Review of exhaled nitric oxide in chronic obstructive pulmonary disease

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Received 8 November 2011
Accepted for publication 24 January 2012
Published 7 June 2012
Online at stacks.iop.org/JBR/6/047101

Abstract

The up-regulation of nitric oxide (NO) by inflammatory cytokines and mediators in central and peripheral airway sites can be easily monitored in exhaled air (FENO). It is now possible to estimate the predominant airway site of increased FENO i.e. large versus peripheral airway/alveoli, and its potential pathologic and physiologic role in obstructive lung disease. In asthma, six double-blind, randomized, controlled algorithm trials have reported only equivocal benefits of add-on measurements of FENO to usual clinical guideline management including spirometry. Significant design issues, as emphasized by Gibson, may exist. However, meta-analysis of these six studies (Petsky et al 2012 Thorax 67 199–208) concluded that routine serial measurements of FENO for clinical asthma management does not appear warranted. In COPD including chronic bronchitis and emphysema, despite significant expiratory airflow limitation, when clinically stable as well as during exacerbation, FENO, $j_{\text{awNO}}$ and $C_{\text{ANO}}$ may all be normal or increased. Furthermore, the role of add-on monitoring of exhaled NO to GOLD management guidelines is less clear because of the absence of conclusive doubleblind, randomized, control trial studies concerning potential clinical benefits in the management of COPD.

Abbreviations

$C_{\text{ANO}}$ peripheral/small airway/alveolar nitric oxide concentration
cNOS constitutive nitric oxide synthase
eNOS endothelial nitric oxide synthase (NOS3)
$F_{\text{ENO}}$ fraction exhaled nitric oxide
iNANC inhibitory non-adrenergic non-cholinergic
iNOS inducible nitric oxide synthase (NOS2)
$j_{\text{awNO}}$ large/central airway maximal nitric oxide flux
nNOS neuronal nitric oxide synthase (NOS1)

Introduction

We have previously emphasized that nitric oxide (NO) is a gaseous signaling molecule produced by resident cells e.g. airway epithelial cells, airway and circulatory endothelial cells, and trafficking inflammatory cells in both large and peripheral airways/alveoli [1]. The fractional concentration of exhaled NO ($F_{\text{ENO}}$) can be easily measured and is well-established in clinical research. However, the incorporation of $F_{\text{ENO}}$ into clinical practice is currently undergoing critical assessment. This paper generously builds upon our previous review on the role of $F_{\text{ENO}}$ in COPD [1] with the goal to update the reader...
with the potential diagnostic and therapeutic role of NO in COPD, evaluate new ways to model NO exchange in the large (\(j'_{awNO}\)) and small airways/alveoli (\(C_{ANO}\)) that may identify the anatomic location of NO production, and then to review published \(F_{ENO}\) measurement in COPD.

**NO as a mediator of inflammatory airway disease**

Endogenous nitric oxide (NO) plays an important role in regulating airway and vascular function and is generated by three isoenzymes of NO synthase (NOS) that are differentially regulated and expressed in the airways and appear to play different pathophysiologic roles [1, 2].

Barnes *et al* [1] have previously noted that all NOS isoenzymes convert L-arginine to L-citrulline with the generation of NO. Constitutive NOS (cNOS) isoenzymes include neuronal NOS (nNOS, NOS1) and endothelial NOS (eNOS, NOS3), both of which are presumed to play a local regulatory role, such as neurotransmission (NOS1) and regulation of local blood flow (NOS3). Inducible NOS (iNOS, NOS2) is not constitutively expressed but is induced by inflammatory and infectious stimuli and produces large amounts of NO which may have a proinflammatory effect [1]. However, the clear distinction between constitutive and inducible isoenzymes has been blurred by the recognition that cNOS may be inducible, whereas NOS2 may be constitutively expressed in some conditions [1]. Nevertheless, these distinct NOS isoenzymes are regulated by different genes and have different physiological and pathologic functions. There are important species differences such that corticosteroids directly suppress NOS2 in rodent cells but do not directly inhibit NOS2 expression in human airway epithelial cells [3]. The increase in NO in exhaled breath in asthma is presumed to originate from increased NOS2 expression in the airway epithelial cells and macrophages, although cNOS isoenzymes may also contribute. Endothelial NOS3 is expressed in endothelial cells of the bronchial and pulmonary circulation and plays a role in regulating vascular flow [4]. It is also expressed in alveolar endothelial cells and airway epithelial cells throughout the respiratory tract. NOS3 expression is reduced in peripheral lung of patients with COPD, especially in severe disease due to alveolar wall destruction as a result of emphysema [5]. Neuronal NOS is localized to cholinergic nerves in the airways and mediates inhibitory non-adrenergic non-cholinergic (i-NANC) neural bronchodilatation, acting as a functional antagonist of its co-transmitter acetylcholine [6]. It is also expressed in airway epithelial cells and type I pneumocytes, and there is evidence that its expression and activity is increased in peripheral lungs of COPD patients as a result of oxidative stress [7]. NOS1 may contribute to the increase in peripheral NO in COPD and severe asthma that has been reported [8, 9].

Increased inducible NOS2 expression is found in airway epithelial cells of patients with asthma and is reduced by inhaled corticosteroids (ICS) [10]. Increased NOS2 expression is also found in peripheral lung and small airways in patients with COPD [5, 11]; however, there was no effect of high-dose ICS on exhaled nitric oxide [9]. Furthermore, it is unlikely that the inflammation that induces NOS2 is steroid resistant in patients with COPD [9]. Oxidative stress generates superoxide anions and in combination with NO may result in the formation of the highly reactive species peroxynitrite, which is increased in exhaled breath condensate of COPD patients [12] and may account for the increased tyrosine nitration found in peripheral lungs by immunocytochemistry [11]. The formation of peroxynitrite removes NO from the gaseous phase so that its concentration in the airways is reduced when there is a high level of oxidative stress, as in COPD patients. Selective inhibitors of NOS2 reduce \(F_{ENO}\) in asthmatic patients and even in normal subjects [13–15], but have less effect in COPD patients, indicating that the increased peripheral NO may derive from NOS1 as well as NOS2 in these patients.

**Modeling of NO excretion in the lungs (see figures 1 and 2)**

George *et al* [1] have emphasized the principles that formed the foundation of the two-compartment model (i.e. an airway compartment and an alveolar compartment) of NO exchange, first reported in 1998 [16] and later confirmed by four additional independent research groups [17–20].

In the simplest form, the two-compartment model can be characterized by two parameters: a maximum flux of NO from the large airway compartment \(j'_{awNO}\), nl s\(^{-1}\), airway generations 1–16 and a steady state mean distal airway/alveolar concentration of NO \(C_{ANO}\) (ppb) (see figure 1). A series of experimental algorithms characterized by measuring \(F_{ENO}\) at different constant exhalation flows have been presented and reviewed [21]. While the simplicity of the initial two-compartment model is a tremendous strength, recent work has demonstrated that axial back-diffusion of NO in the gas phase (i.e. NO back-diffusing from airways toward the alveolar region against the direction of exhalation) cannot be neglected [22–25]. Incorporating axial gas phase back-diffusion of NO produces a two-compartment model with more complex governing equations, and modified algorithms to characterize \(j'_{awNO}\) and \(C_{ANO}\) have been presented and reviewed [26, 27]. For example, a simple method used widely to determine \(j'_{awNO}\) and \(C_{ANO}\) is to regress a line through a plot of the elimination rate of NO versus the exhalation flow, and the intercept and slope are estimates of \(j'_{awNO}\) and \(C_{ANO}\), respectively (‘slope–intercept’ method) (see figure 2) [16]. When axial back-diffusion of NO in the gas phase is considered, NO from the airway tree diffuses back (back-diffusion) into the alveolar region where it can falsely elevate the estimate of \(C_{ANO}\) and depress the estimate of \(j'_{awNO}\). Thus, the modified algorithm using the slope–intercept method still uses the slope to estimate \(C_{ANO}\), but subtracts a term proportional to the airway flux \((j'_{awNO}/0.53)\) to account for axial diffusion; similarly, the estimate for \(j'_{awNO}\) remains the intercept but is multiplied by a factor (1.7) to account for the loss to the alveolar region [26].

Clinical interest in modeling NO exchange dynamics, which has the ability to discriminate between large airway NO flux \((j'_{awNO})\) and the distal alveolated airways and alveolar region \((C_{ANO})\), remains strong due to the potential clinical utility of measuring and potentially determining the
Figure 1. Schematic of the two-compartment model. During exhalation, a steady state mean alveolar or peripheral concentration ($\overline{C}_{ANO}$) enters the airway compartment (net transfer is convection minus diffusion) where upon additional NO is transferred from the airway walls. The alveolar or peripheral concentration represents the acinar region of the lungs (Weibel generations 17–23). The airway compartment represents the oropharynx and Weibel generations 1–16, and considers the increasing surface area per unit volume of the airway tree (i.e. trumpet shape). The contribution from the airways depends on the exhalation flow and is the sum of two terms: $\overline{J}_{awNO} = \overline{J}_{awNO} - \overline{D}_{awNO} \cdot C_{NO}$. $\overline{J}_{awNO}$ is the mean maximum airway flux (picoliters/second). $\overline{D}_{awNO}$ is the mean airway diffusing capacity (or conductance). $C_{NO}$ is the airway compartment gas phase NO concentration which depends on axial volumetric position ($V$), and the airway compartment volume is $V_{aw}$ (T: trachea; BR: bronchus; BL: bronchiole; TB: terminal bronchiole; RB: respiratory bronchiole; AD alveolar duct; AS: alveolar sac). The axial diffusion component takes into account the diffusion of NO in the gas phase from the airways (high concentration) to the alveolar region (low concentration) in accordance with Fick’s laws of diffusion. The gradient for diffusion is in the opposite direction of the exhaled flow. Thus, by taking into consideration the axial diffusion of NO, one can correct for the ‘back-diffusion’ of airway NO and resultant contamination of the alveolar region. Reprinted from [78] with permission from Elsevier.

Figure 2. Illustrative data demonstrating the elimination rate versus flow technique to estimate the mean alveolar concentration and maximum airway flux. The steady or plateau nitric oxide concentration can be measured at a series of constant exhalation flows, and then the elimination rate (pL s$^{-1}$, product of concentration and flow) can be plotted as a function of exhalation flow (mL s$^{-1}$). For flows greater than $\sim$50–100 mL s$^{-1}$ in adults, this relationship is approximately linear (solid circles), as predicted by the two-compartment model with axial diffusion. The slope minus a term proportional to the airway flux is an estimate of the mean alveolar concentration ($\overline{C}_{ANO}$ (pL s$^{-1}$)/(mL s$^{-1}$) = ppb), while the intercept is proportional to the mean maximum airway flux of NO ($\overline{J}_{awNO}$. pL s$^{-1}$). Specific values for the coefficients ‘$a$’ and ‘$b$’ are described in Condorelli et al [26] and depend on the flow range utilized. Reprinted from [78] with permission from Elsevier.

predominant site and source of inflammation [1]. From the practical viewpoint, FENO obtained at 50 ml s$^{-1}$ has been presumed to be a surrogate of large central airway NO flux ($\overline{J}_{awNO}$). However, we have recently reported that the increase in FENO at 50 ml s$^{-1}$ in normals, age $>60$ yr, was related to an increase in $C_{ANO}$ whereas $\overline{J}_{awNO}$ remains relatively constant with ageing [28]. There is no simple surrogate for determining $\overline{J}_{awNO}$ versus peripheral NO production ($C_{ANO}$), therefore FENO needs to be obtained at multiple expiratory flow rates and using the aforementioned algorithm [25–27] to determine $\overline{J}_{awNO}$ and $C_{ANO}$.

Exhaled NO as marker of airway inflammation in asthma

FENO measurements have been considered a surrogate for eosinophilic airway inflammation, especially in asthma. However, correlations with sputum eosinophils are modest. In the largest population studied, the $r^2$ value was 0.26, $p = 0.001$; the sensitivities and specificities for clinically significant eosinophilia were around 70–75% [29] and $r^2 = 0.29$, $p = 0.007$ with tissue eosinophilia in treated asthmatics with severe refractory asthma [30]. However, in most mild asthmatics, high FENO at 50 ml s$^{-1}$ ($>45$ ppb) has been regarded as a marker for steroid responsiveness [31, 32] including improvement in spirometry and airway hyper-responsiveness [33]. Alternatively, we have noted that exacerbations in chronic moderate-to-severe asthmatics maintained on ICS and LABA but not oral corticosteroids had similar spirometric improvement to add-on oral corticosteroid irregardless of the baseline FENO level including normal value at 50 ml s$^{-1}$ [34]. Increased FENO...
may also occur with eosinophilic bronchitis [35]. The ATS clinical practice guideline for exhaled nitric oxide in asthma concluded that add-on FENO monitoring provides potential easier detection of eosinophilic airway inflammation, and likelihood of corticosteroid responsiveness [36].

Using asthma treatment guidelines including spirometry [37] several investigators [38–43] have addressed the clinical role of add-on monitoring of exhaled NO at a single expiratory airflow rate (FENO), usually at 50 ml s\(^{-1}\), to help guide inhaled and/or systemic corticosteroid dosing in clinically stable and unstable children [38, 39, 41, 43] and adults [40, 42, 43] with asthma. Results of these six double-blind, randomized, control trials [38–43] were equivocal. Gibson [44] has raised multiple issues related to study design that challenge the validity of the conclusions. However, after analyses of the trials [38–43] Petsky et al [45] concluded that routine serial measurements of FENO for clinical asthma management does not appear warranted.

**Exhaled NO as marker of airway inflammation in COPD**

COPD is an inflammatory disease of both large and small airways and alveoli that are predominantly mediated by cytokines and interleukins via neutrophilic cellular pathways [46]. Papi et al [47] have noted that in COPD patients with severe exacerbation, airway neutrophilia is increased irregardless of bacterial versus viral etiology, whereas increased eosinophilia usually reflects a viral source. Rutgers et al [48] also described increased airway neutrophils and eosinophils in clinically stable COPD patients who underwent bronchialveolar lavage and large airway biopsy. However, Rutgers et al [49] noted normal values for exhaled NO and only a modest correlation (\(r^2 = 0.4, p = 0.009\)) between sputum eosinophils and exhaled NO.

In stable COPD, FENO measurements need to be obtained in non-smokers to avoid misleading reduction in FENO. When measured at a single expiratory flow rate, FENO has been elevated [50–52] or normal [2] and increased with exacerbations [51–53]. Papi et al [54], Kunisaki et al [55], Dummer et al [56], de Laurentis et al [57] and Antus et al [58] reported that an elevated FENO in COPD may also be a variable signal for increased spirometric response to ICS, although the correlation was poor (\(r^2 = 0.19, p < 0.001\)) [58]. In a randomized trial, Siva et al [59] successfully used sputum eosinophils compared to standard care as a tool to reduce severe COPD exacerbations. In that study sputum eosinophils and FENO were not associated, possibly due to the interaction of ICS and concurrent smoking, both of which independently suppress FENO. Despite these conflicting findings, measurement of large airway NO flux (\(j_{awNO}\)) and distal airway/peripheral lung NO (\(C_{ANO}\)) via modeling [16, 26, 27] may potentially detect increased large airway NO flux, and allow selection of individual COPD patients with severe expiratory airflow limitation who may benefit from ICS. Using the two-compartment NO model as previously described [16] and with correction for NO axial back-diffusion [26, 27] we [34, 60, 61], Berry et al [62], van Veen et al [63], Brindici et al [8], Kerckx et al [27], Mahut et al [64], Paraskakis et al [65], Lehtimaki et al [66] and Shin et al [67] have noted increased \(j_{awNO}\) and increased or normal \(C_{ANO}\) in mild and moderate-to-severe clinically stable asthmatics [34]. Using similar NO modeling [16] with and without correction for NO axial back-diffusion [26, 27] Hogman et al [68], Lehtimaki et al [69], Lehouk et al [70] and Brindici et al [9] reported normal [70] and increased \(j_{awNO}\) as well as normal [69, 70] and increased \(C_{ANO}\) [9, 68] in clinically stable COPD patients compared to controls. However, in the study by Brindici et al [9] their healthy non-smoking controls had a mean age of 45 yr compared to their COPD cohort with a mean age of 62 yr. This may have confounded their findings, since we have noted that normal younger subjects, age <60 yr, have significantly lower values for \(C_{ANO}\) compared to older normals, age ≥ 60 yr [28]. However, in a subsequent study, Brindici et al [5] reported increased mRNA expression and activity of NO synthase isoenzyme nNOS, and not iNOS, in COPD peripheral lung tissue that reflected the severity of the disease. While Hogman et al [68] used age-matched controls, neither Hogman et al or Brindici et al [9] corrected for axial back-diffusion of NO from large airways to peripheral lung [26, 27]. This underestimates large airway NO flux (\(j_{awNO}\)) and overestimates \(C_{ANO}\). After correction, \(j_{awNO}\) will increase and \(C_{ANO}\) will decrease [26, 27]. Alternatively, Verbanck et al [71] have suggested that peripheral airway constriction would block axial back-diffusion leading to overestimation of \(j_{awNO}\) and underestimation of \(C_{ANO}\). We [72] recently compared clinically stable COPD patients to age-matched controls using the two-compartment model [16] and after correcting for NO axial back-diffusion [26] found normal values for \(j_{awNO}\) and \(C_{ANO}\). Furthermore, we [72] noted that moderate dose but not low-dose ICS could suppress normal values for \(j_{awNO}\). Previously, Lehouk et al [70] and Roy et al [73] also noted normal \(j_{awNO}\) and \(C_{ANO}\) in COPD patients compared to age-matched healthy, older, non-smoking subjects, but without correcting for NO axial back-diffusion [73].

With respect to therapeutic intervention, in the TORCH 3 yr double-blind, placebo controlled trial in moderate–severe COPD patients, Jenkins et al [74] noted that inhaled salmeterol/fluticasone propionate reduced moderate-to-severe exacerbations and improved health status and FEV\(_1\) (L) across GOLD [75] Stage 2 and 3 and reduced mortality in Stage 2. Meta-analysis by Drummond et al [76] in 11 eligible randomized controlled trials (14 426 participants) reported that ICS therapy did not affect 1 yr all-cause mortality but was associated with higher incidence of pneumonia. In a similar meta-analysis including 16 996 COPD patients since 2008, Singh et al reached similar conclusions that use of ICS >24 weeks was associated with a significant increase in serious pneumonia without affecting an increase in death [77]. In conclusion, in COPD, the role of add-on FENO monitoring to therapeutic intervention [74–77] is less clear with respect to clinical benefits, especially in the absence of conclusive double-blind, randomized, control studies. Therefore, routine monitoring of FENO in COPD [75] is less-established than in asthma as noted in the recent ATS Clinical Guidelines [36].
Summary

The up-regulation of NO by inflammatory cytokines and mediators in central and peripheral airway sites can be easily monitored in exhaled air (FENO). It is now possible to estimate the predominant airway site of increased FENO i.e. large versus peripheral airway/alveoli, and its potential pathologic and physiologic role in obstructive lung disease. In asthma, six double-blind, randomized, controlled, algorithm trials [38–43] have reported that routine serial measurements of FENO are helpful in the management of COPD. Empirical use by the British Thoracic Society [44] may exist. However, meta-analysis including spirometry. Significant design issues as emphasized by Gibson [45] may exist. However, meta-analysis including spirometry. Significant design issues as emphasized by Gibson [44] may exist. However, meta-analysis including spirometry. Significant design issues as emphasized by Gibson [44] may exist. However, meta-analysis including spirometry.

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