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Ayşe BAYSAL

Nitric Oxide I: Advances in the Measurements for Clinical Applications

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Department of Cardiovascular Surgery, Division of Cardiovascular Anesthesia, V.K.V. American Hospital, Nişantaşı, Istanbul - TURKEY

Abstract: Nitric oxide (NO) is a colorless gas with high affinity to ferrous hemoproteins. This simple molecule is a powerful messenger in the body. This study reviews a literature search using keywords and abstracts in English through Medline. Recent advances in the therapeutic uses of inhaled nitric oxide in lung diseases and other pathologic states urges the efficient and convenient use of nitric oxide in hospital practice. In addition, methods of inhaled nitric oxide delivery, chemiluminescence and electrochemical analyzers were reviewed. The performance characteristics of different equipment were compared. The novel methods of nitric oxide measurements that utilize simple and cheaper equipment were discussed. This study provides necessary information prior to a discussion on various clinical applications of inhaled nitric oxide for therapeutic uses.

Key Words: nitric oxide, inhaled nitric oxide measurements, electrochemical analyzer, chemiluminescence analyzer

Introduction

Nitric oxide (NO) is a multipotent endogenous messenger molecule that is extensively involved in the regulation of vascular tone. Gaseous nitric oxide was considered as an environmental pollutant for decades. Its properties for the dilation of blood vessels were identified by Furchgott and Zawadzki in 1980 (1). In the early 1990s nitric oxide's role in biological systems came to be appreciated. "Science" journal declared nitric oxide as the molecule of the year in 1992. The importance of this molecule was confirmed when in 1998 the Nobel Prize for medicine was awarded to L.J. Ignarro and two other pharmacologists: R. F. Furchgott and F. Murad. Our current understanding is that NO is a ubiquitous substance that not only plays a major role in vasodilation, but also affects the physiology of all organ systems. Excellent recent review articles on NO have been published (2,3). This review will emphasize the measurement methods of NO, including delivery systems of inhaled NO to patients. The adverse effects and toxicity of nitric oxide clinical applications will be reviewed in a subdivision of the subsequent study that will mainly discuss the therapeutic uses of nitric oxide.

The Nobel Prize committee cited Furchgott's 1980 experiment that showed a drug, acetylcholine, could widen blood vessels, but only if the layer of cells lining the vessel was intact.(1) From this, Furchgott concluded that those cells, the endothelium, must produce some signals that relaxed the smooth muscle cells in blood vessels. Furchgott called this signal "endothelium-derived relaxing signal," or EDRF. But it was Ignarro who concluded, after a brilliant series of analyses in 1986, that nitric oxide was the elusive EDRF (4). Murad was cited for showing that the gas could regulate important cellular functions (5).

Occurring throughout the body, nitric oxide performs a number of regulatory tasks, including battling infections, preventing the formation of blood clots, acting as a signal molecule in the nervous system and controlling blood flow to a number of organs. Ignarro discovered the principle that allowed pharmaceutical researchers to create the anti-impotency drug Viagra (6).

Materials and Methods

Medline databases were searched using the internet at the PubMed-National Library of Medicine (U.S.A.) medical database site. The keywords were searched for in the period from January 1, 1990 through December 29, 2000. The restrictions were English language and English abstracted articles. The literature was reviewed and the different methods of inhaled nitric oxide delivery techniques were compared.

Biology and Mechanism of Action: Nitric oxide is a colorless gas with high lipid and water solubility. It is produced endogenously in the endothelium from L-arginine by the enzyme NO synthase (NOS). L-arginine and oxygen serve as substrates for NOS to produce nitric oxide and L-citrulline (7). Nitric oxide has high binding affinity for iron and iron-containing compounds. The physiologic actions of NO are mediated by the activation of the soluble guanylate cyclase to form cyclic guanosine monophosphate (c-GMP), which causes vascular smooth muscle relaxation by decreasing the concentration of free calcium in the smooth muscle cytosol and resulting in vasodilatation (8). Once endogenous NO is released into the bloodstream, it reacts with iron in the hemoglobin. This leads to the conversion of hemoglobin to methemoglobin, which is converted to hemoglobin and nitrite or nitrate by methemoglobin reductase. The latter two products are nonvasoactive and excreted by the kidneys (9). The half–life of nitric oxide in vivo is 0.1 to 5 seconds and is limited by its concentration and the availability of oxygen, superoxide anion, protein, and hemoglobin. Nitric oxide has several pathways of metabolic inactivation. In the gas phase, nitric oxide mixed with oxygen results in the formation of nitrogen dioxide. In the liquid phase, nitric oxide interacts with superoxide anion radical (O_2^-) to produce peroxynitrite ($OONO^-$), which is cytotoxic. Peroxynitrite damages DNA, induces lipid peroxidation and reacts with proteins. It also reacts with the phenolic residues of tyrosine, resulting in the formation of nitrotyrosine (10).

Physiological Effects: Nitric oxide has a wide spectrum of physiological activity. Its formation in vascular endothelial cells, in response to chemical stimuli and to physical stimuli, such as shear stress, maintains a vasodilator tone that is essential for the regulation of blood flow and pressure. NO produces systemic and pulmonary vascular relaxation and controls cardiac output and the distribution of coronary, cerebral, and renal circulation (11). It plays a central role in the inflammatory cascade, inhibits leukocyte adhesion and modulates smooth muscle cell proliferation (12). Nitric oxide is involved in host defense and immunologic response. NO generation was first observed in activated macrophages, where it contributes to their cytotoxicity against tumor cells, bacteria, viruses or other invading organisms. Cytotoxic actions also result from its inhibitor actions on key enzymes in the respiratory chain. NO inhibits platelet adhesion and aggregation and prolongs bleeding time. In the central nervous system, NO functions as a neurotransmitter in many physiological functions, including the formation of memory, coordination between neuronal activity and blood flow and modulation of pain. It modulates carbon dioxide responsiveness of the cerebral vasculature (13). In the peripheral nervous system, in the nonadrenergic and noncholinergic neurons, nitric oxide relaxes smooth muscle cells of the gastrointestinal tract, bronchial tree and penile corpora cavernosa (14). NO exhibits both pro- and anti-inflammatory effects and is also involved in the modulation of ischemia/reperfusion tissue injury (15). It is implicated in myometrial relaxation in pregnancy and nitroglycerin therapy for tocolysis.

NO Measurement Methods and The Comparison of Different Methods for Safe and Accurate Inhaled Nitric Oxide (iNO) Delivery Systems: Nitric oxide measurements in human subjects can be divided into two subgroups:

1) Inhaled nitric oxide measurements: Inhaled NO (iNO) is a selective pulmonary vasodilator predominantly found in the vascular beds supplying ventilated alveoli; thereby improves ventilation/perfusion (V/Q) relationships and reduces venous admixture in the pulmonary vasculature, NO is rapidly absorbed by hemoglobin, neutrophils, and platelets, preventing any significant systemic vasodilatation. For delivery: A) Delivery system including ventilator,circuits with appropriate modules for iNO delivery. B) iNO measurement by chemiluminescence and electrochemical techniques.

A) Systems for delivery of NO: In this study, the review of Medline literature showed 138 cited abstracts about delivery systems in the English language. Various systems to administer inhaled nitric oxide have been used in patients and experimental animals. A lung model to evaluate five NO delivery systems during mechanical ventilation with various ventilatory patterns was prepared (16). Four different injection systems were mentioned; a) NO injected either into the inspiratory circuit 90 cm proximal to the Y piece, b) directly at the Y piece, c) delivered either continuously or d) only during the inspiratory phase (16). Alternatively, NO was mixed with air using a blender and delivered to the high-pressure air inlet of the ventilator. Nitric oxide concentration was measured from the inspiratory limb of the ventilator

circuit and the tracheal level using rapid- and slow-response chemiluminescence analyzers. The ventilator was set for constant-flow volume control ventilation, pressure control ventilation, pressure support ventilation, or synchronized intermittent mandatory ventilation. Tidal volumes of 0.5 L and 1 L were evaluated with inspiratory times of 1 s and 2 s. The system that premixed NO proximal to the ventilator was the only one that maintained constant NO delivery regardless of ventilatory pattern. The other systems delivered variable NO concentrations during pressure control ventilation and spontaneous breathing modes. Systems that injected a continuous flow of NO delivered peak NO concentrations greater than the calculated dose. These variations were not apparent when a slow-response chemiluminescence analyzer was used. NO delivery systems that inject NO at a constant rate, either continuously or during inspiration only, into the inspiratory limb of the ventilator circuit produce highly variable and unpredictable NO delivery when inspiratory flow is not constant. Such systems may deliver a very high NO concentration to the lungs, which is not accurately reflected by measurements performed by slow-response analyzers (17).

Continuous flow delivery of nitric oxide into the circuit of a phasic-flow ventilator results in marked inspiratory nitric oxide concentration fluctuation that is not detected by a slow-response chemiluminescence analyzer. Moreover, nitric oxide concentration fluctuation can influence the accuracy of the chemiluminescence measurements. These effects can be diminished by using additional mixing chambers to facilitate a stable gas concentration. As these mixing volumes increase, the contact time of nitric oxide with oxygen, and an increase of nitrogen dioxide, has to be taken into account (18,19). The application of NO close to the endotracheal tube is associated with a much faster response of the actual inspired NO concentration to dosing changes and shows the lowest NO_2 formation. In order to avoid toxic NO_2 concentrations, an upper limit of 40 ppm (parts per million) NO is recommended for continuous NO inhalation (20,21). The generation of NO_2 in the ventilator circuit is directly proportional to the concentration of NO and O_2 and inversely proportional to the TGF (total gas flow) and MV (minute ventilation) but uninfluenced by the TGF/MV ratio. NO at 80 ppm, but not 20 or 40 ppm in FiO_2 (fraction of inspired oxygen) of 0.6, generates toxic NO_2 irrespective of TGF, MV or TGF/MV ratios (22). To overcome the shortcomings, several techniques have been developed to synchronize the delivery of nitric oxide with inspiration, thereby decreasing the time that nitric oxide can react with oxygen and more closely matching nitric oxide delivery to patient flow demands. The reviewed techniques were the delivery through a nebulizer-type injection mechanism (23), the blending with air or nitrogen before delivery into the inspiratory port of the ventilator (24,25), and the injection through the digitally controlled nitric oxide intake valves (19).

The Ohmeda INOvent Equipment, Nitric Oxide delivery system (Datex-Ohmeda, Madison, WI, USA) is an integrated, single unit designed for the delivery and monitoring of inhaled nitric oxide (iNO). It uses an inspiratory flow sensor to inject a synchronized and proportional nitric oxide (NO) flow into the mechanical ventilator circuit, and was also reviewed in the literature (26,27). This system should deliver a constant NO concentration independent of ventilator mode, minute ventilation, fraction of inspired oxygen, or ventilator brand. It should also minimize NO_2 formation. NO delivery by the Ohmeda INOvent delivery system and a premixing NO delivery system were compared using two ventilators (Puritan-Bennett 7200 and Servo 900C). NO concentrations were measured within the trachea of an attached lung model using a fast-response chemiluminescence NO analyzer. NO concentrations were also measured in the inspiratory limb using the electrochemical analyzer of the Ohmeda INOvent. For three NO concentrations (2, 5 and 20 ppm), the ventilators were set for constant flow volume control ventilation, pressure control ventilation, and spontaneous breathing with pressure support ventilation or synchronized intermittent mandatory ventilation. Different tidal volumes (300, 500, 750 and 1,000 mL) and inspiratory times (1 and 2 s) were evaluated. NO2 formation for both ventilators and delivery systems were evaluated at 20 ppm and 95% FiO2. Regardless of ventilatory pattern, both systems delivered a constant NO concentration. The Ohmeda INOvent delivery system provides a constant NO concentration independent of the ventilatory pattern, and NO_2 formation is minimal (26,27).

Concentrations of nitric oxide and oxides of nitrogen should be monitored continuously during inhaled NO delivery. In breathing systems, nitrogen dioxide production is increased with high nitric oxide and FiO_2,

and with lower minute ventilation and total gas flow in the systems with higher internal volume (Siemens Servo 300; Siemens, Iselin, NJ, USA) (28).

2) Exhaled nitric oxide measurements: In human breath nitric oxide is produced mainly by the nasopharynx and sinuses (29). The pulmonary fraction of exhaled nitric oxide (eNO) comes chiefly from the alveolar epithelium and macrophages. The reference values for eNO were obtained in a study of 159 healthy children from Padua, Italy (30). The tidal breath method with a chemiluminescence analyzer was used for the measurements and no steady state levels were recorded. The mean concentration of endogenous NO in orally exhaled gas was 8.7 ppb (parts per billion) with no gender difference. The mean value of nasal NO concentration was 216 ppb. These levels were independent of age, gender and lung function and can be used to monitor airway inflammation in asthmatic children (30,31).

The presence of asthma (32) or the rejection of a transplanted lung (33) leads to an increase in pulmonary eNO. The clinical utility of eNO measurement as an aid in the assessment of asthma in the emergency department was studied. A total of 52 adult patients with acute asthma, 53 age and sex-matched controls and 8 patients with stable asthma were enrolled (32). A significant difference between the eNO levels of acute asthma patients and controls was observed. Some 23 of the 53 patients with acute asthma versus 2 of the controls had an eNO level higher than 15ppb. The eNO concentration correlated with FEV1% (forced expiratory volume in one second) and the blood eosinophil count in the group of 60 patients with acute and stable asthma. No relation between eNO levels and serum IgE, self-reported smoking or glucocorticoid use were reported. It is concluded that the measurement of eNO is a promising tool for assessing acute asthma (32-36).

B) iNO measurement by chemiluminescence and electrochemical techniques:

Currently, chemiluminescence analyzers constitute the analytical standard for monitoring concentrations of nitric oxide and other oxides of nitrogen in the clinical setting. The performance characteristics of several commercially available chemiluminescence analyzers were compared recently (17). Accurate analysis of NO in a continuous flow system was only possible with the most rapid response analyzer (270B NOA).

On the other hand, electrochemical detectors are cheaper, smaller, and sufficiently sensitive to monitor nitric oxide concentrations in the range between 5 ppm and 80 ppm (23). Different pressure and varying ranges of humidity influence the performance of electrochemical detectors and clinicians should be aware of this possible hazard. Electrochemical monitors use a simple electrochemical principle for the detection of NO. The monitored air passes through a plastic membrane into a liquid acid electrode. The electrolyte solution contains the sensing electrode (anode), a counter electrode (cathode) and a reference electrode. The voltage between the anode and cathode is kept constant and the current flowing through the sensor is proportional to the amount of reacting NO (37).

The Sensor Stik (Model 4586, Exidyne Instrumentation Technologies, Exton, Pennsylvania, USA) consists of a modular electrochemical sensor in a cylindrical tube with a liquid crystal display. The monitor weighs about 540 g without the energy supply and runs on a separate DC energy supply (at least 24 V). The sensor is capable of operating continuously for more than 12 months in the presence of 50 ppm NO (38). The electrochemical reaction at the electrode is as follows:

$$NO + 2H_2O \rightarrow HNO_3 + 3H^+ + 3e^-$$

The PAC II (Drager AG, Lubeck, Germany) is a compact personal monitor, which contains the sensor unit, the liquid crystal display and a power unit (9 volt battery). The weight of the complete monitor is 280 g. The electrochemical reaction at the electrode is as follows:

$$NO + H_2O \rightarrow NO_3 + 2H^+ + 2e^-$$

The PAC II is ready for operation a few seconds following turning it on. No time consuming calibration is necessary. Although both NO monitors provided an accurate and reproducible method for measuring gaseous NO, the Sensor Stik could not detect rapid decreases in NO levels; therefore it is not recommended for clinical use (38). Measurements of NO regression analysis showed a close relationship between the two analyzers (38). Electrochemical devices are more likely to be used in the clinical setting because of their smaller size and quiet operation (38).

One of the new electrochemical monitors was developed by Carter et al. (39). (Printer NO; Micro Medical Limited, Chatham, Kent, England. Also sold as

Sensor NO; Sensormedics Corporation, California, USA.). Printer NO was compared to a chemiluminescence analyzer (42 H Thermo Environmental Instruments Inc., Franklin, Massachusetts, USA). The printer NO is small and is easy to use and to calibrate. It has sufficient accuracy to be of clinical use in the administration of NO (39).

A comparison of electrochemical detector (ECD) versus chemiluminescence detector (CLD) monitoring techniques showed agreement between the instruments within approximately 2 ppm, with the ECD averaging a higher reading than the calculated or CLD measured values. A 2 ppm discrepancy between the instruments is considered to be clinically acceptable. The instruments could be used interchangeably for clinical purposes to measure NO, and the ECD was preferable to the CLD for measuring NO_2 (40).

A micro carbon electrode for monitoring NO: This new NO probe is a combination of a micro carbon fibre working electrode (10 micron diameter), a platinum counter electrode and a silver/silver chloride (Ag/AgCl) reference electrode. The carbon fibre working electrode is covered with a Nafion cation exchange membrane (41). The NO to N_2O reduction current peak is about 1.35 V against the Ag/AgCl electrode. The current outputs are linearly related to dissolved NO concentrations in the 2-10 micromolar range. The Nafion membrane prevents interference by NO and amino acids at physiological pH (pH: 7.4). To improve sensitivity, pulse amperometry was used. Charge outputs are linear to the dissolved NO in the 50-350 nanomolar range. The carbon fiber electrode has the potential of being miniaturized to a smaller electrode, which may enable the detection of NO released from the subendothelial space (41).

On-line recording of NO in breath by laser magnetic resonance: Laser magnetic resonance spectroscopy is a sensitive and isotope-selective technique for determining low concentrations of gaseous free radicals with high time resolution. This technique is used to analyze the NO concentration profile while simultaneously measuring the flow and expired volume during several single breathing cycles (42).

Conclusion

Over the last ten years, significant discoveries about the biology and physiology of nitric oxide were presented to the scientific world. These findings encouraged clinicians to demonstrate the beneficial effects of nitric oxide on their patients. Nitric oxide is a selective pulmonary vasodilator with minimal adverse effects. Precise dosing and careful monitoring would help in the conduct and validation of randomized trials. The standard method is chemiluminescence analysis. Electrochemical systems were recently reported in the literature. They are relatively inexpensive and are regularly used for personal or industrial NO monitoring. As nitric oxide gains wider use in hospitals, clinicians must be familiar with its pharmacophysiology, monitoring and delivery systems. Its various clinical applications and side effects, to ensure safe and effective therapy, will be discussed in a subsequent study.

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