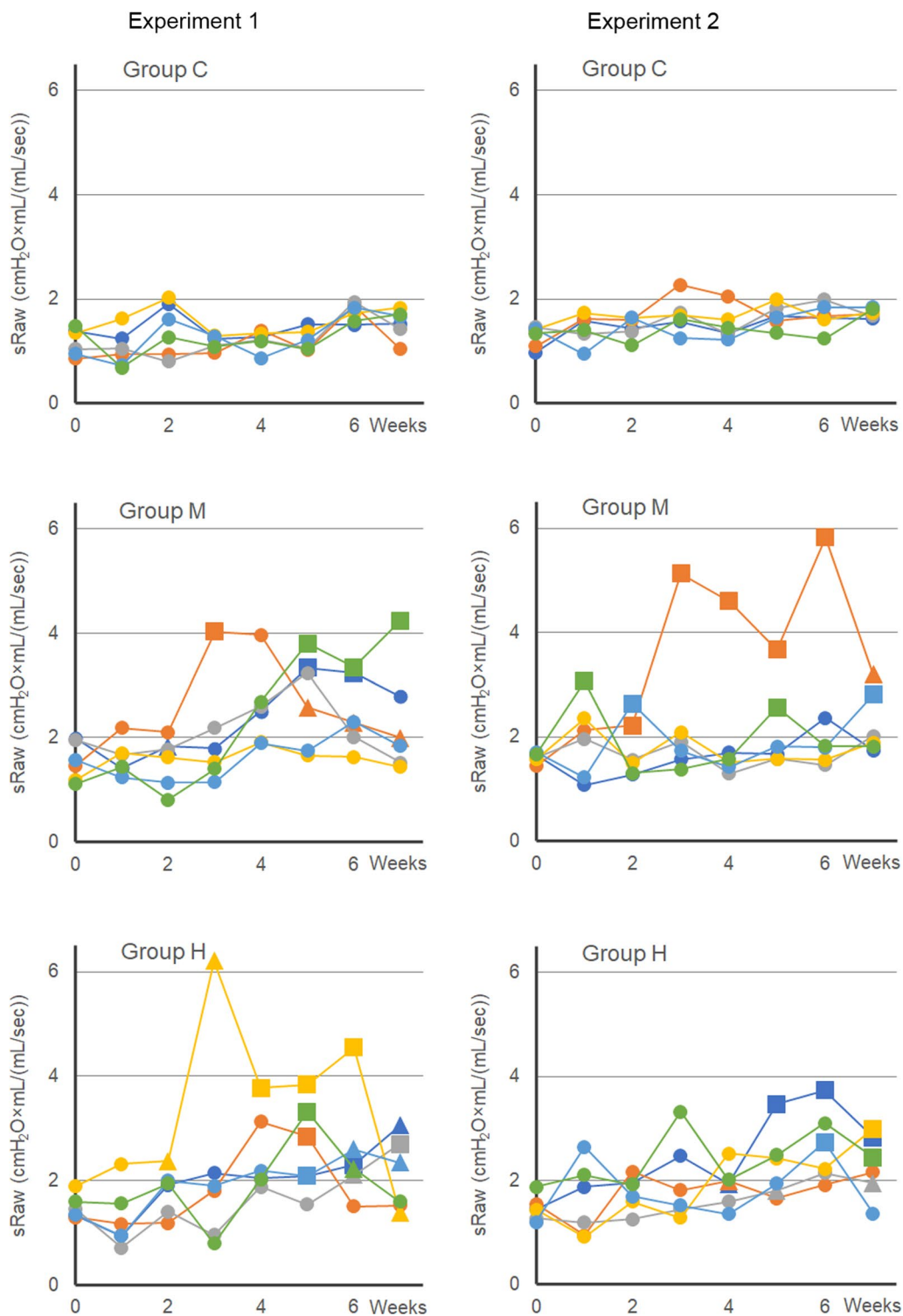
Fig. 3 Change in sRaw and grade of respiratory waveform for each animal over time. Data for 0 weeks of exposure are averages of sRaw of the 3-week preliminary housing period. Grade of respiratory waveform is indicated as follows: circle, normal; triangle, affected; square, strongly affected.

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Table 2 Change in sRaw for each group over time after exposure to HONO

Weeks of exposure	Group C		Group M		*p* value[b]	Group H		*p* value[b]	ANOVA
	n[a]	Mean ± SD	n[a]	Mean ± SD		n[a]	Mean ± SD		*p* value[c]
1	12	1.24 ± 0.37	12	1.79 ± 0.58	*0.034	12	1.44 ± 0.64	0.565	0.056
2	12	1.44 ± 0.37	12	1.65 ± 0.51	0.395	12	1.78 ± 0.36	0.103	0.157
3	12	1.42 ± 0.37	12	2.16 ± 1.19	0.197	12	2.14 ± 1.45	0.213	0.196
4	12	1.36 ± 0.28	12	2.30 ± 1.04	†0.006	12	2.20 ± 0.66	*0.015	†0.006
5	12	1.44 ± 0.32	12	2.44 ± 0.87	†0.002	12	2.46 ± 0.76	†0.002	†0.001
6	12	1.70 ± 0.21	12	2.47 ± 1.22	0.067	12	2.59 ± 0.85	*0.031	*0.035
7	12	1.63 ± 0.22	12	2.27 ± 0.83	*0.028	12	2.19 ± 0.63	0.058	*0.030

Data are presented as the mean ± SD ($cmH_2O \times mL/(mL/s)$) for Experiments 1 and 2. Dunnett's multiple comparison was applied for comparison with the control following ANOVA
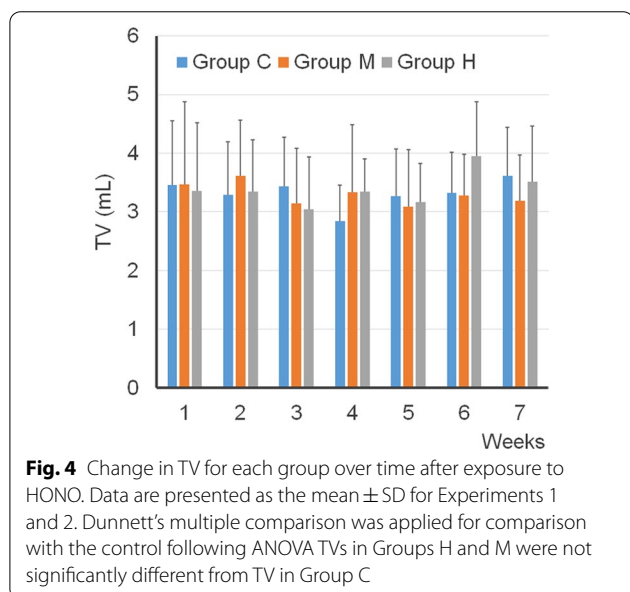
[a] Number of animals

[b] *P* value for comparison with the control

[c] *P* value for ANOVA

* $p < 0.05$

† $p < 0.01$

Fig. 4 Change in TV for each group over time after exposure to HONO. Data are presented as the mean ± SD for Experiments 1 and 2. Dunnett's multiple comparison was applied for comparison with the control following ANOVA TVs in Groups H and M were not significantly different from TV in Group C

Fig. 5 Change in PEF for each group over time after exposure to HONO. Data are presented as the mean ± SD for Experiments 1 and 2. Dunnett's multiple comparison was applied for comparison with the control following ANOVA PEFs in Groups H and M were not significantly different from PEF in Group C

concentration ratios of Groups H/M for HONO and NO. A possible reason for the lower NO_2 concentrations in Experiment 2 could be that the humidity in Experiment 2 was higher than that in Experiment 1, which could have resulted in the formation of HONO from NO_2 in equilibrium. HONO is water soluble, but NO_2 and NO are insolubule in water. Water soluble HONO is more readily absorbed by animals than non water soluble NO_2 and NO, which may have resulted in a reduction in all nitrogen oxide concentrations. Thus, Group M in Experiment 2 would have produced more HONO than Group M in Experiment 1, despite the HONO values

being lower. Generally, in animal exposure experiments, the HONO concentration is measured after the animals have absorbed the exposed HONO through respiration and body hair. Consequently, it is likely that the animals in Group M in Experiment 2 inhaled more HONO than those in Group M in Experiment 1, and that the effect on respiratory function was observed from the first week of HONO exposure. In the general environment, the weather conditions under which equilibrium reactions produce HONO from NO_2 may be an important factor associated with asthma symptoms.

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Our previous experiments on rats [7] and guinea pigs [21] utilized the highest possible concentrations of HONO to indicate clear biological effects at a single time point observation. The NO_2 ranged from 0.3 to 0.7 ppm in the HONO exposure groups in those experiments, and there was no basis for ignoring the NO_2 effects. Therefore, in those reports we discussed that HONO with secondary products of NO_2 and NO adversely affects respiratory function and pulmonary emphysema-like alterations without inflammation. We believe that the previous discussion stating that "HONO with secondary products of NO_2 and NO adversely affects the lungs" should be modified to "HONO adversely affects the lungs."

The respiratory waveforms indicative of increased airway resistance were reproducible in the two experiments, with some animals in Group M exhibiting such respiratory waveforms earlier than in Group H. The respiratory waveform results in Group M suggest that some animals experience short-term effects of airway resistance at low concentrations of HONO. The respiratory waveforms in Group H suggest that high concentrations of HONO affect airway resistance in most guinea pigs. However, the reason for the later effects on the respiratory waveforms in Group H compared to the waveforms in Group M is unclear. Whether NO can delay the effects of HONO on the respiratory waveforms requires further investigation.

HONO significantly increased sRaw, but no significant effects on TV and PEF were detected. These results are consistent with the results of previous HONO exposure rat experiment [7] showing increased LRung but no effects on Cdyn. Our results suggest that HONO specifically enhances respiratory resistance.

NO_2 is often considered as the most important nitrogen oxide affecting respiratory function. However, according to the results of Kobayashi & Miura, 12 weeks of exposure to NO_2 above 2 ppm was required to produce a significant increase in sRaw, whereas, in our experiment, exposure to HONO at 0.66 ppm increased sRaw after only 4 weeks. It may be difficult to explain the relationship between nitrogen oxides and asthma by HONO alone because of the discrepancy that Group M observed the respiratory waveforms indicating increased airway resistance earlier than Group H. However, we believe that HONO is an important nitrogen oxide that increases airway resistance at low concentrations and short exposure periods without inflammatory alterations. Although the concentration of HONO that affects airway resistance is lower than the effective concentration of NO_2, the HONO concentration in the environment is also lower than the environmental concentration of NO_2 [10, 20]. The LOAEL ratios of HONO/NO_2 are greater than the concentration ratios of HONO/NO_2 in outdoor [20], but comparable to the concentration ratio of HONO/NO_2 in indoor [10]. We believe that both the exposure period and concentration of HONO are important when comparing the effects of HONO to NO_2. The present results suggest that HONO is the most important nitrogen oxide affecting asthma and COPD indoors.

HONO is readily decomposed to NO and the OH radical by sunlight at < 400 nm [32, 33]. Therefore, HONO reach the maximum concentration during the night in an urban atmosphere. Moreover, in the indoor environment, HONO is produced directly by combustion processes such as gas appliances or kerosene heaters [34, 35]. Therefore, the effects of HONO might be considered as an indoor pollution rather than air pollution. Thus, in the homes of asthma and COPD patients, measures to reduce HONO indoors may help reduce symptoms.

Conclusion

The nitrogen oxide concentrations in Groups H and M in Experiment 2 were lower than those in Groups H and M in Experiment 1, although the experimental settings, such as reagent concentrations, were the same in both experiments. However, the effects on respiratory function were similar in Experiments 1 and 2. These results suggest that HONO has a more negative impact on respiratory function than NO_2 and that the LOAEL of HONO was less than 0.66 ppm. Some animals in Group M showed respiratory waveforms indicative of increased respiratory function earlier than in Group H. This finding indicates that some animals may experience short-term respiratory effects from low HONO concentrations. These results suggest that HONO affects asthma and COPD symptoms at a lower concentration and shorter exposure time than NO_2. We believe that HONO is the most significant nitrogen oxide impacting asthma and COPD inside due to the fact that HONO concentrations are frequently higher indoors than outdoors.

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generation system. SA: methodology of animal exposure system. All authors read and approved the final manuscript.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The procedures were approved by the committee on animal care of the Osaka Institute of Public Health, and all experiments were performed in accordance with guidelines and regulations of the Osaka Institute of Public Health.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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