Operator Safety Zone During the Nitric Oxide Inhalation Vasoreactivity Test for Pulmonary Hypertension Patients

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Abstract  Background and Objective: Acute vasoreactivity testing using inhaled nitric oxide (NO) is performed to determine the treatment strategy in patients with pulmonary hypertension. Operators of the test are exposed to NO and nitrogen dioxide (NO2) to the same extent during the test procedure, but the safety zone for operators in the catheterization laboratory is unclear. This study aimed to clarify the safety zone for operators during the NO inhalation test. Methods: The mask was placed on the examination table in the catheterization laboratory. NO was added using the INOvent® (INO-Therapeutics, LLC), which was attached to an oxygen line and delivered NO to the mask. During simulated NO inhalation tests (n = 10) in a catheterization laboratory, NO and NO2 concentrations were measured at nine specified locations: at the mask outflow port (E0); 25 cm (E25) and 50 cm (E50) from the mask outflow port; heights of 25 cm (A25) and 50 cm (A50) above E0 and E25; and a height of 25 cm (B25) below E0 and E25. During NO inhalation testing conducted in 5 patients, concentrations of NO and NO2 were measured at E0 and E25A50. The Steel-Dwass and Student t-tests were used to perform intergroup analyses. Results: NO-E0A25 (0.00 ± 0.00 ppm), NO-E25A25 (0.01 ± 0.10 ppm), NO-E0A50 (0.00 ± 0.00 ppm), and NO-E25A50 (0.00 ± 0.00 ppm) were significantly lower than NO-E0 (17.01 ± 0.22 ppm), NO-E25 (4.06 ± 0.47 ppm), and NO-E50 (1.90 ± 0.39 ppm) (p < 0.01, all comparisons). NO2 concentrations were less than 0.1 ppm at all nine locations. NO and NO2 concentrations were not affected by room temperature (20, 24, or 28°C) or the flow rate of oxygen (15 or 10 L/min). During NO inhalation testing in patients, NO-E25A50 (0.20 ± 0.45 ppm) was significantly lower than NO-E0 (7.20 ± 0.23 ppm, p < 0.01); however, no significant difference in the NO2 concentration was seen between NO2-E0 (0.220 ± 0.179 ppm) and NO2-E25A50 (0.180 ± 0.205 ppm). Conclusions: An operator safety zone in NO vasoreactivity testing is a zone >25 cm in all directions from the mask for NO inhalation. The concentrations of NO and NO2 to which an operator is exposed in the clinical setting are within the permissible exposure limits defined by the National Institute for Occupational Safety and Health.

Keywords: nitric oxide, vasoreactivity testing, pulmonary hypertension, catheterization laboratory, safety zone.


1. Background

Pulmonary hypertension (PH) is a pathological condition in which the pulmonary artery pressure is persistently increased by various causes, and it is an intractable disease with a poor prognosis that sequentially progresses to right ventricular failure [1, 2]. Although the quality of life and survival rate of patients with PH have improved greatly by the development of PH-specific treatments, a large number of patients still have a poor outcome. The acute vasoreactivity test with nitric oxide (NO) inhalation is useful for determining the prognosis of patients with pulmonary arterial hypertension [3–7] and for selecting a therapeutic strategy. However, the NO inhalation test exposes personnel conducting the test to the risk of untoward effects of NO, including headache, dizziness, sore throat, cough, and dyspnea. NO is released into the air from a patient’s mask during an NO inhalation test, and it reacts with oxygen, resulting in conversion to a more toxic substance, nitrogen dioxide (NO2), with a half-life in the body of 5 to 10 seconds [8, 9]. The National Institute for Occupational Safety and Health (NIOSH) and other organizations currently recommend that the time-weighted average (TWA) of NO and NO2 should be 25 ppm or lower and 1.0 ppm or lower, respectively, for persons who work 8 hours per day. To our knowledge [8, 10], no study has reported changes in NO and NO2 levels in room air during NO inhalation tests or provided precautions for medical personnel during the test.

Thus, the present study aimed to clarify the safety zone for operators of NO inhalation tests by determining NO and NO2 levels in different locations during NO inhalation tests in a catheterization laboratory.

2. Methods

The present study was designed as an environmental survey in a catheterization laboratory, and hence did not require review by the ethics committee of Sapporo Medical University. The present study was conducted in accordance with the World Medical Association Declaration of Helsinki.

To standardize the protocol and to avoid risks to patients, we used a simulated NO inhalation test (a test in a standard clinical setting without a patient) to collect data, and we verified the find-
ings from the simulation tests in routine clinical examinations of NO inhalation testing conducted in patients with PH.

2.1 Testing room
The experiments were conducted in a catheterization laboratory used for daily clinical practice. The catheterization laboratory had an area of 36 m², and the room air was ventilated 10 times/hour at a rate of 1970 m³/hour. The room temperature was set to 24°C, which was the same as that in normal clinical conditions. The room pressure of the catheterization laboratory was maintained at the same level as atmospheric pressure (range 756.6 to 764.3 mmHg). The experiment was performed on an examination table for an angiography test at a height of 90 cm from the floor.

2.2 Study materials
The equipment and materials used in the study are shown in Table 1. NO gas was supplied through the INOvent® (INO-Therapeutics, LLC). The INOvent® steadily delivers an accurate concentration of NO gas by adjusting the flow rate in accordance with inspired airflow. It monitors the inspired oxygen concentration (FiO₂) and concentrations of dosed NO and NO₂ gas through the attached monitors. It is also equipped with a high concentration alarm. For NO inhalation, the INOflo® (a specific NO gas for INOvent®, 800 ppm for inhalation; INO-Therapeutics, LLC) was diluted by the INOvent®, and the gas was delivered to a manual NO dosing system attached to the INOvent®. In this study, NO gas was added to an oxygen gas line (100% oxygen at a flow rate of 15 L/min) at a concentration of 20 ppm and delivered to the mask. Concentrations of NO and nitrogen dioxide (NO₂) are measured at the outflow port of the mask, mask outflow port (E0), 25 cm (E25) and 50 cm (E50) from the mask outflow port, heights of 25 cm (A25) and 50 cm (A50) above the examination table (at E0 and E25), and a height of 25 cm (B25) below the examination table (at E0 and E25).

2.3 Experimental methods
2.3.1 Experiment 1-1
The experimental system is shown in Fig. 1. A reservoir mask was placed on the examination table for NO inhalation testing, and NO gas was added at a concentration of 20 ppm to 100% oxygen and delivered to the mask. Concentrations of NO and nitrogen dioxide (NO₂) are measured at the outflow port of the mask, mask outflow port (E0), 25 cm (E25) and 50 cm (E50) from the mask outflow port, heights of 25 cm (A25) and 50 cm (A50) above the examination table (at E0 and E25), and a height of 25 cm (B25) below the examination table (at E0 and E25).

Table 1 Equipment used and study materials.
<table>
<thead>
<tr>
<th>Equipment and materials</th>
<th>Model</th>
<th>Manufacturer’s information</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO gas supply system</td>
<td>INOvent®</td>
<td>INO-Therapeutics, LLC, USA (SN: CCCJ00416)</td>
</tr>
<tr>
<td>NO for inhalation</td>
<td>INOflo® for inhalation (800 ppm)</td>
<td>INO-Therapeutics, LLC, USA (Lot: 13FP-0169)</td>
</tr>
<tr>
<td>Exhaust emission tester</td>
<td>Testo 350-XL</td>
<td>TESTO K.K., Japan (SN: 01438795)</td>
</tr>
<tr>
<td>Non-rebreathing oxygen face mask</td>
<td>HT-1095</td>
<td>Avion Medical Spolko zoo, Poland (Lot: 15102655)</td>
</tr>
</tbody>
</table>

![Fig. 1](image-url)
No and NO₂.

2.3.2 Experiment 1-2
Concentrations of NO or NO₂ were measured 10 times at E₀, E₂₅, E₅₀, and E₂₅A₅₀ at room temperatures of 20°C (4°C below the routine clinical condition) and 28°C (4°C above the routine clinical condition) to clarify the degree at which diffusion of NO or NO₂ from the out/flow port of the mask would be affected by room temperature.

2.3.3 Experiment 1-3
When the flow rate of 100% oxygen, to which NO gas was added at a concentration of 20 ppm, was changed from 15 L/min to 10 L/min, concentrations of NO and NO₂ were measured 10 times at E₀, E₂₅, E₅₀, and E₂₅A₅₀.

2.3.4 Experiment 2
During routine clinical examinations of NO inhalation testing in patients with PH, concentrations of NO and NO₂ were measured at E₀ and E₂₅. Five patients underwent NO inhalation testing. Concentrations of NO-E₀, NO-E₂₅A₅₀, NO₂-E₀, and NO₂-E₂₅A₅₀ were measured 10 minutes after the delivery of NO was started.

2.4 Statistical analysis
Data are expressed as a mean ± standard deviation. Data were analyzed using the Pharmaco Basic software program (Scientist Press Co., Ltd.). Intergroup comparisons in experiment 1-1 and experiment 1-2 were conducted using the Steel-Dwass test (two-sided), and differences with \( p < 0.05 \) were considered significant. Results of experiment 1-3 and experiment 2 were compared using the Student’s t-test (two-sided), and differences with \( p < 0.05 \) were considered significant.

3. Results

3.1 Comparisons of NO and NO₂ concentrations
As shown in Fig. 2, NO-E₀ (17.01 ± 0.22 ppm), NO-E₂₅ (4.06 ± 0.47 ppm), and NO-E₅₀ (1.90 ± 0.39 ppm) were significantly lower than NO-E₀. NO was undetectable at the other sampling points: NO-E₀A₂₅, NO-E₂₅A₂₅, NO-E₀A₅₀, NO-E₂₅A₅₀, NO-E₀B₂₅, and NO-E₂₅B₂₅. There was a significant difference between NO-E₂₅ and NO-E₅₀, and NO-E₀A₂₅, NO-E₂₅A₂₅, NO-E₀A₅₀, NO-E₂₅A₅₀, NO-E₀B₂₅ and NO-E₂₅B₂₅ were lower than NO-E₅₀ (\( p < 0.01 \)).

In contrast to the NO concentration, there were no significant differences in NO₂ concentrations between all sampling points (Fig. 3): NO₂-E₀ (0.022 ± 0.063 ppm), NO₂-E₂₅ (0.008 ± 0.039 ppm), NO₂-E₅₀ (0.004 ± 0.028 ppm), NO₂-E₀A₂₅ (0.012 ± 0.048 ppm), NO₂-E₂₅A₂₅ (0.008 ± 0.039 ppm), NO₂-E₀A₅₀ (0.006 ± 0.034 ppm), NO₂-E₂₅A₅₀ (0.006 ± 0.034 ppm), NO₂-E₀B₂₅ (0.008 ± 0.039 ppm), and NO₂-E₂₅B₂₅ (0.006 ± 0.034 ppm).

3.2 Comparisons of NO and NO₂ concentrations at different room temperatures
In experiment 1-2, the NO concentration at three different room temperatures were compared. There were no significant differences in the NO concentration among room temperatures of 20°C, 24°C, and 28°C at all the sampling points: NO-E₀ (17.09 ±
0.038 ppm, 17.01 ± 0.22 ppm, and 17.11 ± 0.40 ppm); NO-E25 (3.99 ± 0.54 ppm, 4.06 ± 0.47 ppm, and 4.04 ± 0.57 ppm); and NO-E50, (1.78 ± 0.75 ppm, 1.90 ± 0.39 ppm, and 1.74 ± 0.76 ppm). NO was undetectable at E25A50 at any room temperature. Additionally, no significant differences in the NO2 concentration among room temperatures of 20°C, 24°C and 28°C were found at all the sampling points: NO2-E0 (0.022 ± 0.044 ppm, 0.022 ± 0.063 ppm, and 0.006 ± 0.034 ppm); NO2-E25 (0.002 ± 0.020 ppm, 0.008 ± 0.039 ppm, and 0.004 ± 0.028 ppm); NO2-E50 (0.012 ± 0.048 ppm, 0.004 ± 0.028 ppm, and 0.006 ± 0.034 ppm); and NO2-E25A50 (0.010 ± 0.044 ppm, 0.006 ± 0.034 ppm, and 0.008 ± 0.039 ppm).

3.3 Comparisons of NO and NO2 concentrations between different flow rates of oxygen gas

In experiment 1-3, the NO concentration was compared between two flow rates of 100% oxygen. No significant differences in the NO concentration between flow rates of 15 and 10 L/min of 100% oxygen were found at all the sampling points: NO-E0 (17.01 ± 0.22 ppm and 17.07 ± 0.29 ppm); NO-E25 (4.06 ± 0.47 ppm and 4.18 ± 0.44 ppm); and NO-E50 (1.90 ± 0.39 ppm and 2.02 ± 0.51 ppm). NO was undetectable at E25A50 at both flow rates of 100% oxygen. There were no significant differences in NO2 concentrations between flow rates of 15 and 10 L/min of 100% oxygen at all the sampling points: NO2-E0 (0.022 ± 0.063 ppm and 0.016 ± 0.055 ppm); NO2-E25 (0.008 ± 0.039 ppm and 0.010 ± 0.044 ppm); NO2-E50 (0.004 ± 0.028 ppm and 0.016 ± 0.055 ppm); and NO2-E25A50 (0.006 ± 0.034 ppm and 0.008 ± 0.039 ppm).

3.4 Comparisons of NO and NO2 concentrations in NO inhalation testing in patients with PH

Table 2 shows the NO and NO2 concentrations in clinical examinations of NO inhalation testing in five patients suspected to have PH, and Fig. 4 shows comparisons of these concentrations. NO-E25A50 (0.20 ± 0.45 ppm, p < 0.01) was significantly lower than NO-E0 (7.20 ± 2.39 ppm). However, there was no significant difference in the NO2 concentration between NO2-E0 (0.220 ± 0.179 ppm) and NO2-E25A50 (0.180 ± 0.205 ppm).

4. Discussion

In the present study, the NO2 concentration was markedly lower than the expected value. NO reacts with oxygen in the atmosphere and is oxidized to NO2, but its oxidation rate depends on the ambient temperature, as well as oxygen and NO concentrations. At room temperature and atmospheric pressure, NO and NO2 exist in an equilibrium mixture, with the percentage of NO2 being about 5% [13]. However, as shown in Fig. 2 and 3, the concentration of NO at the measurement point E0 was approximately 900 times higher than that of NO2. Standard deviations for the NO concentration were smaller compared to those for NO2, which were considerably larger. There are a few possible explanations for these results. First, NO2 could have settled around the lower part of the mask because of its higher specific gravity (1.58) than NO (1.06), and this would account for the relatively low concentration measured in the upper part of the mask.

Underestimation of the NO2 concentration and measurement

<table>
<thead>
<tr>
<th>Examination</th>
<th>NO (ppm)</th>
<th>NO2 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E0</td>
<td>E25A50</td>
</tr>
<tr>
<td>Examination 1</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Examination 2</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Examination 3</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Examination 4</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Examination 5</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

NO, nitric oxide; NO2, nitrogen dioxide; E0, mask outflow port; E25, 25 cm from the mask outflow port; E25A50, 50 cm above the examination table; B25, height of 25 cm below the examination table (at E0 and E25).

Fig. 4 Comparisons of nitric oxide (NO) and nitrogen dioxide (NO2) concentrations in nitric oxide inhalation testing in patients with pulmonary hypertension. A: Comparison of NO concentrations in clinical practice. B: Comparison of NO2 concentrations in clinical practice. NO-E25A50 is significantly lower than NO-E0. However, no significant difference in NO2 concentration is found between NO2-E0 and NO2-E25A50. NS, not significant; E0, mask outflow port; E25, 25 cm from the mask outflow port; A50, 50 cm above the examination table.
variability may have occurred because the measurement point E0 was positioned near the aperture of the outflow port located in the upper part of the mask. Second, the same system was used to measure NO and NO₂ concentrations in the present study. Although data variation in measurements by the Testo 350 XL used in this study is reported to be ±5% or less for both NO and NO₂, the data for variations were based on measurements of a comparatively high concentration of the gases (500 ppm). It is possible that data reproducibility with the Testo 350 XL depends on the concentration of NO₂ and is lower for low levels of NO₂. Third, measurements may have become more vulnerable to technical error when sampling was performed under random airflow around the measurement system.

NO₂ is more toxic than NO [14]. Known acute symptoms induced by NO₂ include sore throat, cough, dizziness, headache, nausea, and chest pain [14]. The American Conference of Governmental Industrial Hygienists and Japan Society for Occupational Health set the TWA value of 3 ppm as the permissible exposure limit for workers working for 8 hours per day and 40 hours per week on average, and set the short-term exposure limit to 5 ppm for workers working for short periods intermittently (e.g., 15 minutes × 4 times) [8, 10]. Few reports have measured NO₂ concentrations during NO inhalation testing in an examination room, and the status of NO₂ exposure for operators is currently unknown. The present study demonstrated that the concentration of NO₂ to which operators are exposed does not reach a hazardous concentration, unless the test is performed under special conditions. However, one study has shown the development of asymptomatic pulmonary dysfunction with the inhalation of NO₂ at 0.3–0.5 ppm, which is lower than the aforementioned limits, and induction of asthmatic attacks in asthmatic patients [14]. We detected NO₂ at a distance of 50 cm from a reservoir mask at a concentration similar to that inside the reservoir mask, although the concentration did not exceed the TWA permissible exposure limit. The results indicate that an operator can be exposed to NO₂ and adverse events may occur due to NO₂ exposure even at a sufficient distance from the patient subjected to an NO inhalation test. The results also suggest that the risk of adverse events increases if an operator has allergic diseases or if the time of examination is prolonged under specific conditions. Since operators are exposed to NO₂ during NO inhalation testing, despite the concentration being within the allowable range, it is necessary to be aware of the potential risk of adverse events and the countermeasures.

The present findings showed the distance from a reservoir mask at which the operator’s NO exposure level decreased significantly. The NO concentration was significantly decreased at a height of 25 cm above the mask compared to that at the surface of the examination table. Our results are explained by the fact that NO is slightly heavier than air. Additionally, our results confirmed that the concentration of NO or NO₂ to which an operator is exposed in the clinical setting is within the permissible limit during NO inhalation testing, because the mouth and nose of an operator or operating room worker is at a height of more than 25 cm, which is usually more than 50 cm above the level of a reservoir mask. Verifying changes in NO and NO₂ levels in room air during NO inhalation can provide important information for establishing a standardized protocol to ensure operators’ safety during acute vasoreactivity tests in the future.

The present study has several limitations. First, the study protocol did not consider concentrations of NO and NO₂ contained in expired air from operators or operating room nurses. The amount of NO₂ may be affected by expired air from operating room workers when it is higher than usual. Second, the disturbance of airflow by the movement of operators and the arrangement of large equipment in an examination room cannot be neglected in the clinical setting. Further studies performed under various conditions are needed to affirm the operator safety zone in the clinical setting.

5. Conclusions

We measured concentrations of NO and NO₂ in a catheterization laboratory during NO inhalation testing and showed that an operator safety zone in NO vasoreactivity testing is a zone more than 25 cm in all directions from the mask for NO inhalation. The concentrations of NO and NO₂ to which an operator is exposed in the clinical setting are within the permissible exposure limits defined by NIOSH.

Conflicts of interest

We declare no conflicts of interest.

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