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Modelling the production of nitric oxide within the human airways

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Modelling the production of nitric oxide within the human airways. R.A. Jörres. ©Eur Respir J 2000.
ABSTRACT: The measurement of exhaled nitric oxide (NO) is well established for monitoring of airway inflammation in bronchial asthma. It is known that the concentration of NO determined as steady state (plateau) value at a constant expiratory flow rate depends on the flow rate chosen. This suggests that the exhaled NO is released within the conducting airways, whereas alveolar NO levels are negligible. The processes involved can be described through a lung model comprising an alveolar compartment and an airway compartment thought of as a pipe. This concept has been formulated mathematically and the models proposed in the literature are essentially equivalent. NO plateau levels obtained at different flow rates allow the estimation of 1) an effective airway wall NO concentration that represents the driving force for NO release, 2) an airway diffusing capacity for NO which depends on factors impeding or facilitating NO transport, including an increase in NO-producing surface area. Clinical studies will have to assess whether the knowledge of these or related parameters offers a significant advantage over the determination of exhaled NO at a single flow rate.
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The measurement of exhaled nitric oxide (NO) has gained much popularity in the last few years [1] and this success resides mainly on two facts. On the one hand the exhaled NO seems to provide a sensitive marker of airway inflammation in asthma [2] and on the other hand its measurement is easy and fast, provided the required equipment is available. Irrespective of any controversy about the role that NO plays in asthma [3], there are a number of studies that demonstrated the concentration of exhaled NO to be associated with other more or less indirect markers of airway inflammation such as the percentage of eosinophils in induced sputum or the degree of nonspecific airway hyperresponsiveness [4].

Meanwhile, the methodological problems that mainly arose from contamination by nasal NO have been solved, and a standardized procedure of measurement has evolved, which is documented in recent recommendations [1]. This procedure involves expiration against a resistance at a constant pressure over ambient pressure, to ensure closure of the vellum and to achieve a constant flow rate. Whereas the vellum closure is an effective measure to exclude the nasal NO, the requirement of a constant flow rate allows the concentration of exhaled NO to reach a plateau value that is maintained over the major part of expiration [5].

The plateau values depend on the expiratory flow rate, with lower values occurring at higher flow rates [5]. Therefore, any information about the level of exhaled NO is only meaningful, if the corresponding flow rate is also given. In the literature, flow rates of up to 250 $mL \cdot s^{-1}$ have been chosen, but recent recommendations favour a lower value of 50 $mL \cdot s^{-1}$ in adults [1]. This is based on the argument that the contribution arising from the airways is larger at lower flow rates; therefore, in principle flow rates as low as possible should be used (see below). Of course there is a trade-off between the aim to measure at a low expiratory flow rate and the patient′s ability to master the corresponding breathing manoeuvre with sufficient accuracy. Intermediate flow rates such as 50 $mL \cdot s^{-1}$ seem to fulfil both of these requirements. The assertion that higher flow rates yield more stable readings is probably hampered by the fact that the increase in stability just reflects a decrease in sensitivity to assess bronchial NO production.

As NO values are commonly evaluated in a phenomenological manner, the flow dependence has been considered as a factor that interferes with measurements and therefore requires standardization to a fixed expiratory flow rate. Only a few authors have devoted their studies to the question of, which sort of information might be

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concealed within the flow dependence. This information, if sensible and worth investigating, would favour measurements over a range of expiratory flow rates instead of measurements at a single selected rate.

The step towards modelling

Owing to the fact that the diffusing capacity of the lung for NO is very high [6], it is to be considered unlikely *a priori*, that the exhaled NO arises from the alveolar space. Indeed, attempts to determine the origin of exhaled NO, which utilized either a comparison between the profiles of exhaled NO and carbon dioxide [7] or direct measurements by an intrabronchial catheter [8], have demonstrated that the exhaled NO originates primarily in the airways and not the alveolar space. This is also suggested by the fact that NO levels rapidly reach plateau values [5], since gases released in the alveolar space, such as CO_2, typically show a sloping curve and never reach a level that is to be called constant. The hypothesis that the exhaled NO has its source within the airways, also offered an explanation for the flow dependence, and this explanation was proposed at the time when flow dependence was first recognized [5, 9].

When the airways are considered as simple tubes, through which air is led at expiration, a higher flow rate produces a lower NO level, simply because it corresponds to a shorter transit time through the tube and thus a shorter time available to pick up NO from the airway wall. If there is no depletion of NO within the wall, steady state conditions will be reached rapidly and this will lead to plateau values that depend on flow rate.

Quite in contrast to the standard models of gas exchange, as used for CO_2 and O_2, the essentially new feature of this concept was, that gas exchange occurs within the airways themselves and is controlled by flow rate. The airways are not simply conducting tubes which mediate the flow of gases exchanged in the alveoli. Accordingly, processes occurring in the alveoli do not play a major role - these processes primarily depend on time and not on flow rate. One of the models, that were initially proposed, involved NO production and absorption in a peripheral compartment without explicit reference to the concept outline above [10], but later approaches invariably incorporated a bronchial and an alveolar compartment as well as expiratory flow rate as essential ingredients [11, 12].

Model approaches

The basic ideas outlined above have been sketched in a qualitative fashion by SILKOFF *et al.* [5] and worked out numerically at about the same time [9, 11, 13]. Part of these contributions are currently available only in abstract form [9, 13], others were based on rather general transport models [11, 14], that incorporated a multitude of adjustable parameters to account for processes potentially involved in NO transport. Therefore these models significantly added to our understanding but were not particularly suited for the analysis of measured data.

A concise formulation of a simplified model, contracted to a small number of physiologically plausible parameters, as well as useful proposals for their estimation have later been published by SILKOFF *et al.* [12], and the terminology adopted here does refer to this work. To avoid a wealth of technical detail the present article does not aim to supply a stringent mathematical formulation that can be found in the original papers, but is an attempt to give some insight into the physical and physiological reasoning underlying the models, and potentially to support their interpretation and application.

Elements of the nitric oxide transfer model

It is suggested to set up a lung model in the form of two compartments linked to each other. The first one is the alveolar compartment and the second one the bronchial compartment comprising the conducting airways as modelled by a single tube (fig. 1). Air from the alveoli is led through the airways at expiration and is enriched by NO from the airway walls during its passage. The local release of NO is proportional to the concentration difference between airway wall and lumen, as suggested by Fick′s law, which is known to be valid for a broad range of diffusion processes.

Hence, at each point of the tube there are two factors determining the local NO concentration. The first one arises from the flow that brings along NO-containing air from the part of the airway tube distal to the site under consideration. The second, is given by the local diffusion of NO from the airway wall into the lumen. According to this, the concentration at the outlet of the tube, *i.e.* the level of exhaled NO, is the cumulative result of longitudinal transport plus lateral diffusion of NO over the whole length. The initial value for this process is given by the NO concentration in the alveolar air that enters the tube (fig. 1).

Noteworthy enough, and as a consequence of its quite general character, the model bears close analogies to models encountered in other areas, *e.g.* to that of a heated tube through which fluid is led, which is warmed up during its passage [12]. The transfer of heat depends on the temperature gradient between the wall and the fluid, whereby the temperature of the fluid leaving the tube is the analogue of the concentration of exhaled NO.

Airway wall nitric oxide concentration

The source that supplies the flux of NO into the lumen is given by the concentration of NO in the airway wall (C_W). This concentration is expressed as the level of NO, that

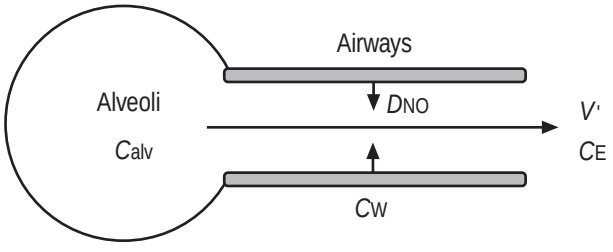

Fig. 1. – Two-compartment lung model for the analysis of exhaled nitric oxide. Air from the alveolar space, with NO concentration C_{alv}, is passed through the airways at flow rate $\dot{V}$. During passage, NO diffuses from the airway walls, where a fixed NO concentration of C_W is maintained, into the airway lumen. At the outlet of the bronchial tube the concentration of exhaled NO, that has accumulated, is C_E. The release of NO from the airway walls follows the local concentration gradient between wall and airspace according to Fick′s diffusion law. It can be characterized by the respective overall NO diffusing capacity of the airways, which is denoted by D_{NO}.

would be reached within the airspace after an infinitely long time of breathholding, or equivalently, at zero flow rate. Under these circumstances, the NO will be distributed between the airway tissue and the airway lumen according to the appropriate partition coefficients. This reasoning implies, that through measurements at zero flow rate, or possibly through extrapolation from very low flow rates, it might be possible to estimate the NO concentration within the airway wall.

Owing to the fact that the bronchial system has been lumped into a single tube, the airway wall concentration, which represents the driving force for NO diffusion, is to be considered as an average over the bronchial tree. It is denoted by C_W [12]. More refined analyses, in which this concentration is allowed to vary locally and in which additional compartments are introduced, show that the airway wall concentration is the result of an equilibrium between NO production through cellular sources and NO depletion including its diffusive loss into the airways [14]. C_W might therefore also be termed the "effective bronchial mucosal NO concentration".

Alveolar nitric oxide concentration

In contrast to breathholding, it can also be imagined that the expiratory flow is made extremely rapid, tending towards infinity. Under these circumstances there is virtually no time to pick up NO from the airway walls. Therefore, the NO concentration achieved within the alveolar airspace is transmitted unchanged to the outlet of the tube, *i.e.* measured at the mouth. As a consequence extremely high flow rates, or possibly extrapolation from the highest flow rates that are achievable in reliable measurements, might allow to estimate the alveolar NO concentration, C_{alv}.

Due to the high diffusing capacity for NO, it is reasonable to expect this value to be low. However, it seems worth mentioning that, even without the knowledge of diffusing capacity, this conclusion can be arrived at solely through analysis of the concentration-flow-curve, which shows a steep fall towards low values at high flow rates (fig. 2). For practical purposes, the concentration might even be assumed to be constant and very small, *e.g.* ~5 ppb, or zero [12].

Nitric oxide transport characteristics

As shown above, the airway wall NO concentration, C_W as well as the alveolar NO concentration, C_{alv}, are parameters that are directly measurable only under ideal conditions, either at zero flow rate, when equilibrium has been established after prolonged breathholding, or at infinitely high flow rates.

This reasoning relies on general principles and does not need a mathematical formulation. However, to estimate the airway wall and alveolar NO concentration from experimental data, requires extrapolation from the limited flow range available. A close inspection of data, that show exhaled NO *versus* flow rate, reveals that this extrapolation cannot be performed using standard tools such as straight lines. There appears to be no unique way to extend the curve to both sides of the measured values, and this is the site where the mathematical model enters. The quantitative analysis of the transport processes allows the derivation of a formula for extrapolation that is based on physical insight and therefore, distinguished from other approaches.

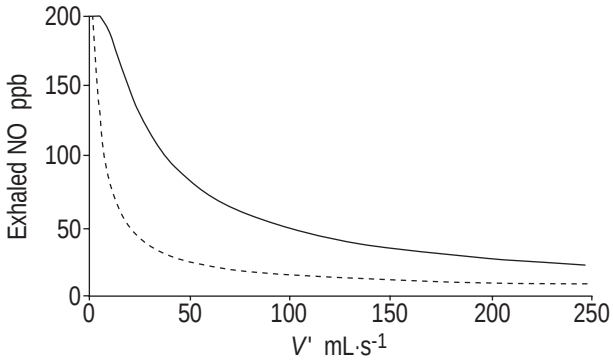

Fig. 2. – Two plots of plateau (steady state) levels of exhaled nitric oxide, C_E, *versus* expiratory flow rate ($\dot{V}$) (for convenience depicted in $mL \cdot s^{-1}$). At zero flow rate, the average NO concentration in the airway wall, C_W (200 ppb), is achieved, whereas for flow rate tending towards infinity the alveolar concentration, C_{alv} (5 ppb), is approached. The curve between these two values is given by the NO diffusing capacity, (D_{NO}). When the expiratory flow rate, $\dot{V}$, is equal to 1.44 D_{NO}, the flow-dependent part of exhaled NO has decreased to 50% of its initial value ($D_{NO}/\dot{V}=1n(2)$, see second term in Equation 2). Therefore, with larger values of the diffusing capacity, D_{NO}, *i.e.* with improved NO diffusion, the curve declines less steeply. Two examples with different D_{NO} (25 (—) and 5 $nL \cdot s^{-1} \cdot ppb^{-1} \times 10^{-3}$ (- - -) but equal C_W and C_{alv} (in ppb) are shown. The values chosen are indicated at the curves.

Of course there are different possibilities regarding the physicochemical mechanisms involved. One might argue that NO is released into the airway lumen at a constant rate like the fumes from a chimney. Then the amount of NO released would depend only on the time available for passage through the tube and the concentration of exhaled NO would be inversely proportional to the expiratory flow rate. This argument was implicitly associated with the notions of "NO excretion", "NO release" or "NO output", expressed as flow rate times NO concentration. However, measurements have clearly shown that NO output is not constant [5]. Instead, inspection of plots of NO concentration *versus* flow rate demonstrates that curves are more flat than a hyperbola. This suggests that the amount of NO released decreases at low flow rates, *i.e.* high NO concentrations within the airspace, and increases at high flow rates, *i.e.* low NO concentrations. At the same time, this observation is a strong argument, that the transport of NO is driven by concentration gradients, which depend on NO concentrations in the airway lumen.

Nitric oxide diffusing capacity of the airways

Indeed, according to its physicochemical properties, the release of NO is likely to occur by diffusion. Phenomenologically this process can be characterized by a diffusion constant describing the flux of NO from the airway walls into the lumen. This value represents an overall measure of bronchial diffusion characteristics and is termed "NO diffusing capacity of the airways" (D_{NO}) [12].

Two lungs may have the same airway wall NO concentration, C_W, and the same alveolar NO concentration, C_{alv}. Then the concentration-flow curves should start at the same NO level at zero flow and should approach the same limit for flow rate tending towards infinity. However, airway diffusing capacity for NO might differ between the

two lungs. A lower airway diffusing capacity implies that the transfer of NO into the lumen is impeded. It therefore takes more time to release a given amount of NO from the airway wall into the airspace; conversely, in a given time less NO is released. As a consequence the lung, which shows a smaller airway diffusing capacity, exhibits a steeper fall of its concentration-flow curve from its initial value, although the limiting value for extremely high flow rates remains the same (fig. 2). This suggests that the relative slope of the concentration-flow curve is a measure of the airway diffusing capacity for NO.

Diffusing capacity (D_{NO}) can be shown to depend on a number of factors including the magnitude of chemical conversion as well as competing transport processes - most of them representing sinks for NO. It should decrease with increasing thickness of airway wall fluid layers, such as mucus, that impede diffusion, and it should increase with increasing surface area available for NO release. It seems reasonable to expect that the surface area becomes smaller during bronchoconstriction, and this could explain the reduction of NO levels after methacholine inhalation [15]. At the same time the airway surface area involved in NO production might be increased in patients with asthma as compared to healthy subjects, as far as airway inflammation associated with NO production extends to more peripheral airways. Although all of these issues are at present highly hypothetical and require further study, the D_{NO}, must be considered as a potentially important characteristic of airway inflammation, in addition to the airway C_W.

Mathematical formulation of the model

The mathematical formulation of the processes described above leads to familiar forms of transport equations, expressed as (linear) partial differential equations; versions of these can be found in the original papers [11, 12]. Owing to the rationale of the present work to give an illustrative account of NO models, only the basic formula, that results for a two-compartment NO transfer model, will be given. If C_W denotes the average effective NO concentration in the airway wall, C_{alv} the average concentration in the alveolar airspace, D_{NO} the overall diffusing capacity of the airways for NO, and "exp" the exponential function, then the level of exhaled NO, C_E, at a constant expiratory flow rate, V', is given by:

$$C_E = C_W(1 - \exp(-D_{NO}/V')) + C_{alv} \exp(-D_{NO}/V') \quad (1)$$

In this formula the concentrations C_E, C_W and C_{alv} can be expressed in ppb, the flow rate V' in $L \cdot s^{-1}$ and the diffusing capacity D_{NO} in $nL \cdot s^{-1} \cdot ppb^{-1} \times 10^{-3}$ [12] (fig. 2).

The ratio D_{NO}/V' becomes larger the smaller V', is. Thus for flow rate towards zero, the exponentials also tend towards zero. As a consequence the second term involving C_{alv} becomes very small, emphasizing the minor role of the alveolar NO at low flow rates. The expression in parentheses becomes one, demonstrating that at low flow rates only NO from the airway wall, C_W, contributes to the exhaled NO. Conversely, when V' tends towards infinity, the ratio D_{NO}/V' tends towards zero and the exponential towards one. This demonstrates that at high flow rates C_E measures predominantly the alveolar NO level, C_{alv}. Therefore, Equation 1 exhibits all the qualitative properties that have been worked out above.

Is the model oversimplified?

Since the assumptions, that underly Equation 1, obviously involve major simplifications, it might be asked whether this formula is altered, when the model is rendered more realistic and in particular inhomogeneities along the bronchial tree are allowed for. A detailed mathematical analysis shows that inhomogeneities in the diffusing capacity of the airway wall lead to precisely the same equation, with D_{NO} replaced by a weighted average of local airway diffusing factors. In contrast, inhomogeneities in the airway wall concentration of NO, C_W, produce an additional term, which however is likely to be small in most circumstances.

Noteworthy enough, further refinement through inclusion of additional compartments, that represent diffusion barriers, chemical conversions *etc.*, also leads to Equation 1. This finding suggests that the parameters are resultant from multiple processes involved; they might be termed "effective" to indicate their phenomenological character. In either case the model seems to be fairly robust and efficient provided it is kept in mind that the parameters are average values over the bronchial tree, just as it is true, for example, for body plethysmography and airway resistance.

Estimation of model parameters

The model described above is conceptually valuable for understanding the mechanisms that determine the level of exhaled NO. Furthermore, it also provides a framework for unification of different approaches to NO measurement. In particular, it illustrates how flow dependence is accomplished and how measurements at different flow rates might yield additional information that is not visible in data measured at a single flow rate. Since C_{alv} always appear to be small, the respective term in the formula given above might be neglected or a constant value of 3–5 ppb might be inserted.

Parameters can be estimated directly using standard nonlinear fitting procedures that are available in all major commercial statistics packages. These procedures are commonly based on the least squares approach. If data at different flow rates show different variance, this can be taken into account through weighted least squares procedures. To illustrate the dependence on parameters, Equation 1 can be put into the equivalent form:

$$C_E = C_W - (C_W - C_{alv}) \times \exp(-D_{NO}/V') \quad (2)$$

When Equations 1 or 2 is multiplied by the flow rate V', the NO output, *i.e.* the amount of NO exhaled per unit time is obtained; it is denoted by q' [12]:

$$q' = V' \times C_E \quad (3)$$

This has been used in approximate linear regression analyses of q' against C_E to obtain estimates of D_{NO} and C_W using a limited range of data [12]. However, if all

parameters of the model are to be obtained with sufficient accuracy, still multiple measurements over a wide range of flow rates are needed, as in the direct approach through Equations 1 or 2.

Potential applications

The models offer the opportunity to estimate their parameters from measured data; therefore it is tempting to make use of this additional information in clinical or experimental studies. As models have been introduced rather recently, there has not been much work trying to reveal, whether parameters such as C_W, or D_{NO} provide information that adds to that supplied by measurements at a fixed flow rate. It has been suggested that the increase in bronchial diffusing capacity for NO in patients with asthma as compared to normal subjects indicates, that their NO production reaches farther into the lung periphery than in normal subjects [12]. C_W but not D_{NO} has been found to be reduced after treatment with inhaled beclomethasone in patients with asthma, and the maximum release rate of NO, as given by the product of C_W and D_{NO}, was correlated with baseline lung function and the degree of airway hyperresponsiveness. This prompted the authors to associate this parameter with an upregulation of NO production in nonadrenergic, noncholinergic nerves [12]. Despite their currently speculative nature, these hypotheses certainly open up fascinating perspectives for future studies.

It would also be challenging to test the role of airway diffusing capacity for NO through intervention studies, in which inflammation is selectively altered within central or peripheral airways. There has been one study which proposed a method for the determination of the bronchial volume, from which the exhaled NO is derived [13]; this method, relying upon so-called step responses, requires a fast NO analyser. A recent pilot study aimed at detecting a potential difference in NO production volumes after inhalation of low doses of beclomethasone either by conventional, CFC-driven, or by ultrafine, HFA-driven, aerosol [16]. There was a decrease in volume after HFA aerosol, which, however, did fail to reach statistical significance. But probably the approach deserves further study.

In other cases, the parameter estimates turned out to be either closely correlated with NO levels determined at a fixed flow rate or they posed difficulties owing to a lack of numerical robustness, *e.g.* when three parameters were estimated from five measurements [17]. The last issue appears to require special caution, since all models involve some sort of extrapolation, whose results critically depend on the reliability as well as on the range of values that have been measured. Even transformations that attempt to linearize data are not *a priori* guaranteed to exclude a bias; this is well known, *e.g.* from elementary models in enzyme chemistry. Furthermore, some of the advantages of the NO measurement, particularly those regarding cooperation and time, are lost, if lengthy and demanding measurements are needed. However, the situation might be not so much different from that encountered in the early times of body plethysmography, and time will tell.

Conclusion

The measurement of exhaled NO offers an easy way to assess one aspect of airway inflammation through a particular marker. Mathematical models, which incorporate bronchial NO production may help in the understanding of the conditions, under which NO is produced and NO production is altered in asthma. The models in particular postulate a relationship between the NO concentrations or NO output measured at the mouth and basic characteristics of the NO production system. All of the models that have been proposed share similar features and are to a large extent mathematically equivalent. They are based on physically sound principles such as common diffusion and transport equations and describe the data fairly well. Therefore, it seems justified to consider the issue of NO production within the airways as one that can be tackled with regard to modelling. Future studies will have to assess whether the additional information provided by the models is worthwhile to be assessed in clinical and experimental settings.

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