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EDITORIAL

Measuring exhaled nitric oxide: not only a matter of how – but also why – should we do it?

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The biological functions of nitric oxide are so diverse and complex that it is now becoming increasingly difficult to delineate briefly the physiological roles and pathophysiological implications of this seemingly simple messenger molecule [1]. In respiratory medicine, NO can either be viewed as a paracrine factor (derived from endothelium, epithelium, nerves, inflammatory cells, *etc*), a therapeutic gas or a marker of inflammation [2]. Amongst other paracrine factors, the endothelium-derived NO has, in its own right, a central role to play in the modulation of pulmonary vascular tone [3]. As a gaseous molecule, NO has been extensively investigated in clinical settings and used as inhalational therapy to relieve pulmonary hypertension and/or refractory hypoxaemia in adults and infants [4]. Although there are many questions that still remain to be properly answered [5], the use of inhaled NO has undoubtedly revived interest in molecules that can selectively reduce both pulmonary vascular resistance and intrapulmonary shunt. As a radical molecule, NO is highly reactive and readily combines with an array of biological molecules, ranging from reactive oxygen species to haeme moiety containing proteins [6]. This explains why measurement of NO in biological systems was often fraught with difficulties in the early days [7]. Since 1991, however, measurement of *in vivo* NO production in humans have been proven to be technically feasible by means of *ex vivo* manoeuvres, *i.e*. by sampling the exhaled breath and analysing it for NO content using a chemiluminescent NO analyser [8]. As the technique is noninvasive, it was immediately applied to patients, especially those with bronchial asthma, to assess endogenous production of NO by the lung [9–11]. Soon, the accumulating evidence suggested that measurement of exhaled NO could be viewed as a new lung function test [12] to monitor airway inflammation in asthma [13] and other conditions associated with inflammation of the respiratory tract [14]. It is still difficult to know the actual source of the endogenous NO that is detected in the exhaled air [15]. As NO is synthesized by many lung cells, it could originate from virtually anywhere in the respiratory tract, from alveolar space to the nose. Several recent and carefully conducted studies have clearly shown how the techniques of measurement are likely to affect the amount and origin of exhaled NO [16–20]. This prompted the European Respiratory Society, in 1997, to issue specific recommendations for the measurement of exhaled and nasal NO [21], an initiative which was followed in 1998 by the American Thoracic Society.

In contrast with the ongoing technical debate on how to measure exhaled NO, it seems that a consensus has been reached on the diagnostic value of NO measurement in asthma. Compelling evidence clearly demonstrates that asthmatic subjects who are not treated with inhaled glucocorticoids have on average higher amounts of exhaled NO as compared with healthy controls [9–14, 18, 21]. The strength of the evidence, and hence the validity of the finding, is based on both practical and theoretical grounds. Higher levels of exhaled NO in asthma have been consistently found by several independent investigators using different techniques of measurement [18, 22, 23]. Theoretical considerations are also consistent with the raw data. NO is synthesized by a group of three enzymes, called NO synthases (NOS). Each isoform is differentially distributed in organs and tissues, with a preferential expression of the constitutive NOS I in neurons, the inducible NOS II in inflammatory cells and the constitutive NOS III in vascular endothelium [1]. All three isoforms have been detected in lung cells. In particular, the inducible NOS II is markedly expressed in asthmatic airways [24]. Unlike the constitutive neuronal NOS I and endothelial NOS III which synthesize NO only in minute amounts to meet physiological demands, expression of inducible NOS II by asthmatic epithelial cells leads to a massive synthesis of NO, thus explaining high levels of exhaled NO in asthma. Moreover, the inhibitory effect of glucocorticoid hormones on NO production [25–28] is supported by the molecular links between inducible NOS II, nuclear factor-κB (NF-κB) (a key transcription factor in asthma [29]) and the genomic effect of the glucocorticoids [30]. Briefly, binding of the hormone to its intracellular receptor leads to the transcription of the inhibitory subunit (IκB) which normally impedes NF-κB binding to the promoter regions of inflammatory genes, including the gene encoding inducible NOS II [30].

It is probably true that exhaled NO is increased in asthma as a result of airway inflammation. There are several practical issues that can be inferred from this observation. Because NO production is increased in inflammatory diseases, measuring exhaled NO can therefore be viewed as a noninvasive, though indirect, means of detecting inflammation in the respiratory tract. Because NOS II is induced by several inflammatory cytokines (*e.g*. interleukin-1β, tumour necrosis factor-α, interferon-γ), monitoring exhaled NO is also an elegant way to assess the efficacy of anti-inflammatory agents, assuming that the more potent the

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medication, the lower the amount of exhaled NO. Consistent with this contention is the observation of a dose-dependent inhibitory effect of inhaled glucocorticoids on exhaled NO in asthmatic patients [27]. Although of considerable interest, the measurement of exhaled NO may be difficult to interpret in some instances, and there are a few questions that still need to be answered.

The first question relates to the sensitivity and specificity of the measurement of exhaled NO in detecting airway inflammation. There are at least two categories of patients in whom airways inflammation does not seem to be associated with high levels of exhaled NO. These are smokers [31] and patients with cystic fibrosis (CF) [32] who consistently have reduced exhaled NO in comparison with healthy subjects. NO and carbon monoxide are two major components of tobacco smoke. Both are putative inhibitors of NOS activity. Inhibition of NOS by cigarette smoke occurs at a post-translational level and probably accounts for the reduced NO production in smokers. The mechanism of reduced exhaled NO in CF patients is not known. Circumstantial evidence however suggests that accelerated oxidation, rather than reduced production, of NO is a likely mechanism responsible for the relatively low level of exhaled NO in CF patients [33]. Also, not all medications with putative anti-inflammatory properties result in a reduced exhaled NO in asthmatic patients. For example, it has been shown that the sulphidopeptide leukotriene receptor antagonists do not alter endogenous production of NO in asthmatic patients [34].

The second question relates to the physiology of endogenous NO with respect to its bronchodilatory effect. There is evidence to suggest that maintaining a certain level of NO production within the tracheobronchial tree is critical in modulating bronchial tone, especially when the airways are stimulated by bronchoconstrictor stimuli. Firstly, inhibition of NO production by various L-arginine analogues often aggravates bronchial hyperreactivity to various agents, including histamine [35], methacholine [36] and bradykinin [36]. Basal bronchomotor tone, however, does not seem to be affected by NOS inhibitors [37, 38]. Secondly, exhaled NO is increased during upper respiratory tract viral infections [39], but this increased production seems to be beneficial for asthmatic patients. This was first suggested by the demonstration that experimental virus-induced airway hyperresponsiveness was related to a relative deficiency in NO [40]. The protective role of NO in this condition is further supported by the recent finding that the greater an increase in exhaled NO, the lesser the severity of airway hyperresponsiveness to histamine following experimental rhinovirus infection in asthmatic subjects [41]. A stimulus such as nebulized spa water also causes an increase in exhaled NO which parallels an improvement of lung function in asthmatic children [42]. Evidence is therefore accumulating to suggest that endogenous NO is not only beneficial, but it may also be of particular importance when the bronchi are harmed by exogenous factors.

Such factors may simply be a strenuous exercise with its associated hyperventilation, which may cause exercise-induced asthma in susceptible individuals [43]. In this issue of the Journal, Therminarias *et al*. [44] have shown that airway obstruction is associated with a relative de-crease in NO output during exercise in eight healthy subjects breathing cold (-10°C) as compared with ambient (22°C) air. Due to the bronchodilatory effect of NO, it is tempting to speculate that such a relative deficiency in NO production may favour the occurrence of airway obstruction in these subjects. This protective effect is further supported by the recent demonstration that the release of endogenous NO by kinins inhibits the bronchoconstriction induced by cold air inhalation in guinea pigs [45]. We must, however, keep in mind that in the study conducted by Therminarias *et al*. [44], as in other studies [46–50], there is an increase from baseline of the actual NO output during exercise, even with cold air breathing. The explanation is therefore likely to be far more subtle than the mere hypothesis of a relative deficiency of a bronchodilator agent (namely endogenous NO) being responsible for the occurrence of bronchial obstruction.

There has been, during the last decade, a great deal of discussion about the putative beneficial and/or deleterious effect of NO. Even when NO seems to play a protective role as shown by Therminarias *et al*. [44], we still have dif-ficulty in putting forward an explanation for such a putative beneficial effect. Biological mechanisms underlying the ac-tion of NO in the human body are usually a complex matter. It is likely that the same conclusion also applies to the respiratory system. If complexity often appeals to the scientists, we now understand why NO has become such as appealing topic for chest physicians during the last 10 years.

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