NITRIC OXIDE IN EXHALED AIR IS A NEW MARKER OF
AIRWAY INFLAMMATION

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Gazi Medical Journal 1998; 9 (Suppl 1) : S25-S30

Nitric oxide (NO) is produced by many cells within the respiratory tract and may play an important signalling role in the physiological control of airway function and in the pathophysiology of airway diseases (1,2). Endogenous NO is generated from the amino acid L-arginine by the enzyme NO synthase (NOS), of which three distinct isoforms exist: constitutive isoforms are found in endothelial cells (eNOS) and neurons (nNOS) and are physiologically activated by a rise in intracellular calcium; and a third isoform (iNOS) is induced in several cell types by exposure to proinflammatory cytokines and endotoxin and its induction is blocked by glucocorticoids. All three isoforms have been detected in the human respiratory tract (3-7).

Gustafsson and colleagues first demonstrated that NO can be detected in the exhaled air of animals and normal human subjects (8). Furthermore, exhaled NO is increased in patients with inflammatory diseases of the airways, such as asthma (9-11) and bronchiectasis (12), derived from the lower respiratory tract (10,13) and may be modulated by inhaled corticosteroids (14,15).

This suggests that exhaled NO may provide a non-invasive means of monitoring inflammation in the respiratory tract and the measurement of exhaled NO has attracted increasing interest. However, interpretation of exhaled NO measurements may be difficult and there is a wide variation in the reported levels of NO in exhaled air, suggesting that technical factors are important.

How is NO in exhaled air measured?

Most studies have measured exhaled NO by chemiluminescence and detection depends on the photochemical reaction between NO and ozone generated in the analyser (Fig. 1). The specificity of exhaled NO measurements by chemiluminescence has been confirmed using gas chromatography-mass spectroscopy. Several NO analysers are now commercially available, but may need to be converted for on-line measurement of NO in exhaled air. Most analysers are sensitive to 1 part per billion (ppb) of NO and this is adequate for studies of exhaled air. NO may be detected by direct expiration into the analyser or by collection into a reservoir for later analysis.

Several technical factors may affect the measurement of exhaled NO. Breath-holding increases NO levels, due to accumulation of NO in the upper or lower respiratory tracts (13). High concentrati-
ons of NO have been detected in the paranasal sinuses (16). This has suggested that exhaled NO may largely reflect NO derived from the upper airways, rather than the lower airways. Thus manoeuvres that block the upper respiratory tract reduce exhaled NO concentrations and lower levels of NO are recorded from the lower respiratory tract in patients with tracheostomies that exclude the upper respiratory tract (17). However expiration against a resistance prevents any nasal contamination, as this leads to isolation of the nasopharynx from the oropharynx by the soft palate (9). Thus, with a slow expiration against resistance the levels of NO measured at the mouth are identical to those measured directly from the lower airways via a bronchoscope (13) (Fig. 2). During quiet tidal breathing, however, there may be nasal contamination of the exhaled NO as there is a communication between the nasopharynx and oropharynx. This means that collection of expired air in a reservoir for later analysis may result in higher levels of NO and that any increase in NO from the lower respiratory tract will be blunted.

Source of NO in exhaled air

The source of NO in the lower respiratory tract is not yet certain. Studies with perfused animal lungs suggest that exhaled NO originates at the alveolar surface, rather than from the pulmonary circulations (18), and may be derived from eNOS expressed in the alveolar walls of normal lungs. Studies in ventilated perfused lungs of guinea pigs show that exhaled NO is reduced during perfusion with calcium-free solutions, suggesting that NO is derived from a constitutive NOS. Airway epithelial cells may also express both eNOS and nNOS and therefore contribute to NO in the lower respiratory tract.

In inflammatory diseases, it is likely that the increase in exhaled NO is due to induction of iNOS. Indeed increased NOS activity has been demonstrated in lung tissue of patients with asthma, cystic fibrosis and obliterative bronchiolitis (19). In asthmatic patients biopsies obtained by fiberoptic biopsy have demonstrated increased expression of iNOS in airway epithelial cells in comparison with non-asthmatic airways. In vitro pro-inflammatory cytokines, that are found in increased amounts in the airway of asthmatic patients, increase the expression of iNOS in cultured human airway epithelial cells (20-22). Furthermore, glucocorticoids inhibit the induction of iNOS in epithelial cells in vitro (20, 21), and in vivo (23), and reduce exhaled NO levels in asthmatic patients to normal (15). iNOS may be expressed in alveolar macrophages and other inflammatory cells. Thus, there is some evidence for iNOS expression in macrophages of affected lung in bronchiectasis (5).

Simultaneous measurement of expired CO₂ and NO demonstrate that exhaled NO precedes the peak value of CO₂ (end-tidal), suggesting that NO is derived from airways rather than alveoli (13) (Fig. 3; Fig. 4). Direct sampling via fiberoptic bronchoscopy in asthmatic patients shows a similar elevation of NO in trachea and main bronchi to that

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Fig. 2: Exhalation against a resistance causes closure of the soft palate and thus prevents contamination of exhaled air with NO that is present in high concentrations in the nose.

Fig. 3: Trace of NO and carbon dioxide (CO₂) concentration during expiration in a normal subject (NO=7.1 ppb).
Fig - 4: Trace of NO and CO2 in a patient with asthma (20.4 ppb), recorded at the mouth, thus indicating that the elevated levels in asthma are derived from the lower airways (10, 13).

Functional importance of exhaled NO

Exhaled NO may be a useful non-invasive marker of airway inflammation, but it may also play a physiological and pathophysiological role. The high concentrations of NO generated in the paranasal sinuses may have a sterilising effect in the sinuses and upper respiratory tract, since NO is toxic to bacteria, parasites and viruses. NO derived from the lower respiratory tract may also contribute to host defence and the fact that iNOS can be rapidly expressed in airway epithelial cells provides a rapid non-specific defence mechanism in the respiratory tract. Knock-out mice that lack the iNOS gene have a marked increase in susceptibility to infection (24, 25).

NO in the respiratory tract may also have an effect on the bronchial and pulmonary circulations (26, 27). NO is a potent vasodilator and the increased production of NO in asthmatic airways may underlie the hyperaemia seen in asthmatic airways. NO may play an important role in perpetuating asthmatic inflammation (28) as it not only increases airway blood flow and therefore plasma exudation, but it may also tip the balance in favour of Th2 cells. Since NO may be chronically produced by epithelial cells when iNOS has been induced (and possibly also by inflammatory cells), chronic asthmatic inflammation may be perpetuated and amplified (1).

Although endogenous NO appears to be the major bronchodilator neurotransmitter in humans, high concentration of inspired NO have only weak bronchodilator effects (29, 30), so it is unlikely that endogenous NO is an important determinant of airway calibre. Indeed marked inhibition of endogenous NO production by nebulised NOS inhibitors has no detectable effect on airway function, even in patients with asthma (31, 32).

Clinical relevance of exhaled NO

Asthma

There is now persuasive evidence that levels of NO are increased in association with airway inflammation and are decreased with anti-inflammatory therapy. Several studies have reported an elevation of exhaled NO in patients with asthma (9, 10). However, exhaled NO is not elevated in asthmatic patients treated with inhaled steroids (9, 15).

After inhaled allergen challenge in asthmatic patients there is no change of exhaled NO during the early bronchoconstriction response, but a progressive elevation during the late response (33). In patients who have no late response to allergen (single responders), there is no change in exhaled NO throughout the study period, suggesting that increased NO is associated with the inflammatory late response and may be a reflection of iNOS expression in response to inflammatory cytokines. In sensitised guinea pigs allergen challenge is associated with increased NO production during the late response and this is preceded by iNOS mRNA expression (34). By contrast, there is no increase in exhaled NO after bronchoconstriction induced by histamine (direct effect on airway smooth muscle) or by adenosine (via activation of airway mast cells (35).

There is also an increase in exhaled NO during exacerbations of asthma (36) and when the maintenance dose of inhaled glucocorticoids is reduced (14). Indeed, the level of exhaled NO increased before any change in lung function or increase in symptoms, suggesting that exhaled NO may be provide early detection of poor control.

Bronchiecstasy

An increase in exhaled NO is not specific to asthma and is also found in bronchiecstasy and the level of NO is related to the extent of disease, as measured by a computerised tomography score (12). As in asthma, the elevation of exhaled NO is not seen in patients treated with inhaled steroids. This
suggests that exhaled NO in bronchiectasis may reflect active inflammation in the lower airways and may be used to monitor disease activity.

**Nasal disease**

High concentrations of NO have been detected in the nose of normal individuals (13, 37) and very high concentrations in the paranasal sinuses (16). Elevated nasal NO has been described in patients with allergic and perennial rhinitis, may reflect allergic inflammation in the nose, and may be modulated by nasal glucocorticosteroids (38).

**Vascular disease**

In patients with pulmonary hypertension, secondary to systemic sclerosis, there is a reduction in exhaled NO compared to normal subjects and to patients with interstitial lung disease without pulmonary hypertension (39). This may be a reflection of the reduced eNOS expression described in patients with pulmonary hypertension (40). The reduced endogenous production in the vessels of patients with pulmonary hypertension may contribute to the vasoconstriction of pulmonary vessels and to the increased proliferation of vascular smooth muscle cells in this condition (2).

**Effects of Therapy**

Exhaled NO levels are significantly lower in patients with asthma and bronchiectasis who are treated with inhaled glucocorticoids, suggesting that inhaled steroids reduce exhaled NO (9, 12, 36). An oral glucocorticoid prednisolone (30 mg for 3 days) has no effect on exhaled NO in normal individuals, but decreases the elevated levels of exhaled NO in asthmatic patients (31). This suggests that the exhaled NO in normal subjects is derived from constitutive NOS (unaffected by steroids), whereas the elevated levels in asthma are derived from iNOS, which is inhibited by glucocorticoids. In asthmatic patients a double-blind study of inhaled budesonide shows a progressive reduction in exhaled NO down to normal values after three weeks of therapy (15). The reduction in exhaled NO is progressive and may reflect direct inhibitory effects of glucocorticoids on induction of iNOS, via a direct blockade of the transcription factor nuclear factor-kappa B (NF-κB) and an indirect effect due to reduced synthesis of the proinflammatory cytokines that lead to iNOS expression in airway epithelial cells. Biopsy studies have confirmed that iNOS expression in asthmatic airway epithelial cells is reduced in patients of inflammatory mediators tumor necrosis factor-α, IL-6 and NO production in the lower airway (41). Methylprednisolone given prior the institution of CPB reduced the endogenous production of all three of these inflammatory mediators.

By contrast, neither short-acting nor long-acting inhaled β2-agonists reduce exhaled NO in asthmatic patients (42), adding further support to the view that exhaled NO may be useful in assessing anti-inflammatory effect of inhaled asthma treatments.

NO may perpetuate asthmatic inflammation, which may be addressed by the use of NO synthase inhibitors, such as N \(^G\) - monomethyl-L-arginine (L-NMMA) and N \(^O\) - nitro-L-arginine methyl ester (L-NAME). These NOS inhibitors reduce exhaled NO in normal and asthmatic subjects yet have no effect on airway function when given as a single dose (31, 32). More chronic treatment will be required to demonstrate whether NO contributes to the persistence of asthmatic inflammation. In animal studies, NO synthase inhibitors are very effective in controlling inflammation in septic shock (43-45). While L-NMMA and L-NAME are non-selective inhibitors of eNOS and iNOS, aminoguanidine or isothioureas have some selectivity for iNOS (46). Inhalation of aminoguanidine has no effect on exhaled NO in normal subjects, but significantly reduces exhaled NO in patients with asthma (32), adding further support to the view that the elevated exhaled NO in asthma is derived from iNOS.

**Summary**

The measurement of exhaled NO has excited considerable interest as it may provide a simple non-invasive means of measuring airway inflammation. There is now persuasive evidence that levels of NO are increased in association with airway inflammation and are decreased with anti-inflammatory treatments. Correlation of exhaled NO with more direct measurements of inflammation in the airways, such as induced sputum, bronchoalveolar lavage and bronchial biopsies, is needed. The great advantage of exhaled NO is that the measurement is completely non-invasive and can therefore be performed repeatedly and also in children and patients with severe airflow obstruction where more invasive techniques are not possible. The measurement, however, is not specific and exhaled NO is increased in inflammation due to asthma, bronchiectasis
(12) and respiratory tract infections (47). This means that absolute values are less important than serial measurements in individual patients. The value of this approach has been demonstrated in asthmatic patients where the dose of inhaled steroid is changed, resulting in increased levels when the dose is reduced and lower levels when the dose is increased (14). Because exhaled NO is reduced by anti-inflammatory treatments, it may be useful for monitoring whether therapy is adequate. The technique may also have application in the monitoring of anti-inflammatory effects of new anti-asthma drugs, such as selective phosphodiesterase inhibitors, leukotriene antagonists and synthesis inhibitors and immunomodulators (48). Because the measurement is precise and reasonably reproducible, it may facilitate the measurement of dose response effects with anti-inflammatory treatments, that is difficult at present.

The currently available analysers for exhaled NO are expensive, but in the future it is likely that technological advances will make it possible to miniaturise these analysers, so that they are portable and may even be used at home in conjunction with peak flow meters. This may lead to their application in epidemiological studies and this may be a useful screening measurement for community studies.

Although we have discussed exhaled NO, other volatile substances may also be detected in exhaled air. Thus, ethane and pentane, which are volatile products of lipid peroxidation, and hydrogen peroxide, may be used to detect oxidant stress in the respiratory tract and may also be useful as markers of inflammation (49, 50). There is little doubt that this is a rapidly expanding area of research.

REFERENCES


