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Validation of a simple method assessing nitric oxide and nitrogen dioxide concentrations

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Abstract Monitoring of nitric oxide (NO) and nitrogen dioxide (NO_2) is a prerequisite for the clinical use of NO. Chemiluminescence, the reference method, cannot be used as a routine in clinical practice in view of its cost and other restraints. This study was performed to evaluate a device using an electrochemical method (Polytrons NO and NO_2, Dräger®). Forty-nine simultaneous measurements of NO and various oxides of nitrogen (NO_x) concentrations by the two apparatus were performed. NO measurements by means of these two methods are very well correlated ($r = 0.96$; $p < 10^{-5}$). The mean difference according to the method of Bland and Altman was 2.8 ± 1.7 ppm, with the limits of agreement at -0.6 and $+6.2$ ppm (confidence interval of 95%). There was also a good correlation between measurements of NO_2 obtained via Polytrons and NO_x via chemiluminescence ($r = 0.84$; $p < 10^{-5}$). However, NO_2 measurements obtained via Polytron may be insufficient to exclude potential toxicity of NO_2 due to the inability to detect measurements in the ppb-range. This study demonstrates that devices designed for industrial purposes (Polytrons NO and NO_2, Dräger®) can be used for clinical purposes.

Key words Nitric oxide · Monitoring · Chemiluminescence assay · Electrochemical assay

Introduction

The pulmonary vasodilator effect of inhaled nitric oxide (NO) has been well established in animals [1, 2] and in patients with primary pulmonary hypertension [3]. The inactivation of NO by hemoglobin explains its very short half life in blood and therefore inhaled NO produces a selective pulmonary vasodilatation while systemic vascular resistances remain unchanged [1–4]. All these properties are stressed when inhaled NO is discussed as an alternative to intravenously infused vasodilators for treatment of secondary pulmonary hypertension involved in various diseases [3].

However, monitoring of NO and various oxides of nitrogen (NO_x) concentrations in the airway is a prerequisite for the clinical use of NO. The reference method, represented by chemiluminescence, cannot be used in clinical practice, owing to its high price and lack of practical qualities (large apparatus, repeated calibrations required before each utilization). The aim of the study was to assess NO and NO_2 analyzers developed for industrial use (Polytrons, Dräger) and to compare them with the reference method of chemiluminescence (Analyser AC2, Environment SA).

Materials and methods

The reference method for nitrogen oxides concentration measurements is chemiluminescence (Analyser AC2, Environment SA; Poissy, France). NO reacts with ozone in the reaction chamber,

leading to the release of photons which are detected by a photomultiplier tube. The range of measurement is 0.1 – 100 ppm (parts per million). Response time is less than 30 s at 25 °C. Using chemiluminescence, NO_2 is not measured directly, but as a difference between NO and NO_x measurements. NO_x measurement is obtained by reducing higher nitrogen oxides to NO in a catalytic furnace before gas introduction into the reaction chamber. Higher nitrogen oxides, such as N_2O_3 and N_2O_5, are not stable at room temperature and normal pressure. Thus, the difference between NO_x and NO measurement is correlated to the presence of NO_2 in the sampled gas and can be considered as a specific NO_2 measurement.

The NO detector (Polytron NO, amplifier model 83 11 395 and transducer model 68 07 670, Dräger Industrie SA, Strasbourg, France) has an electrochemical probe to measure NO. This probe consists of 3 electrodes immersed in an acid electrolytic solution. A constant voltage between measurement electrodes and the reference electrode is maintained by a potentiostat, the voltage being chosen to oxidize NO at the anode as in the following equation $NO + H_2O \rightarrow NO_2 + 2\,H^+ + 2\,e^-$. The magnitude of the electric current is directly proportional to NO content of the sample of air analyzed. The measurement range of the apparatus runs from 0.1 to 50 ppm. Resolution of the digital LCD is 0.1 ppm. Response time is less than 40 s at 25 °C. During the starting up of the electrochemical cell, calibration is done in air (apparatus zero) and at different NO concentrations (gas calibration).

The NO_2 detector (Polytron NO_2: amplifier model 83 11 579 and transducer model 45 95 082, Dräger Industrie SA, Strasbourg, France) has an electrochemical probe to measure NO_2. The air that has to be analyzed diffuses through a plastic membrane into the electrolytic solution of the probe, where 3 electrodes are immersed. A constant voltage between measurement electrodes and the reference electrode is maintained by a potensiostat, the voltage being chosen to oxidize NO_2 at the anode according to the equation $NO_2 + 2H^+ + 2e^- \rightarrow NO + H_2O$. The magnitude of the current is directly proportional to NO_2 content in the sample of air analyzed. The range of measurement runs from 0.1 to 50 ppm. Resolution of digital LCD is 0.1 ppm. Response time is less than 60 s at 25 °C. During the starting up of electrochemical cell, calibration is done in air (apparatus zero) and at different concentrations of NO_2 (gas calibration).

NO was provided in tanks containing 90 ppm or 225 ppm NO diluted in N_2 (Compagnie Française des Produits Oxygénés, Paris, France).

Different calculated quantities of humidified air at 20 °C were mixed extemporaneously in a container (5 l) with NO/N_2 to obtain 5 gas mixtures containing respectively 10, 15, 20, 25 and 30 ppm of NO at T0 (initial mixture of gas). Simultaneous measurements of NO concentration (AC2) and Polytron), NO_x (AC2) and NO_2 concentrations (Polytron) were performed at T0, T5 (5 min after gas mixing), T10 (10 min after gas mixing), T15 (15 min after gas mixing) and T30 (30 min after gas mixing). The same measurements were performed using the same mixtures of pure oxygen and NO/N_2. Data were analyzed using regression analysis and the Bland and Altman method [5].

Results

A total of 49 paired measurements of NO concentrations performed by chemiluminescence (Analyser AC2) and by electrochemical technique (Polytron) were obtained with air/NO/N_2 (25 pairs of measurements) and with pure oxygen/NO/N_2 (24 pairs of measurements) mixtures. Considering all pairs of measurements, the correlation of

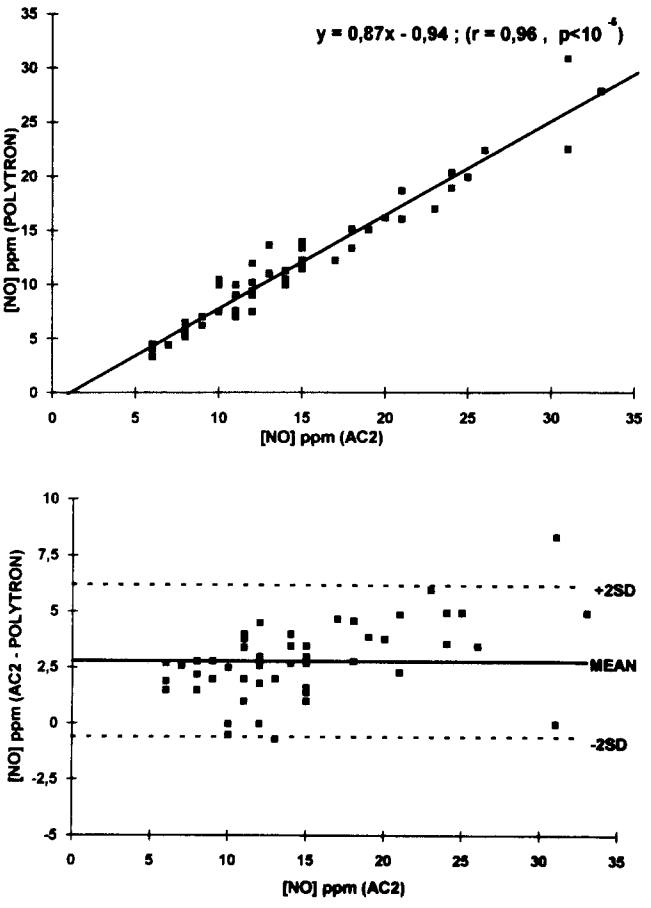

Fig. 1a Regression analysis for the relationship between [*NO*] measured by AC2 and [*NO*] measured by Polytron. ($y = 0.87\,x - 0.94$; $r = 0.96$, $p < 10^{-5}$). **b** Agreement of [*NO*] measurements Polytron with AC2 (Bias: 2.8 ppm; SD: 1.6 ppm)

electrochemical technique with chemiluminescence was $r = 0.96$ ($p < 10^{-5}$). The y-intercept was at −0.94 ppm. The mean difference according to the method of Bland and Altman was 2.8 ± 1.7 ppm, with the limits of agreement at −0.6 and +6.2 ppm (confidence interval of 95%) (Fig. 1 b). The standard error of differences was 0.24 ppm. During air/NO/N_2 mixture, the correlation of electrochemical technique and chemiluminescence was $r = 0.98$ ($p < 10^{-5}$) (Fig. 2a). The mean difference was 3.5 ppm and the standard deviation 1.6 ppm (Fig. 2b). During $O_2/NO/N_2$ mixture, the correlation between the two methods was $r = 0.96$ ($p < 10^{-5}$) (Fig. 2c), the mean difference was 2.15 ppm and the standard deviation was 1.6 ppm (Fig. 2d).

A total of 49 paired measurements of NO_x concentrations by chemiluminescence (Analyser AC2) and NO_2 concentrations by electrochemical technique (Polytron) were obtained with air/NO/N_2 and with pure oxygen/NO/N_2 mixtures. Considering all pairs of measure-

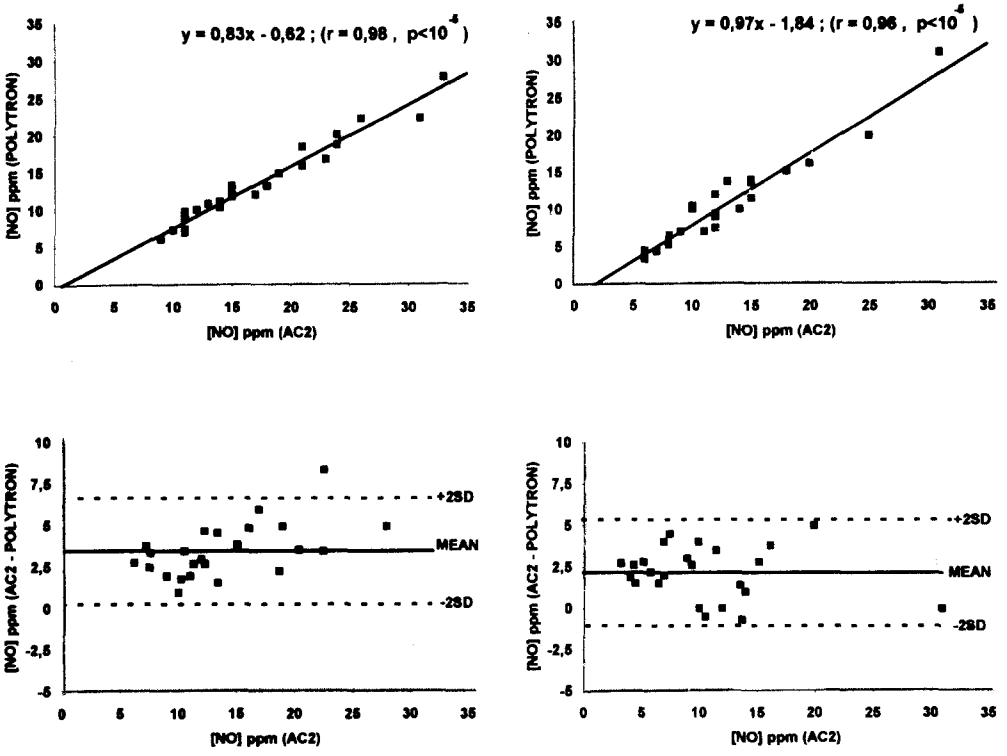

Fig. 2a Regression analysis for the relationship between [*NO*] measured by AC2 and [*NO*] measured by Polytron under air mixture ($y = 0.83\,x - 0.62$; $r = 0.98$, $p < 10^{-5}$). **b** Agreement of [*NO*] measurements Polytron with AC2 (Bias: 3.5 ppm; SD: 1.6 ppm) under air. **c** Regression analysis for the relationship between [*NO*] measured by AC2 and [*NO*] measured by Polytron under oxygen mixture ($y = 0.97\,x - 1.84$; $r = 0.96$, $p < 10^{-5}$). **d** Agreement of [*NO*] measurements Polytron with AC2 (Bias: 2.15 ppm; SD: 1.6 ppm) under O_2

ments, the correlation of electrochemical technique with chemiluminescence was $r = 0.84$ ($p < 10^{-5}$). The y-intercept was at 0.31 ppm. During air/NO/N_2 mixture, the correlation between NO_2 (Polytron) and NO_x (AC2) was $r = 0.64$ ($p < 10^{-2}$) (Fig. 3b). During $O_2/NO/N_2$ mixture, the correlation was $r = 0.85$ ($p < 10^{-5}$) (Fig. 3c).

Discussion

Our study shows that NO concentration can be measured either by chemiluminescence, the reference method, or by an electrochemical method within the concentration range used in clinical practice. A good correlation exists for NO measurement between the two methods. Measurement accuracy is satisfactory for clinical practice, since the bias is 2.8 ppm. In a hyperoxic setting, frequently necessary in patients with acute respiratory failure, the correlation is unaffected and the measurement bias (2.15 ppm) remains small.

In clinical practice, the electrochemical devices need regular calibration (once a month) with a standard gas mixture. The electrochemical cell must be replaced every eighth month if the apparatus is used frequently. The polytrons has been set up in a portable unit including an alarm and a rechargeable battery (12 h life), which allows immediate readings. An optimal sampling rate (about 0.5 l/min) was ensured by a pump placed between the breathing circuit and the polytron unit. The NO and NO_2 concentrations can be measured in the inspired or expired air mixture although large amounts of NO and NO_2 are absorbed in the lung, making measurements on expired gas erroneous. With the Bland and Altman plot, the Polytron slightly underestimated NO measured via chemiluminescence in the range of (0.1 – 50 ppm) with a bias of 2.15 ppm in hyperoxic setting. Consequently, we cannot measure NO concentration in the parts per billion range (ppb) with the electrochemical device and in the range of (0 – 1000 ppb), chemiluminescence has to be used.

Chemiluminescence measures nitric oxides (NO_x, $X > 1$) without distinguishing NO_2 from other nitrogen oxides, whereas the electrochemical method measures only NO_2. However a good correlation exists between both measurements ($r = 0.84$). This value does not change in the hyperoxic setting and therefore the clinician can be alerted by the monitoring to the presence of toxic products. Although we had detected some concentrations below 1 ppm of NO_2 (0.6 ppm) with the NO_2 Polytron, chemiluminescence is more sensitive for low NO_2 and NO concentrations.

Practical modalities of NO administration into the respiratory tract have not yet been clarified. Inhaled NO can be used in spontaneously breathing patients, by means of a Douglas bag [3]. In mechanically ventilated patients NO can be used either before the gas inlet [4, 6] or after gas outlet by means of a flowmeter or a sprayer connected after the ventilator [7, 8]. Whatever method is

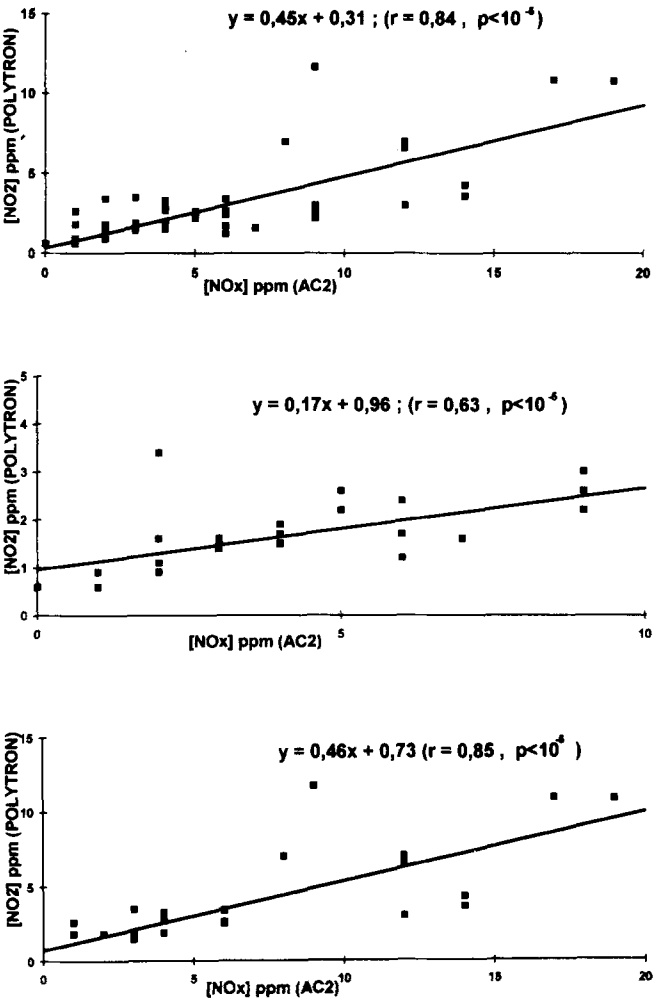

Fig. 3a Regression analysis for the relationship between [*NO*] measured by AC2 and [NO_2] measured by Polytron (y = 0.45 x +0.31; $r = 0.84$, $p < 10^{-5}$). **b** Regression analysis for the relationship between [NO] measured by AC2 and [NO_2] measured by Polytron under air (y = 0.17 x+0.96; $r = 0.63$, $p < 10^{-5}$). **c** Regression analysis for the relationship between [NO_x] measured by AC2 and [NO_2] measured by Polytron under oxygen (y = 0.46 x+0.73; $r = 0.85$, $p < 10^{-5}$)

used, NO and NO_2 should be measured for the following reasons:

– if oxygen is present inside the tank (i.e., a Douglas bag), NO concentration varies over time due to an oxidative reaction. This reaction increases NO_2 and other nitric oxide concentrations. The magnitude and rapidity with which this oxidation takes place are dependent upon O_2 concentration and contact time of NO with O_2 [9].

– NO can be administrated before the respirator [4, 6]. This process allows a good O_2/air/NO mixture and avoids variations in the final NO concentration and changes in minute ventilation. But it risks the production of higher NO_2 concentration by lengthening the contact time between O_2/NO and therefore the extent of oxidation. Although a soda lime adsorber can scavenge NO_2 from inspired gas [6], it is advisable to monitor NO/NO_2 concentrations.

– continuous injection or supply of NO downstream from the respirator is a simple way of administration. This process reduces the time for oxidation, however NO concentrations measured on both sides of the endotracheal tube are different from theoretical values depending especially on I/E ratio, respiratory rate and NO injection inflow [7]. The NO administration after Y piece and especially in the endotracheal catheter cannot be used because we cannot monitor a pre-inspired gas mixture, and could accidentally expose the patient to dangerous NO_2 levels.

Comparison of clinical trials using various amounts of NO would be facilitated if NO concentration at a precise site of measurement was known [10, 11].

In different studies concerning inhaled NO, the production of measured NO_2 was insignificant and without quantifiable adverse effects [1–4, 8]. However NO_2 production has to be taken into account, since a low concentration (0.3 ppm) can lead to respiratory [12] and immune system impairment [13]. In patients requiring exposure to NO over a long time, delayed toxicity is not excluded, since NO_2 tends to accumulate [14] and trigger nitrosylation reactions that are potentially carcinogenic [15]. In healthy subjects it is difficult to identify serious adverse effects from inhaling low levels of NO_2 [16]. On the other hand, during acute lung injury toxic oxygen concentrations [18] and high airway pressures are present [17]. Whether low levels of NO_2 in such clinical situations may be more dangerous is not known. In addition it could be suspected that an additive toxic effect on the alveolocapillary membrane from NO_2 exposure [19, 20] may be present during acute lung injury.

The emergence of inhaled NO within the therapeutic panoply is very promising. As with any new technology, the benefit/risk ratio has to be assessed. This assessment requires a reliable and reproducible standardized system of administration and of monitoring as well. The Polytrons system is a simple and ergonomic alternative to apparatus using chemiluminescence, the reference method in measuring nitric oxides. However, NO_2 measurements obtained via Polytrons may be insufficient to exclude potential toxicity of NO_2 due to the inability for measurements in the ppb-range.

References

1. Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WM (1991) Inhaled nitric oxide. A selective pulmonary vasodilatator reversing hypoxic pulmonary vasoconstriction. Circulation 83: 2038–2047
2. Fratacci MD, Frostell C, Chen TY, Wain JC, Robison DR, Zapol WM (1991) Inhaled nitric oxide. A selective pulmonary vasodilatator of heparin protamine vasoconstriction in sheep. Anesthesiology 75:990–998
3. Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Stone D, Wallwork J (1991) Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. Lancet 338: 1173–1174
4. Girard C, Lehot JJ, Pannetier JC, Filley S, Ffrench P, Estanove S (1992) Inhaled nitric oxyde after mitral valve replacement in patients with chronic pulmonary artery hypertension. Anesthesiology 77:880–883
5. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet I:307–310
6. Stenqvist O, Kjelltoft B, Lundin S (1993) Evaluation of a new system for ventilatory administration of nitric oxide. Acta Anaesthesiol Scand 37: 687–691
7. Moutafis M, Lawkoune JD, Fischler M (1992) NO_2 concentration during inhalation of NO in pigs. Anesthesiology 77:A1230
8. Rossaint R, Falke KJ, Lopez F, Slama K, Pison U, Zapol WM (1993) Inhaled nitric oxide for the adult respiratory distress syndrome. N Engl J Med 328: 399–405
9. Foubert L, Fleming B. Latimer R, Jonas M, Oduro A, Borland C, Higenbottam T (1992) Safety guidelines for use of nitric oxide. Lancet 339:1615–1616
10. Roberts DJ, Polaner DM, Lang P, Zapol WM (1992) Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. Lancet 340:818–819
11. Kinsella JP, Neish SR, Shaffer E, Arman SH (1992) Low-dose inhalational nitric oxide in persistent pulmonary hypertension of the newborn. Lancet 340:819–820
12. Morrow PE, Utell MJ, Bauer MA, Smeglin AM, Frampton MW, Cox C, Speers DM, Gibb FR (1992) Pulmonary performance of elderly normal subjects with chronic obstructive pulmonary disease exposed to 0.3 ppm nitrogen dioxide. Am Rev Respir Dis 145:291–300
13. Davis JK, Davidson MK, Schoeb TR, Lindsey JR (1992) Decreased intrapulmonary killing of Mycoplasmapulmonis after short-term exposure to NO_2 is associated with damaged alveolar macrophages. Am Rev Respir Dis 145:406–411
14. Goldstein E, Peek NF, Parks NJ, Hines HH, Steffey EP, Tarkington B (1977) Fate and distribution of inhaled nitrogen dioxide in rhesus monkeys. Am Rev Respit Dis 115:403–412
15. Stamler JS, Singel DJ, Loscalzo J (1992) Biochemistry of nitric oxide and its redox-activated form. Science 258: 1898–1902
16. Frampton MW, Morrow PE, Cox C, Gibb FR, Speers DM, Utell MJ (1991) Effects of nitrogen dioxide exposure on pulmonary function and airway reactivity in normal humans. Am Rev Respir Dis 143:522–527
17. Dreyfuss D, Basset G, Soler P, Saumon G (1985) Intermittent positive-pressure hyperventilation with high inflation pressures produces pulmonary microvascular injury in rats. Am Rev Respir Dis 132:880–884
18. Klein J (1990) Normobaric pulmonary oxgen toxicity. Anesth Analg 70: 195–207
19. Patel JM, Block ER (1986) Nitrogen dioxide-induced changes in cell membrane fluidity and function. Am Rev Respir Dis 134:1196–1202
20. Rasmussen TR, Kjaergaard SK, Tarp U, Pedersen OF (1992) Delayed effects of NO_2 exposure on alveolar permeability and glutathione peroxidase in healthy humans. Am Rev Respir Dis 145: 654–659