Nitric oxide and the respiratory system

G. Himashree*, Deepak Dass, P. K. Banerjee and W. Selvamurthy

Defence Institute of Physiology and Allied Sciences, Defence Research and Development Organization, Lucknow Road, Timarpur, Delhi 110 054, India

Nitric oxide (NO) is a significant biologically active molecule; its role in controlling cellular and organ functions, especially the respiratory, cardiovascular, nervous and immune system, has been well established. In the respiratory system, this molecule is responsible for maintaining pulmonary vascular integrity. It improves arterial oxygenation, which may be associated with its action on the distribution of blood flow in the lungs. This property is the basis for inhaled nitric oxide (INO) being employed in the treatment of high altitude pulmonary edema (HAPE), acute respiratory distress syndrome and persistent pulmonary hypertension of the newborn. The combined use of NO and oxygen has cumulative effect on the pulmonary haemodynamics and gas exchange. Soldiers of the Indian army deployed at high altitude are prone to develop acute mountain sickness and HAPE. Inhalation of NO is preferred for the treatment of these patients. Clinical application of INO has been frequently tempered by difficulties in safe and accurate INO delivery. NO inhalation has a number of short-term and long-term conflicting effects, and is still at the experimental stage. The fundamental outline of a delivery system should be able to provide for safe gas delivery and accurate gas analysis or monitoring. Clinical interest in the identification of exhaled nitric oxide (ENO) as a marker of diseases is mounting, notably with reference to inflammatory airway diseases. Further studies and standardization of ENO testing are needed to turn these findings into a reliable diagnostic tool.

Nitric oxide – Physiological perspective

Nitric oxide in our body

NITRIC oxide (NO) is endogenously produced within the vascular endothelium and functions as a natural vasodilator (Figure 1). Its discovery led to the awarding of the Nobel Prize in medicine to Furchgott, Ignarro and Murad in 1998. They identified NO as a signal molecule in the nervous system, as a weapon against infection and as a modulator of blood pressure. NO, one of the smallest (30 kDa) biosynthetic products, is made by enzymes that are among the largest (150 kDa) and most complicated. NO has emerged as an important signal and effector molecule in the immune, nervous and cardiovascular systems. Three isoforms of NO synthetase (NOS) have been characterized. These generate NO from l-arginine in a stepwise monooxygenase reaction that, in turn, generates *N*-hydroxy-l-arginine as an intermediate. The mammalian systems in which NO synthesis was first demonstrated were the vascular endothelium, the brain and activated macrophages. The three isoforms of the NOS enzyme have been characterized, purified and referred to as neuronal (nNOS), inductible (iNOS) and endothelial (eNOS). The NO stimulates intracellular cyclic guanine monophosphate which causes vascular smooth-muscle relaxation (Figure 2). This vasodilatation is terminated by the action of phosphodiesterases (PDEs) that degrade the guanine monophosphate. PDE-5 is found in high concentration in the lung and is also a powerful degrader of guanine monophosphate in vascular smooth muscle. Milrinone, which is a PDE-3 inhibitor, thus enhances the action of endogenous NO leading to pulmonary, as well as systemic vasodilatation.

Fate of inhaled NO

After NO is inhaled and passes through the lungs into the blood stream of a patient, it is soon deactivated (Figure 3).

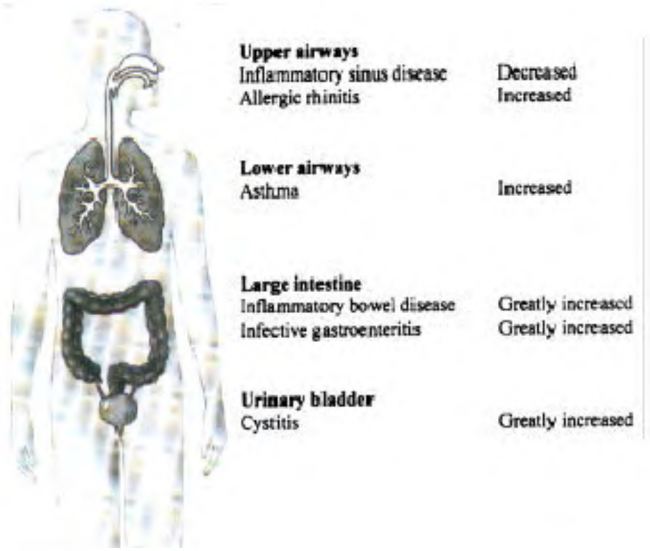

Figure 1. Various locations where airborne NO has been measured in health and disease.

*For correspondence. (e-mail: himashree@yahoo.com)

This is because NO quickly reacts with the iron-containing pigment (haemoglobin) of the red blood cell that functions to transport oxygen from the lungs to the tissues. Haemoglobin inactivates NO and hence, when it is carried to the rest of the body, it does not cause vasodilatation beyond the lung area. This is in sharp contrast to some of the other pulmonary vasodilator drugs that not only cause vasodilatation of blood vessels in and around the lungs, but also cause vasodilatation throughout the body. This can potentially lead to a serious decrease in the blood pressure of a patient. In the lungs and the upper airways, air-filled spaces coexist with highly perfused tissues. The alveolar surface acts as a medium for exchange of molecules such as oxygen and carbon dioxide, depending on their solubility in hydrophilic and lipophilic phases, and on their ability to bind to plasmatic or cellular components (e.g. haemoglobin). Exogenous NO, inhaled for the treatment of acute respiratory distress syndrome (ARDS) or pulmonary hypertension, causes vasodilatation of the pulmonary vasculature, and then binds to haemoglobin. Part of the endogenous NO synthesized in cells of the upper and lower airways is released in the gas phase and then exhaled, like CO_2.

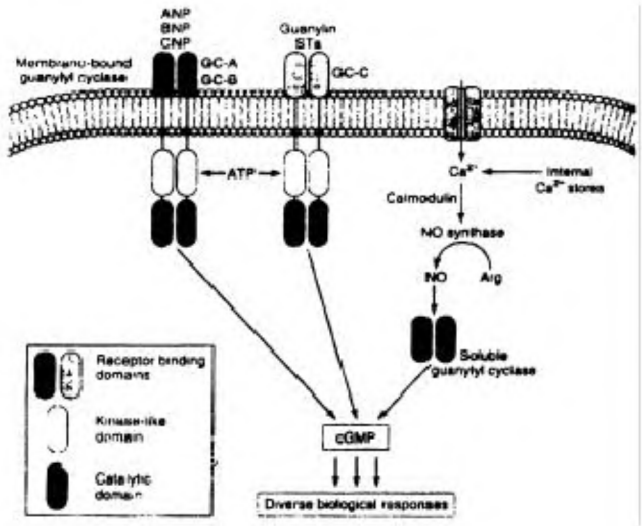

Figure 2. Mechanism of biological action of NO in tissues.

In recent years, scientific and clinical interest has focused on the identification and evaluation of exhaled NO (ENO) as a marker for several diseases. The discovery of vasodilatory effect of NO led to the possible role of inhaled NO (INO) as a means of treating pulmonary hypertension. Its brief duration of action limits its effects to the pulmonary vasculature and only to those vessels perfusing ventilated alveoli. Therefore, it can improve oxygenation of the blood by increasing the perfusion of the ventilated alveoli as well as reducing pulmonary vascular resistance. The role of INO in treating primary pulmonary hypertension of the newborn has been well established[1–4] (Figure 4).

Chemical properties of NO

NO is known as a common environmental pollutant and contaminant during the manufacturing process of nitrous oxide. NO is normally manufactured from the reaction of sulphur dioxide with nitric acid. NO is a component

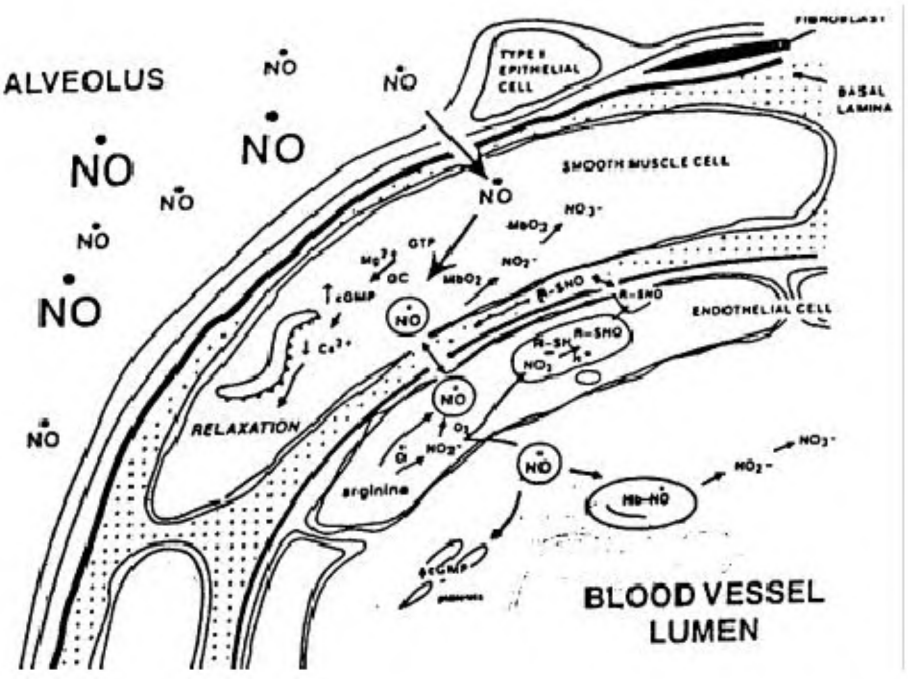

Figure 3. Fate of inhaled NO.

of smog that can be measured in the atmospheric air in urban areas at 10 to 100 parts per billion (ppb). NO being poorly soluble in water, when injected into a physiologic buffer, more than 80% immediately appears in the head space. The formation of NO_2 from the reaction of NO and O_2 depends on the concentration of the NO and O_2. NO gas is fairly stable at low concentration in room air. The small size (molecular weight 30 Da) and high lipid solubility of NO makes it readily membrane-permeable. Once within the cell, it can bind to transition metals such as Fe and Cu in enzymes. In blood, NO rapidly binds to albumin and haemoglobin[5].

High-altitude pulmonary edema and INO

Vigorous young men are most susceptible to high-altitude pulmonary edema (HAPE). Other risk factors include strenuous exercise, cold weather and recent respiratory-tract infection. The symptoms and signs are cough, tachypnea, tachycardia, orthopnea, cyanosis, rales, and frothy pink sputum. Symptoms usually begin two to four days after a rapid ascent, often during night-time rest. Without the administration of oxygen, rest, and descent to a lower altitude, death may result. Radiographic findings are variable but usually consist of fluffy, perihilar infiltrates with sparing of the lung bases and periphery[6]. Lung-perfusion scans obtained before and after the inhalation of NO showed that the gas diverted the pulmonary blood flow from edematous regions of the lung to nonedematous regions in patients of HAPE. It is likely that the INO had a greater vasodilating effect in the nonedematous regions of the lung, where it could more easily enter the alveoli. In contrast, no such redistribution of perfusion in response to NO was seen in subjects resistant to HAPE. Thus, exaggerated hypoxic pulmonary vasoconstriction resulting in ventilation–perfusion mismatch appears to be an important mechanism in the pathophysiology of HAPE[7]. Inhalation of NO is beneficial in the treatment of neonatal pulmonary hypertension, but its effect on HAPE has not been reported earlier[8]. It has also been suggested that HAPE develops due to stress failure in capillaries[9] and over-distended, relatively thin-walled pulmonary arteries[10,11].

NO exerts its effect on the pulmonary vascular bed at the following two sites: (a) muscular pulmonary arterial vessels and (b) capillary bed. Patients of HAPE probably have a defect in the NO-mediated vasodilatation and the capillary-bed permeability. A defect in NO synthesis may be the causative factor for increased leaking of water, proteins and cells rather than due to capillary rupture[12]. It has also been seen that low concentration NO inhalation is more effective in treating HAPE than the prevailing conventional treatment[13].

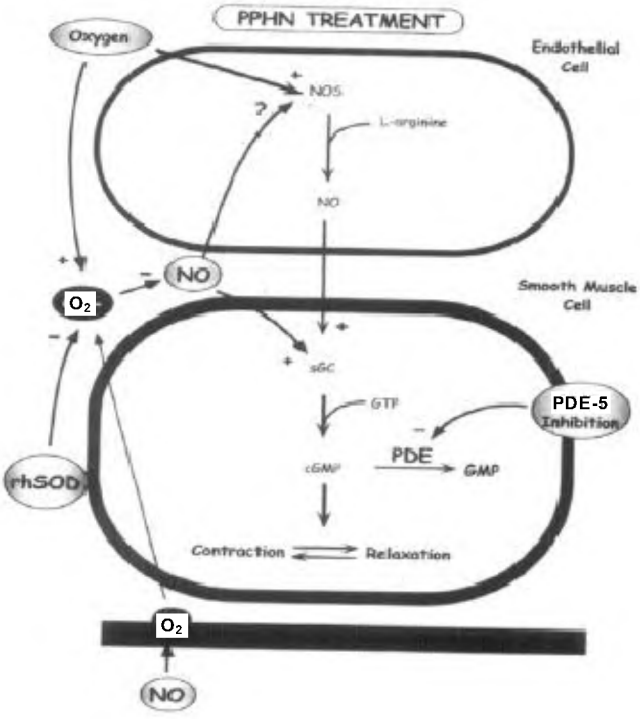

Figure 4. Potential points of N-cGMP signalling pathway that may allow therapeutic intervention and enhancement of effect in persistent pulmonary hypertension of the newborn.

Indian scenario

Presently, the Indian army is deployed at heights up to 6700 m (22,000 ft). The incidence of high altitude (HA) maladies is on the rise. Presently, the incidence of HAPE is 0.3 to 0.4%, but because of the increasing number of troops being deployed under the prevailing geopolitical situation, the total number of patients has increased almost threefold. Currently, the treatment modality of HAPE is aimed at immediate relief of symptoms and evacuation of a patient to a lower altitude. This includes oxygen administration, disprin, diamox, recompression in a pressurized chamber and de-induction to lower altitude. However, INO therapy would reduce the morbidity and mortality of HAPE and also help the organization to re-induct the acclimatized and trained manpower back to the place of duty, thereby saving wastage of trained manpower. INO is a selective pulmonary vasodilator for which the mechanism of action involves guanylyl cyclase activation leading to production of cyclic guanosine monophosphate and subsequent smooth-muscle relaxation[1,3,4]. Several studies have suggested that INO improves oxygenation[14–20] and revealed the efficacy of inhaled NO therapy for HAPE. However, most of these are pilot-studies which necessitate the investigation of this modality of treatment in further detail[12,13,21,22].

A significant study by Dellinger *et al.*[23] assessed the safety, physiological and clinical effects of various doses of NO over a 28-day period. An acute response to NO

was defined as a 20% increase in P_aO_2 in the first 4 h. The primary long-term outcome of this group was indicated by the number of days these patients could stay without the ventilator in this 28-day period of observation. A longer period off the ventilator was considered to be a better outcome. Twenty-four per cent of patients in the placebo group had showed a 20% increase in P_aO_2 in the first 4 h, compared to 60% of patients receiving NO. Mortality in the placebo and NO groups was 30% (ref. 23).

Virtually every study found that inhaled NO (i) induced a redistribution of blood flow to those areas of lungs which were well ventilated; (ii) reduced the blood pressure in pulmonary circulation in the arteries and (iii) improved oxygen levels in the blood. INO is a selective pulmonary vasodilator in patients with pulmonary hypertension which causes an increase in right ventricular load, and in case severe or an acute increase in pressure occurs, it may result in right ventricular failure. The successful role of INO in weaning patients off cardiopulmonary bypass, when they had developed acute elevations in pulmonary artery pressure, has been well reported.

NO accounts for many of the physiological effects of vasodilating drugs such as nitroglycerine; a drug commonly used to treat high blood pressure. Recent studies have found that excess NO production in the body plays a role in the massive vasodilatation and low BP associated with septic shock syndrome. In this disorder, there is Gram-negative bacterial infection and the endotoxin released by the bacteria causes a severe degree of vasodilatation, known to be mediated by NO. Since NO exists in gaseous form, it can be administered to the pulmonary vessels as an inhaled gas, and inhaled NO selectively dilates blood vessels in only those lung segments that are actively participating in gas exchange (oxygen and carbon dioxide) at the alveolar–capillary level. In other words, this increases the blood flow to areas of the lung where oxygen is being provided and thus improves oxygen levels in the body. This is known as ventilation–perfusion (V/Q) matching.

Recent advances in NO and HAPE

NO was not the first agent discovered that causes pulmonary vasodilatation. There are several other drugs that are known vasodilators which have been in the market for several years. These include the aforementioned nitroglycerine and nitroprusside. The shortcoming of these types of drugs is that they increase the pulmonary blood flow to all lung segments, including those that are not well-ventilated. This further inhibits oxygen delivery to the blood stream because the capillaries which are in contact with the alveoli are dilated, and do not provide or contain oxygen[21,24]. INO improves survival in the rat model of HAPE[21]. Alveolar haemorrhages may occur in patients on INO. Several studies have suggested that INO improves oxygenation[4,14,16–20,22,25,26].

More insight into the pathophysiology of HAPE has been provided by Sartori *et al.*[27] and Bärtsch[28]. In these studies, the role of trans epithelial sodium transport and subsequently water clearance has been ascertained to have a significant role in the progression of HAPE. Inhaled Salmeterol has been cited as being beneficial in HAPE, affronting the mechanism of development of HAPE. Also, the role of venoconstriction in the pathobiology of HAPE has been highlighted. The hallmark of HAPE is an exaggerated hypoxic pulmonary vasoconstriction. Pulmonary hypertension is crucial for the development of HAPE[29–31]. Initial symptoms of dyspnea, cough, weakness and chest tightness appear usually within 1–3 days after the arrival to HA. Common physical signs are tachypnea, tachycardia, rales and cyanosis. The treatment of choice is immediate descent. When this is impossible and supplemental oxygen is not available, current treatment with Nifedipine is recommended until descent is possible[32–34]. Although Ca^{++} blockers are currently in use for prophylaxis and treatment of HAPE, INO is a promising treatment modality[35].

NO and genetic markers

A defect of NO synthesis in the lungs in the case of HAPE has been suggested to contribute to its exaggerated pulmonary hypertension. Several polymorphisms have been identified in the gene encoding eNOS, which is a key enzyme responsible for NO synthesis, some of which were reported to be associated with vascular disorders. Both polymorphisms of the eNOS gene were significantly associated with HAPE. A genetic background may underlie the impaired NO synthesis in the pulmonary circulation of HAPE-susceptible subjects. These polymorphisms could be genetic markers for predicting the susceptibility of HAPE[36].

Adverse effects of INO

The adverse effects of NO at higher concentrations are now known to a certain extent[5,37,38]. However, more information is required for the accepted protocol of 15 ppm NO and 50% oxygen in our ethnic population. After studying these adverse effects, there may be certain modifications recommended in the present treatment schedule.

NO is an antioxidant, and in high doses its prooxidant effects are more pronounced. NO inhalation has a number of short-term and long-term potential adverse effects, and is still at the experimental stage in premature infants. Consequently, there is a need for further clinical studies monitoring the long-term consequences of this therapy. NO acts as a second messenger activating a number of cytokines, inducing apoptosis, thus suggesting a close relationship between NO, oxidative stress, regulation of growth and inflammation[39]. The potential side effects of

NO may lead to systemic hypotension and methaemoglobinemia[7]; abnormal liver enzymes; myopathy and agitation; lung haemorrhage and coagulation disorder; and renal dysfunction[23]. One of the potential adverse side effects for patients who receive NO therapy is the formation of methaemoglobin. Methaemoglobin is haemoglobin that cannot release the oxygen that it is carrying, nor can it combine with more oxygen. Therefore, it impairs the ability of blood to deliver oxygen to the tissues. This is a rare complication because the body contains certain chemicals and enzymes that convert methaemoglobin back to haemoglobin. Nevertheless, blood levels should be closely monitored.

Infants who receive INO therapy should be monitored according to institutionally-derived protocols designed to avoid the potential toxic effects associated with INO administration. These effects include methaemoglobinemia (secondary to excess NO concentration), direct pulmonary injury (attributable to excess levels of nitrogen dioxide), and ambient air contamination[40,41]. The normal level of methaemoglobin is 1.7% (ref. 42). NO is believed to help regulate muscle tone in the arteries of the lungs, but it could also cause excessive bleeding[43]. NO also has a role to play in inhibition of platelet aggregation. As a result, patients on INO show a tendency for prolonged bleeding and a prolongation in bleeding time, when tested[44–46]. There are many other clinical applications of INO[47–50]. INO reduces the extent to which extracorporeal membrane oxygenation is needed in neonates with hypoxemic respiratory failure and pulmonary hypertension[47]. Inhaled NO is effective in improving oxygenation in patients with ARDS[48]. NO merits further study and may represent a life-saving therapy in this select patient population[49].

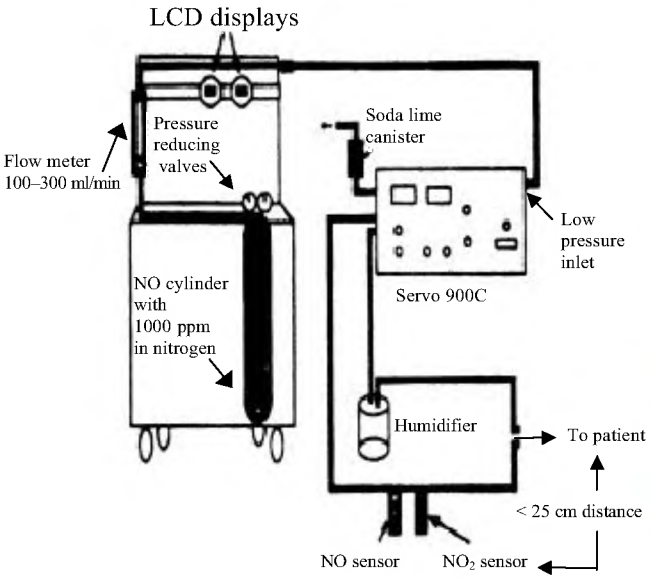

Figure 5. Scheme for monitoring and delivering inhaled NO.

Gas delivery systems

Soldiers deployed at HA are prone to develop AMS, HAPE and other diseases due to malacclimatization (Figure 5). Inhaled NO is the choice of treatment in patients with HAPE. Patients suffering from HAPE are spontaneously breathing patients, whereas commercially available systems work in conjunction with a ventilator[12,26] (Figure 6). Hence there is a need for an indigenous delivery system, especially for patients with HAPE. The attempt to design and develop an indigenous delivery system will enable us to deliver NO at the earliest to patients and reduce the morbidity and mortality in Indian soldiers deployed at HA.

The basic design and goal of a delivery system is to provide a system for safe gas delivery and precision gas analysis. While delivering the gas through a ventilator, either a continuous or intermittent flow of NO is fed into the inspiratory limb of the ventilator tubing. The rate of NO gas flow is controlled to maintain the desired concentration of NO. The concentration of NO, which is being delivered to the patient, is constantly monitored by a sensor which is placed in the ventilator tubing close to the patient. This sensor or sample line is connected to an analyser that displays NO, NO_2 (discussed in further detail later) and possibly oxygen levels. Usually, the displayed NO and NO_2 readings are measured in parts per million (ppm).

Safety concerns

As with any drug, there are legitimate safety and toxicity concerns regarding the use of inhaled NO. Inhaling high levels of NO (5000 to 20,000 ppm) can be lethal, causing a severe and acute accumulation of fluid in the lungs (pulmonary edema) and methaemoglobinemia. However, there is little evidence of such toxicity when the concentration is kept in the normal range (1 to 80 ppm). Animals have breathed the gas in concentrations of 10 to 40 ppm, for six days to six months, without evidence of toxicity. Virtually all patients receiving NO will also receive O_2.

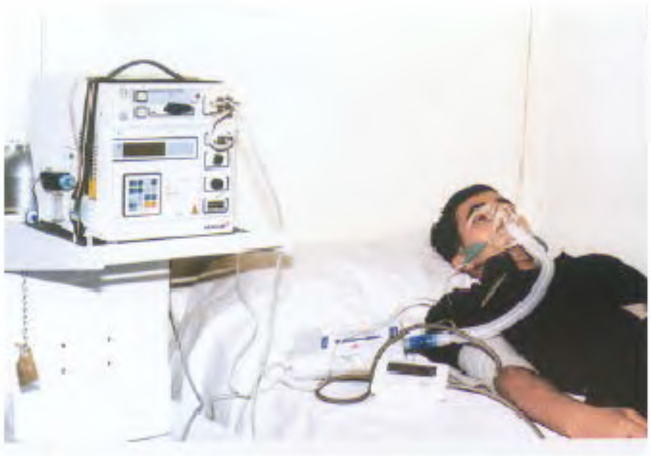

Figure 6. Patient of HAPE being treated with NO.

ARDS patients usually require high levels of O_2. The by-product of NO and O_2 yields nitrogen dioxide (NO_2), a highly toxic gas. Although Occupational Safety and Health Administration (OSHA) has set the safety limit for NO_2 at 5 ppm, some investigators have found that prolonged exposure to even 2 ppm of NO_2 can be injurious to the lungs. The amount of NO_2 produced is dependent upon the levels of NO and O_2, and the duration for which they are mixed together prior to inhalation. Therefore, the lowest dose of NO and lowest concentration of O_2 that achieve the desired effect are used. NO is usually fed into the ventilator tubing as close to the patient as possible, limiting the mixing time between O_2 and NO. All delivery systems monitor NO_2 levels continuously. Newer delivery systems have been designed to limit NO_2 production or inhibit its delivery to the patient; but situations may occur where the NO dose, the O_2 concentration, or both, may have to be reduced.

Dedicated NO-equipped ventilators are now available commercially, but are not yet common in clinical practice. With other ventilators, there is no standardized procedure for the administration or monitoring of NO. The use of NO in conjunction with a simple time-cycled, pressure-regulated, flow-generating ventilator attached to a model infant-sized lung has been described. In this model the ventilator generates a stream of air, which acts as a vehicle to carry the predetermined dose of NO to the lung model. It is recommended that, when used as therapy, NO levels in inspired gases should always be measured[50]. It is also suggested that all compressed air methods using tap water have charcoal filters at the compression site and the gases be assessed periodically for oxidants[51].

ENO as a marker of disease

HAPE is a life-threatening condition occurring in predisposed subjects at altitudes above 2500 m. It is not clear whether, in addition to haemodynamic factors and defective alveolar fluid clearance, inflammation plays a pathogenic role in HAPE, which is not preceded by airway inflammation. Low levels of ENO may be related to altered pulmonary NO synthesis and/or transport and clearance. In HAPE-prone subjects, a defect in pulmonary epithelial NO synthesis may contribute to exaggerated hypoxic pulmonary vasoconstriction, leading to pulmonary edema. NO is naturally produced in the body in the upper and lower airways at 100 to 1000 ppb, and is present in cigarette smoke at 400 to 1000 ppm. Clinical research has found that the concentration of ENO is increased during exercise and in patients with asthma[52]. ENO correlates with several variables of airway inflammation and may be useful in asthma therapy.

An exaggerated hypoxic pulmonary vasoconstriction is associated with the development of HAPE. Susceptibility to HAPE may be related to decreased production of NO, an endogenous modulator of pulmonary vascular resistance, and that a decrease in ENO could be detected during hypoxic exposure. There is decreased pulmonary NO production in HAPE-susceptible subjects during acute hypoxia that may contribute, among other factors, to their enhanced pulmonary vascular response[26,53]. In HAPE patients, a decline in pulmonary epithelial NO synthesis may contribute to exaggerated hypoxic pulmonary vasoconstriction and in turn to pulmonary edema[54]. Everyone exhales small amounts of NO, but it seems that asthmatics produce more of this gas by-product. Smooth muscle cells make up the muscle fibres that surround the small airways of the lungs, and neutrophils have also been associated with asthma. It has been found that during an attack, asthmatics produce additional amounts of ENO in the lower airways. This correlation of ENO and asthma has also been confirmed in children with asthma. This finding is especially important considering the difficulty in diagnosing asthma in very young children. ENO production is directly related to airway inflammation[55–58]. HA natives have a higher level of ENO. The advantage of high concentration of NO in the lungs seems to offset ambient hypoxia by enhancing the uptake of oxygen from the lungs, which presumably improves delivery of oxygen to the peripheral tissues[59].

Analysis of ENO

NO has an unpaired electron and is therefore a radical. Although not as unstable as many other radicals, NO does react readily with oxygen, and has a short half-life. NO is mainly measured by indirect techniques, with the exception of in-line chemiluminescence. Since NO is an endothelium-derived relaxing factor (EDRF) and it inhibits platelet aggregation, both vascular tissue and platelets can be used as indicators of NO production by other cells. Haemoglobin and other haem proteins (which absorb NO to form paramagnetic detectable nitroso-haem products) and superoxide dismutase (which prolongs NO action by preventing its breakdown to other nitrogen oxides) are then used to modulate pharmacological effects. Another indirect method of measurement is the spectrophotometric determination of nitrite and nitrate (two nitrogen oxides rapidly formed during NO oxidation). There is now considerable evidence that the effects of NO in some physiological processes are mediated through the activation of guanylate cyclase, resulting in an increased level of cyclic guanylate monophosphate (cGMP) in the target cell. Therefore, cGMP accumulation in cultured cells is frequently used as a model to study the ability of cells to produce NO. A class of L-arginine analogues is known to inhibit NO synthetase in either a competitive or irreversible way, and are therefore useful for exploring the role of L-arginine-dependent NO production. Recent observations suggest that this metabolic pathway is present in different pulmonary cell types, and that it might play a role in various regulatory mechanisms in the airways and

lung tissues[48]. ENO, which requires a chemiluminiscence analyser, is now known to be a sensitive index for certain diseases like asthma, and other inflammatory diseases of the airways. It is not clearly known if ENO can predict the systemic NO status as well, alone, or in combination with blood and/or urinary nitrates and nitrites[60–64].

Whether ENO predicts the acclimatization efficiency, susceptibility and prognosis to HA malady has to be studied; and if it can, we have a simple screening tool for assessing the fitness of Indian troops for HA deployment. Future studies should include the effect of some known NOS-inducing drugs, like nitrates, L-Arginine and Sildenafil on the exhaled NO patterns and predict their probable utility in ameliorating or preventing HA maladies and other clinical conditions marked by pulmonary vasoconstriction.

NOS-inducing drugs

Currently available intravenous pulmonary vasodilators such as epoprostenol, milrinone or nitroglycerine also cause systemic vasodilatation. Therefore, the resulting systemic hypotension limits the efficacy of these agents. This is especially pertinent for patients with cardiac diseases, where coronary perfusion may be adversely affected by a decline in perfusion pressure. Increase in pulmonary vascular resistance can greatly complicate the post-operative recovery of patients who have undergone cardiac surgery[2]. Milrinone is a PDE-3 inhibitor which enhances the effect of endogenous NO leading to pulmonary and systemic vasodilatation. Sildenafil (Viagra, Pfizer Laboratories, NY) is a selective inhibitor of PDE-5; the iso-enzyme that hydrolyses guanine monophosphate in the corpus cavernosum, thereby potentiating the effect of locally produced NO and maintaining vasodilatation. It has been hypothesized that because of the high concentrations of PDE-5 in the lung, Sildenafil might also potentiate pulmonary vasodilatation[2]. Several case reports now exist suggesting that Sildenafil is effective in ameliorating rebound pulmonary hypertension following the withdrawal of INO in children after congenital heart surgery, and in facilitating weaning of INO and Milrinone in a teenager following placement of a biventricular assist device. Sildenafil may be an effective oral substitute for INO. Sildenafil, at a daily dose of 500 mg in divided doses, significantly reduced pulmonary artery pressure[2,65].

Conclusion

NO is gaining a lot of significance in modern medicine, especially with reference to pulmonary physiology and maladies of the respiratory system. It is one of the simplest biomolecules synthesized and regulated by a complex enzyme NOS. Its vital role in maintaining pulmonary vascular integrity has been firmly established. INO is now being considered as the cornerstone in treatment of clinical conditions which involve pulmonary hypertension. Studies are being conducted with accent on INO as a treatment modality of HAPE. However, INO treatment is constrained with a few known adverse effects. This makes it mandatory to have a safe and reliable delivery system that delivers a prescribed dose with minimal NO_2 formation. ENO is a useful non-invasive indicator of NO levels of the pulmonary vasculature. Its role in inflammatory airway diseases is being widely studied. ENO will emerge as a useful indicator of HA tolerance and acclimatization.

1. Ignarro, I. J., Buga, G. M., Wood, K. S., Byrns, R. E. and Chaudhuri, G., Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 9265–9269.
2. Michelakis, E., Tymchak, W., Lien, D., Webster, L., Hashimoto, K. and Archer, S., Oral sildenafil is an effective and specific pulmonary vasodilator in patients with pulmonary arterial hypertension: comparison with inhaled nitric oxide. *Circulation*, 2002, **105**, 2398–2403.
3. Palmer, R. M., Ferrige, A. G. and Moncada, S., Nitric oxide release accounts for the biologic activity of endothelium-derived relaxing factor. *Nature*, 1987, **327**, 524–526.
4. Frostell, C., Fratacci, M. D., Wain, J. C., Jones, R. and Zapol, W. M., Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation*, 1991, **83**, 2038–2047.
5. Anne Greenough, Nitric oxide – Clinical Aspects. *Care Crit. Ill*, 1995, **11**, 143–146.
6. Ward, M. P., Milledge, J. W. and West, J. B. (eds), *High Altitude Medicine and Physiology*, Chapman & Hall, London, 1989.
7. Jerome and Severinghaus, J. W., *High altitude pulmonary edema. N. Engl. J. Med.*, 1996, **334**, 662.
8. Frostell, C. G., Blomqvist, H., Hedenstierna, G., Lundberg, J. and Zapol, W. M., Inhaled nitric oxide selectively reverses human hypoxic pulmonary vasoconstriction without causing systemic vasodilation. *Anesthesiology*, 1993, **78**, 427–435.
9. West, J. B., Tsukimoto, K., Mathieu-Costello, O. and Prediletto, R., Stress failure in pulmonary capillaries. *J. Appl. Physiol.*, 1991, **70**, 1731–1742.
10. Whayne, T. F. Jr. and Severinghaus, J. W., Experimental hypoxic pulmonary edema in the rat. *J. Appl. Physiol.*, 1968, **25**, 729–772.
11. Severinghaus, J. W., Transarterial leakage: a possible mechanism of high altitude pulmonary oedema. In *High Altitude Physiology: Cardiac and Respiratory Aspects* (eds Porter, R. and Knight, J.), Churchill Livingstone, London, 1971, pp. 61–77.
12. Scherrer, U. *et al.*, Inhaled nitric oxide for high-altitude pulmonary edema. *N. Engl. J. Med.*, 1996, **334**, 624–629.
13. Wang, W., Zhang, X. and Ma, Y., Low-concentration nitrous oxide inhalation in the treatment of high-altitude pulmonary edema. *Zhonghua Jie He He Hu Xi Za Zhi*, 1998, **21**, 212–214.
14. Day, R. W., Lynch, J. M., White, K. S. and Ward, R. M., Acute response to inhaled nitric oxide in newborns in respiratory failure and pulmonary hypertension. *Pediatrics*, 1996, **98**, 698–705.
15. Barefield, E., Karle, V. A., Phillips, J. B. III and Carlo, W. A., Inhaled nitric oxide in term infants with hypoxemic respiratory failure. *J. Pediatr.*, 1996, **129**, 279–286.
16. Roberts, J. D. Jr. *et al.*, Inhaled nitric oxide and persistent pulmonary hypertension of the newborn. *N. Engl. J. Med.*, 1997, **336**, 605–610.
17. Kinsella, J. P. *et al.*, Randomized, multicenter trial of inhaled nitric oxide and high-frequency oscillatory ventilation in severe, persistent pulmonary hypertension of the newborn. *J. Pediatr.*, 1997, **131**, 55–62.
18. Wessel, D. L. *et al.*, Improved oxygenation in a randomized trial of inhaled nitric oxide for persistent pulmonary hypertension of the newborn. *Pediatrics*, 1997, **100**, E7.
19. Davidson, D. *et al.* and the INO/PPHN Study Group. Inhaled nitric oxide for the early treatment of persistent pulmonary hypertension of

the term newborn: a randomized, double-masked, placebo-controlled, dose-response, multicenter study. *Pediatrics*, 1998, **101**, 325–334.
20. The Franco-Belgium Collaborative NO Trial Group. Early compared with delayed inhaled nitric oxide in moderately hypoxaemic neonates with respiratory failure: a randomized controlled trial. *Lancet*, 1999, **354**, 1066–1071.
21. Omura, A., Roy, R. and Jennings, T., Inhaled nitric oxide improves survival in the rat model of high-altitude pulmonary edema. *Wilderness Environ. Med.*, 2000, **11**, 251–256.
22. Anand, I. S. *et al.*, Effects of inhaled nitric oxide and oxygen in high-altitude pulmonary edema. *Circulation*, 1998, **98**, 2441–2445.
23. Dellinger, R. P. *et al.*, Effects of inhaled nitric oxide in patients with acute respiratory distress syndrome: Results of a randomized phase trial. *Crit. Care Med.*, 1998, **26**, 15–23.
24. Lunn, R., Subspecialty clinics: Anesthesiology; Inhaled nitric oxide therapy. *Mayo Clin. Proc.*, 1995, **70**, 247–255.
25. Rossaint, R., Falke, K. J., Lopez, F., Slama, K., Pison, U. and Zapol, W. M., Inhaled nitric oxide for the adult respiratory distress syndrome. *N. Engl. J. Med.*, 1993, **328**, 399–405.
26. Neonatal Inhaled Nitric Oxide Study Group, Inhaled nitric oxide in full-term and nearly full-term infants with hypoxic respiratory failure. *N. Engl. J. Med.*, 1997, **336**, 597–604.
27. Sartori, C. *et al.*, Salmeterol for the prevention of high altitude pulmonary edema. *N. Engl. J. Med.*, 2002, **346**, 1631–1636.
28. Bärtsch, P., Maggiorini, M., Ritter, M., Noti, C., Vock, P. and Oelz, O., Prevention of high-altitude pulmonary edema by nifedipine. *N. Engl. J. Med.*, 1991, **325**, 1284–1289.
29. Maggiorini, M., Mountaineering and altitude sickness. *Ther. Umsch.*, 2001, **58**, 387–393.
30. Klocke, D. L., Decker, W. W. and Stepanek, J., Altitude-related illnesses. *Mayo Clin. Proc.*, 1998, **73**, 988–992.
31. Bärtsch, P., High altitude pulmonary edema. *Annu. Rev. Med.*, 1996, **47**, 267–284.
32. Hultgren, H. N., High-altitude pulmonary edema: current concepts. *Adv. Exp. Med. Biol.*, 1995, **381**, 15–25.
33. Ladner, E., Schobersberger, W., Sparr, H. and Pall, H., High altitude pulmonary edema at a medium height. A case report. *Anaesthesist*, 1994, **43**, 183–186.
34. Hackett, P. H., Roach, R. C., Hartig, G. S., Greene, E. R. and Levine, B. D., The effect of vasodilators on pulmonary hemodynamics in high altitude pulmonary edema: a comparison. *Md. Med. J.*, 1993, **42**, 641–645.
35. Oelz, O., Maggiorini, M., Ritter, M., Noti, C., Waber, U., Vock, P. and Bärtsch, P., Pathophysiology, prevention and therapy of altitude pulmonary edema. *Schweiz. Med. Wochenschr.*, 1992, **122**, 1151–1158.
36. Droma, Y. *et al.*, Positive association of the endothelial nitric oxide synthase gene polymorphisms with high altitude pulmonary edema. *Circulation*, 2002, **43**, 978–987.
37. Heidi, J. and Dalton, Inhaled nitric oxide: Medical miracle or passing fad? *Respir. Care*, 1998, **43**, 978–987.
38. Anggard, E., Nitric oxide: Mediator, murderer and medicine. *Lancet*, 1994, **343**, 1199–1206.
39. Saugstad, O. D., Does nitric oxide prevent oxidative mediated lung injury? *Acta Paediatr.*, 2000, **89**, 905–907.
40. Finer, N. N. and Barrington, K. J., Nitric oxide for respiratory failure in infants born at or near term (Cochrane Review). In *The Cochrane Library*, Issue 1, Update Software, Oxford, 1999.
41. Barrington, K. J. and Finer, N. N., Inhaled nitric oxide in preterm newborn infants with respiratory failure (Cochrane Review). In *The Cochrane Library*, Issue 1, Update Software, Oxford, 1999.
42. Scott, E. M., The relation of diaphorase of human erythrocytes to inheritance of methemoglobinemia. *J. Clin. Invest.*, 1960, **39**.
43. Hogman, M., Frostell, C., Arnberg, H. and Hedenstierna, G., Bleeding time prolongation and NO inhalation. *Lancet*, 1993, **341**, 1664–1665.
44. Samama, C. M. *et al.*, Inhibition of platelet aggregation by inhaled nitric oxide in patients with acute respiratory distress syndrome. *Anesthesiology*, 1995, **83**, 56–65.
45. Cheung, P. Y., Salas, E., Etches, P. C., Phillipos, E., Schulz, R. and Radomski, M., Inhaled nitric oxide and inhibition of platelet aggregation in critically ill neonates. *Lancet*, 1998, **351**, 1181–1182.
46. Bell, P. R. F., Bleeding time prolongation and NO inhalation. *Lancet*, 1993, **341**, 1664.
47. Clark, R. H. *et al.* (Clinical Inhaled Nitric Oxide Research Group), Low-dose nitric oxide therapy for persistent pulmonary hypertension of the newborn. *N. Engl. J. Med.*, 2000, **342**, 469–674.
48. Ruhnau, B. K., Iversen, S. and Qvist, J., Nitric oxide inhalation therapy in patients with acute respiratory distress syndrome. A retrospective analysis. *Ugeskr. Laeg.*, 2000, **162**, 331–334.
49. Nakagawa, T. A., Johnston, S. J., Falkos, S. A., Gomez, R. J. and Morris, A., Life-threatening status asthmaticus treated with inhaled nitric oxide. *J. Pediatr.*, 2000, **137**, 119–122.
50. Jones, P. R., Hooper, R. H., Stopp, N. and Moore, N. A., Nitric oxide delivery to the lung: a model. *Eur. J. Anaesthesiol.*, 2000, **17**, 160–167.
51. Thibeault, D. W., Rezaiekhaligh, M. H., Ekekezie, I. and Truog, W. E., Compressed air as a source of inhaled oxidants in intensive care units. *Am. J. Perinatol.*, 1999, **16**, 497–501.
52. Griese, M., Koch, M., Latzin, P. and Beck, J., Asthma severity, recommended changes of inhaled therapy and exhaled nitric oxide in children: a prospective, blinded trial. *Eur. J. Med. Res.*, 2000, **5**, 334–340.
53. Busch, T. *et al.*, Hypoxia decreases exhaled nitric oxide in mountaineers susceptible to high-altitude pulmonary edema. *Am. J. Respir. Crit. Care Med.*, 2001, **163**, 368–373.
54. Duplain, H., Sartori, C., Lepori, M., Egli, M., Allemann, Y., Nicod, P. and Scherrer, U., Exhaled nitric oxide in high-altitude pulmonary edema: role in the regulation of pulmonary vascular tone and evidence for a role against inflammation. *Am. J. Respir. Crit. Care Med.*, 2000, **162**, 221–224.
55. David, G. and Tinkelman, D. G., Exhaled nitric oxide and bronchial reactivity during and after inhaled beclomethasone in mild asthma, *J. Asthma*, The Official Journal of the Association for the Care of Asthma, Edited by National Jewish Medical and Research Center, Denver, Colorado, 1999, **36**, 618–622.
56. Artlich, A., Hagenah, J. U., Jonas, S., Ahrens, P. and Gortner, L., Klinik für Pädiatrie, Lübeck, Germany, Exhaled nitric oxide in childhood asthma. *Eur. J. Pediatr.*, 1996, **155**, 698–701.
57. Nelson, B. V., Sears, S., Woods, J., Ling, C. Y., Hunt, J., Clapper, L. M. and Gaston, B., Expired nitric oxide as a marker for childhood asthma. *J. Pediatr.*, 1997, **130**, 423–427.
58. Marlene, G. and Gerlach, H., Exhaled nitric oxide. *Respir. Care*, 1999, **44**, 349–357.
59. Cynthia, M. B. *et al.*, Pulmonary nitric oxide in mountain dwellers. *Nature*, 2001, **414**, 411–412.
60. Avital, A., Uwyyed, K., Berkman, N., Godfrey, S., Bar-Yishay, E. and Springer, C., Exhaled nitric oxide and asthma in young children. *Pediatr. Pulmonol.*, 2001, **32**, 308–313.
61. Chambers, D. C. and Ayres, J. G., Effects of nitrogen dioxide exposure and ascorbic acid supplementation on exhaled nitric oxide in healthy human subjects. *Thorax*, 2001, **56**, 774–778.
62. Jones, S. L., Kittelson, J., Cowan, J. O., Flannery, E. M., Hancox, R. J., McLachlan, C. R. and Taylor, D. R., The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. *Am. J. Respir. Crit. Care Med.*, 2001, **164**, 738–743.
63. Yamamoto, Y. *et al.*, Role of airway nitric oxide on the regulation of pulmonary circulation by carbon dioxide. *J. Appl. Physiol.*, 2001, **91**, 1121–1130.
64. Montuschi, P., Kharitonov, S. A. and Barnes, P. J., Exhaled carbon monoxide and nitric oxide in COPD. *Chest*, 2001, **120**, 496–501.
65. Mychaskiw, G., Sachdev, V. and Heath, B. J., Sildenafil (Viagra) facilitates weaning of inhaled nitric oxide following placement of a biventricular-assist device. *J. Clin. Anesth.*, 2001, **13**, 218–220.

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